Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts

Support Information

Influence of Poultry Litter Land Application on the Concentrations of Estrogens in Water and Sediment within a Watershed

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Thirty two Pages

Six Tables

Four Figures

I. Experimental Section

Site Description. The Upper Satilla watershed is located in southeastern Georgia and occupies a total area of 2,922 km^2 with 440 poultry houses, and agricultural land accounts for approximately 23% of its land use (Fig. 1 and Table 1) (Vereen et al. 2007). The study area has dense dendritic stream networks with riparian wetlands along streams (Fig. S1). Small tributaries such as Seventeen Mile River, Hurricane Creek, and Pudding Creek flow into the Satilla River. Douglas (population 11,437) is the largest city in the study area.

We used the ArcGIS software to estimate the cotton/pasture land area in the drainage area of each sampling site based on the data collected from the USDA Farm Service Agency, ground truth surveys, and aerial photographs of the watershed. We first derived the catchment of each site from the USGS/DNR Hydrologic units (12-digit) map which delineated the Upper Satilla watershed into small subwatersheds (USGS/DNR 2001), and then combined it with 2008 Georgia Cropland Data Layer released by USDA in 2009 (USDA-NASS-RDD) to estimate the area of cotton/pasture lands in the drainage area of each site. The data is listed in Table 1.

Ten sample sites were selected to represent different land use and three more sites were chosen to collect samples from STP influent, effluent, and a pond through which the STP effluent passes before entering the river (Table 1) (Vereen et al. 2007). Site 1 was Rocky Creek with little or no agricultural activity. It was used as a control site in this study. Sites 2 and 3 were nested in subwatersheds where poultry litter was applied but few poultry houses existed. Sites 4 and 5 were located in subwatersheds where poultry litter was applied and large numbers of poultry houses existed. Sites 6 and 13 were sewage treatment plant influent and effluent, respectively. The effluent samples were collected at the point of discharge prior to flowing into the stream. Site 7 was from a pond where the sewage treatment plant effluent passes through.

Other sites were located along the main channel of the Satilla River and major tributaries. Sites 8 and 9 were on Seventeen Mile River. Sites 10 and 11 were on the Satilla River, respectively above and below the confluence with Seventeen Mile River, and Site 12 was on the main channel of the Satilla River, below the confluence of the Satilla River and Hurricane Creek. Sites 1, 2, 3, 4, 5, 8, and 10 were all upstream of the sewage treatment plant while Sites 7, 9, 11, and 12 were downstream of the sewage treatment plant.

Stream Flow Rate. We estimated flow rates of the studied streams based on the USGS real-time streamflow data (http://waterdata.usgs.gov/ga/nwis/current?type=flow&group_key=NONE). The flow rates for all sites but site 11 and 12 were estimated using the data directly from the staff gage in the streams. The site 11 flow rate was estimated by the flow rate data of a USGS station in Satilla River at GA 158, Georgia (02226362). For site 12, we estimated the flow rate by a station located at Waycross, GA (02226500), immediately downstream to site 12, with data adjusted by drainage areas, i.e. we divided the data by the Waycross drainage area (3108 km^2) and times the site 12 drainage area (2922 km^2).

Analysis of Water Quality Parameter. Two approximately 1-L of water samples were collected in sterile glass bottles from the middle of the stream channel. One was used for estrogen analysis described in the next section, and the other used for water quality measurements described following. Temperature (ºC), pH, dissolved oxygen (DO, mg/L), and turbidity (nephlometric turbidity units, NTU) were determined immediately using an YSI® 6600 Multiparameter Sonde (YSI Inc. Yellow Springs, OH). The water samples were stored on ice and transported to the laboratory and then stored in refrigerators within 6 hours of collection. The water samples were then immediately analyzed for chloride (Cl), solid, nitrate-nitrogen (NO3-N),

ammonia-nitrogen (NH4-N), orthophosphate, total phosphorus (total P), and total nitrogen (total N) at the University of Georgia's National Environmentally Sound Production Agriculture Laboratory Water Quality Laboratory using methods from the APHA Standard Reference guide for Water and Wastewater analyses (Eaton et al. 2005).

Sample Preparation for Analysis of Estrogens in Water. Aqueous samples were first filtered through 1.0-µm pore size glass fiber filters (Millipore GF/AFPF). The filters were dried at 103^{o}C to achieve constant weight prior to use. The filters with retained suspended solids were freeze dried and then weighed again. The weight difference before and after the filtration was used to estimate the mass of suspended solids and then to calculate the concentration of suspended solids (Table S2). The filters with suspended particles were then used for subsequent analysis of estrogen concentrations by methods described in the next section.

The filtrate was added with a few drops of 3 M H_2SO_4 to adjust the pH below 2 to improve recovery. Each subsample was then divided into two 400-mL replicate subsamples. Each subsample was loaded at approximately 5 mL/min into a C_{18} cartridge for extraction. Before loading, the cartridge was conditioned with 5 mL of methanol, 5 mL of deionized water, and then 5 mL of deionized water. After loading, the cartridge was washed with 5 mL of water and dried for 5 min. Each solid phase extraction cartridge was kept in a freezer at -20^{o}C until eluted. The estrogens were eluted into a 10-mL test tube using 5 mL of methanol. The test tube was placed in a 40^{o}C water bath and blown dry under a gentle stream of nitrogen gas. The sample was then reconstituted in 1 mL dichloromethane/hexanes (v/v, 1:1).The extract was then loaded onto a florisil cartridge for cleanup by a modified method reported by Chang et al. 2009. The cartridge was conditioned with 5 mL dichloromethane/methanol (v/v, 4:1) and then dichloromethane/hexanes (v/v, 1:1) prior to use. After loading, the cartridge was rinsed with 10

mL of dichloromethane/hexanes (v/v, 1:1), and the estrogens were eluted using 6 mL dichloromethane/methanol (v/v, 4:1). The solutions were evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1 ml methanol.

Sample Preparation for Analysis of Estrogens in Sediment and Suspended Particles. A 5-g freeze dried sediment sample was extracted with 10 mL extraction solvent dichloromethane/methanol (v/v, 2:1) in a 30-mL glass tube. The slurry was placed in an ultrasonic bath (Cavitator Ultrasonic Cleaner) for 30 min, and then centrifuged at 3000×g for 5 min. The supernatant was collected, and the extraction procedure was then repeated twice with 5 mL extraction solvent each time. The three extracts were combined and evaporated under a stream of nitrogen gas in a 40^{o}C water bath, then reconstituted in 10 mL hexanes.

The extract was transferred to a florisil cartridge that had already been rinsed with 5 mL hexanes. After the extract passed through the cartridge, 30 mL of hexanes was used to wash out the impurities. The cartridge was then eluted with 5 mL of methanol to collect the target compounds. The eluant was completely evaporated and reconstituted in 1 mL of methanol.

For suspended particle samples, the same extraction procedure was adopted except that each filter with retained suspended particles was first extracted with 6 mL solvent followed by two extractions with 3 mL solvent each.

LC/MS Analysis. A LC/MS procedure was used to determine estrogen concentrations. An HPLC (Waters Corp, Alliance 2690) with an Ascentic C_{18} reversed phase column (250 × 4 mm, 5 μm; Supelco, St. Louis, MO) was utilized to separate the estrogens. A mass spectrometer (Waters Micromass QuattroMass) with an electrospray source operated in the negative ion mode was used to detect the estrogens. The selected ion monitoring mode was used to identify and quantify estrogens. The LC-MS parameters were optimized. The capillary voltage was -2.98 kV,

cone voltage -53 kV, the source temperature 100^{o}C, and desolvation temperature 300^{o}C. The nebulizer was set at 36 L/h and desolvation gas at 367 L/h. The HPLC gradient consisted of water and acetonitrile at flow rate of 0.3 ml/min. Acetonitrile was 35% at 0 min and increased linearly to 65% at 21 min, held at 65% for 9 min, and increased to 100% at 31 min, then held at 100% for 4 min. A 4-min equilibration at 35% of acetonitrile was used at the end of each run.

Target compounds were identified by comparing the retention times (within 5%) with the standards by confirmation ions in the SIM: *m/z* = 269 for E1, *m/z* = 271 for E2, *m/z* = 287 for E3, and *m/z* = 295 for EE2. The retention time for E1 is 31.58 min, for E2 is 26.11 min, for E3 is 14.15 min, and for EE2 is 29.49 min. Each estrogen in sample was quantified by a calibration standard curve containing five points ($r^2 \geq 0.999$). Selected chromatograms for an un-spiked aqueous sample are shown in Fig. S3 which illustrates the typical retention times of the four estrogens.

Quality Control. For each aquatic sample, replicate were made by dividing one sample into two subsamples. Each sediment sample had replicates. Quality control samples include field blanks, trip blanks, and method blanks that were prepared along each sampling time, which were analyzed together with all other samples that were collected at the same sampling time. During LC/MS analysis, a mixture standard solution containing each chemical at 10 μg/L was analyzed to assure the accuracy and stability of the instrument. One solvent blank was measured after runs of every 10 samples to ensure that the instrument is free from contamination.

The recovery of the analytical method was calculated by the measured concentration of each estrogen in a spiked sample minus its concentration measured in the sample without spiking and then divided by the added concentration of this estrogen. To calculate the recovery for each estrogen in aquatic samples, one extra sample was collected with each sample batch and spiked

with a 0.9-mL mixture standard solution containing each estrogen at 0.2 mg/L to a 900-mL aquatic sample. The final concentration was 200 ng/L for each estrogen in the spiked aqueous sample. The recovery for solid samples was assessed by spiking a mixture standard containing 0.3 μg of each tested estrogen into a 3-g sediment sample. The spiked samples were subjected to the same sample treatment and analysis procedures as described above.

Detection limit was calculated as three times of the standard deviation, at zero concentration, of a blank sample in 20 independent measurements. Quantitation limits was defined as 3 times of the detection limits. The detection and quantitation limits of all target chemicals in water and solid samples are listed in Table S1.

Estrogen Concentrations and Water Quality Parameter. Many water quality parameters were measured and documented (Table S3). The results of ANOVA suggested there is no significant difference among sites in temperature, DO, pH, turbidity, solid, and total P, whereas NO_3-N, chloride, NH_4-N, orthophosphate, and total N changed with sampling sites (Table S7). The concentration of NO_3-N was higher in Site 3, 4, 5 than Site 9, 11, 8, 12, and 2 ($P < 0.05$). Total N concentrations ranged from 0.62 to 1.26 mg/L. Site 1 contains lowest mean concentration of total N while Site 4 was constantly above 1 mg/L ($P < 0.05$). The comparison of water quality parameters (Table S7) between pre-closure time (February to August 2009) and post-closure time (September 2009 to March 2010) reveals that the NO_3-N, NH_4-N, and total N were lower in post-closure samples than in pre-closure samples ($P < 0.05$).

Poultry litter contains a significant amount of phosphorus, part of which is present in orthophosphate form. Phosphate tends to attach to solid soil particles, so it tends to be retained by soil. However, when litter is applied on the surface of pasture, contact between litter and soil is limited, and the phosphates can be carried into stream waters with storm runoff. Soil erosion of

poultry litter amended fields can potentially introduce a considerable amount of particulate phosphate to streams (Pierson et al., 2001). Our regression analysis shows that estrogen concentrations seemed to increase as orthophosphate concentration increases at site 8 (R^2=0.9) and 11 (R^2=0.45) (Fig. 3.6). Animal wastes contain high concentration of NH_4-N that can easily transfer into water with runoff. The relationship between NH_4-N and estrogen concentrations was found at 11 (R^2=0.62) but not at site 8 (R^2=0.38). It needs to be noted that the above regression analysis only include those data above detection limits. If we included estrogen concentrations below detection limits into the regression analysis, no linear relationship was found between any water quality parameters and estrogen concentration. Therefore, whether orthophosphate or NH_4-N can be used as indicators of estrogen concentration cannot be determined based on the data we collected.

Phase Distribution of Estrogens. The Log Kd values for natural estrogens are listed in Table S8. The averaged LogKd values for E1 (3.55 ± 0.50) are in good agreement with other studies, while the Log Kd values for E2 and E3 were 2- to 3-fold higher than some other studies (Koh et al, 2009; Holthaus et al, 2002). But our results are close to the results reported by (Carballa et al. 2008) and (Braga et al. 2005).

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II. Tables and Figures

Table S1. Detection limits, quantitation limits, and analytical recoveries of estrogens in water and solid samples.

Compound	DL_w	QL_w	DL_s	QL_s	$Recovery_w$	$Recovery_s$
	ng/L	ng/L	ng/g	ng/g	% ± SD	% ± SD
E1	0.21	0.63	0.040	0.12	66 ± 5.9	89 ± 4.9
E2	0.87	2.6	0.16	0.48	96 ± 8.7	101 ± 2.0
E3	0.63	1.9	0.12	0.36	68 ± 6.6	74 ± 2.6
EE2	1.0	2.9	0.20	0.60	98 ± 8.0	91 ± 7.8

DL: detection limit. QL: quantitation limit. W: water sample. S: solid sample. SD: standard deviation.

Table S2 Concentrations of estrogens in water and suspended particle samples, stream flow rates, and concentrations of suspended particles in water samples.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Feb09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.01	2.50
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	0.754
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.07	1.21
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.30	1.80
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.23	4.53
6	10.2	67.2	125	bdl	bdl	bdl	bdl	bdl	na	73.5
7	5.68	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	6.97
8	7.06	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.69	3.93
9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	bdl	10.4	bdl	bdl	bdl	bdl	bdl	bdl	1.47	2.07
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.66	na
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.63	1.63
13	ns	ns	ns	ns	ns	ns	ns	ns	na	ns
Mar09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.02	7.90
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	5.45
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.08	2.70
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.45	34.3
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.25	5.00
6	bdl	8.89	4.63	bdl	bdl	bdl	bdl	bdl	na	30.9
7	7.72	7.32	bdl	bdl	bdl	bdl	bdl	bdl	na	4.32
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.81	5.47
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.86	4.05
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.60	3.12
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.99	3.94
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	5.06	3.71
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	2.31

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
MarS09										
1	bdl	62.0	bdl	bdl	555	19554	bdl	bdl	0.026	2.55
2	bdl	11.2	bdl	bdl	bdl	939	676	bdl	na	22.7
3	bdl	20.0	bdl	bdl	8.03	297	52.5	bdl	0.13	54.4
4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
5	bdl	bdl	bdl	bdl	26.2	bdl	bdl	bdl	0.34	17.3
6	19.1	63.0	19.6	4.62	bdl	105	bdl	bdl	na	78.8
7	ns	ns	ns	ns	ns	ns	ns	ns	na	ns
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.25	13.4
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.33	14.4
10	bdl	36.5	bdl	bdl	bdl	bdl	bdl	bdl	2.68	14.2
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	6.66	25.5
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	8.44	25.7
13	11.8	3.07	7.73	bdl	bdl	bdl	bdl	bdl	na	165
Apr09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.36	1.82
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	1.47
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.80	1.95
4	bdl	6.52	bdl	bdl	bdl	bdl	bdl	bdl	8.14	1.44
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.57	1.18
6	ns	ns	ns	ns	ns	ns	ns	ns	na	ns
7	2.18	2.74	bdl	bdl	bdl	bdl	bdl	bdl	na	92.0
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	17.30	14.2
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	18.40	4.13
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	16.17	6.63
11	bdl	22.7	bdl	bdl	bdl	bdl	Bdl	bdl	92.02	1.08
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	116.60	2.59
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	0.112

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
May09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.39	31.4
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	8.61
3	bdl	21.9	bdl	bdl	bdl	bdl	bdl	bdl	1.92	7.00
4	bdl	bdl	bdl	bdl	125	3832	bdl	bdl	9.61	3.93
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	6.20	5.70
6	2.92	30.8	81.7	bdl	7.94	37.3	bdl	bdl	na	87.5
7	bdl	bdl	bdl	bdl	bdl	182	bdl	bdl	na	25.8
8	bdl	15.5	bdl	bdl	bdl	bdl	bdl	bdl	17.42	6.64
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	19.53	9.48
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	39.36	9.73
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	97.90	6.86
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	124.06	8.73
13	bdl	bdl	bdl	bdl	45.3	bdl	bdl	bdl	na	13.2
Jun09										
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	bdl	bdl	bdl	bdl	bdl	104	135	bdl	0.02	18.6
4	bdl	bdl	bdl	bdl	bdl	Bdl	bdl	bdl	0.08	13.0
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	11.6	15.5	54.8	bdl	12.2	48.3	26.4	bdl	na	40.5
7	2.74	bdl	bdl	bdl	29.7	118	bdl	bdl	na	18.0
8	bdl	bdl	bdl	bdl	bdl	247	bdl	bdl	0.18	11.7
9	bdl	bdl	bdl	bdl	32.7	199	bdl	bdl	0.19	13.3
10	3.27	bdl	bdl	bdl	27.5	bdl	bdl	bdl	0.38	4.37
11	1.25	8.56	34.4	bdl	bdl	bdl	bdl	bdl	0.95	4.47
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.48	5.00
13	3.26	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	16.3

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Jul09										
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	2.70	12.9	bdl	bdl	23.5	148	bdl	bdl	0.06	15.3
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	12.7	8.20	23.7	7.60	bdl	51.6	19.8	bdl	na	56.9
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	25.2
8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.26	5.58
11	1.70	6.40	bdl	bdl	bdl	bdl	bdl	bdl	0.66	1.12
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.22	4.97
13	3.60	bdl	bdl	5.50	bdl	bdl	bdl	bdl	na	6.04
Aug09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.01	0.744
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	21.9	bdl	bdl	bdl	bdl	bdl	bdl	0.59	8.78
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	46.4	25.2	47.2	bdl	bdl	bdl	bdl	bdl	na	168
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	4.70
8	3.71	13.0	bdl	bdl	bdl	7.78	bdl	bdl	1.25	3.54
9	8.09	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.33	7.83
10	bdl	9.33	bdl	bdl	bdl	bdl	bdl	bdl	2.68	8.71
11	2.25	4.30	9.27	bdl	bdl	277	bdl	bdl	6.67	6.46
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	8.55	24.9
13	8.19	13.4	bdl	bdl	bdl	bdl	bdl	bdl	na	15.3

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Sep09										
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.1	1.88
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	11.3	30.1	65.1	5.19	bdl	6.62	bdl	bdl	na	167
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	25.5
8	bdl	bdl	23.0	bdl	bdl	bdl	167	bdl	0.21	12.1
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.23	0.538
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.45	4.56
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.13	0.216
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.29	3.47
13	bdl	bdl	bdl	5.69	bdl	bdl	bdl	bdl	na	1.68
Oct09										
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.02	4.73
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	7.23	15.7	75.7	bdl	bdl	bdl	bdl	bdl	na	92.5
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	0.162
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.04	8.07
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.05	1.1
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.09	0.371
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.23	0.158
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.72	0.753
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	5.28

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Nov09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0	14.7
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	bdl	12.3	bdl	bdl	bdl	bdl	bdl	bdl	0.02	2.05
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	3.12	14.9	84.7	bdl	bdl	bdl	bdl	bdl	na	141
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	2.58
8	bdl	bdl	18.9	bdl	bdl	bdl	bdl	bdl	0.05	60.7
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.05	6.35
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.1	15.4
11	bdl	13.1	bdl	bdl	bdl	bdl	bdl	bdl	0.25	1.09
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.64	40.0
13	6.59	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	2.96
Dec09										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.01	3.40
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.47	13.0
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.11	
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.87	7.56
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	20.2	45.8	27.2	8.60	15.0	13.3	bdl	bdl	na	173
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	3.63
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.01	6.15
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.95	2.64
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.94	1.72
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	9.80	1.24
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.83	10.1
13	8.80	19.8	bdl	bdl	bdl	bdl	bdl	bdl	na	5.32

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Jan10										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.05	1.11
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.43	4.16
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.23	1.52
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.95	1.30
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.85	0.223
6	25.3	29.7	61.2	bdl	bdl	bdl	bdl	bdl	na	96.6
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	2.23
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.21	7.32
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.15	9.15
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.33	0.323
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	10.76	0.656
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	16.03	0.745
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	1.21
Feb10										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.26	0.639
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.85	1.37
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.91	3.78
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.11	3.02
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	3.73	2.49
6	26.5	bdl	37.6	5.24	bdl	bdl	bdl	bdl	na	81.4
7	bdl	bdl	bdl	bdl	280	bdl	bdl	bdl	na	1.73
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	8.73	4.89
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	9.27	3.63
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	18.67	3.16
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	46.44	1.30
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	81.55	4.47
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	1.31

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S2. Continuation from before.

Month	Water (ng/L)				SS (ng/g)				Flow Rate	SS conc.
Site	E1	E2	E3	EE2	E1	E2	E3	EE2	(m^3/s)	(mg/L)
Mar10										
1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.10	0.573
2	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.98	0.772
3	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.45	0.875
4	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.05	1.87
5	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	1.60	2.76
6	4.07	bdl	5.75	bdl	bdl	bdl	bdl	bdl	na	93.7
7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	17.3
8	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.35	8.44
9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	4.62	10.3
10	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	9.31	9.01
11	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	23.16	1.10
12	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	30.35	2.30
13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	na	5.17

ns: no sample. SS: suspended particles. Conc.: concentration. na: not applicable. MarS09: March storm 2009. bdl: below detection limit.

Table S3. Water quality parameters.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Feb09													
1	11.42	1.42	0.00	9.05	2.10	0.02	6.87	0.008	0.019	0.09	0.40	61.8	21.3
2	12.75	0.85	0.00	9.32	2.23	0.00	13.29	0.025	0.002	0.04	0.81	291	81.8
3	10.81	1.14	0.00	13.50	4.77	0.02	16.85	0.007	0.023	0.26	0.61	181.4	100
4	10.95	2.18	0.00	13.30	1.34	0.10	18.19	0.015	0.097	0.07	0.86	223.8	230
5	12.60	0.84	0.00	9.04	2.76	0.06	14.49	0.057	0.057	0.05	0.79	139.4	108
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	52000	5200000
7	17.84	1.04	0.00	11.56	21.05	4.71	118.22	0.855	0.170	0.90	4.33	121.8	99
8	9.73	2.25	0.00	13.52	1.41	0.05	13.38	0.014	0.016	0.06	0.67	176.4	70
9	8.74	1.95	0.00	14.14	1.78	0.03	13.33	0.016	0.009	0.06	0.62	109.4	54
10	9.36	2.15	0.00	9.47	1.67	0.02	15.30	0.003	0.014	0.01	0.74	132.6	66
11	9.25	2.51	0.00	14.33	1.44	0.03	16.59	0.035	0.011	0.08	0.67	79.4	70
12	9.55	2.39	0.00	9.84	1.54	0.03	15.64	0.038	0.014	0.34	0.67	109.4	54
13	18.16	0.67	56.10	11.59	1.38	13.28	122.66	1.075	0.069	1.43	10.13	2187	901
Mar09													
1	13.94	11.26	4.00	7.86	3.32	0.00	6.69	0.017	0.021	0.05	0.50	344.4	110
2	15.84	5.51	4.00	7.79	7.00	0.01	14.97	0.056	0.117	0.06	1.23	142.4	108
3	15.15	7.19	4.00	7.92	3.00	0.02	16.36	0.008	0.126	nd	nd	215.2	200
4	18.60	139.65	1.10	nd	3.08	0.05	16.42	0.077	0.167	nd	nd	49.2	66
5	15.90	9.24	5.60	7.99	3.31	0.06	17.04	0.105	0.117	0.40	0.97	305.8	310
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0	0
7	20.25	12.95	1.50	nd	21.65	1.60	96.88	0.795	0.132	0.98	2.38	172.4	182
8	17.54	142.68	0.80	nd	4.00	0.06	14.46	0.021	0.158	0.04	1.01	140.6	88
9	17.58	154.06	1.20	nd	3.67	0.08	14.47	0.023	0.151	0.03	1.09	229	100
10	18.44	181.23	1.40	nd	4.49	0.03	15.88	0.017	0.111	0.08	1.21	120.2	88
11	18.74	204.55	0.30	nd	3.61	0.04	17.49	0.076	0.059	0.40	0.98	24.4	40
12	18.67	207.63	1.80	7.76	4.50	0.04	16.68	0.057	0.060	0.12	0.92	31.6	34
13	20.16	10.93	578.5	7.77	1.41	5.57	125.03	0.251	0.071	0.35	5.02	10.4	90

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
MarS09													
1	15.81	5.97	12.90	5.65	5.16	0.05	5.09	0.018	0.032	0.04	0.75	754	570
2	nd	nd	nd	nd	24.03	0.08	6.48	0.050	0.050	0.09	0.87	7701	5100
3	16.21	6.51	112.9	7.34	26.36	0.26	8.33	0.051	0.071	0.08	1.00	5794	5700
4	16.99	0.02	42.60	6.33	28.87	0.12	8.79	0.066	0.053	0.11	1.16	14136	TNTC
5	16.20	4.35	611.6	5.50	15.45	0.60	10.10	0.179	0.078	0.23	1.70	14136	TNTC
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	17300	190909.1
7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
8	16.59	0.34	30.10	6.54	15.32	0.12	7.47	0.020	0.033	0.05	0.82	12033.	TNTC
9	16.64	0.03	22.00	6.42	14.13	0.08	5.88	0.011	0.032	0.04	0.87	17328.	TNTC
10	17.31	0.00	38.00	6.84	18.43	0.23	8.43	0.100	0.045	0.14	1.21	17328.	TNTC
11	17.61	0.01	19.80	6.48	14.91	0.12	10.36	0.039	0.040	0.07	0.96	6488	TNTC
12	18.02	0.00	28.00	6.29	24.25	0.11	8.37	0.031	0.036	0.06	0.88	17328.	TNTC
13	19.22	0.01	57.30	6.99	24.34	1.10	23.19	2.532	2.131	3.18	3.90	111985	180000
Apr09													
1	15.37	9.03	2.00	7.22	3.51	0.02	4.08	0.015	0.029	0.01	0.53	544.6	173
2	16.24	5.35	6.30	7.31	4.93	0.02	7.19	0.069	0.277	0.06	1.20	222.4	81.1
3	16.08	6.35	5.60	7.77	3.74	0.27	9.78	0.012	0.178	0.02	1.07	148.8	117
4	19.00	5.85	5.20	6.93	3.44	0.16	8.00	0.100	0.192	0.09	1.28	146	135
5	16.27	7.87	4.30	7.41	4.57	0.11	7.63	0.078	0.120	0.10	1.14	262.6	171
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	217800	2100000
7	23.36	8.41	2.80	7.63	73.18	2.44	68.14	0.280	4.620	0.48	6.19	313	1300
8	17.68	6.46	345.6	7.74	6.66	0.12	7.84	0.050	0.130	0.03	1.04	86	72.1
9	17.60	6.16	9.10	7.61	6.36	0.12	7.72	0.038	0.136	0.03	1.07	74	27
10	21.58	9.67	0	7.76	9.87	0.08	7.61	0.024	0.170	0.03	1.17	86	63.1
11	18.46	5.45	338.7	7.17	5.65	0.08	7.47	0.048	0.095	0.05	1.05	20	54
12	18.72	5.59	5.80	6.84	5.79	0.07	7.35	0.031	0.076	0.04	1.01	20	36
13	21.13	8.73	786.2	7.50	2.91	4.85	85.60	0.027	6.885	0.13	8.73	1607	3700

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity UnitNO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
May09													
1	20.93	7.52	234.9	7.02	5.42	0.09	6.63	0.021	0.051	0.01	0.77	324.8	118
2	21.63	5.47	13.10	6.65	7.19	0.17	6.44	0.067	0.120	0.09	1.10	357	220
3	21.41	6.11	98.70	7.14	4.52	0.32	10.41	0.017	0.097	0.02	0.99	151.8	191
4	22.04	5.29	7.70	6.91	3.79	0.11	6.95	0.125	0.073	0.14	1.25	204.4	260
5	21.53	6.10	7.00	6.64	6.75	0.07	8.96	0.036	0.046	0.09	1.10	434.4	700
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	193500	600000
7	25.59	5.66	6.20	7.23	23.05	1.99	25.54	0.488	0.146	0.61	1.48	48.6	390
8	21.41	6.05	227.1	7.35	4.31	0.09	8.40	0.029	0.050	0.06	1.07	176.8	164
9	21.41	5.71	10.70	7.02	4.42	0.09	8.20	0.016	0.040	0.05	1.07	202.8	230
10	22.05	5.51	8.70	6.96	4.96	0.11	7.07	0.050	0.054	0.10	1.17	278.2	270
11	22.16	4.74	5.50	6.71	2.48	0.06	7.85	0.019	0.033	0.06	1.14	176	106
12	22.51	4.70	5.80	6.55	2.76	0.06	7.82	0.014	0.028	0.07	1.11	99.2	82
13	23.83	8.08	32.70	7.18	7.59	2.44	29.86	0.508	0.096	0.72	3.03	167.2	1545
Jun09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	25.49	1.69	8.60	5.89	16.05	0.03	12.45	0.026	0.106	0.02	0.97	nd	nd
4	26.86	1.66	7.00	6.72	12.95	0.04	10.37	0.110	0.211	0.12	1.74	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	30.69	8.82	192.8	6.47	15.51	17.37	91.86	1.823	17.302	2.03	32.80	nd	nd
8	27.56	2.25	34.00	5.94	2.80	0.04	11.84	0.037	0.401	0.01	1.28	nd	nd
9	25.29	3.65	8.60	5.90	4.20	0.16	26.43	0.153	0.143	0.15	0.98	nd	nd
10	nd	nd	nd	nd	6.19	0.03	12.13	0.065	0.471	0.03	1.41	nd	nd
11	28.40	3.02	nd	6.90	0.55	0.10	13.27	0.084	0.103	0.07	1.32	nd	nd
12	30.29	3.85	nd	5.67	3.57	0.10	13.77	0.077	0.082	0.06	1.16	nd	nd
13	28.31	7.05	199.7	6.30	4.40	17.75	98.93	1.647	0.042	1.61	16.74	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Jul09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	26.41	2.73	15.40	6.69	20.85	0.02	9.09	0.077	0.024	0.16	1.45	19.4	72.1
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	313000	3500000
7	27.26	7.06	7.00	7.93	30.47	3.93	99.95	1.439	0.027	1.43	1.33	41	1273
8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
9	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
10	24.92	1.76	2.30	6.28	2.51	0.04	7.46	0.057	0.181	0.01	1.03	12.6	19.7
11	26.68	3.94	1.80	5.50	4.69	0.07	9.57	0.055	0.040	0.03	1.16	23.6	46
12	27.36	4.92	2.90	5.60	5.67	0.04	9.88	0.067	0.027	0.03	0.96	22	16.4
13	27.41	8.01	0.20	8.13	8.76	9.57	99.39	1.081	0.033	1.19	7.67	880	1364
Aug09													
1	24.55	4.34	6.60	5.48	2.26	0.02	12.19	-0.005	0.008	0.02	0.63	54.4	56
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	24.99	4.54	1.70	5.87	2.71	0.18	11.20	0.044	0.038	0.07	1.40	209.8	310
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	410600	4900000
7	27.86	5.88	4.00	7.10	50.88	4.89	42.90	0.170	0.232	0.35	5.37	341	1909
8	24.18	4.68	4.40	6.56	3.46	0.06	8.04	0.037	0.020	0.02	0.97	172.5	164
9	24.15	4.45	3.90	6.10	3.03	0.05	8.89	0.011	0.013	0.02	0.93	71.4	173
10	24.52	4.83	2.10	5.75	2.96	0.11	9.27	0.002	0.028	0.02	1.18	123.6	104
11	25.29	5.02	7.40	5.52	4.84	0.05	14.38	0.020	0.023	0.04	1.03	111.2	270
12	26.67	5.26	4.10	5.70	6.78	0.08	10.48	0.031	0.040	0.06	1.24	116	470
13	28.08	7.86	6.70	8.06	2.27	1.15	54.97	0.115	0.003	0.14	1.01	5710	5700

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Sep09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	27.04	1.22	9.90	6.47	3.34	0.02	24.99	0.112	0.175	0.10	1.15	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	26.82	2.95	9.50	6.95	16.21	9.40	66.35	1.002	0.152	1.03	6.35	nd	nd
8	25.88	4.74	18.10	6.28	9.49	0.03	11.08	0.047	0.067	0.01	1.17	nd	nd
9	27.28	6.57	2.90	7.05	10.26	0.15	45.71	0.649	0.007	0.66	0.62	nd	nd
10	27.33	2.13	11.70	5.96	4.60	0.11	9.67	0.041	0.031	0.04	1.16	nd	nd
11	28.26	5.05	3.70	5.96	2.33	0.08	9.86	0.060	0.036	0.05	1.16	nd	nd
12	28.00	5.88	8.50	5.87	4.32	0.10	10.38	0.060	0.033	0.11	1.07	nd	nd
13	27.32	8.58	0.60	7.82	0.39	14.54	73.67	0.869	0.016	0.88	13.32	nd	nd
Oct09													
1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	23.55	3.26	11.30	6.67	9.53	0.01	23.26	0.082	0.164	0.05	1.26	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	24.36	2.73	2.90	7.15	3.76	5.88	58.81	0.880	8.583	0.96	12.56	nd	nd
8	22.40	4.37	30.00	6.14	156.45	0.01	5.92	0.031	0.016	0.02	1.21	nd	nd
9	21.14	4.33	10.70	6.74	25.81	0.03	40.40	0.506	0.006	0.46	0.60	nd	nd
10	21.94	2.31	5.90	6.37	6.39	0.03	6.34	0.045	0.089	0.03	0.91	nd	nd
11	23.68	3.86	16.50	6.02	3.22	0.06	10.09	0.084	0.054	0.06	0.92	nd	nd
12	23.93	5.91	5.40	5.94	2.71	0.02	8.94	0.061	0.027	0.04	0.72	nd	nd
13	25.39	8.07	1.60	7.77	1.44	2.66	68.95	0.108	3.035	0.13	4.66	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Nov09													
1	18.23	6.81	5.80	6.58	5.53	0.01	15.56	0.023	0.018	0.00	0.65	nd	nd
2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
4	18.64	4.56	20.40	6.77	14.23	0.04	21.91	0.032	0.110	0.06	1.13	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	21.08	4.57	12.50	7.20	134.00	2.77	52.33	0.567	1.572	0.60	4.28	nd	nd
8	18.38	2.86	7.40	6.77	6.97	0.02	13.00	0.044	0.017	0.05	0.95	nd	nd
9	18.49	4.39	4.40	6.82	17.13	0.02	21.67	0.106	0.014	0.13	0.71	nd	nd
10	17.22	3.60	3.20	6.56	2.43	0.03	8.07	0.035	0.015	0.02	0.80	nd	nd
11	18.93	7.15	3.50	6.92	1.47	0.02	18.34	0.042	0.024	0.04	0.63	nd	nd
12	19.48	8.22	3.00	6.80	1.21	0.02	18.22	0.034	0.013	0.03	0.57	nd	nd
13	22.89	8.84	3.00	7.90	1.87	1.03	90.66	0.767	0.011	0.77	0.68	nd	nd
Dec09													
1	13.99	8.97	6.90	6.12	2.98	0.35	11.49	0.027	0.019	0.02	0.66	nd	nd
2	14.23	3.73	22.00	5.73	8.42	0.02	9.12	0.033	0.020	0.01	0.81	nd	nd
3	13.80	7.33	9.80	6.39	4.10	0.09	15.19	0.009	0.013	0.01	0.74	nd	nd
4	11.93	7.97	18.70	6.68	6.11	0.21	14.97	0.019	0.026	0.02	1.02	nd	nd
5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	17.46	7.42	23.20	6.58	9.79	3.81	22.17	0.211	0.776	0.81	4.61	nd	nd
8	11.44	8.22	10.50	6.09	8.72	0.02	10.95	0.025	0.015	0.04	0.68	nd	nd
9	11.91	7.43	8.70	5.99	7.93	0.04	11.71	0.010	0.017	0.05	0.75	nd	nd
10	12.68	8.15	15.10	6.61	8.65	0.24	11.65	0.008	0.019	0.00	1.00	nd	nd
11	13.50	8.18	18.70	6.24	8.68	0.07	11.22	0.015	0.016	0.02	0.84	nd	nd
12	13.71	8.55	18.10	6.51	9.05	0.09	12.34	0.023	0.016	0.04	0.77	nd	nd
13	19.84	9.65	11.00	7.21	10.15	4.88	45.09	0.230	0.307	0.32	5.14	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Jan10													
1	2.80	13.13	4.00	7.21	13.58	0.42	7.09	0.027	0.014	0.00	0.69	nd	nd
2	1.98	11.65	2.50	6.66	12.10	0.12	14.28	0.038	0.020	-0.01	0.73	nd	nd
3	1.08	13.26	3.10	8.77	13.71	0.82	13.61	0.014	0.038	0.00	1.06	nd	nd
4	2.63	12.23	6.80	7.41	12.68	1.10	13.76	0.009	0.033	0.01	1.46	nd	nd
5	2.31	12.88	4.30	6.68	13.33	0.41	14.66	0.026	0.025	0.01	0.97	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	9.93	12.66	4.20	7.06	2.19	4.11	46.72	0.036	0.065	0.11	3.92	nd	nd
8	2.64	13.47	3.10	6.67	1.20	0.19	11.84	0.007	0.016	0.00	0.63	nd	nd
9	2.93	13.35	3.20	6.50	1.30	0.19	11.82	0.028	0.022	0.00	0.64	nd	nd
10	2.17	13.03	4.30	6.99	1.55	0.33	11.90	0.002	0.031	0.01	0.86	nd	nd
11	2.61	13.12	4.00	6.40	1.61	0.21	12.39	0.017	0.021	0.01	0.84	nd	nd
12	2.65	12.57	4.00	6.31	1.65	0.19	12.37	0.001	0.019	0.00	0.82	nd	nd
13	14.00	10.76	0.80	7.48	0.67	4.58	57.75	0.003	0.010	0.04	4.41	nd	nd
Feb10													
1	9.28	10.93	17.00	6.33	1.93	0.26	5.45	0.031	0.039	0.01	0.73	nd	nd
2	9.64	8.74	3.40	5.40	1.81	0.09	9.00	0.044	0.041	0.03	0.92	nd	nd
3	9.15	10.33	8.60	7.89	2.82	0.61	9.43	0.018	0.045	0.01	1.05	nd	nd
4	10.81	9.16	7.90	6.03	3.10	0.52	9.44	0.023	0.061	0.04	1.26	nd	nd
5	10.82	10.08	4.40	6.18	2.79	0.26	9.94	0.037	0.048	0.05	1.06	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	16.11	8.95	4.30	6.90	3.40	4.43	26.19	0.091	0.635	0.13	5.28	nd	nd
8	10.43	10.68	7.10	6.70	3.17	0.18	8.73	0.017	0.038	0.02	0.70	nd	nd
9	11.23	10.46	7.60	6.53	2.53	0.16	8.75	0.012	0.038	0.02	0.76	nd	nd
10	10.66	9.28	6.90	6.30	2.55	0.23	8.80	0.008	0.046	0.08	0.83	nd	nd
11	10.73	8.88	5.50	5.99	2.34	0.16	8.64	0.009	0.041	0.03	0.90	nd	nd
12	10.90	8.93	5.60	5.95	2.10	0.14	8.63	0.022	0.042	0.02	0.93	nd	nd
13	15.07	10.87	117.6	6.79	0.94	4.03	32.85	0.034	0.122	0.03	4.10	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S3. Continuation from before.

Month Site	T °C	DO mg/L	Turb NTU	pH	Solid mg/L	NO_3-N mg/L	Cl mg/L	OrthoP mg/L	NH_4-N mg/L	Total P mg/L	Total N mg/L	*E. coli* MPN	*fecal coli* MPN
Mar10													
1	7.74	11.62	4.50	6.18	1.72	0.11	4.84	0.026	0.022	0.00	0.48	nd	nd
2	8.04	10.71	3.50	5.85	1.93	0.02	9.13	0.043	0.024	0.02	0.75	nd	nd
3	7.47	10.56	6.40	7.95	3.74	0.48	10.28	0.019	0.034	0.01	0.89	nd	nd
4	8.91	10.63	28.00	6.21	3.50	0.57	10.04	0.020	0.040	0.03	1.20	nd	nd
5	8.95	10.78	6.10	6.49	2.90	0.26	11.15	0.043	0.030	0.03	1.01	nd	nd
6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
7	13.35	11.32	16.80	6.79	34.50	3.18	27.44	0.071	0.440	0.12	3.92	nd	nd
8	9.18	10.68	6.00	6.70	2.48	0.16	9.04	0.021	0.026	0.01	0.65	nd	nd
9	10.01	11.61	52.00	6.72	2.70	0.15	9.17	0.017	0.022	0.02	0.71	nd	nd
10	8.79	10.37	26.90	6.37	3.10	0.23	9.29	0.016	0.026	0.01	0.85	nd	nd
11	9.52	9.94	6.10	6.04	2.71	0.13	8.94	0.016	0.026	0.02	0.88	nd	nd
12	9.49	9.85	10.50	6.08	2.85	0.12	8.92	0.037	0.031	0.02	0.81	nd	nd
13	14.95	11.22	4.20	7.13	1.81	6.67	47.60	0.064	0.022	0.04	6.18	nd	nd

T: temperature. DO: dissolved oxygen. Turb: turbidity. NTU: Nephelometric Turbidity Unit. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen. Total P: total phosphorus. Total N: total nitrogen. TNTC: too numerous to count. nd: not determined. ns: no sample.

Table S4. Concentrations of estrogens in sediment.

Month	Sediment (ng/g)			
Site	E1	E2	E3	EE2
Jan09				
1	bdl	bdl	bdl	bdl
2	bdl	bdl	bdl	bdl
3	bdl	bdl	0.97	bdl
4	1.06	bdl	bdl	bdl
5	bdl	bdl	1.74	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns
Apr09				
1	bdl	bdl	bdl	bdl
2	2.41	bdl	4.95	bdl
3	bdl	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl
5	bdl	bdl	0.75	bdl
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	1.13	bdl	0.23	bdl
9	0.28	bdl	1.62	bdl
10	0.31	bdl	0.34	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns

bdl: below detection limit. ns: no sample.

Table S4. Continuation from before.

Month	Sediment (ng/g)			
Site	E1	E2	E3	EE2
Jun09				
1	ns	ns	ns	ns
2	ns	ns	ns	ns
3	0.17	bdl	bdl	bdl
4	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	0.24	bdl	0.3	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns
Oct09				
1	ns	ns	ns	ns
2	ns	ns	ns	ns
3	ns	ns	ns	ns
4	bdl	bdl	bdl	bdl
5	ns	ns	ns	ns
6	ns	ns	ns	ns
7	ns	ns	ns	ns
8	bdl	bdl	bdl	bdl
9	bdl	bdl	bdl	bdl
10	bdl	bdl	bdl	bdl
11	bdl	bdl	bdl	bdl
12	bdl	bdl	bdl	bdl
13	ns	ns	ns	ns

bdl: below detection limit. ns: no sample.

Table S5. P values result from ANOVA on the means by time, or means by site.

*: significant difference at the 0.05 probability level. nd: not determined.
The "means by site" is defined as the sum of the concentrations of one estrogen of all sampling times divided by the number of sample times. The "means by time" is equal to the sum of the concentrations of one estrogen of all agricultural site (all sites except the sites 6, 7, and 13 that are associated with STP) divided by the number of agricultural site.

Chemical	P-value					
	Stream water		Suspended Particles		Sediment	
	Month	Site	Month	Site	Month	Site
E1	0.009*	0.473	0.558	0.414	0.380	0.284
E2	0.041*	0.568	0.579	0.392	nd	nd
E3	0.417	0.325	0.477	0.192	0.555	0.127
EE2	nd	nd	nd	nd	nd	nd

Table S6. P values result from ANOVA on the means of the sum of each estrogen concentration averaged by sampling times of pre-closure against that of post-closure periods.

Close Event	p-value			
	E1	E2	E3	EE2
STP Influent [a]	0.248	0.0503	0.659	0.439
Agricultural Sites [b]	0.0225*	0.00650*	0.934	nd

*: significant difference at the 0.05 probability level.
[a]: the means of the sum of each estrogen concentration averaged by sampling times at Sites 6 reflecting the influence of broiler processing plant.
[b]: the means of the sum of each estrogen concentration averaged by sampling times at Sites 1, 2, 3, 4, 5, 8, 9, 10, 11, and 12, reflecting the influence of poultry houses.
nd: not determined.

Table S7. P values result from ANOVA on the means of water quality parameters over all sampling times for each site and estrogen concentrations summed for pre-closure against that for post-closure periods

P	T	DO	pH	Turb	Solid	NO_3-N
Site	0.0772	0.980	0.627	0.524	0.569	0.0012*
	< 0.0001*	0.0827	0.9883	0.0577	0.672	0.0001*

P	Cl	OrthoP	NH_4-N	Total P	Total N
Site	0.00650*	0.0482*	0.0252*	0.123	< 0.0001*
	0.0624	0.681	< 0.0001*	0.0521	< 0.0001*

*: significant difference at the 0.05 probability level. T: temperature. DO: dissolved oxygen. Turb: turbidity. NO_3-N: nitrate-nitrogen. Cl: chloride. OrthoP: orthophosphate. NH_4-N: ammonia-nitrogen Total P: total phosphorus. Total N: total nitrogen.

Table S8. Log K_d values estimated in this study and reported by other references

Estrogen	Our study	Koh et al.[a]	Carballa et al.[b]	Braga et al.[c]	Holthaus et al.[d]
E1	2.63-4.03	1.99-2.53	2.77-2.90	2.73	nd
E2	2.39-5.50	1.11-2.78	4.02-4.54	~3.50	1.32-2.09
E3	2.68-3.86	1.46-2.79	nd	nd	nd
EE2	nd[e]	2.00-3.35	nd	nd	1.28-2.41

[a] Koh et al. 2009. [b] Carballa et al. 2008. [c] Braga et al. 2005. [d] Holthaus et al. 2002.
[e] nd: not determined.

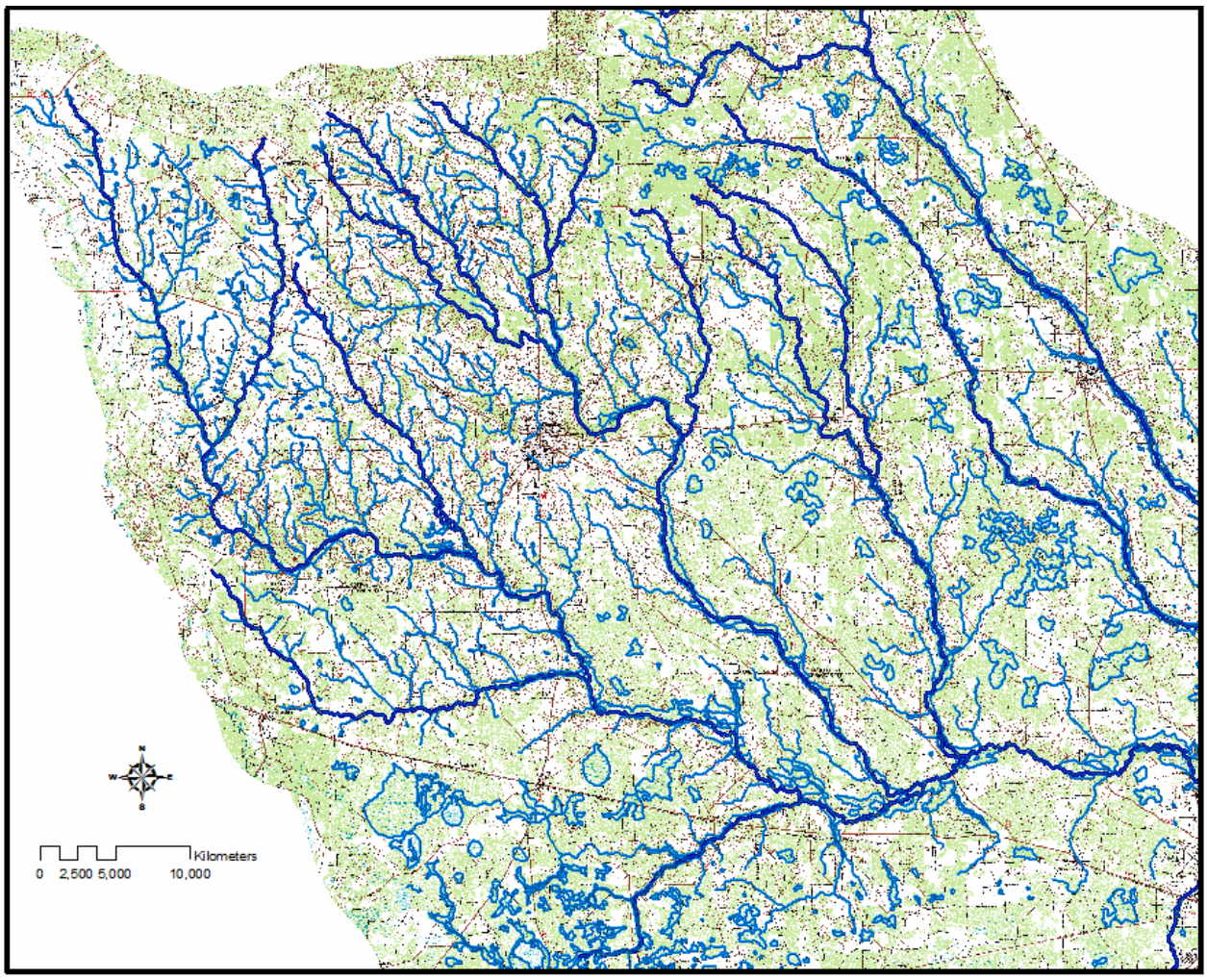

Fig. S1. Map of Upper Satilla Watershed, Gerogia, USA with topographic and small streams information.

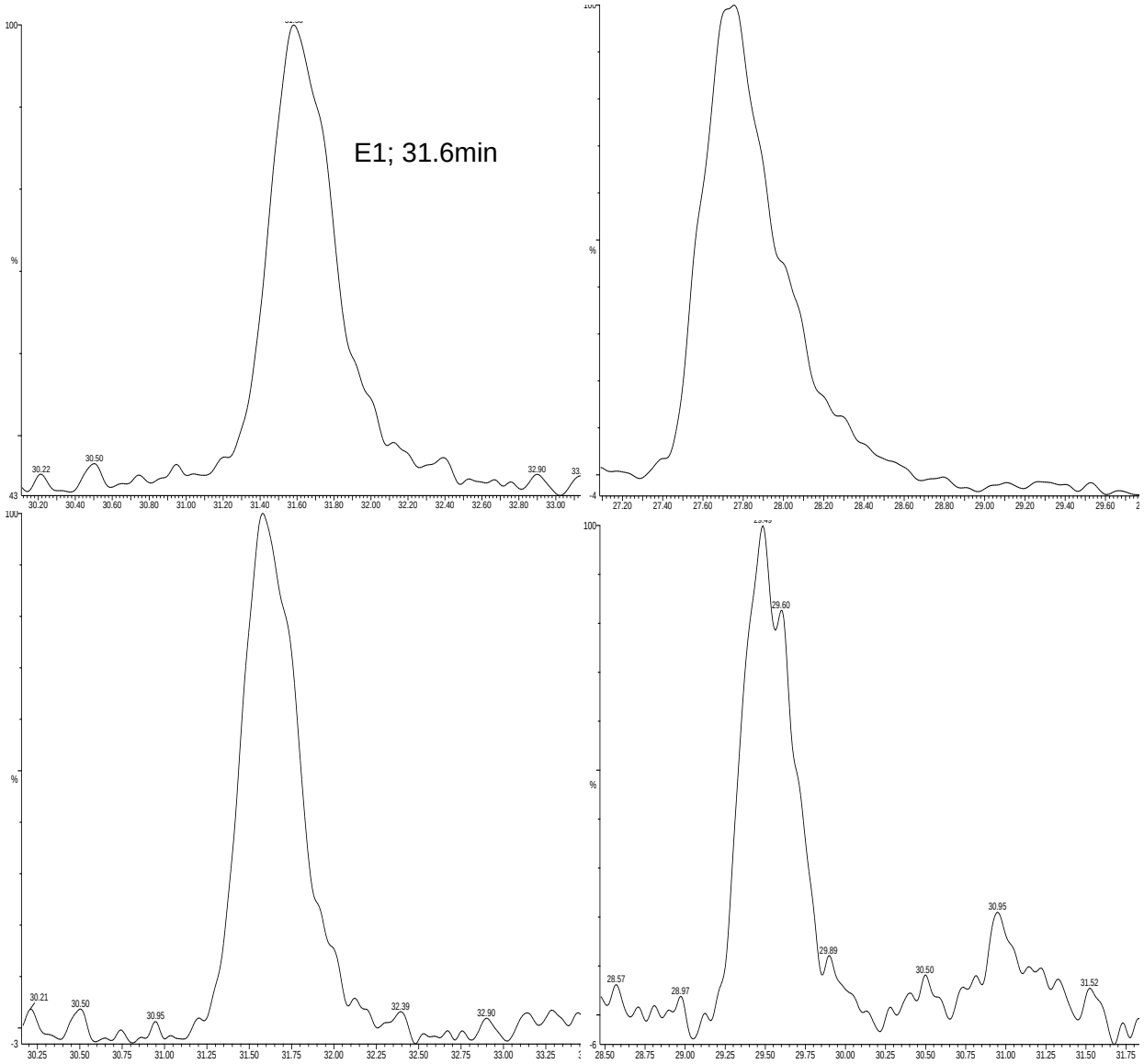

Fig. S2. Typical chromatograph and retention times of E1, E2, E3, and EE2. The concentration of E1 is 12.7 ng/L, E2 is 15.7 ng/L, E3 is 54.8 ng/L, and EE2 is 7.63 ng/L in this figure.

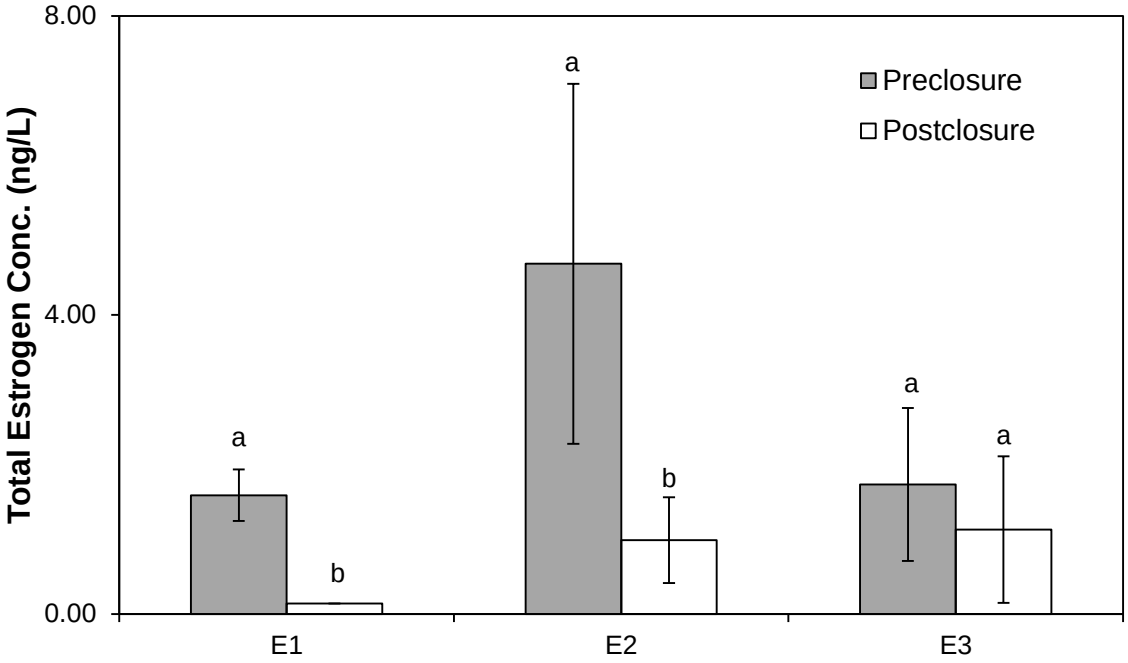

Fig. S3. Means of the sum of each natural estrogen in all agricultural sites averaged by the number of sampling times during pre-closure and post-closure times with 95% confidence intervals. Means followed by different letters indicates a significant difference at $P \leq 0.05$.

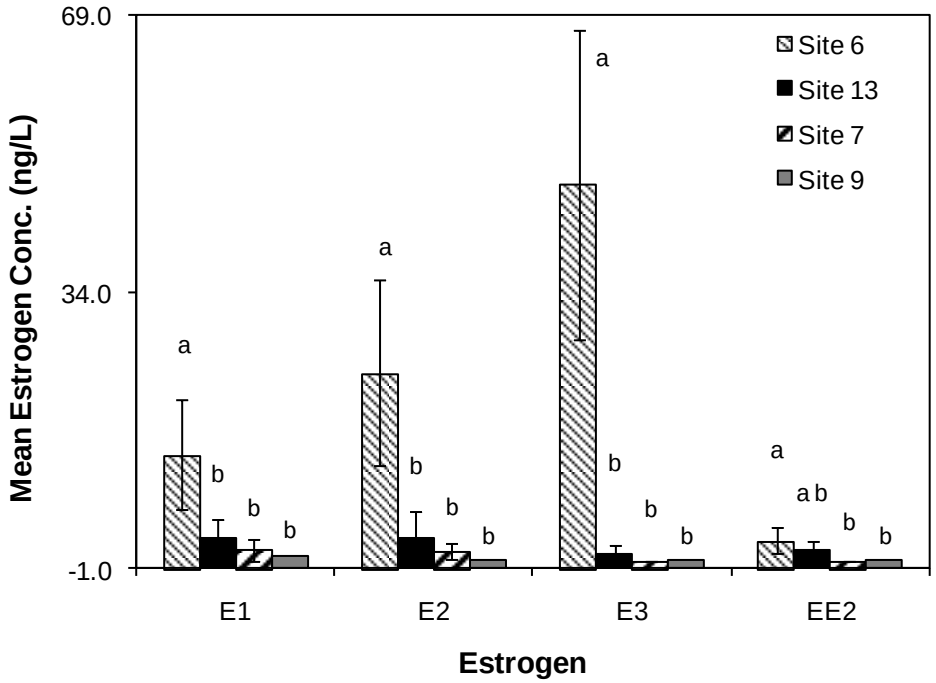

Fig. S4. Mean concentrations and 95% confidential intervals of each estrogen at Site 6, 7, 9, and 13 averaged by sampling times. Means followed by different letters indicates a significant difference at $P \leq 0.05$.