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

Supplementary Information

Identifying sources of emerging organic contaminants in a mixed use watershed using principal components analysis

M. Ekrem Karpuzcu[a*], David Fairbairn[b], William A. Arnold [c], Brian Barber[d], Elizabeth Kaufenberg[b], William Koskinen[e], Paige Novak[c], Pamela Rice[e], Deborah Swackhamer[a]

[a] University of Minnesota, Water Resources Center, 1985 Buford Ave., St Paul, MN 55108, United States

[b] University of Minnesota, Water Resources Science Graduate Program, 1985 Buford Ave, Saint Paul, MN, 55108, United States

[c] University of Minnesota, Department of Civil, Environmental, and Geo-Engineering, 500 Pillsbury Drive SE, Minneapolis, MN, 55455, United States

[d] University of Minnesota, Department of Soil, Water, and Climate, 1902 Dudley Ave, Saint Paul, MN, 55108, United States

[e] United States Department of Agriculture, Agricultural Research Service, 1991 Upper Buford Circle, University of Minnesota, Saint Paul, MN, 55108

*Corresponding author: mkarpuzc@umn.edu, (510) 637-9705

Summary: This file contains 20 pages, 3 Tables and 14 Figures.

1. Details of Sampling Procedure

Water samples were collected by submerging a clean amber glass or stainless steel container into the stream to a depth of 10-20cm. Samples were sealed with Teflon-lined lids and transported on ice to the laboratory, where they were refrigerated at 4°C until processing. Corollary physical and chemical data were acquired using *in situ* sondes (YSI Inc, Yellow Springs, OH; Hach Hydromet, Loveland, CO), YSI flowmeters, and from three existing USGS monitoring stations. Samples (2 L) were filtered using 0.7µm glass-fiber filters, spiked with a surrogate standard, and processed via solid phase extraction (SPE) using an Autotrace 280 (Dionex, Sunnyvale, CA)

equipped with Oasis HLB cartridges (Waters, Milford, MA). Extraction solvents were acetonitrile and acetonitrile/0.1% formic acid. The eluate was collected in glass culture tubes and evaporated under nitrogen to near-dryness. Following evaporation, the sample was reconstituted to 1.5mL of acetonitrile and ultrapure water (1:1), transferred to amber LC vials, and stored at 0°F until analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2. Chemical Standards and Materials

HPLC-grade solvents, formic acid, and CEC standards were purchased from Fisher Scientific (Waltham, MA) and Sigma Aldrich (St. Louis, MO). Deuterated surrogate and internal standards were purchased from CDN Isotopes (Pointe-Claire, Canada). Individual stock solutions (5-50 mg/L) were prepared in acetonitrile. These were mixed to create a stock solution of all CECs that was serially diluted to working standard levels spanning four orders of magnitude. Ultrapure water was produced by a Milli-Q Advantage A10 system (EMD Millipore, Inc., Billerica, USA). Prior to use, all field, laboratory, and storage equipment was cleaned with CEC-free soap and water, triple rinsed with ultrapure water, methanol-rinsed, and (for glassware and metals) baked at 400 °C for 3 hours.

3. LC/MS Analysis

Shimadzu (Kyoto, Japan) high performance liquid chromatograph (HPLC), with an Agilent C8 2.1 X 150mm X 5µm film thickness Zorbax column and Eclipse Plus C8 2.1 X 12.5mm X 5µm film thickness narrow bore guard column was used for analyte separation. The HPLC was coupled to an Applied Biosystems (Carlsbad, CA) API 3200 triple quadrupole mass spectrometer using turbo spray (ESI) in scheduled Multiple Reaction Monitoring Mode (MRM) in either positive or negative mode for compound identification. The column was maintained at 40˚C. The mobile phase was gradient, 80% water (0.1% formic acid) and 20% Acetonitrile (ACN)

(0.1% formic acid) to 80% ACN at 15 min and held at 80% to 20 min, 90% ACN to 24 min with column re-equilibration to 20% ACN from 24 to 30 min. Flow rate of 0.2mL min^{-1} used for all runs. The sample injection volume was 50µL. Samples were maintained at 15°C in the auto sampler to minimize decomposition. Tuning parameters were optimized for each analyte by direct infusion.

4. Data QA/QC

Method reporting limits (MRLs, Table S1) were established using published US EPA and USGS methods and the minimum compound mass that consistently produced a signal-to-noise ratio of at least nine in order to ensure appropriateness across numerous analytical runs. Calibration curves were generated using eight standard levels across four orders of magnitude of analyte concentration. R-squared values were greater than 0.99 for all detected analytes. Each analytical run included laboratory spikes to assess analytical accuracy and precision, and laboratory blanks to assess contamination and instrument carryover. Procedural spikes and blanks were used to assess recovery and contamination resulting from sample processing. Field blanks were included to assess potential contamination resulting from sample collection, handling, storage, and processing. Spiked environmental samples were used to assess matrix interference. If a laboratory blank response was greater than 20% of that in an associated environmental sample, data were flagged and reviewed. If a laboratory blank response was above 50% of an associated environmental sample, the data was reported as "non-detect". Analytical recoveries for all detected analytes were between 67%-179% in analytical spikes and 36-150% in spiked matrix water samples.

Table S1 Distribution of the number of water samples collected at each site and each season

Site / Season	Spring	Early Summer	Late Summer	Fall	Winter
Bear Creek	2	9	8	7	2
SFZR-Golf Course	2	9	8	7	2
Willow Creek	2	8	8	8	2
WWTP-US	1	3	2	2	0
WWTP-DS	2	8	8	8	2
WWTP-EFF	2	3	1	2	1

Table S2 Method Reporting Limits (MRLs) for water grab samples

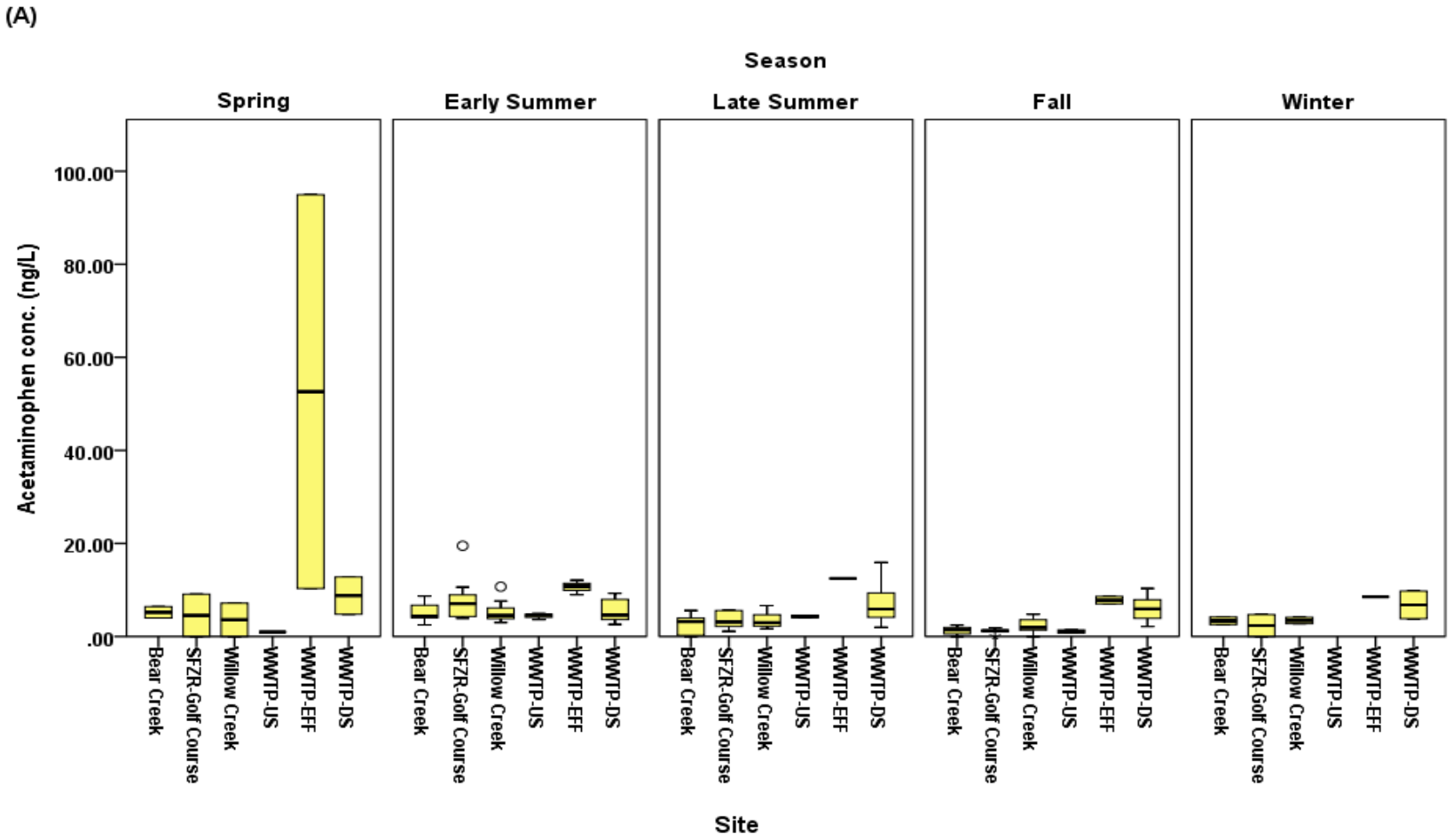
Compound	Method Reporting Limit (MRL, ng/L)
Acetaminophen	0.6
Acetochlor	0.9
Atrazine	0.3
Caffeine	0.6
Carbamazepine	0.1
Carbaryl	0.5
Cotinine	2.0
Daidzein	0.5
DEET	6.4
Erythromycin	166.9
Iprodione	14.4
Metolachlor	0.9
Sulfamethoxazole	8.5

Table S3 Results of Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity for PCA

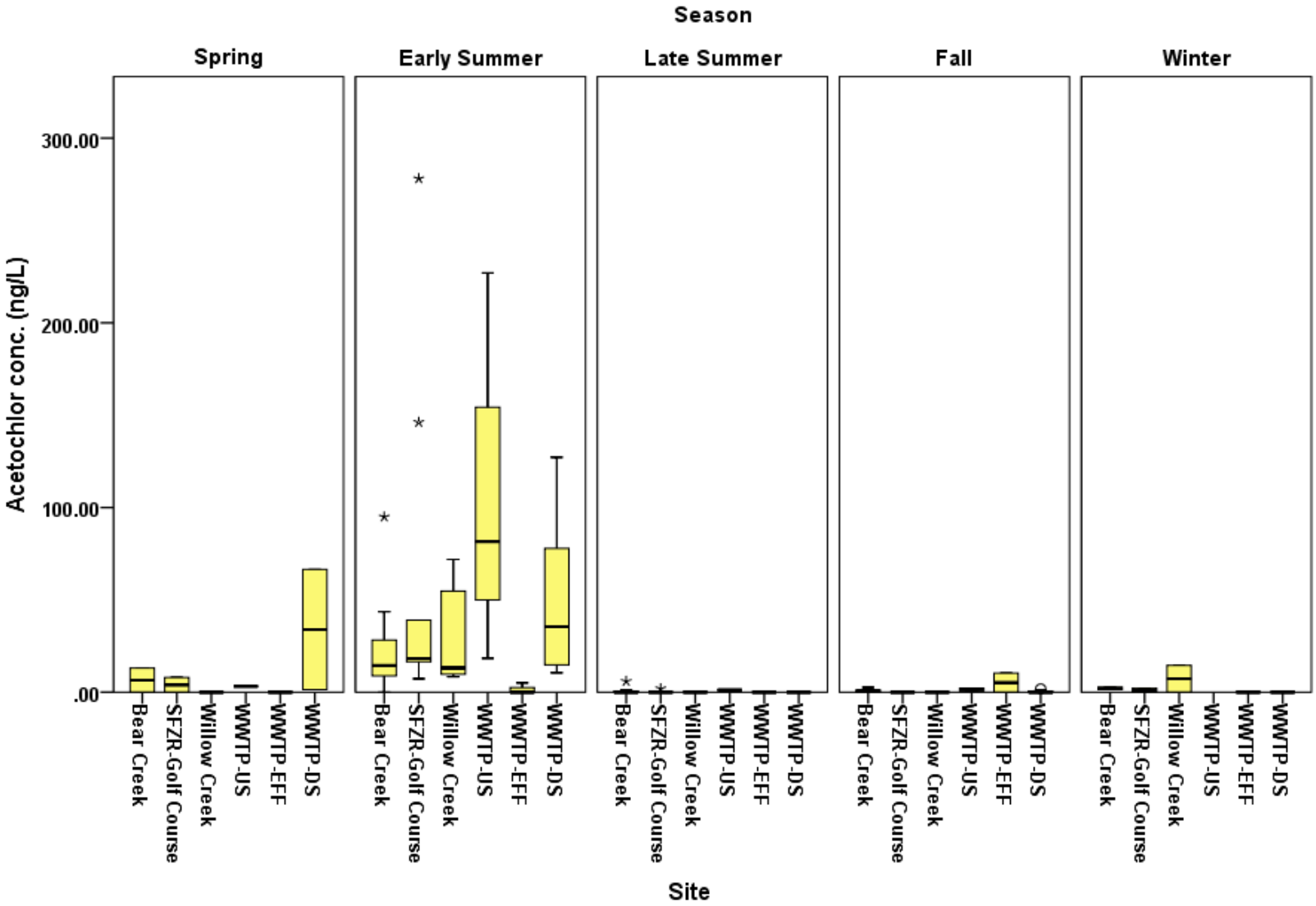
KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.669
Bartlett's Test of Sphericity	Approx. Chi-Square	356.123
	df	45
	Sig.	.000

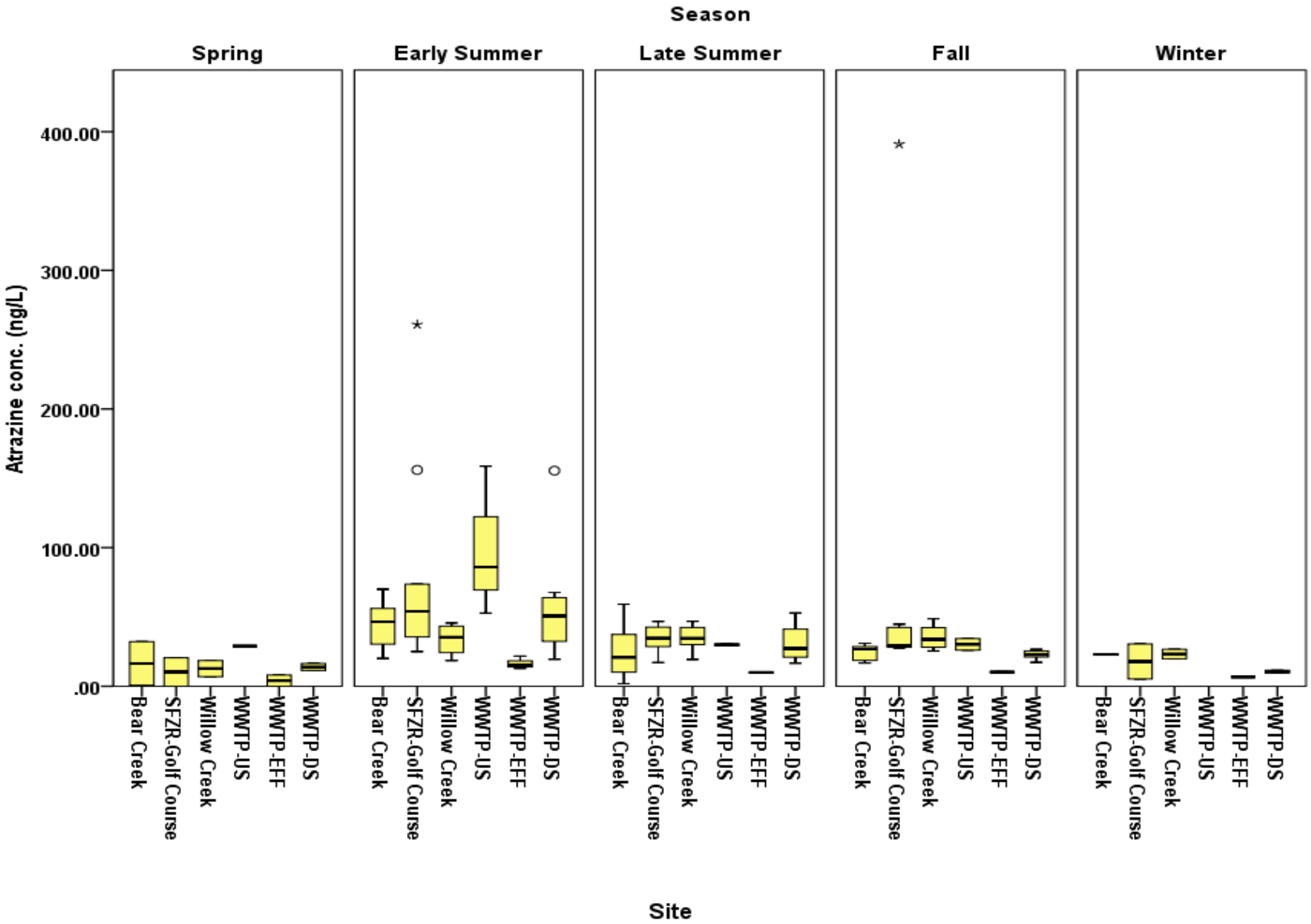
Figure S1 Concentration profiles of target analytes at each site and sampling season: (A) Acetaminophen, (B) Acetochlor, (C) Atrazine, (D) Caffeine, (E) Carbamazepine, (F) Carbaryl, (G) Cotinine, (H) Daidzein, (I) DEET, (J) Erythromycin, (K) Iprodione, (L) Metolachlor, (M) Sulfamethoxazole. WWTP-US = upstream of South Fork Zumbro River (SFZR)-Wastewater treatment plant, WWTP-DS = downstream of SFZR-Wastewater treatment plant, WWTP-EFF = effluent from SFZR-Wastewater treatment plant. Boxplots represent the minimum, lower quartile, the median, upper quartile and the maximum. The length of the box is defined as interquartile range (IQR). Values which exceed three IQRs are denoted by asterisks and represent extreme values. Values which are between one and a half and three IQRs are denoted by empty circles and represent outliers.



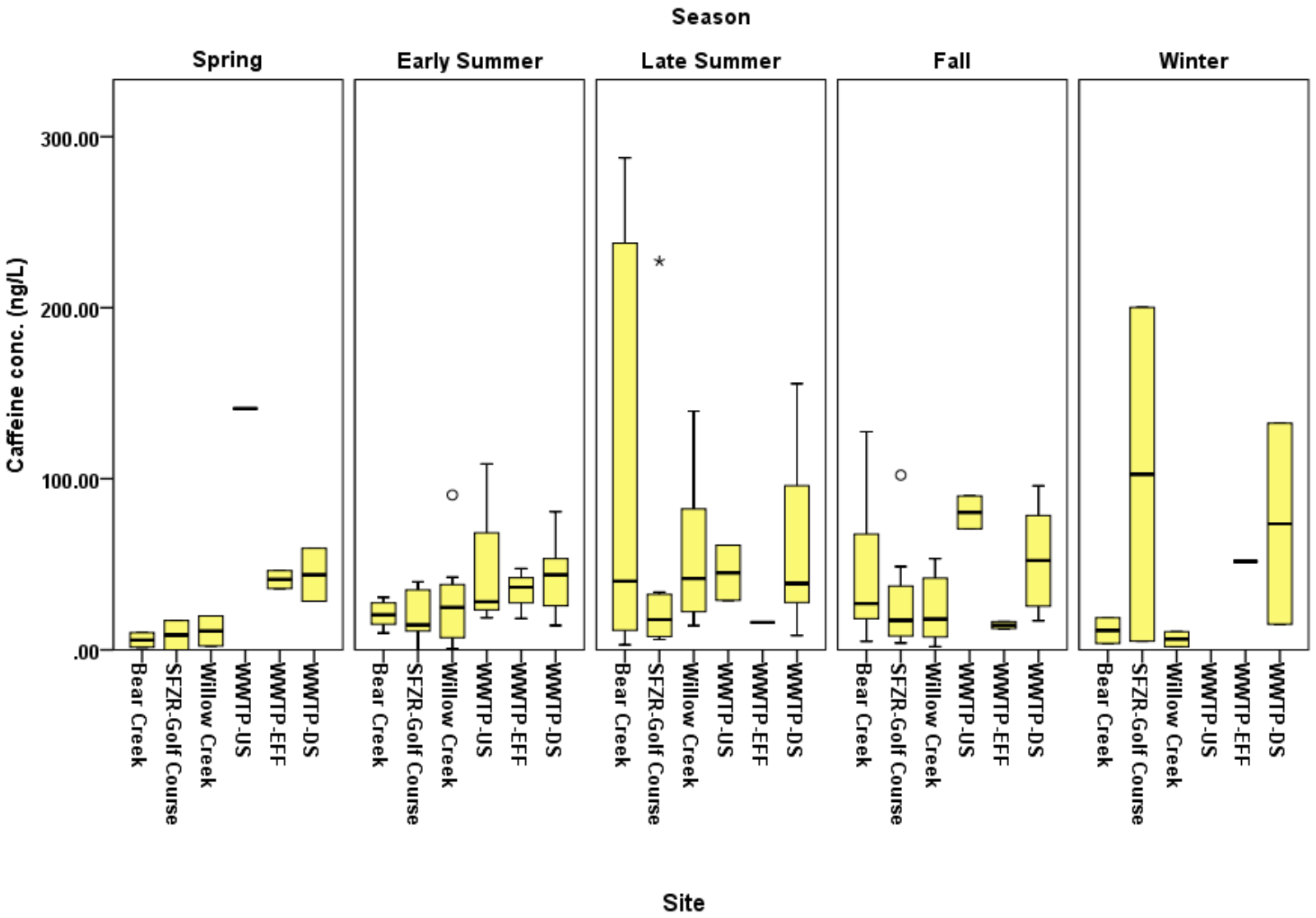
(B)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Acetochlor conc. (ng/L)
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

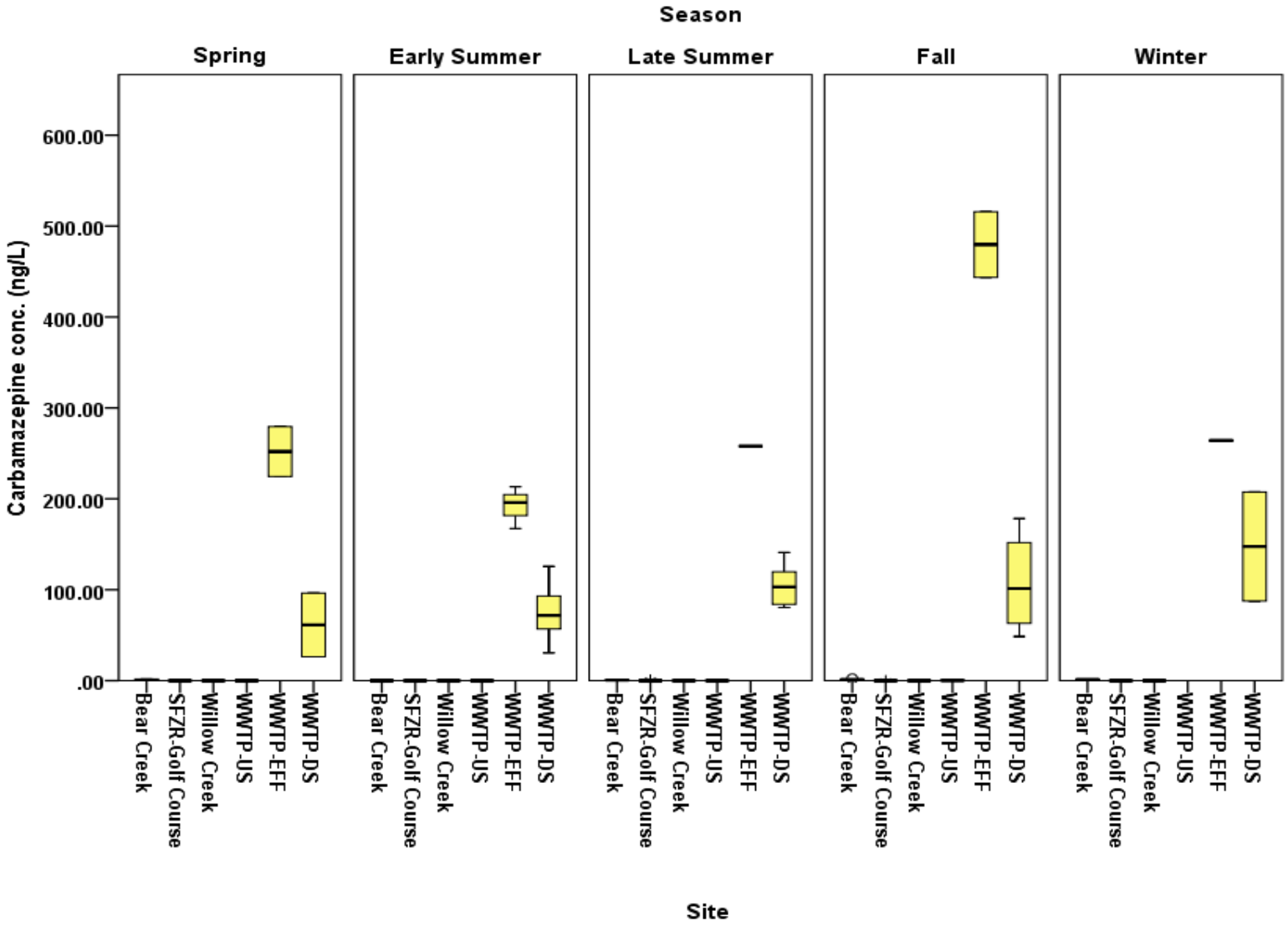
(C)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Atrazine conc. (ng/L)
400.00
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

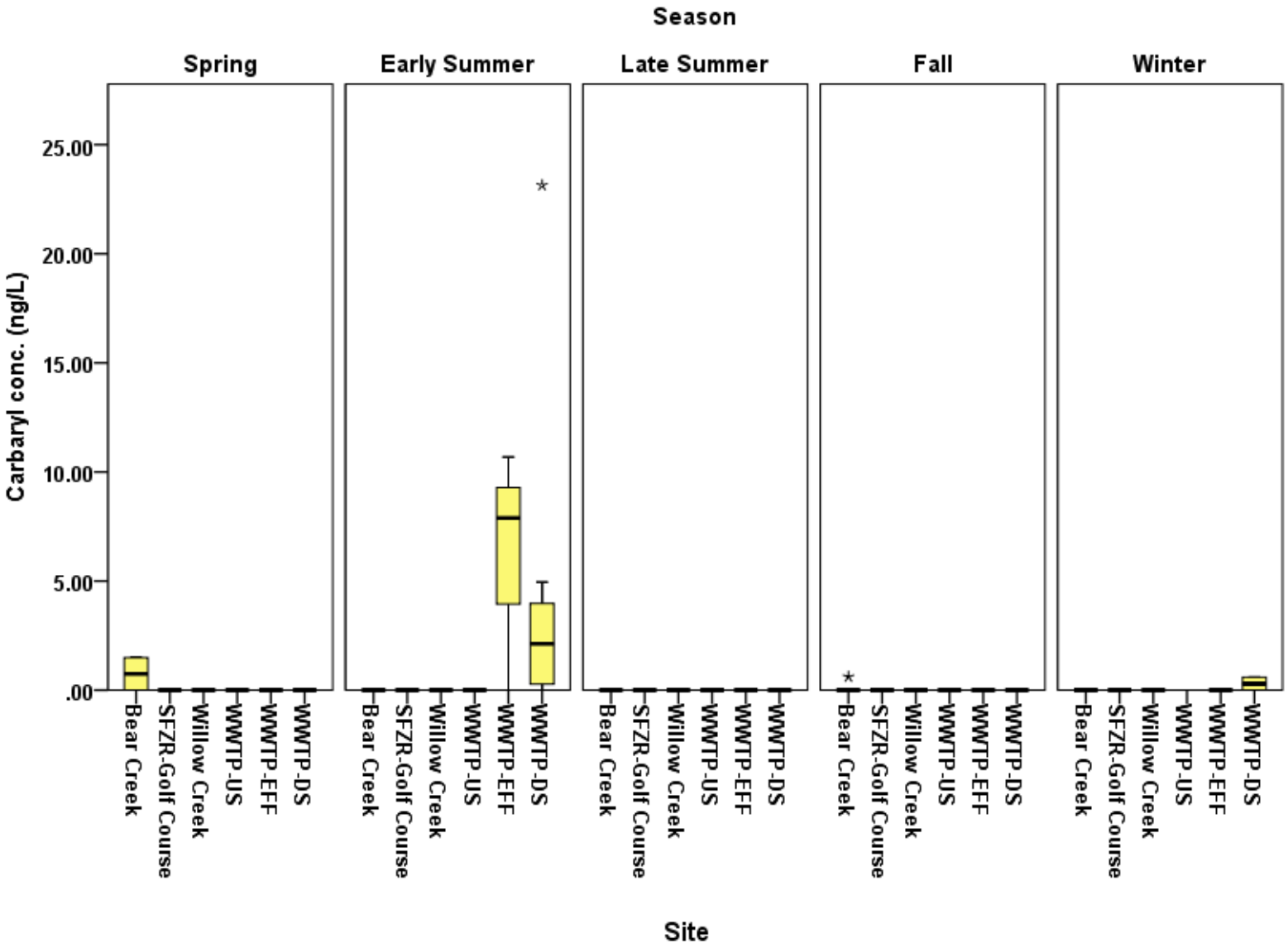
(D)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Caffeine conc. (ng/L)
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

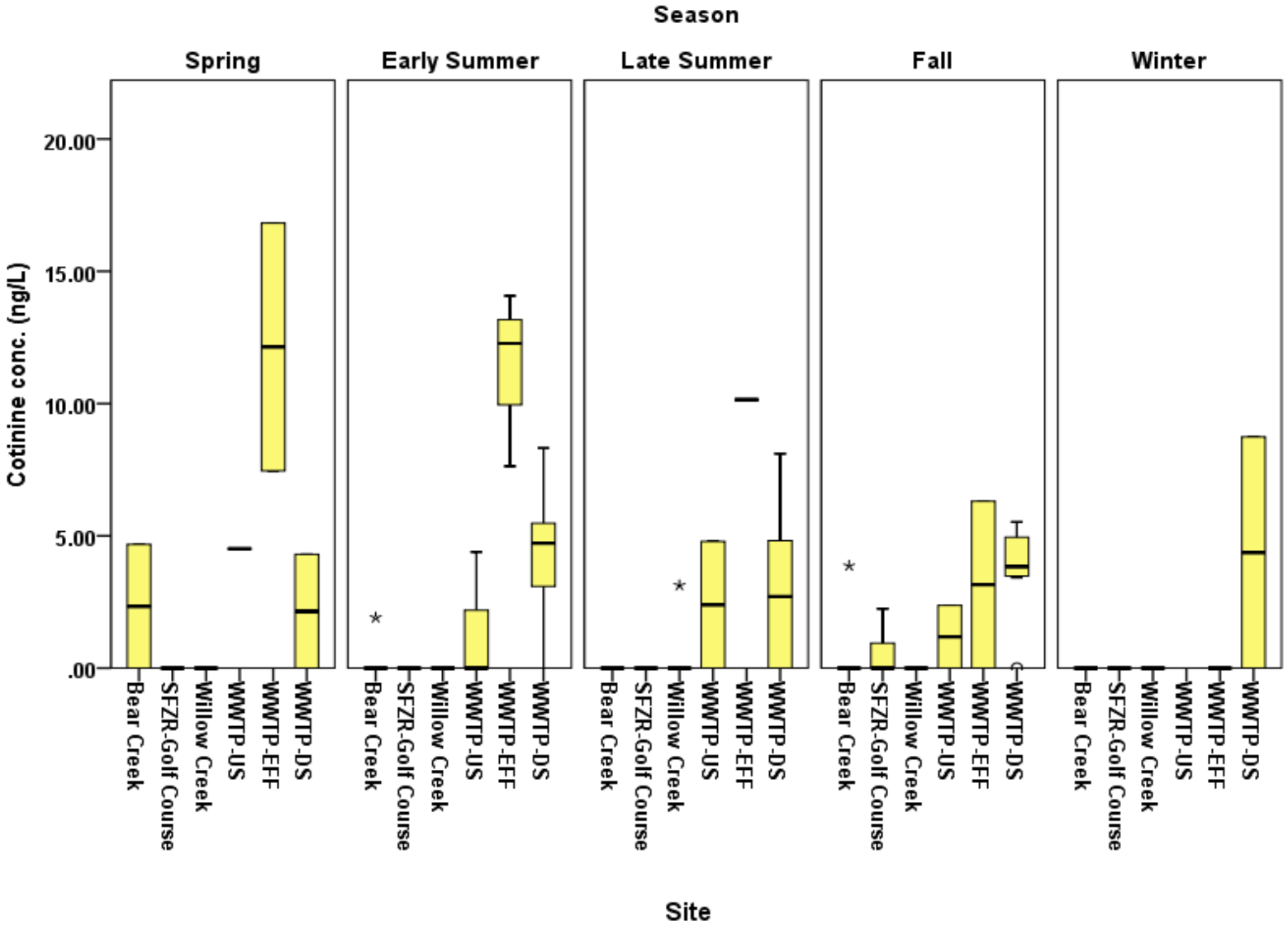
(E)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Carbamazepine conc. (ng/L)
600.00
500.00
400.00
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

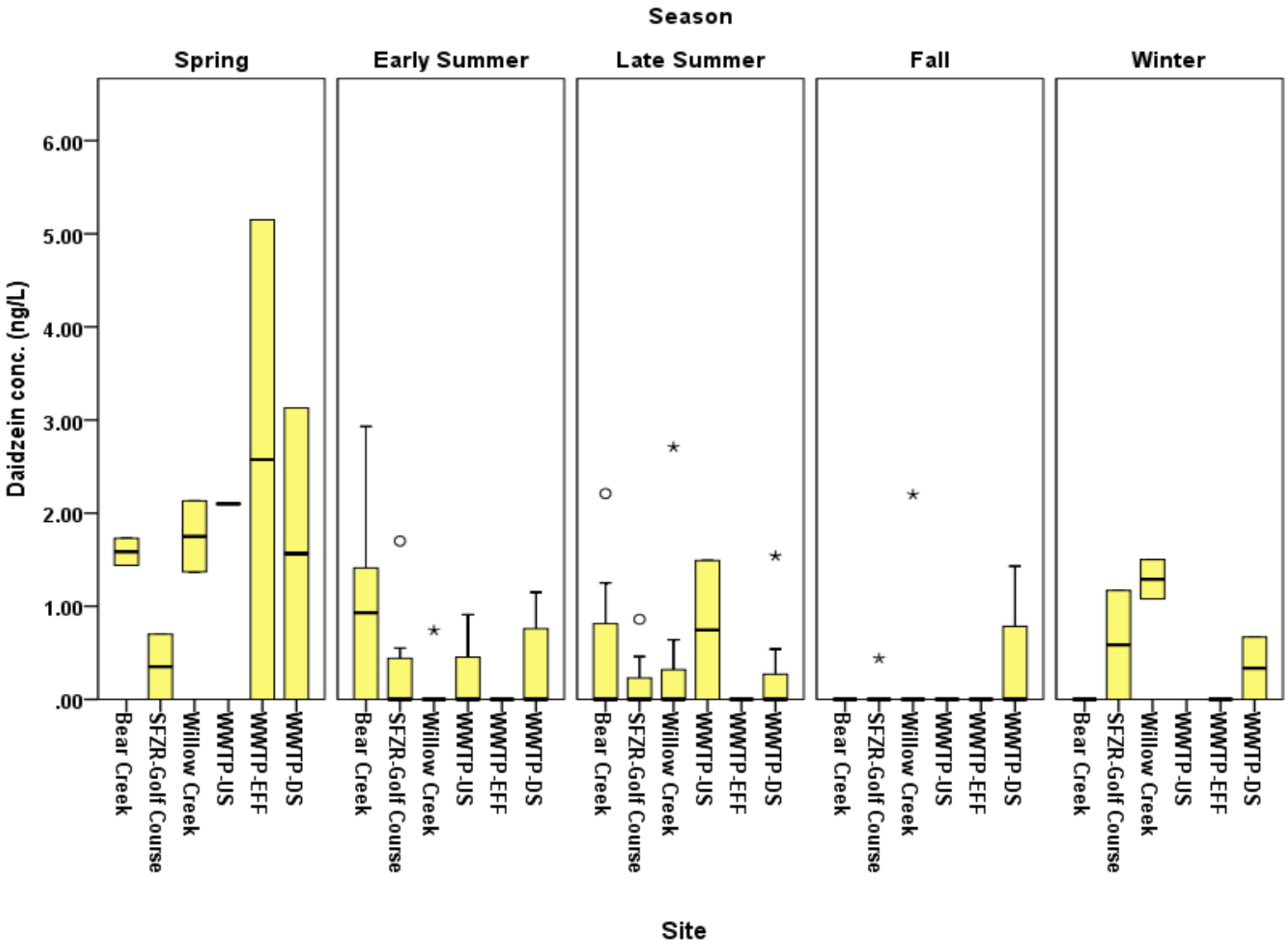
(F)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Carbaryl conc. (ng/L)
25.00
20.00
15.00
10.00
5.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

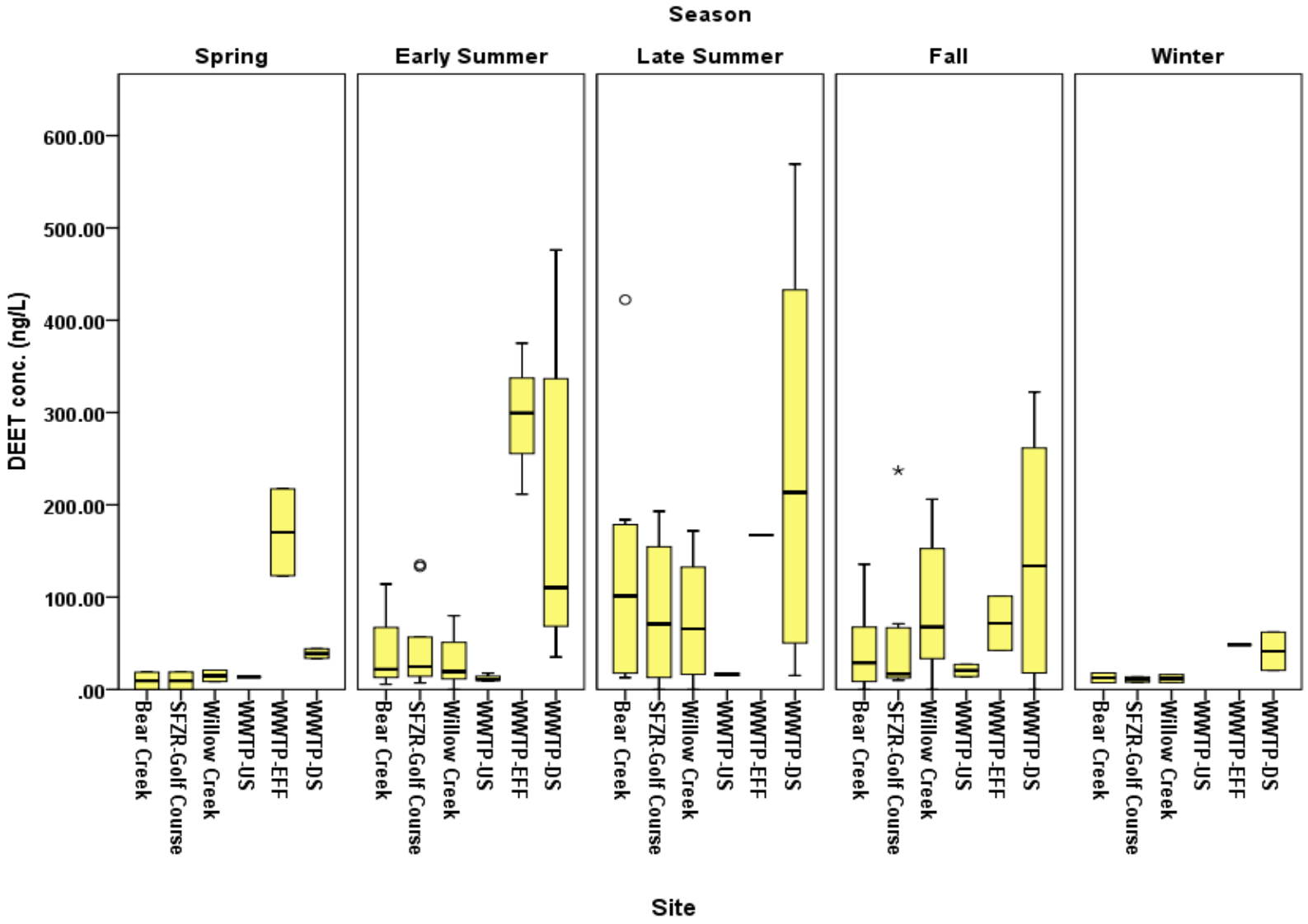
(G)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Cotinine conc. (ng/L)
20.00
15.00
10.00
5.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

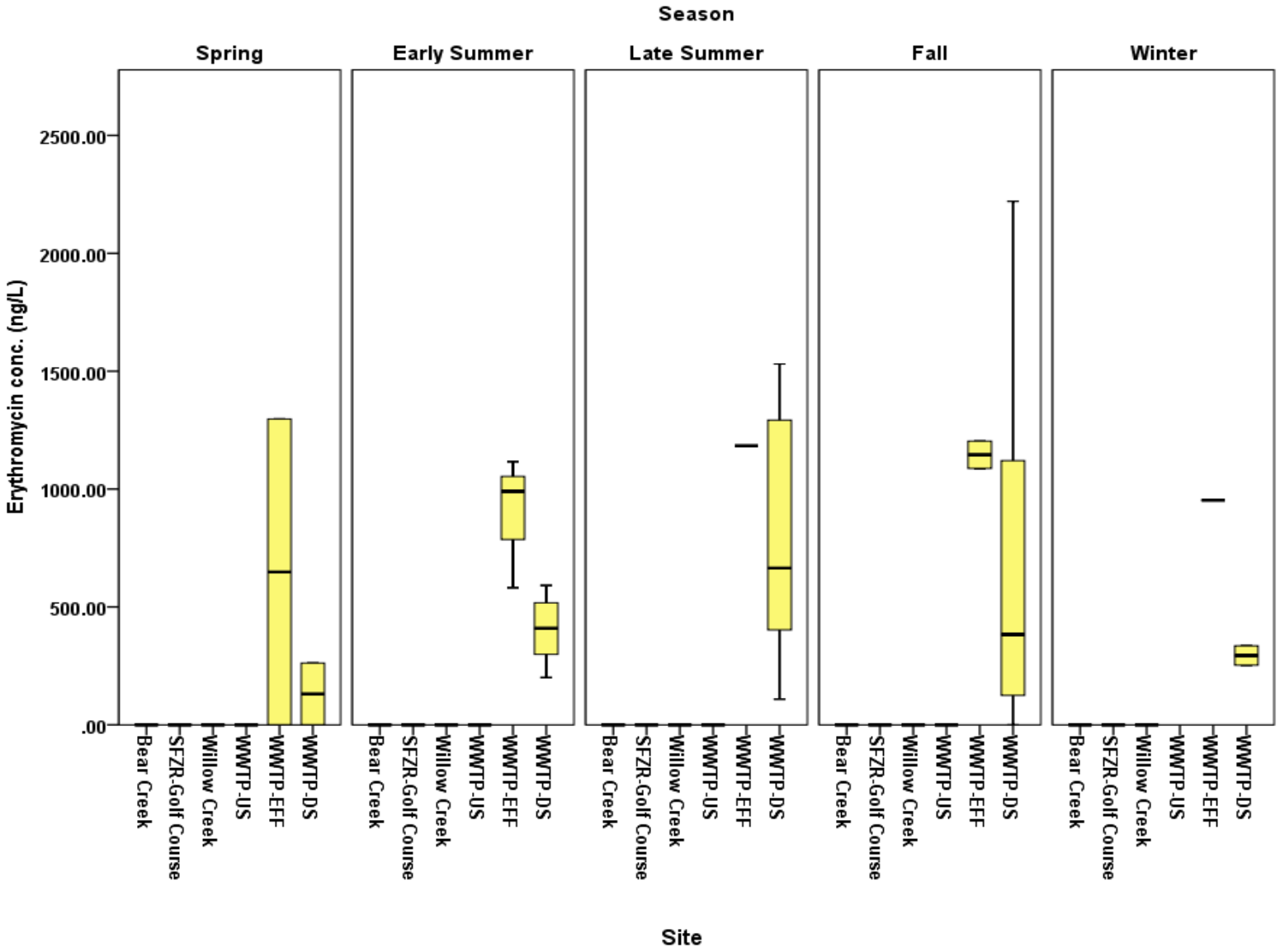
(H)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Daidzein conc. (ng/L)
6.00
5.00
4.00
3.00
2.00
1.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

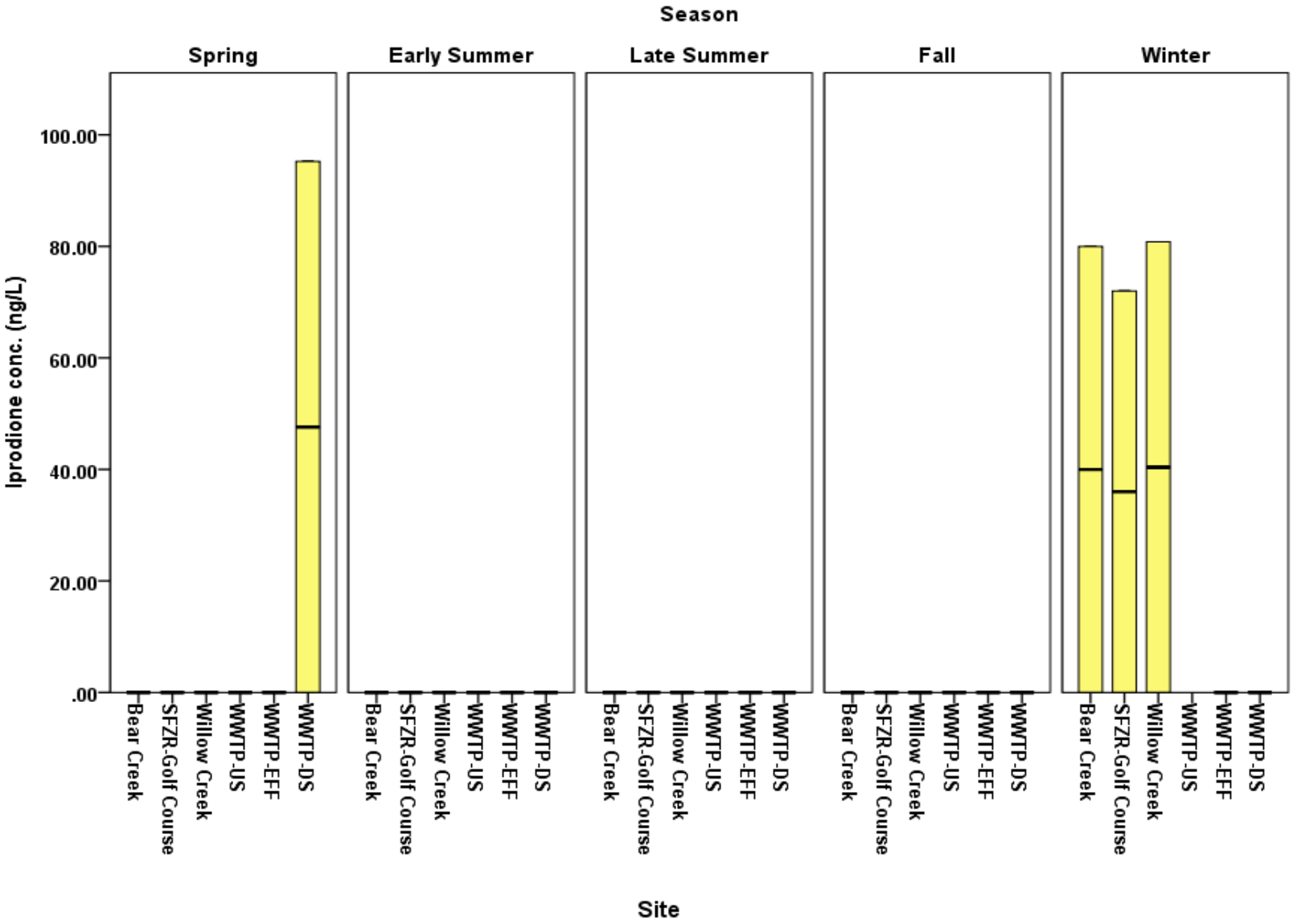
(I)


Season
Spring
Early Summer
Late Summer
Fall
Winter
DEET conc. (ng/L)
600.00
500.00
400.00
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

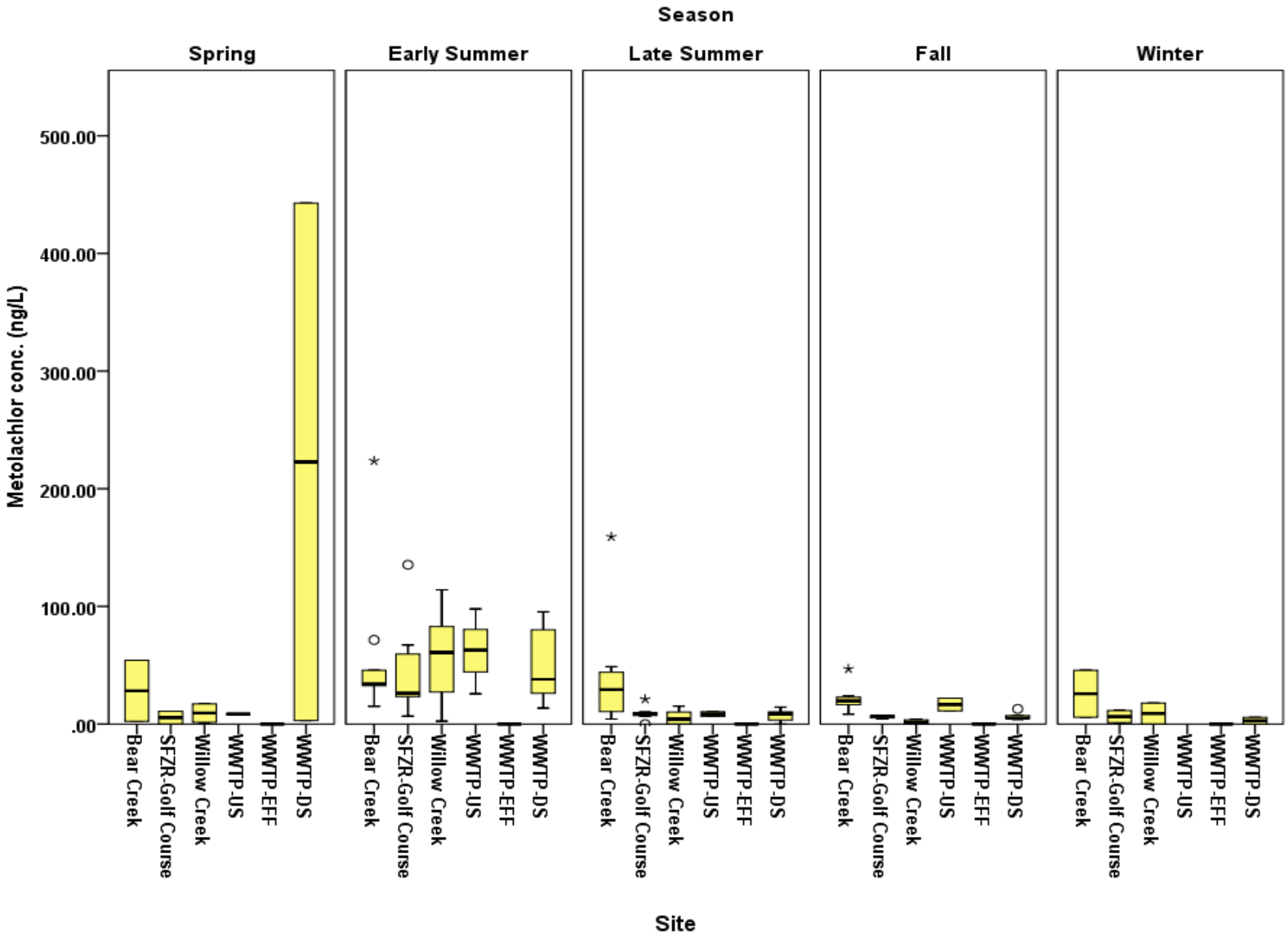
(J)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Erythromycin conc. (ng/L)
2500.00
2000.00
1500.00
1000.00
500.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

(K)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Iprodione conc. (ng/L)
100.00
80.00
60.00
40.00
20.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

(L)


Season
Spring
Early Summer
Late Summer
Fall
Winter
Metolachlor conc. (ng/L)
500.00
400.00
300.00
200.00
100.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

(M)

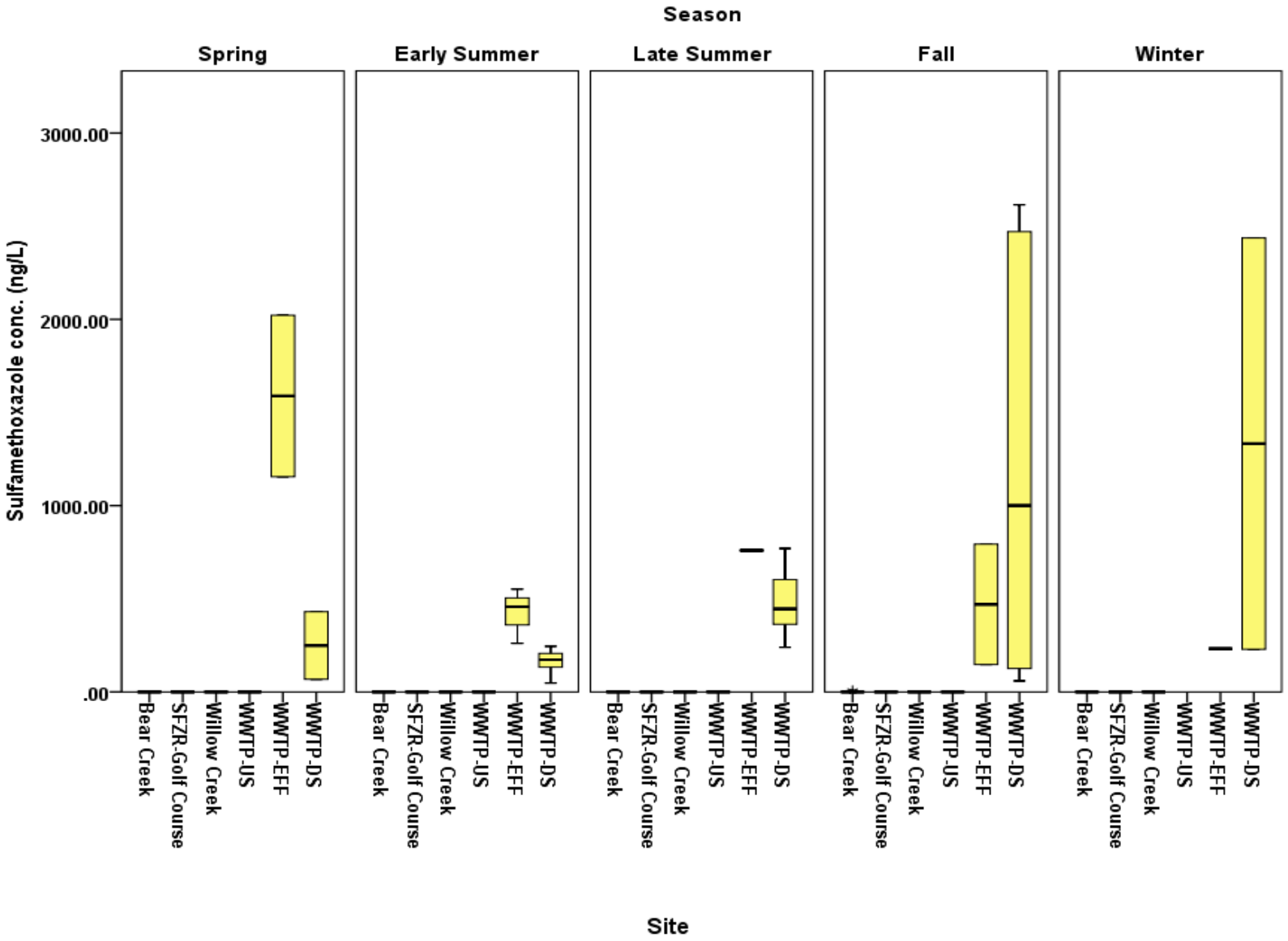

Season
Spring
Early Summer
Late Summer
Fall
Winter
Sulfamethoxazole conc. (ng/L)
3000.00
2000.00
1000.00
.00
Bear Creek
SFZR-Golf Course
Willow Creek
WWTP-US
WWTP-EFF
WWTP-DS
Site

Figure S2 Scree plot showing Eigenvalues of each principal component in decreasing order.