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Chemical contaminants in water and sediment near fish nesting sites in the Potomac River basin: Determining potential exposures to smallmouth bass (*Micropterus dolomieu*)

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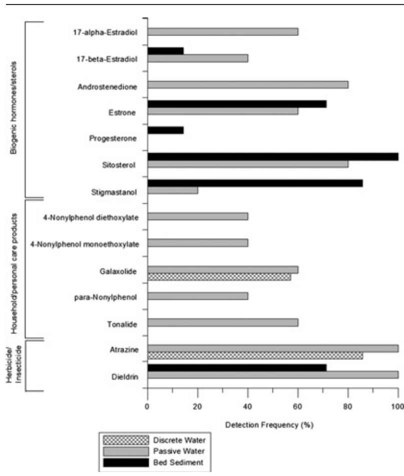
HIGHLIGHTS

- ► Water and bed sediment samples were collected at seven active smallmouth bass nesting sites.
- ► A total of 135 chemicals were detected in at least one of the samples collected.
- ► A significant positive relation between intersex in SMB and atrazine in the water column above the fish nests was observed.
- ► A significant positive relation between intersex in SMB and total hormone/sterol in bed sediment at the nests was observed.

GRAPHICAL ABSTRACT



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ABSTRACT

The Potomac River basin is an area where a high prevalence of abnormalities such as testicular oocytes (TO), skin lesions, and mortality has been observed in smallmouth bass (SMB, *Micropterus dolomieu*). Previous research documented a variety of chemicals in regional streams, implicating chemical exposure as one plausible explanation for these biological effects. Six stream sites in the Potomac basin (and one out-of-basin reference site) were sampled to provide an assessment of chemicals in these streams. Potential early life-stage exposure to chemicals detected was assessed by collecting samples in and around SMB nesting areas. Target chemicals included those known to be associated with important agricultural and municipal wastewater sources in the Potomac basin. The prevalence and severity of TO in SMB were also measured to determine potential relations between chemistry and biological effects.

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A total of 39 chemicals were detected at least once in the discrete-water samples, with atrazine, caffeine, deethylatrazine, simazine, and iso-chlorotetracycline being most frequently detected. Of the most frequently detected chemicals, only caffeine was detected in water from the reference site. No biogenic hormones/sterols were detected in the discrete-water samples. In contrast, 100 chemicals (including six biogenic hormones/sterols) were found in a least one passive-water sample, with 25 being detected at all such samples. In addition, 46 chemicals (including seven biogenic hormones/sterols) were found in the bed-sediment samples, with caffeine, cholesterol, indole, para-cresol, and sitosterol detected in all such samples.

The number of herbicides detected in discrete-water samples per site had a significant positive relation to TO_{rank} (a nonparametric indicator of TO), with significant positive relations between TO_{rank} and atrazine concentrations in discrete-water samples and to total hormone/sterol concentration in bed-sediment samples. Such significant correlations do not necessarily imply causation, as these chemical compositions and concentrations likely do not adequately reflect total SMB exposure history, particularly during critical life stages.

1. Introduction

Reports of wild fish exhibiting signs of endocrine disruption have become increasingly prevalent (Bjerregaard et al., 2006; Hinck et al., 2009; Hinfray et al., 2010; Blazer et al., 2012; Brown et al., 2011). Characteristics include the development of intersex gonads (Purdom et al., 1994; Jobling et al., 1998), the expression of the egg-yolk protein vitellogenin by male fish (Harries et al., 1997; Rodgers-Gray et al., 2000), and the promotion or suppression of secondary sex characteristics (Vajda et al., 2008). Since 2003, fish mortalities and a high prevalence of intersex or testicular oocytes (TO) have been observed in smallmouth bass (SMB, *Micropterus dolomieu*) within the Potomac basin (Blazer et al., 2007; Iwanowicz et al., 2009; Blazer et al., 2010). These observations have caused substantial public concern because the Potomac River is an important source of drinking water for the Washington, DC area and SMB is an economically important sport fish species in the larger Chesapeake Bay watershed (Jenkins and Burkhead, 1994).

Research has shown that exposure to select chemicals can cause deleterious effects to fish (Mills and Chichester, 2005; Kidd et al., 2007; Yonkos et al., 2010; Vajda et al., 2011). Thus, chemical exposure is one plausible explanation for observances of TO and other biological effects in SMB in the Potomac basin. Various suburban/urban sources, including wastewater treatment plants (WWTPs), combined sewer overflows, and septic discharges, as well as agriculture sources (e.g. crop and livestock production) exist within this basin. Multiple chemical contaminants have been documented in these sources (Jelic et al., 2011; Pal et al., 2010; Rostkowski et al., 2011; Sim et al., 2011; Weyrauch et al., 2010; Phillips et al., 2012).

Previous research explored the possible relation between fish health and chemical exposures in the Potomac basin (Alvarez et al., 2009; Blazer et al., 2010; Iwanowicz et al., 2009). Expanding upon this earlier work, a network of six stream sites (plus one reference stream) was sampled to provide a more comprehensive assessment of chemical contaminants in water and bed sediment in the Potomac basin. Biological effects (e.g. prevalence and severity of TO, plasma vitellogenin induction in male fish) as well as land use attributes were evaluated during the same time period at the study sites (Blazer et al., 2012). Linking indicators of SMB reproductive health to contaminant composition and concentration were accomplished by: (1) strategically selecting streams to represent a range of previously observed TO abnormalities and a gradient of agricultural and urban land use, (2) placing sampling points within each stream in areas where exposures of sensitive life stages were likely to occur (i.e., SMB nesting areas), and (3) collecting environmental samples during the spawning season. This paper describes the chemical results obtained from water and bed-sediment samples collected for this study and evaluates potential relations between chemical occurrence and the prevalence and severity of TO in SMB.

2. Materials and methods

A network of six stream sites in the Potomac basin was selected and sampled in 2007 to provide sites with a range of previously observed SMB TO and agricultural and urban land use (Fig. 1, Table 1). The Gauley River (WV4) was used as an out-of-basin reference site as no appropriate reference stream was identified within the Potomac basin. Previous monitoring indicated a low prevalence and severity of SMB TO at WV4 (Blazer et al., 2007, 2012).

2.1. Basin characteristics

Select basin characteristics were compiled for land areas upgradient of the stream sampling sites (Table 1). Land use was acquired from the 2001 National Land Cover Dataset (Homer et al., 2007). Human population was derived from the 2000 decennial census (United States Census Bureau, 2010).

2.2. Field methods

A combination of discrete-water, passive-water, and bed-sediment samples was collected in and around SMB nesting areas at each site. The exact locations of the SMB nesting areas were identified by local expertise and observation of male SMB guarding the nests. The purpose of sampling at SMB nesting sites was to provide chemical data to determine potential exposures to eggs and newly hatched fish, life stages shown to be most susceptible to induction of TO in other species (Koger et al., 2000; Krisfalusi and Nagler, 2000; Liney et al., 2005). Sampling of both water and bed sediment in these nesting areas provides a more comprehensive assessment of SMB exposures to chemicals than conducted previously.

A single, discrete-water sample was collected at each identified SMB nesting site just below the water surface ($<$0.25 m). The water depth at all sites was less than 1 m. In most cases, the water velocities were near zero at the nesting sites. This approach was used to determine water chemistry to which SMB eggs, newly hatched fish, and adult fish guarding the nest were exposed. Water temperature, pH, specific conductance, and dissolved-oxygen concentration were measured at a single point at each site near where the water samples were collected. All samples were chilled immediately upon collection and shipped to participating laboratories for analysis.

To complement the discrete-water and bed-sediment samples, passive-sampling devices were deployed as a basic screening tool to determine chemicals that may be present in the streams episodically or that are transported in concentrations too low to be detected by conventional sampling techniques (Alvarez et al., 2005). Two types of devices were used for this study: semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS). SPMDs are designed to monitor for lipid soluble (non-polar)

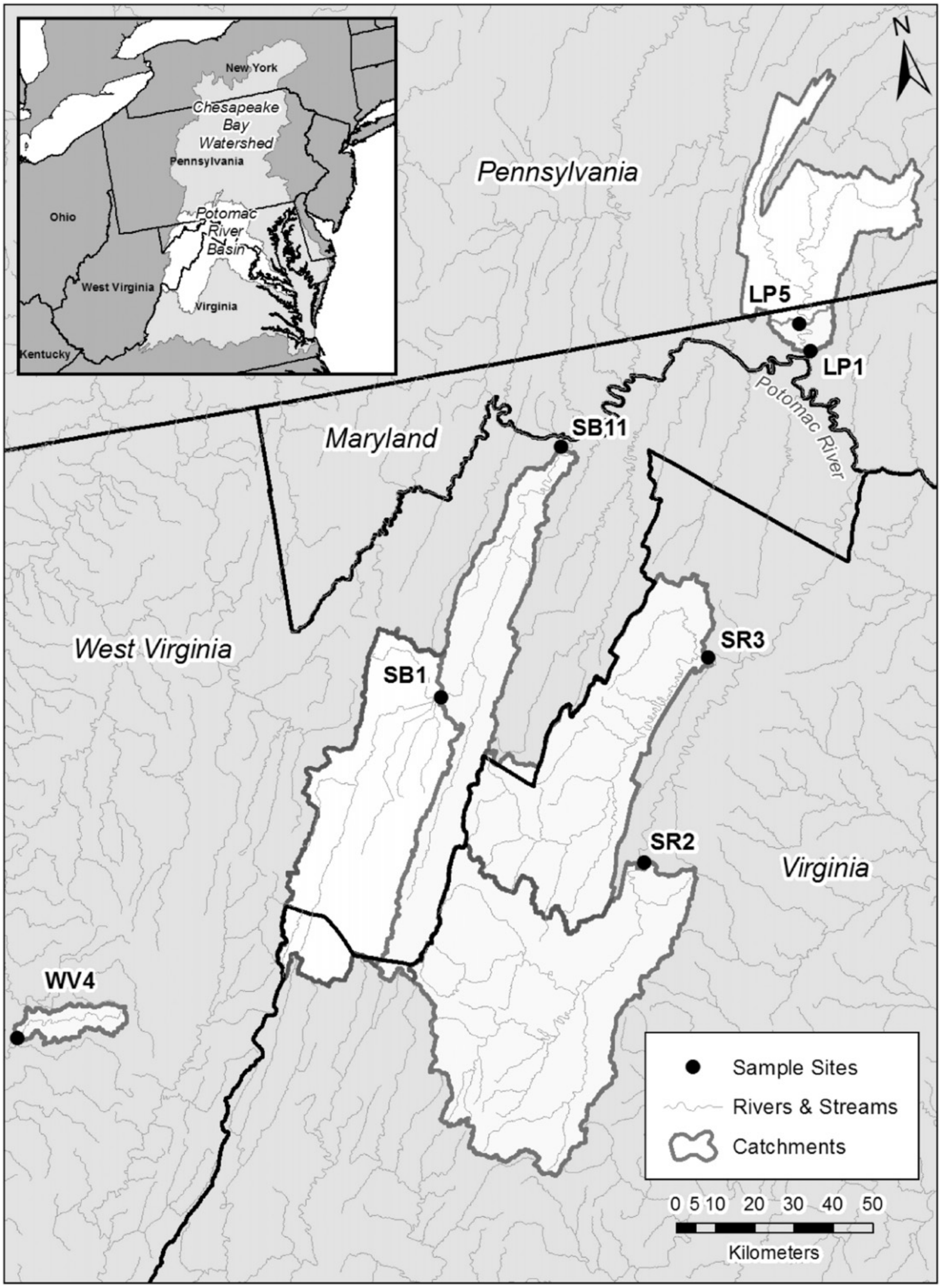

Fig. 1. Location of six stream sites in the Potomac basin, which is part of the Chesapeake Bay wastershed (SB1, South Branch, Petersburg; SB11, South Branch, Springfield; SR2, Shenandoah, South Fork; SR3, Shenandoah, North Fork; LP5, Conococheague Creek (upper); LP1, Conococheague Creek (lower)), and the out-of-basin reference site (WV4, Gauley River).

chemicals to mimic uptake by organisms (Petty et al., 2000). POCIS are designed to monitor more polar or hydrophilic chemicals than the SPMDs (Alvarez et al., 2005). The SPMD and POCIS devices were deployed together for approximately 30 days at the downstream end of the identified nest area in each stream. No passive-sampler results were obtained from the South Branch Springfield site (SB11) because the devices were vandalized. Results from the North Fork Shenandoah site (SR3) previously were reported as part of a larger water quality study of the Shenandoah drainage (Alvarez et al., 2008).

A stainless-steel ladle was used to scoop fine-grained sediment from around the rocks present on the bed surface. Surficial, bed-sediment material was sampled to best represent potential exposures to SMB. Bed sediment was collected from inside the nesting area to the extent possible. The ladle containing collected bed sediment was slowly raised to the water surface to minimize the loss of fine-grained material during

Table 1
Land use and fish intersex metrics for the study sites.

Site	Human population[a]	Catchment area (ha)	Percent land cover[b]			Percent intersex[c]	Intersex severity[c]	TO_{rank}[e]	Estrogenicity ng E2Eq/POCIS[c]
			Urban	Ag	Forest				
Gauley River (WV4)	1,157	18,698	3.9	0.5	95.1	11	0.03	1.0	0
South Branch Petersburg (SB1)	15,067	219,944	3.8	16.4	79.4	82	1.20	3.5	3.6
South Branch Springfield (SB11)	29,003	382,132	3.8	15.2	80.4	74	0.63	2.0	Not available
Shenandoah North Fork (SR3)	67,426	241,004	6.6	32.7	60.2	100	1.62	5.0	4.1
Shenandoah South Fork (SR2)	187,303	336,559	11.1	35.9	52.6	100	2.10	5.5	5.1
Conococheague Creek (upper) (LP5)	88,707	135,000	9.2	32.6	37.5	100[d]	2.10[d]	–	2.6[f]
Conococheague Creek (lower) (LP1)[g]	100,239	145,446	12.4	50.3	35.8	88	1.10	3.5	9.1

[a] Estimated number of people from the US Census 2000, apportioned by percent of census tract in catchment.
[b] Homer et al. (2007).
[c] Blazer et al. (2012).
[d] Iwanowicz et al. (2009).
[e] TO_{rank} was calculated by ranking sites for both percent intersex and intersex severity and taking the average of the two ranks.
[f] Alvarez et al. (2009).
[g] Site downstream of WWTP.

sampling. Collected bed sediment was placed in a stainless-steel stock pot where it was composited, homogenized, and split among the various containers required for analysis. The ladle and stock pot were pre-cleaned with soap and tap water, rinsed with methanol, rinsed with organic-free water, and rinsed with native water prior to the collection of each bed-sediment sample. All samples were chilled immediately upon collection and shipped to participating laboratories.

All SMB were captured by boat electroshocking. SMB were processed and reproductive endpoints and histological evaluations were conducted according to protocols provided in the companion paper describing the biological component of this study (Blazer et al., 2012). To capture both the prevalence and severity of intersex occurrence with a single variable, both biologic endpoints were ranked and the average of the two ranks (TO_{rank}) was used to assess the relation with the chemical data (Table 1).

2.3. Analytical methods

A total of 200 target chemicals were measured in single, discrete-water samples collected from the seven sampling sites using six analytical methods including a filtered water liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for antibiotics (Meyer et al., 2007), a whole water gas chromatography tandem mass spectrometry (GC/MS/MS) method for synthetic and biogenic hormones (Foreman et al., 2012), a filtered water gas chromatography/mass spectrometry (GC/MS) method for pesticides (Sandstrom et al., 2001), a filtered water liquid chromatography/mass spectrometry (LC/MS) method for pharmaceuticals (Furlong et al., 2008), an unfiltered water GC/MS method for wastewater indicator compounds (Zaugg et al., 2006), and an unfiltered water derivatization GC/MS method for alkylphenol ethoxycarboxylates and metal complexing agents (Barber et al., 2000).

A total of 161 target chemicals were measured in single, passive-water samples collected at five sites. All extracts from the passive-water samplers were prepared using established procedures (Alvarez et al., 2008, 2009). Extracts were analyzed for antibiotics (Meyer et al., 2007); synthetic and biogenic hormones (Foreman et al., 2012); pharmaceuticals (Furlong et al., 2008; Schultz and Furlong, 2008); wastewater indicator compounds (Zaugg et al., 2006); agricultural pesticides, halogenated insecticides, polychlorinated biphenyls (PCBs); and polycyclic aromatic hydrocarbons (PAHs) (Alvarez et al., 2008). In addition, POCIS extracts were screened for total estrogenicity using the yeast estrogen screen (YES) (Alvarez et al., 2009). One issue in using passive samplers in a quantitative approach is having a method available to correct for in situ exposure conditions (e.g. water flow rates, water temperature) which are known to affect uptake rates (Harman et al., 2011). While this issue has been overcome with SPMDs through the use of performance reference compounds, this approach is largely not available for POCIS. Thus, for this study, these passive samplers were used to determine only the presence of the target compounds in the dissolved phase as uptake rates were not available for all target chemicals to calculate their concentration in water using these devices.

A total of 130 target chemicals were measured in the single bed-sediment samples collected from seven sites using four separate analytical methods. Pressurized solvent extraction (PSE) and GC/MS were used to determine wastewater indicator compounds (Burkhardt et al., 2006) and halogenated compounds (Zaugg et al., in press). PSE with GC/MS/MS was used for synthetic and biogenic hormones in sediment (Lee et al., 2011). A previous PSE approach was combined with LC/MS/MS to determine pharmaceuticals in sediment (Kinney et al., 2006).

2.4. Quality assurance protocol

A field quality assurance protocol was used to determine effects, if any, of field equipment and procedures on chemical concentrations in environmental samples. For discrete-water samples, a single field blank, field duplicate, and matrix spike were collected. The field blank was prepared using laboratory-grade organic free water and was subjected to the same sample processing, handling, and equipment as the corresponding discrete-water sample. The field blank sample was analyzed for all target compounds. The field duplicate was collected, processed and analyzed along with the routine environmental water sample and was used to assess variation in ambient concentrations. The field duplicate results confirmed the presence or absence of the target compounds measured in the discrete-water samples, with the exception of nonylphenol monoethoxycarboxylate (NP1EC) that was below the reporting limit (<5.0 μg/L) in the environmental sample and just above the reporting limit (5.4 μg/L) in the duplicate. For 12 analytes having a confirmed detection in both the environmental sample and the corresponding field replicate, the relative percent difference (RPD) ranged from 0 to 31% (median RPD = 4%). The field matrix spike consisted of an additional discrete-water sample that was spiked with 73 chemicals in the laboratory. Spike recoveries ranged from 6 to 113% with a median recovery of 82.6%. Five compounds had spike recoveries less than 40%: fluoxetine (6%), tetrachloroethylene (20%), sulfamethoxazole (33%), d-limonene (34%), and thiabendazole (38%).

Quality control samples for the passive samplers included fabrication (laboratory) blanks, field blanks, surrogate recovery spikes, and procedural recovery checks. Fabrication blanks were SPMDs and POCIS constructed concurrently with the field samplers and stored at <−20 °C in air-tight containers until processing commenced. Field blanks were SPMDs and POCIS exposed to the air at each site during the deployment and retrieval operations only. Compounds detected in the five field blanks included methylsalicylate (in 3 blanks), *N*,*N*-diethyl-m-toluamide (DEET) (4), benzophenone (3), para-nonylphenol (2), galaxolide (2), cholesterol (5), and stigmastanol (1). For the SPMDs, 20 of the 33 organochlorine pesticides had trace levels (generally <1 ng/SPMD) present in the field blanks. Results from the passive samplers were censored as non-detects if the regular value was less than the mean of the field blank values plus three times the standard deviation of the blank values (Alvarez et al., 2008). Radiolabeled surrogates, ^{14}C phenanthrene, ^{14}C diazinon, and ^{3}H ethynylestradiol, were used to monitor performance of the POCIS processing steps. Recoveries ranged from 84% (^{14}C diazinon) to 99% (^{3}H ethynylestradiol). Chemicals reported in the study fell within acceptable recovery limits as determined by each cooperating laboratory.

A field duplicate bed-sediment sample was collected that confirmed the presence or absence of 117 of the 129 chemicals measured. Most unconfirmed detections were for compounds with low concentrations near the reporting levels. For the 14 regular/duplicate pairs having a confirmed chemical detection, the RPD ranged from 0 to 181% (median RPD = 29%). The greater number of unconfirmed detections and median RPD for the bed-sediment field duplicate compared to the discrete-water field duplicate reflects the greater chemical heterogeneity of bed sediment compared to stream water and the increased difficulty in analyzing chemicals in bed sediment compared to water.

2.5. Statistical comparisons

Statistical tests were used to determine the significance of relations between water chemistry and the observances of TO in SMB at the study sites (Table 1). No SMB were able to be collected at LP5 during this study. Thus, only the remaining six sites could be used in the comparisons with discrete-water and bed-sediment samples. Because of the sites missing passive water sample results and the detection-only nature of the results, these data were not included in this statistical analysis. Nonparametric techniques were used because of the presence of left-censored water-quality data (concentrations less than analytical reporting levels). A Spearman's rank correlation (rho) was used to test the monotonic relation between all pair-wise continuous variables (Helsel and Hirsch, 1992). A significance (p) level of 0.05 was used for all statistical tests.

3. Results

A total of 283 chemicals were analyzed among the three collected sample types. There were no detectable concentrations for 148 (52%) of these chemicals in any of the sample types collected (Table 2).

3.1. Discrete-water samples

Of the 200 chemicals measured in the discrete-water samples, 39 (20%) were detected in at least one of the seven samples collected (Table 2). Chemicals detected included 14 prescription/nonprescription pharmaceuticals, seven herbicides, seven industrial/commercial chemicals, five household/personal care products, four insecticides, and two natural fragrances. No biogenic hormones or sterols were detected in the discrete-water samples.

The most frequently detected chemicals included atrazine (herbicide, 86%), caffeine (prescription/nonprescription pharmaceutical, 86%), deethylatrazine (herbicide, atrazine degradate, 86%), simazine (herbicide, 86%), iso-chlorotetracycline (prescription/nonprescription pharmaceutical, antibiotic degradate, 71%), metolachlor (herbicide, 71%), epi-iso-chlorotetracycline (prescription/nonprescription pharmaceutical, antibiotic degradate, 57%), galaxolide (household/personal care product, fragrance, 57%), ethylenediaminetetraacetic acid (EDTA, chelating agent, 57%), tetracycline (prescription/nonprescription pharmaceutical, antibiotic, 57%), and oxytetracycline (prescription/nonprescription pharmaceutical, antibiotic, 43%). Only four chemicals (EDTA, isochlorotetracycline, 4-nonylphenolmonoethoxycarboxylate, and 4-nonylphenoldiethoxycarboxylate) had a maximum concentration that exceeded 1 μg/L (Table 2).

The number of chemicals detected in the discrete-water samples was relatively similar among sampling sites within the Potomac drainage (11 to 16 compounds), whereas only 5 chemicals (caffeine, camphor, DEET, methyl salicylate, and skatol) were detected at the out-of-basin reference site (WV4). In particular, the detection of herbicides was uniform among the in-basin sites (4 to 6 herbicides detected), whereas herbicides were not detected in the out-of-basin reference site. In contrast, select chemical groups had substantially different numbers of compounds detected among the sampling sites. For example, insecticides were only detected in the two sampling sites from Conococheague Creek (LP1 and LP5). Antibiotic compounds were found most frequently at sites SB11, SR3, and SB1. Total concentrations of tetracyclines (parents plus degradates) ranged dramatically among the sampling sites: SB11 (4.7 μg/L) > SR3 (0.85 μg/L) > SB1 (0.21 μg/L) > SR2 (0.076 μg/L) > LP1 (0.005 μg/L) > LP5 (no detections) = WV5 (no detections).

3.2. Passive-water samples

Of the 161 target chemicals measured in the five passive-water samples, 100 (62%) were found in a least one such sample (Table 2). The chemicals detected included 29 industrial/commercial chemicals, 24 insecticides, 20 prescription/nonprescription pharmaceuticals, 10 household/personal care products, six biogenic hormones/sterols, five herbicides, three natural fragrances, two flame retardants, and 1 fungicide. There were 25 chemicals that were found at all five sites where passive samplers were deployed (Table 2).

While there was not a complete overlap for chemicals being measured in both discrete- and passive-water samples, there were 40 compounds that had no detections in any of the discrete-water samples but were detected in at least one passive-water sample. Of particular note, six biogenic hormones/sterols were detected in the passive-water samples but not in the corresponding discrete-water samples (Table 2). At least one biogenic hormone/sterol was found at all five sampling sites with available passive-water samples. This suggests that such compounds were present at concentrations below the reporting levels in the corresponding discrete-water samples or were episodic in nature and missed with the single discrete-water sample. Thus, such passive-water samples provide additional complimentary information on potential chemical exposure beyond that provided by a single discrete-water sample. As expected, there was a direct relation between both the number of estrogenic hormones detected in the passive-water samples per site ($p = 0.005$, Spearman rank correlation) and total estrogenicity based on the YES assay (Table 1).

The number of compounds detected in the passive-water samples varied substantially among sampling sites (WV4 = 39, SB1 = 45, SR2 = 77, LP5 = 82, and LP1 = 87). While it was expected that the fewest number of detected compounds would be found in WV4, the low number of compounds detected in the passive-water samples from SB1 was surprising as no such trend was observed in the discrete-water samples. While current-use insecticides were only detected at two sites in the discrete-water samples (LP1 and LP5), legacy halogenated insecticides were relatively evenly detected in the passive-water samples across all sites (WV4 = 13, SB1 = 13, SR2 = 19, LP1 = 21, and LP5 = 23). In addition, while prescription/nonprescription pharmaceuticals were relatively evenly detected in the passive-water samples at most sampling sites within the Potomac

(WV4 = 1, SB1 = 8, LP5 = 16, SR2 = 16, LP1 = 19), fewer such chemicals were detected in SB1 and only caffeine was detected at WV4.

3.3. Bed-sediment samples

Of the 130 target chemicals measured in the bed-sediment samples, 46 (35%) were detected in at least one of the seven samples collected (Table 2), with 17 chemicals detected in more than 50% of the samples, 12 in more than 70%, and five compounds (caffeine, cholesterol, indole, para-cresol, and sitosterol) detected in all seven bed-sediment samples. The 46 compounds detected included 16 industrial/commercial chemicals, 10 insecticides, seven biogenic hormones/sterols, six flame retardants, two herbicides, two natural fragrances, one fungicide, one household/personal care product, and one prescription/nonprescription pharmaceutical. Because of the complex matrix, however, fewer pesticides and pharmaceuticals were able to be measured in bed-sediment samples. Of particular note, while biogenic hormones/sterols were not detected in the discrete-water samples above reporting levels, four compounds were frequently detected (>70%) in the corresponding bed-sediment samples (Table 2). Biogenic hormones have been documented to have an affinity for binding to sediments (Ying et al., 2002). Previous research has documented the prevalence of a variety of chemicals (including hormones and pharmaceuticals) in bed sediment in the Potomac basin (Reif et al., 2012).

The number of compounds detected in the bed-sediment samples varied among the sites (SB11 = 11, SB1 = 13, SR2 = 16, LP1 = 21, WV4 = 21, SR3 = 26, and LP5 = 29). The number and type of compounds found in bed sediments were substantially different than found with both the discrete- and passive-water samples as one would expect based on the properties of the target chemicals and has been reported

Table 2
Summary results for the 283 organic contaminants measured in environmental samples collected from the network of six streams sites in the Potomac basin and one out-of-basin reference site, 2007. Shaded rows represent chemicals without detections in any sample type. As uptake rates into the passive sampler devices were not available for all chemicals, only frequencies of detection are provided for passive-water samples.

		Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
Compound	CASRN	RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Industrial/commercial chemicals									
1,2-Dimethylnaphthalene	573-98-8	NA	–	–	2.0 - S	0	NA	–	–
1,4-Dichlorobenzene	106-46-7	0.08	0	–	130 - P	0	50	0	–
1-Ethylnaphthalene	1127-76-0	NA	–	–	2.0 - S	0	NA	–	–
1-Methylfluorene	1730-37-6	NA	–	–	2.0 - S	20	NA	–	–
1-Methylnaphthalene	90-12-0	0.1	0	–	2.0 - S	60	50	14	31.8
2,3,5-Trimethylnaphthalene	2245-38-7	NA	–	–	20 - S	20	NA	–	–
2,6-Dimethylnaphthalene	581-42-0	0.2	14	0.008	NA	–	50	57	18.1
2-Methylfluoranthene	33543-31-6	NA	–	–	2.0 - S	40	NA	–	–
2-Methylnaphthalene	91-57-6	0.08	0	–	2.0 - S	60	50	14	47.1
2-Methylphenanthrene	2531-84-2	NA	–	–	2.0 - S	40	NA	–	–
3,6-Dimethylphenanthrene	1576-67-6	NA	–	–	2.0 - S	40	NA	–	–
3-tert-Butyl-4-hydroxyanisole (BHA)	25013-16-5	0.6	0	–	NA	–	150	0	–
4-Methylbiphenyl		NA	–	–	2.0 - S	0	NA	–	–
5-Methyl-1H-benzotriazle	136-85-6	1.8	0	–	510 - P	0	NA	–	–
9-Methylanthracene	779-02-2	NA	–	–	2.0 - S	0	NA	–	–
Acenaphthene	83-32-9	NA	–	–	2.0 - S	100	NA	–	–
Acenaphthylene	208-96-8	NA	–	–	2.0 - S	0	NA	–	–
Anthracene	120-12-7	0.08	0	–	32 - P	40	50	0	–
Anthraquinone	84-65-1	0.16	0	–	64 - P	0	50	29	14.9
Benz[a]anthracene	56-55-3	NA	–	–	2.0 - S	40	NA	–	–
Benzo[a]pyrene	50-32-8	0.12	0	–	2.0 - S	20	50	14	11.1
Benzo[b]fluoranthene	205-99-2	NA	–	–	2.0 - S	40	NA	–	–
Benzo[b]naphtho[2,1-d]thiophene	205-43-6	NA	–	–	2.0 - S	20	NA	–	–
Benzo[b]thiophene	95-15-8	NA	–	–	2.0 - S	0	NA	–	–
Benzo[e]pyrene	192-97-2	NA	–	–	2.0 - S	60	NA	–	–
Benzo[g,h,i]perylene	191-24-2	NA	–	–	2.0 - S	0	NA	–	–
Benzo[k]fluoranthene	207-08-9	NA	–	–	2.0 - S	60	NA	–	–
Biphenyl	92-52-4	NA	–	–	2.0 - S	60	NA	–	–

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Bromoform	75-25-2	0.08	14	0.009	260 - P	0	NA	–	–
Carbazole	86-74-8	0.08	0	–	63 - P	0	50	29	11.2
Chrysene	218-01-9	NA	–	–	2.0 - S	80	NA	–	–
Dibenz[a,h]anthracene	53-70-3	NA	–	–	2.0 - S	0	NA	–	–
Dibenzothiophene	132-65-0	NA	–	–	2.0 - S	20	NA	–	–
Diethyl phthalate	84-66-2	NA	–	–	NA	–	100	14	45.8
Diethylhexyl phthalate	117-81-7	NA	–	–	NA	–	250	0	–
Ethylenediaminetetra-acetic acid (EDTA)	60-00-4	0.5	57	8.1	NA	–	NA	–	–
Fluoranthene	206-44-0	0.08	0	–	2.0 - S	100	50	57	24.6
Fluorene	86-73-7	NA	–	–	2.0 - S	100	NA	–	–
Indeno[1,2,3-c,d]pyrene	193-39-5	NA	–	–	2.0 - S	0	NA	–	–
Isophorone	78-59-1	0.14	14	0.02	64 - P	80	50	0	–
Isopropylbenzene (cumene)	98-82-8	0.1	0	–	64 - P	0	100	0	–
Isoquinoline	119-65-3	0.4	0	–	64 - P	0	100	0	–
Naphthalene	91-20-3	0.1	14	20.03	2.0 - S	100	50	14	17.7
Nitrilotriacetic acid (NTA)	139-13-9	0.5	0	–	NA	–	NA	–	–
para-Cresol	106-44-5	0.18	14	10.05	130 - P	100	250	100	396
Pentachloroanisole (PCA)	1825-21-4	NA	–	–	49 - S	0	0.1	14	0.12
Pentachlorophenol	87-86-5	NA	–	–	510 - P	0	NA	–	–
Perylene	198-55-0	NA	–	–	2.0 - S	60	NA	–	–
Phenanthrene	85-01-8	0.08	0	–	20 - S	100	50	14	61.8
Phenol	108-95-2	0.4	14	0.092	130 - P	80	50	43	63
Pyrene	129-00-0	0.08	0	–	2.0 - S	100	50	43	22.8
Tetrachloroethylene	127-18-4	0.18	0	–	NA	–	NA	–	–
Total PCBs	1336-36-3	NA	–	–	9.1 - S	100	0.1-2	86	0.41
Tri(2-butoxyethyl) phosphate	78-51-3	0.5	0	–	1000 - P	40	150	0	–
Triphenyl phosphate	115-86-6	0.16	0	–	130 - P	0	50	14	8.92
Biogenic hormones/sterols									
11-Ketotestosterone	53187-98-7	0.02	0	–	5.0 - P	0	1	0	–
17-alpha-Estradiol	57-63-6	0.004	0	–	1.0 - P	60	1	0	–
17-beta-Estradiol	50-28-2	0.004	0	–	1.0 - P	40	1	14	0.22
Androstenedione	63-05-8	0.008	0	–	2.0 - P	80	2.6	0	–
Cholesterol	57-88-5	5.0	0	–	510 - P	0	250	100	1940
cis-Androsterone	53-41-8	0.004	0	–	1.0 - P	0	1	0	–
Coprostanol	360-68-9	4.0	0	–	510 - P	0	500	14	68.8
Dihydrotestosterone	521-18-6	0.008	0	–	20 - P	0	1	0	–
Epitestosterone	481-30-1	0.008	0	–	20 - P	0	5	0	–
Equilenin	517-09-9	NA	–	–	NA	–	2.6	0	–
Equilin	474-86-2	NA	–	–	NA	–	5	0	–
Estiol	50-27-1	0.004	0	–	1.0 - P	0	1	0	–
Estrone	53-16-7	0.004	0	–	1.0 - P	60	1	71	0.92
Progesterone	57-83-0	0.02	0	–	5.0 - P	0	5	14	6.38
Sitosterol	83-46-5	2.0	0	–	P	80	500	100	6270
Stigmastanol	19466-47-8	2.0	0	–	510 - P	20	500	86	1080
Testosterone	58-22-0	0.008	0	–	2.0 - P	0	1	0	–

(*continued on next page*)

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Prescription/nonprescription pharmaceuticals (includes their degradates)									
1,7-Dimethylxanthine	611-59-6	0.021	0	–	5 - P	0	4	0	–
Acetaminophen	103-90-2	0.024	29	0.029	5 - P	20	1.5	0	–
Albuterol	18559-94-9	0.014	0	–	5 - P	0	2.2	0	–
Azithromycin	83905-01-5	0.005	14	0.013	1.0 - P	60	NA	–	–
Bupropion	34841-39-9	NA	–	–	0.9 - P	60	NA	–	–
Caffeine	58-08-2	0.015	86	0.076	5 - P	80	2.6	100	25.9
Carbamazepine	298-46-4	0.018	29	0.015	1.0 - P	80	3.3	0	–
Chloramphenicol	56-75-7	0.1	0	–	NA	–	NA	–	–
Chlorotetracycline	57-62-5	0.1	0	–	NA	–	NA	–	–
Cimetidine	51481-61-9	NA	–	–	5 - P	0	1.8	0	–
Ciproflaxacin	85721-33-1	0.005	0	–	NA	–	NA	–	–
Citalopram	59729-33-8	NA	–	–	0.9 - P	60	NA	–	–
Codeine	76-57-3	0.022	0	–	5 - P	0	2.6	0	–
Cotinine	486-56-6	0.028	0	–	5 - P	80	2.6	0	–
Dehydronifedipine	67035-22-7	0.022	0	–	5 - P	20	3.4	0	–
Diltiazem	42399-41-7	0.018	0	–	5 - P	0	2.9	0	–
Diphenhydramine	58-73-1	0.023	14	0.007	5 - P	60	2.7	0	–
Doxycycline	564-25-0	0.1	0	–	NA	–	NA	–	–
Duloxetine	116539-59-4	NA	–	–	0.9 - P	0	NA	–	–
Enrofloxacin	93106-60-6	0.005	0	–	NA	–	NA	–	–
Epi-chlorotetracycline	14297-93-9	0.1	0	–	NA	–	NA	–	–
Epi-iso-chlorotetracycline	NA	0.1	57	0.879	NA	–	NA	–	–
Epi-oxytetracycline	35259-39-3	0.1	0	–	NA	–	NA	–	–
Epi-tetracycline	79-85-6	0.1	29	0.122	NA	–	NA	–	–
Erythromycin	144-07-8	0.008	0	–	1.0 - P	80	3.3	0	–
Erythromycin-H2O	23893-13-2	0.008	0	–	1.0 - P	80	NA	–	–
17-alpha Ethynyl estradiol	57-63-6	0.004	0	–	1.0 - P	0	1	0	–
Fluoxetine	54910-89-3	0.016	0	–	0.9 - P	0	4.3	0	–
Fluvoxamine	54739-18-3	NA	–	–	0.9 - P	0	NA	–	–
Ibuprofen	15687-27-1	0.05	0	–	1.0 - P	20	NA	–	–
Iso-chlorotetracycline	514-53-4	0.1	71	2.483	NA	–	NA	–	–
Lincomycin	154-21-2	0.005	0	–	NA	–	NA	–	–
Lomefloxacin	98079-51-7	0.005	0	–	NA	–	NA	–	–
Mestranol	72-33-3	0.004	0	–	1.0 - P	0	1	0	–
Miconazole	22832-87-7	NA	–	–	5 - P	0	1.9	0	–
Norethindrone	68-22-4	0.008	0	–	2.0 - P	0	1	0	–
Norfloxacin	70458-96-7	0.005	0	–	NA	–	NA	–	–
Norfluoxetine	83891-03-6	NA	–	–	0.9 - P	0	NA	–	–
Ofloxacin	82419-36-1	0.005	0	–	1.0 - P	60	NA	–	–
Ormetoprim	6981-18-6	0.005	0	–	NA	–	NA	–	–
Oxytetracycline	79-57-2	0.1	43	0.648	NA	–	NA	–	–
Paroxetine	61869-08-7	NA	–	–	0.9 - P	0	NA	–	–
Ranitidine	66357-35-5	0.025	0	–	5 - P	0	2.2	0	–
Roxithromycin	80214-83-1	0.005	0	–	1.0 - P	0	NA	–	–
Sarafloxacin	98105-99-8	0.005	0	–	NA	–	NA	–	–
Sertraline	79617-96-2	NA	–	–	0.9 - P	20	NA	–	–
Sulfachloropyridazine	80-32-0	0.005	0	–	1.0 - P	0	NA	–	–
Sulfadiazine	68-35-9	0.1	0	–	NA	–	NA	–	–

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Sulfadimethoxine	122-11-2	0.005	0	–	1.0 - P	80	NA	–	–
Sulfamethazine	57-68-1	0.005	29	0.208	1.0 - P	40	NA	–	–
Sulfamethoxazole	723-46-6	0.005	29	0.018	1.0 - P	60	3.2	0	–
Sulfathiazole	72-14-0	0.02	14	0.04	1.0 - P	0	NA	–	–
Tetracycline	60-54-8	0.1	57	0.519	NA	–	NA	–	–
Thiabendazole	148-79-8	0.025	0	–	5 - P	0	2.1	0	–
trans-Diethylstilbestrol	56-53-1	NA	–	–	NA	–	1	0	–
Trimethoprim	738-70-5	0.005	0	–	1.0 - P	60	2.9	0	–
Tylosin	1401-69-0	0.005	14	0.017	1.0 - P	0	NA	–	–
Venlafaxine	93413-69-5	NA	–	–	0.9 - P	80	NA	–	–
Virginiamycin	21411-53-0	0.005	0	–	1.0 - P	0	NA	–	–
Warfarin	81-81-2	0.019	0	–	5 - P	20	2.5	0	–
Insecticides (includes their degradates)									
1-Naphthol	90-15-3	0.088	0	–	NA	–	NA	–	–
alpha-Hexachlorocyclohexane (alpha-HCH)	319-84-6	NA	–	–	4.0 - S	0	NA	–	–
Azinphos-methyl	86-50-0	0.08	0	–	NA	–	NA	–	–
Azinphos-methyl-oxon	961-22-8	0.042	0	–	NA	–	NA	–	–
beta-Hexachlorocyclohexane (beta-HCH)	319-85-7	NA	–	–	0.2 - S	60	NA	–	–
Carbaryl	63-25-2	0.06	29	0.006	NA	–	NA	–	–
Carbofuran	1563-66-2	0.02	0	–	NA	–	NA	–	–
Chlorpyrifos	2921-88-2	0.005	0	–	2.4 - S	100	0.2	29	0.06
cis-Chlordane	5103-71-9	NA	–	–	2.6 - S	100	0.2	71	0.09
cis-Nonachlor	5103-73-1	NA	–	–	0.2 - S	60	0.1	57	0.02
cis-Permethrin	61949-76-6	0.01	0	–	28 - S	0	NA	–	–
Cyfluthrin	68359-37-5	0.053	0	–	NA	–	0.2	0	–
Cyhalothrin	68085-85-8	NA	–	–	NA	–	0.2	0	–
Cypermethrin	52315-07-8	0.046	0	–	NA	–	NA	–	–
delta-Hexachlorocyclohexane (delta-HCH)	319-86-8	NA	–	—	2.7 - S	80	NA	–	–
Desulfinylfipronil	205650-65-3	0.012	29	0.004	NA	–	NA	–	–
Desulfinylfipronil amide	NA	0.029	0	–	NA	–	NA	–	–
Diazinon	333-41-5	0.005	0	–	NA	–	50	0	–
Diazoxon	962-58-3	0.005	0	–	NA	–	NA	–	–
Dichlorvos	62-73-7	0.013	0	–	130 - P	20	NA	–	–
Dicrotophos	141-66-2	0.084	0	–	NA	–	NA	–	–
Dieldrin	60-57-1	0.009	0	–	0.46 - S	100	0.1	71	0.13
Dimethoate	60-51-5	0.006	0	–	NA	–	NA	–	–
Disulfoton	298-04-4	0.02	0	–	NA	–	NA	–	–
Disulfoton sulfone	2497-06-5	0.014	0	–	NA	–	NA	–	–
Endosulfan I	959-98-8	0.011	0	–	7.9 - S	0	0.2	14	0.01
Endosulfan sulfate	1031-07-8	0.022	0	–	0.2 - S	40	NA	–	–
Endosulfan II	115-29-7	NA	–	–	0.2 - S	20	NA	–	–
Endrin	72-20-8	NA	–	–	4.2 - S	40	NA	–	–
Ethion	563-12-2	0.016	0	–	NA	–	NA	–	–
Ethion monoxon	17356-42-2	0.021	0	–	NA	–	NA	–	–

(*continued on next page*)

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Ethoprophos	13194-48-4	0.012	0	–	NA	–	NA	–	–
Fenamiphos	22224-92-6	0.029	0	–	NA	–	NA	–	–
Fenamiphos sulfone	31972-44-8	0.053	0	–	NA	–	NA	–	–
Fenamiphos sulfoxide	31972-43-7	0.04	0	–	NA	–	NA	–	–
Fipronil	120068-37-3	0.016	14	0.007	NA	–	0.1	43	0.14
Fipronil sulfide	120067-83-6	0.013	14	0.005	NA	–	0.1	29	0.01
Fipronil sulfone	120068-36-2	0.024	0	–	NA	–	NA	–	–
Fonofos	944-22-9	0.006	0	–	NA	–	NA	–	–
Heptachlor	76-44-8	NA	–	–	0.2 - S	60	NA	–	–
Heptachlor epoxide	1024-57-3	NA	–	–	0.2 - S	80	NA	–	–
Isofenphos	25311-71-1	0.011	0	–	NA	–	NA	–	–
lambda-Cyhalothrin	91465-08-6	0.014	0	–	NA	–	NA	–	–
Lindane (gamma-HCH)	58-89-9	NA	–	–	1.6 - S	80	NA	–	–
Malaoxon	1634-78-2	0.039	0	–	NA	–	NA	–	–
Malathion	121-75-5	0.016	0	–	NA	–	NA	–	–
Methidathion	950-37-8	0.009	0	–	NA	–	NA	–	–
Methyl parathion	298-00-0	0.008	0	–	NA	–	NA	–	–
Mirex	2385-85-5	NA	–	–	0.2 - S	0	NA	–	–
o,p'-DDD	53-19-0	NA	–	–	0.2 - S	60	NA	–	–
o,p'-DDE	3424-82-6	NA	–	–	1.4 - S	100	NA	–	–
o,p'-DDT	789-02-6	NA	–	–	6.2 - S	60	NA	–	–
Oxychlordane	27304-13-8	NA	–	–	0.2 - S	40	1	0	–
Paraoxon-methyl	950-35-6	0.019	0	–	NA	–	NA	–	–
p,p'-DDD	72-54-8	NA	–	–	1.1 - S	80	2	0	–
p,p'-DDE	72-55-9	NA	–	–	3.6 - S	100	1	29	0.57
p,p'-DDT	50-29-3	NA	–	–	5.5 - S	100	4	0	–
p,p'-Methoxychlor	72-43-5	NA	–	–	2.0 - S	100	NA	–	–
Phorate	298-02-2	0.02	0	–	NA	–	NA	–	–
Phorate oxygen analog	2600-69-3	0.027	0	–	NA	–	NA	–	–
Phosmet	732-11-6	0.008	0	–	NA	–	NA	–	–
Propargite	2312-35-8	0.02	0	–	NA	–	NA	–	–
Tefluthrin	79538-32-2	0.003	0	–	5.6 - S	100	0.5	0	–
Terbufos	13071-79-9	0.012	0	–	NA	–	NA	–	–
Terbufos oxygen analog sulfone	56070-15-6	0.045	0	–	NA	–	NA	–	–
Tetradifon	116-29-0	NA	–	–	NA	–	0.2	0	–
trans-Chlordane	5103-74-2	NA	–	–	0.2 - S	100	0.2	86	0.05
trans-Nonachlor	39765-80-5	NA	–	–	0.2 - S	100	0.1	86	0.06
trans-Permethrin	61949-77-7	NA	–	–	14 - S	0	NA	–	–
Herbicides (includes their degradates)									
2,6-Diethylaniline	579-66-8	0.006	0	–	NA	–	NA	–	–
2-Chloro-2,6-diethylacetanilide	6967-29-9	0.007	0	–	NA	–	NA	–	–
2-Ethyl-6-methylaniline	24549-06-2	0.01	0	–	NA	–	NA	–	–
3,4-Dichloroaniline	95-76-1	0.005	29	0.009	NA	–	NA	–	–
3,5-Dichloroaniline	626-43-7	0.012	0	–	NA	–	NA	–	–
4-Chloro-2-methylphenol	1570-64-5	0.005	0	–	NA	–	NA	–	–
Acetochlor	34256-82-1	0.006	29	0.015	NA	–	NA	–	–
Alachlor	15972-60-8	0.005	0	–	NA	–	NA	–	–

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
Atrazine	1912-24-9	0.007	86	0.071	260 - P	100	100	0	–
Benfluralin	1861-40-1	0.01	0	–	NA	–	0.2	0	–
Bromacil	314-40-9	0.4	0	–	260 - P	0	500	0	–
Cyanazine	21725-46-2	0.018	0	–	NA	–	NA	–	–
Dacthal (DCPA)	1861-32-1	0.003	0	–	0.8 - S	40	0.2	14	0.01
Deethylatrazine	6190-65-4	0.006	86	0.081	NA	–	NA	–	–
EPTC	759-94-4	0.002	0	–	NA	–	NA	–	–
Hexazinone	51235-04-2	0.026	0	–	NA	–	NA	–	–
Metolachlor	51218-45-2	0.01	71	0.036	64 - P	80	50	0	–
Metribuzin	21087-64-9	0.012	0	–	NA	–	NA	–	–
Molinate	2212-67-1	0.003	0	–	NA	–	NA	–	–
Oxyfluorfen	42874-03-3	0.017	0	–	NA	–	4	0	–
Prometon	1610-18-0	0.01	29	0.011	260 - P	60	50	0	–
Prometryn	7287-19-6	0.006	0	–	NA	–	NA	–	–
Pronamide	23950-58-5	0.004	0	–	NA	–	NA	–	–
Propanil	709-98-8	0.011	0	–	NA	–	NA	–	–
Simazine	122-34-9	0.006	86	0.053	NA	–	NA	–	–
Pendimethalin	40487-42-1	0.02	0	–	NA	–	1	0	–
Tebuthiuron	34014-18-1	0.016	0	–	NA	–	NA	–	–
Terbuthylazine	5915-41-3	0.008	0	–	NA	–	NA	–	–
Thiobencarb	28249-77-6	0.01	0	–	NA	–	NA	–	–
Tribufos	78-48-8	0.035	0	–	NA	–	NA	–	–
Trifluralin	1582-09-8	0.009	0	–	0.02 - S	100	0.2	14	0.02
Household/personal care products									
4-Cumylphenol	599-64-4	0.14	0	–	NA	–	50	0	–
4-n-Octylphenol	1806-26-4	0.16	0	–	NA	–	50	0	–
4-Nonylphenol diethoxylate	84852-15-3	5.0	0	–	1000 - P	40	1000	0	–
4-Nonylphenol monoethoxylate (NP1EO)	9016-45-0	2.0	0	–	510 - P	40	500	0	–
4-Octylphenol diethoxylate	9036-19-5	1.0	0	–	NA	–	50	0	–
4-Octylphenol monoethoxylate	Not available	1.0	0	–	64 - P	0	250	0	–
4-tert-octylphenol	140-66-9	0.1	0	–	32 - P	0	50	0	–
Acetophenone	98-86-2	0.1	0	–	130 - P	100	150	0	–
Benzophenone	119-61-9	0.18	0	–	130 - P	0	50	0	–
d-Limonene	5989-27-5	0.14	0	–	260 - P	0	50	0	–
Ethyl citrate	77-93-0	0.4	0	–	64 - P	60	NA	–	–
Galaxolide (HHCB)	1222-05-5	0.5	57	0.027	63 - P	60	50	0	–
Menthol	1490-04-6	0.2	0	–	510 - P	60	50	0	–
Methoxytriclosan	4640-01-1	NA	–	–	NA	–	6	0	–
Methyl Salicylate	119-36-8	0.18	14	0.014	130 - P	40	NA	–	–
N,N-Diethyl-m-toluamide (DEET)	134-62-3	0.2	29	0.019	64 - P	100	100	0	–
4-Nonylphenolmonoethoxy-carboxylate (NP1EC)	Not available	5.0	29	6.9	NA	–	NA	–	–
4-Nonylphenoldiethoxy-carboxylate (NP2EC)	106807-78-7	1.0	14	2.2	NA	–	NA	–	–

(continued on next page)

Table 2 (*continued*)

Compound	CASRN	Discrete Water (N = 7)			Passive water (N = 5)		Bed sediment (N = 7)		
		RL (μg/L)	Freq (%)	Max (μg/L)	RL (ng/sampler)	Freq (%)	RL (μg/kg)	Freq (%)	Max (μg/kg)
4-Nonylphenoltriethoxy-carboxylate (NP3EC)	Not available	1.0	0	–	NA	–	NA	–	–
4-Nonylphenoltetraethoxy-carboxylate (NP4EC)	Not available	1.0	0	–	NA	–	NA	–	–
para-Nonylphenol	84852-15-3	1.8	0	–	460 - P	40	750	0	–
Tonalide (AHTN)	1506-02-1	0.5	0	–	63 - P	60	50	0	–
Triclosan	3380-34-5	0.2	0	–	510 - P	60	4	43	4.12
Flame retardants									
1,2-bis (2,4,6-Tribromophenoxy) ethane	37853-59-1	NA	–	–	NA	–	0.1	0	–
Dechlorane Plus	13560-89-9	NA	–	–	NA	–	1	29	0.28
PBDE 47	5436-43-1	NA	–	–	NA	–	0.2	0	–
PBDE 66	189084-61-5	NA	–	–	NA	–	0.1	0	–
PBDE 71	189084-62-6	NA	–	–	NA	–	0.1	14	0.02
PBDE 85	182346-21-0	NA	–	–	NA	–	0.1	14	0.04
PBDE 99	60348-60-9	NA	–	–	NA	–	0.2	14	0.32
PBDE 100	189084-64-8	NA	–	–	NA	–	0.1	57	0.1
PBDE 138	182677-30-1	NA	–	–	NA	–	0.1	0	–
PBDE 153	68631-49-2	NA	–	–	NA	–	0.1	0	–
PBDE 154	207122-15-4	NA	–	–	NA	–	0.1	14	0.02
PBDE 183	207122-16-5	NA	–	–	NA	–	0.1	0	–
Pentabromotoluene	87-83-2	NA	–	–	NA	–	1	0	–
Tri(2-chloroethyl)phosphate	115-96-8	0.18	0	–	260 - P	80	100	0	–
Tri(dichlorisopropyl)phosphate	13674-87-8	0.18	0	–	NA	–	100	0	–
Tributylphosphate	126-73-8	0.2	0	–	64 - P	60	50	0	–
Fungicides									
cis-Propiconazole	60207-90-1	0.013	0	–	NA	–	NA	–	–
Hexachlorobenzene (HCB)	118-74-1	NA	–	–	0.3 - S	100	0.1	14	0.09
Iprodione	36734-19-7	0.026	0	–	NA	–	NA	–	–
Metalaxyl	57837-19-1	0.007	0	–	260 - P	0	NA	–	–
Myclobutanil	88671-89-0	0.033	0	–	NA	–	NA	–	–
Pentachloronitrobenzene	82-68-8	NA	–	–	NA	–	0.1	0	–
Tebuconazole	107534-96-3	0.014	0	–	NA	–	NA	–	–
trans-Propiconazole	60207-90-1	0.034	0	–	NA	–	NA	–	–
Natural fragrances									
Camphor	76-22-2	0.1	14	0.037	130 - P	80	50	0	–
Indole	120-72-9	0.14	0	–	64 - P	100	100	100	197
Isoborneol	124-76-5	0.06	0	–	NA	–	50	0	–
Skatol	83-34-1	0.08	14	0.004	64 - P	100	50	57	78.5

RL, reporting level; Freq, frequency of detection, Max, maximum concentration; NA, not analyzed; –, not applicable; S, semipermeable membrane device (SPMD); P, polar organic compound integrative sampler (POCIS); PBDE, pentabromodiphenyl ether.

in the literature (Reif et al., 2012). Such differences are likely driven by variation in the fraction of fine-grained material or percent organic carbon among sampling sites as sorption of organic contaminants typically increases with increasing organic carbon (Schwarzenbach et al., 1993). Neither a size characterization nor percent organic carbon was determined for these samples.

3.4. Relations between chemicals and testicular oocytes

Given the small sample size (N = 6), a statistically significant relation between water chemistry and TO_{rank} was observed for only a few compounds. No significant relation was determined between the total number of chemicals detected per discrete-water sample and TO_{rank} ($p = 0.525$). When the detected compounds were segregated into chemical groups (Table 2) and the total concentration and the total number of compounds detected were used for each chemical group, a significant relation was noted only between the number of herbicides detected per site and TO_{rank} (rho = 0.868, $p = 0.025$). Of the 11 chemicals detected in at least three of the discrete-water samples, only atrazine was significantly related to TO_{rank} (Table 3, Fig. 2A). Similar to the discrete-water results, there was no significant relation ($p = 0.674$) between the number of chemicals detected per bed-sediment sample and TO_{rank}. Upon segregation of detected compounds into chemical groups (Table 2), a strong significant relation (rho = 0.986, $p < 0.001$) was observed between TO_{rank} and total hormone/sterol concentration. Of the 21 chemicals detected in at least three of the bed-sediment samples, four (sitosterol, trans-nonachlor, stigmastanol, and fluoranthene) were significantly related with TO_{rank} (Table 3, Fig. 2). It is important to note, however, that significant correlations to TO_{rank} do not necessarily denote a causal relation. Such causal relations can only be determined with additional research. Of the five chemicals that were significantly related to TO_{rank} (Fig. 2), all were significantly related to each other ($p < 0.05$, Spearman rank correlation) except for fluoranthene. Such inter-correlations provide additional evidence that the significant correlations to TO_{rank} (Fig. 2) may not all be causal in nature.

Table 3
Spearman rank correlations (ρ) between TO_{rank} and chemicals with at least three detections in either discrete-water or bed-sediment samples. Significant correlations are highlighted in bold.

Compound	Discrete water		Bed sediment	
	ρ	p-Value	ρ	p-Value
2,6-Dimethylnaphthalene	–	–	−0.031	0.954
Ethylenediaminetetra-acetic acid (EDTA)	0.647	0.165	NA	–
Fluoranthene	–	–	**−0.832**	**0.04**
para-Cresol	–	–	0.203	0.7
Phenol	–	–	−0.069	0.897
Pyrene	–	–	−0.429	0.396
Total PCBs	NA	–	0.632	0.178
Cholesterol	–	–	0.696	0.125
Estrone	–	–	0.691	0.128
Sitosterol	–	–	**0.986**	**<0.001**
Stigmastanol	–	–	**0.899**	**0.015**
Caffeine	0.029	0.957	0.725	0.103
Epi-iso-chlorotetracycline	0.132	0.803	NA	–
Iso-chlorotetracycline	0.203	0.700	NA	–
Oxytetracycline	−0.092	0.862	NA	–
Tetracycline	0.132	0.803	NA	–
cis-Chlordane	NA	–	0.544	0.264
cis-Nonachlor	NA	–	0.792	0.06
Dieldrin	–	–	0.313	0.545
Fipronil	–	–	0.360	0.483
trans-Chlordane	NA	–	0.627	0.183
trans-Nonachlor	NA	–	**0.925**	**0.008**
Atrazine	**0.899**	**0.015**	–	–
Deethylatrazine	0.667	0.148	NA	–
Metolachlor	0.806	0.053	–	–
Simazine	0.720	0.106	NA	–
Galaxolide (HHCB)	0.609	0.200	–	–
Triclosan	–	–	0.789	0.062
BDE 100	NA	–	0.627	0.183
Indole	–	–	0.783	0.066
Skatol	–	–	0.092	0.862

–, fewer than three detections; NA, not analyzed.

4. Discussion

This study demonstrated that: 1) there generally were a greater number of chemicals detected at sites where adverse biological effects on fish also were observed; 2) there was a benefit of using a variety of sample types to evaluate potential relations between contaminant occurrence and concentration to corresponding observed biological effects, and 3) the collection of water and bed sediment from nesting sites during spawning provides evidence of potential exposure pathways during a critical life stage for SMB. Many detected chemicals have been shown to cause deleterious effects to aquatic organisms including current use pesticides (e.g. atrazine), antibiotics (e.g. tetracycline), steroid hormones (e.g. 17β-estradiol), and chemicals in various personal care products (e.g. triclosan) (Veldhoen et al., 2006; Iwanowicz and Ottinger, 2009; Jobling et al., 2009; Pal et al., 2010; Milla et al., 2011; Quinlan et al., 2011). Additionally, several organohalogen insecticides including dieldrin and DDT, were detected and have been shown to cause endocrine and immune system modulation (Milston et al., 2003; Garcia-Reyero et al., 2006; Zhang and Hu, 2008).

Most prior studies have relied on discrete-water samples to assess water-quality conditions. Results obtained from the passive-water and bed-sediment samples demonstrate that discrete-water samples can underestimate the number of chemicals present in stream systems and potential chemical exposures of SMB. For the 81 chemicals measured in all sample types collected, only 16 were detected in the discrete-water samples, while 49 chemicals were detected in the passive-water samples and 28 were detected in the bed-sediment samples. In fact, the passive-water and bed-sediment samples documented the presence of an additional 40 chemicals that were not identified via discrete-water sampling alone (Table 2). This underestimation is demonstrated graphically using 14 chemicals with potential endocrine disruptive properties (Fig. 3). Only two (atrazine and galaxolide) of these chemicals were detected in the discrete-water samples. In contrast, 13 were detected in the passive-water samples and six were detected in the bed-sediment samples (Fig. 3).

Despite the lower number of detections in discrete-water samples, a significant positive correlation with TO_{rank} was observed for atrazine. It is interesting to note that atrazine, one of the more frequently detected herbicides in streams (Battaglin et al., 2005), previously has been documented to have potential endocrine disruption effects including induction of intersex in amphibians and fish (Hayes et al., 2010; Tillitt et al., 2010; Cragin et al., 2011; Hayes et al., 2011).

Three compounds detected in bed-sediment samples showed a significant positive correlation with TO_{rank}. These included trans-nonachlor (a major constituent of the chlordane insecticide mixture) and two plant sterols, sitosterol and stigmastanol. Trans-nonachlor have been shown to be weakly estrogenic, with an affinity for alligator estrogen receptor-α (Rider et al., 2010) Preparations from wood and soy, containing β-sitosterol, have been shown to be weakly estrogenic, inducing vitellogenin in both in vivo (injection into juvenile male rainbow trout) and in vitro (hepatocyte cultures) tests (Nakari, 2005). Multigenerational exposure of zebrafish (*Danio rerio*) resulted in vitellogenin production and altered sex ratios (Nakari and Erkomaa, 2003). Exposure of viviparous blenny (*Zoarces viviparus*) to β-sitosterol from wood affected embryonic development of larvae and circulating hormone levels of the adults (Mattsson et al., 2001).

Documenting the presence of a complex mixture of chemicals at SMB nesting sites is important. For example, the presence of hormones is especially important considering the low concentrations (ng/L) at which such endocrine-disrupting compounds have been shown to cause adverse biological effects (Young et al., 2004; Kidd et al., 2007). In addition, research has shown the potential for additive effects to be caused by the presence of chemical mixtures (Rajapakse et al., 2002; Brian et al., 2005; Bistodeau et al., 2006; Barber et al., 2007; Sárria et al., 2011). For multiple fish species, the early life-stages have been shown to be the most sensitive for induction of TO by natural

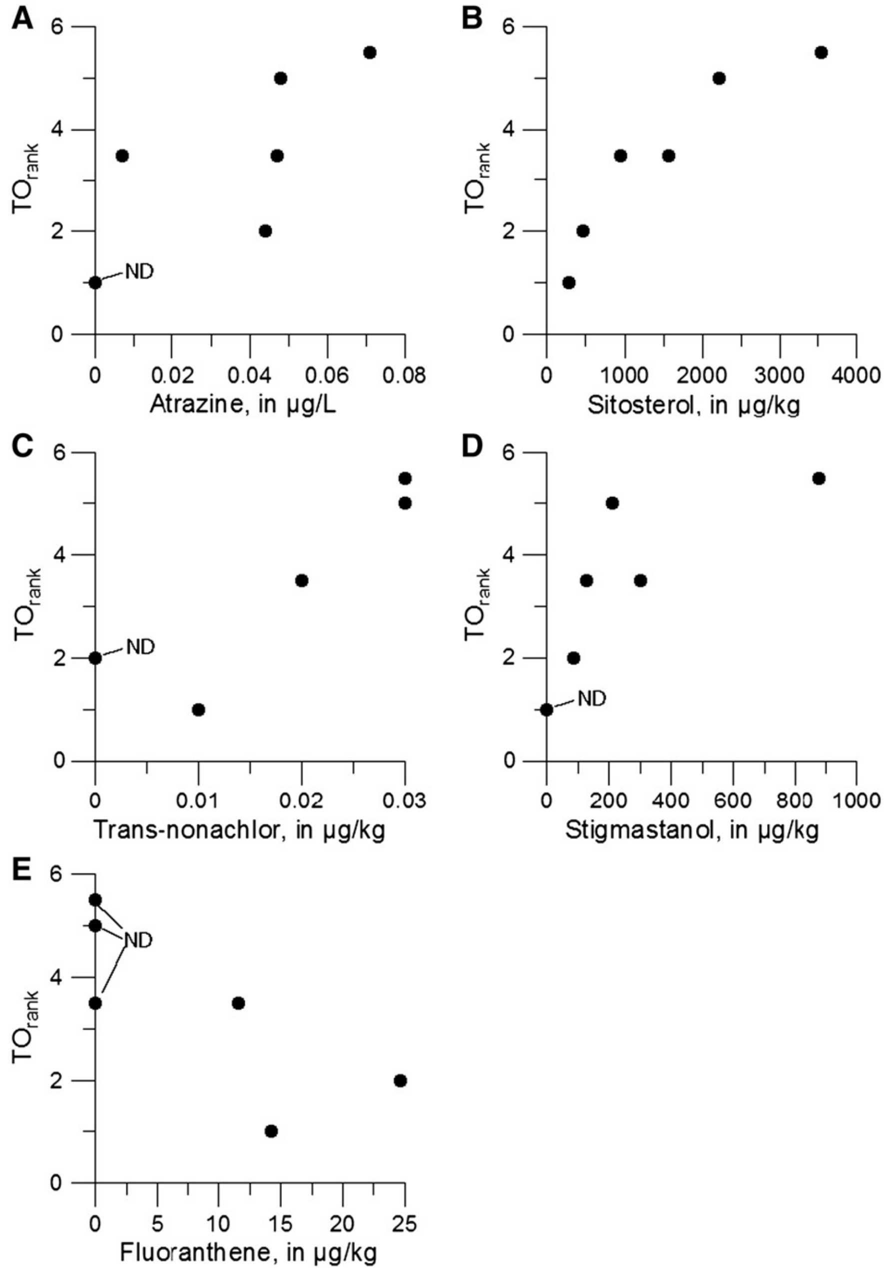

Fig. 2. Scatter plots of TO_{rank} (prevalence and severity of intersex for smallmouth bass combined into a single variable) to: (A) atrazine concentration in discrete-water samples, (B) sitosterol in bed-sediment samples, (C) trans-nonachlor in bed-sediment samples, (D) stigmastonol in bed-sediment samples, and (E) fluoranthene in bed-sediment samples. Site LP5 not included in these plots as fish were not able to be collected at this site. ND identify samples where the chemicals in question were not detected. Corresponding Spearman rank correlations are provided in Table 3.

and synthetic estrogens (Koger et al., 2000; Krisfalusi and Nagler, 2000; Liney et al., 2005). Male SMB create a nest by sweeping the substrate with their caudal fin, court a female, spawn, and then provide parental care for the eggs and fry (Ridgway et al., 1989). Eggs hatch 2–8 days after fertilization and the fry remain in the nest for another 3–11 days before they rise from the nest and begin to feed (Shuter et al., 1980). Hence, during the critical time of sexual differentiation, the fry are potentially exposed to complex chemical mixtures via both water and bed sediment that could lead to the manifestation of TO in adults or making the fish more sensitive to subsequent chemical exposures (Blazer et al., 2012).

Correlations between TO_{rank} and chemicals detected in the passive-water samples were not possible due to the detection-only results. It is noteworthy, however, that total estrogenicity of these passive-water samples was significantly related to the number of estrogen hormones present.

Antibiotics are not only used in animal agriculture to treat diseases, but also often are added to feed to promote growth and improve feed conversion (Gilchrist et al., 2007). Both tetracycline and oxytetracycline exhibit substantial sorption to particles (Tolls, 2001). Thus, these chemicals are generally infrequently detected in streams and at generally low (<0.1 µg/L) concentrations (Kolpin et al., 2002, 2004; Arikan et al., 2008; Focazio et al., 2008). Concentrations of oxytetracycline and tetracycline at >0.5 µg/L (SB11 and SR3) suggest a rapid transport mechanism from an antibiotic source. Neither of these sites were downstream of a WWTP, thus, suggesting a potential agricultural source for these antibiotic concentrations. In a previous assessment of land use attributes and TO at these same sites, the percent agriculture and animal density in the

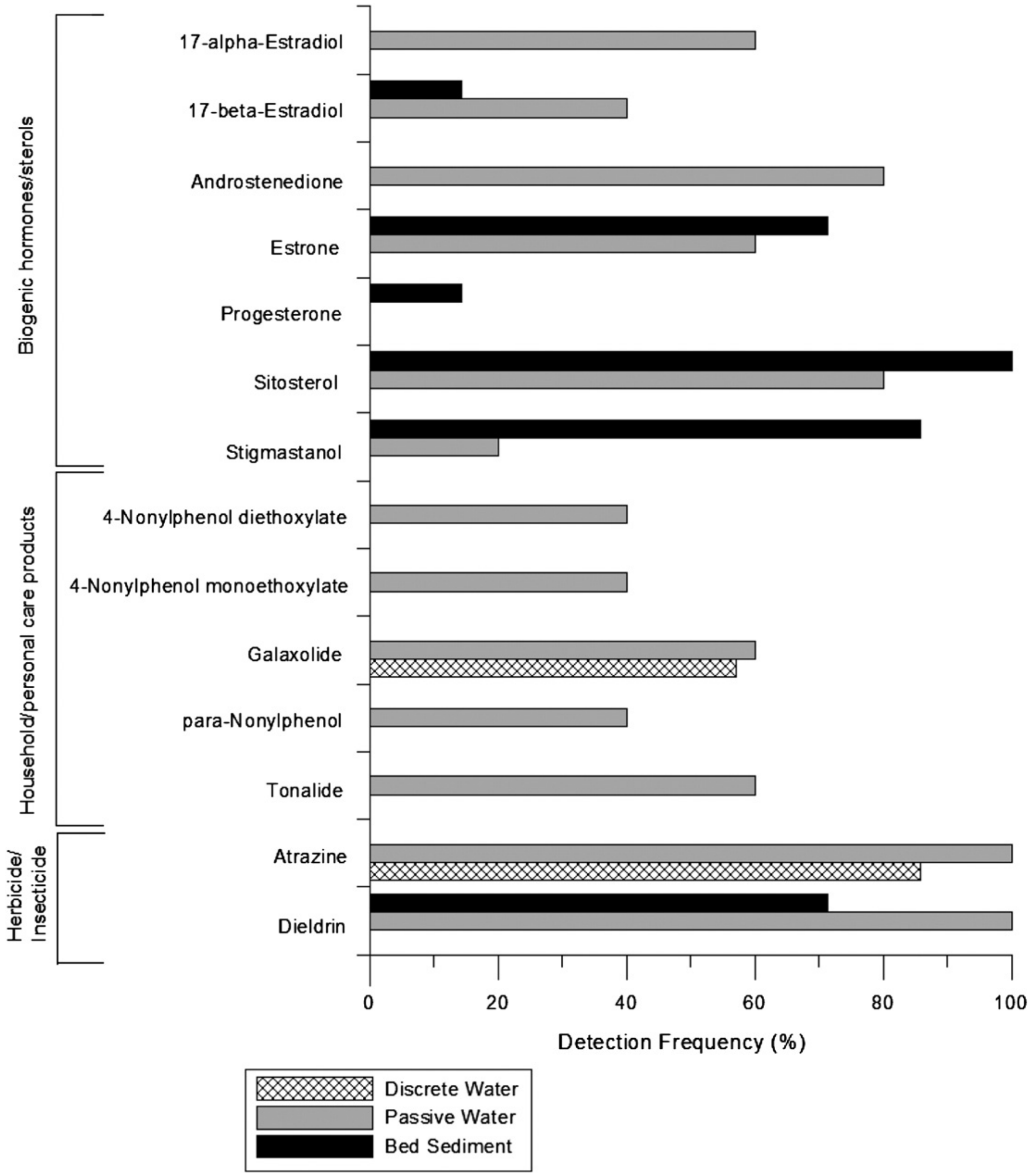

Fig. 3. Frequencies of detection for 14 known or suspected endocrine disrupting chemicals (Koger et al., 2000; Krisfalusi and Nagler, 2000; Nakari and Erkomaa, 2003; Brian et al., 2005; Nakari, 2005; Bistodeau et al., 2006; Garcia-Reyero et al., 2006; Barber et al., 2007; Villeneuve et al., 2007; van der Burg et al., 2008; Hayes et al., 2010; Rider et al., 2010; Simmons et al., 2010; Tillitt et al., 2010) that were measured in all discrete-water, passive-water, and bed-sediment samples.

catchment above the sampling sites were significantly correlated with TO prevalence, while these two attributes plus the total number of animal feeding operations, number of poultry houses, and WWTP flow were correlated with TO severity (Blazer et al., 2012). Previous research has shown endocrine disruption of fish by exposure to contaminants associated with poultry litter (Yonkos et al., 2010) and to water and sediment from agricultural intensive basins (Jeffries et al., 2011).

5. Conclusions

The 135 chemicals detected in water and/or bed-sediment samples associated with active SMB nesting sites can originate from various human and animal waste sources (e.g. WWTP, septic systems, animal feeding operations, crop land). These results, in conjunction with the companion study on the biological assessment of SMB at these same sites (Blazer et al., 2012), provide weight-of-evidence that agricultural activities (e.g. crop and livestock production) are important sources of endocrine disrupting chemical exposures to SMB during critical life stages in the Potomac River basin. Such evidence includes: (1) the detection of 17alpha-estradiol (the primary form of estradiol excreted by bovine); (2) the detection of veterinary pharmaceuticals used in animal agriculture (e.g. tetracycline and sulfonamide antibiotic compounds); (3) a statistically significant relation between dissolved atrazine concentrations in the water column above active SMB nests and TO_{rank}; and (4) a statistically significant relation between biogenic hormone/sterol concentrations in bed sediment associated with active SMB nests and TO_{rank}. This corroborates previous studies in the Chesapeake Bay watershed (Ciparis et al., 2012) which conclude that a more definitive assessment of the relative contributions of contaminants from agricultural activities is key to understanding the underlying factors causing the observed adverse effects to SMB. Such understanding is critical to making scientifically defensible management decisions regarding overall fish health in the Chesapeake Bay region.

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