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Occurrence of Organic Chemicals in Two Rivers Inhabited by Ozark Hellbenders (*Cryptobranchus alleganiensis bishopi*)

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Abstract Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*) populations are in decrease throughout their native range with rare recruitment of young. Increased estrogenic chemical levels and alterations of physicochemical properties in their habitat may play a significant role in this phenomenon. We report here the first systematic, comprehensive study of organic chemical concentrations and physical and nutrient parameters in two rivers containing Ozark hellbender populations. Water samples were collected monthly from August 2003 to November 2004. Concentrations of 21 organic chemicals were determined using gas chromatography–mass spectrometry. Nine organic chemicals were detected. Benzyl butyl phthalate, dibutyl phthalate, bisphenol A, and β-sitosterol were all detected >85% of the time, with median concentrations of 18 to 234 ng/L and maximum concentrations of 198 to 4141 ng/L. Individually, concentrations of nutrients and organic chemicals were much lower than those shown previously in laboratory and field experiments to have reproductive effects on amphibians. Nevertheless, hellbenders are exposed to a variety of chemicals with potential estrogenic effects. Our study establishes the basis to examine the specific effects of the detected concentrations, alone and in combination, on the Ozark hellbenders.

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The Ozark Hellbender (*Cryptobranchus alleganiensis bishopi*), an aquatic salamander, inhabits the White, Spring, Current, and Eleven Point Rivers and tributaries (Rogers et al. 2001; Nickerson & Mays 1973a). It was estimated that the population has decreased approximately 77% during the last 2 decades (Wheeler et al. 2003). Currently the Ozark hellbender is listed as a Missouri State Endangered Species and has been listed as a Federal Candidate by the United States Department of the Interior Fish and Wildlife Service.

Comparison of historic data from the late 1970s against more recent data from 1998 found a shift in size classes and a decrease in population density of Ozark Hellbenders in the Eleven Point River and the North Fork of the White River (Peterson 1985; Wheeler 2003). The length distribution of Ozark Hellbenders collected from the Eleven Point River in the late 1970s was in the range of 300 to 349 mm, although most of the length distribution obtained in 1998 was between 400 and 449 mm. A similar phenomenon was also observed in the North Fork of the White River where historic data showed most of the animals were in the 250- to 449-m size class compared with the 1998 data, which showed a 400- to 500-mm size class. Mean body mass was also different, with heavier animals in 1998 in both rivers. These data indicate that during a period of 20 years, the population structure of Ozark Hellbenders has shifted to older age groups. Apparently, there has been a lack of young animals (Wheeler et al. 2003). To date, it is still unknown whether this phenomenon is being caused by reproductive failure, postreproductive stressors (*e.g.*, predation, starvation), or both.

Several changes in abiotic and biotic factors have been observed in the Hellbender habitat, including habitat degradation, heavy canoe traffic, erosion, predation, and increased gigging activities (Dundee 1971; Humphries & Pauley 2005; Minton 1972; Nickerson & Mays 1973b; Trauth et al. 1992; Smith & Minton 1957; Williams et al. 1981). Excess nutrients in the water have been shown to affect amphibian populations (Berger 1989; Laposata & Dunson 1998; de Solla et al. 2002). Thus far, there has been no systematical investigation on the alteration of physicochemical characters in the Hellbender's habitat, which may have contributed to the decrease of the population.

Furthermore, several rivers in Missouri, including those with Ozark Hellbenders, have been polluted with herbicides, pesticides, and animal and human waste (Adamski 1996; Bell et al. 1997; Miller et al. 2001). Some of these pollutants have been shown to mimic the activity of 17β-estradiol, a sex steroid hormone that plays a major role in secondary sex organ development, behavior, fertility, and reproductive capacity and could affect animal reproductive cycles (Belfroid et al. 1999; Körner et al. 2000). Additional field data have demonstrated that exposure to estrogenic chemicals can cause abnormalities of reproductive organs and their functionality in juvenile alligators (Guillette et al. 1995), feminization in fish from streams receiving discharges from municipal and industrial wastes (Purdom et al. 1984), and masculinization in mosquito fish downstream from pulp and article mills in Florida (Howell et al. 1980). The existence of these chemicals has not been previously investigated in the Ozark Hellbender's habitat.

Thus, the objectives of this study were (1) to systematically identify and monitor the levels of selective pesticides, natural and synthetic estrogens, and industrial chemicals at two study sites in southern Missouri using gas chromatograph–mass spectrometry (GC-MS) analytic methods; (2) to monitor physicochemical properties and nutrient concentrations at the same sites; and (3) to compare current water quality of the study sites with that measured historically.

The significance of the data from this study is threefold. First, this was the first study that used monthly sampling to establish short-term (1+ year) trends in organic chemicals and nutrient loadings, along with seasonal and weather events, in the habitat of Ozark Hellbenders. Second, our study established the basis for further investigations into the potential effects of the identified chemicals on Ozark Hellbenders. Third, this study model is applicable to the Ozark Hellbender subspecies and Eastern Hellbenders (*Cryptobranchus alleganiensis alleganiensis*), whose distribution extends from southwestern and south central New York, west to southern Illinois, south to extreme northeastern Mississippi, the northern parts of Alabama and Georgia, and a disjunct population in east central Missouri.

Materials and Methods

Study Sites

Study sites were established in the lower sections of the Eleven Point River and the North Fork of the White River. The stretches of both rivers are fast moving and spring fed with numerous pools and riffles, and Ozark Hellbenders have been found in the area in the past 3 decades (Wheeler et al. 2003; Mauricio & Huang, unpublished data 2003–2004). The land-use area adjacent to the rivers is deciduous forest. Pastureland and grassland are present in the upland areas. Water samples were collected monthly from August 2003 to November 2004.

Physicochemical Measurement

Physicochemical parameters, nutrients, and organic chemicals were measured in the same reaches of the rivers where animals are found. On-site measurements of dissolved oxygen, specific conductivity, pH, and temperature were performed in the middle of the river, approximately 18 cm lower than the water surface, using a multiparameter meter from Hach (model sensION156; Hach, Loveland, CO). Nephelometric turbidity was also measured on site using a Hach 2100P Turbidmeter. Grab samples were analyzed for alkalinity, bromine, free chlorine, total chlorine, chloride, hardness, total iron, manganese, reactive phosphorus, silica, sulfate, sulfide, total nitrogen, total phosphorus, total organic carbon, and dissolved organic carbon using a Hach DREL/2400 water-quality unit. Water samples for total nitrogen and total phosphorus analyses were preserved with sulfuric acid in the field. Once the samples were ready for analysis in the laboratory, the pH was increased to neutrality.

Organic Chemical Analysis

The chosen analytes of pesticides, herbicides, natural and synthetic estrogens, and industrial chemicals, such as plasticizers and surfactants, are ubiquitous, and most of them have been reported to be estrogenic (Daughton & Ternes 1999; Dube & MacLatchy 2000; Tyler et al. 2000). Water samples for determination of organic chemical concentrations were collected using an automatic sampling device (model 3710; ISCO, Lincoln, NE) set in a 24-hour time-proportional mode. Concentrations of 21 organic chemicals were assessed using solid phase extraction (SPE) and GC-MS. C18 SPE cartridges (1 g; Alltech, Deerfield, IL) were washed sequentially with 6 mL acetone, 10 mL methanol, and 6 mL deionized water. Water samples (4 to 6 L) were passed through the cartridges at a rate of 5 to 10 ml/min and then washed with 6 mL deionized water.

The cartridges were dried by pulling air for 30 minutes under vacuum. Analytes collected on C18 SPE cartridges were eluted with 6 mL acetone (2 x 3 mL). The eluent was passed through a bed of anhydrous Na_2SO_4 to remove residual water. All extracts were evaporated to dryness under a gentle stream of nitrogen in a graduated tube. Before undergoing SPE, each water sample was spiked with 20 µL 10 ng/µL surrogate standards, d_5-atrazine, and d_5-estradiol.

Fifty µL Sylon BFT (99:1 mixture of Bis (trimethylsilyl) trifluoroacetamide plus trimethylchlorosilane; Supelco, Bellefonte, PA) was added to the extract in a glass vial with a Teflon-lined screw cap. After reacting for 30 minutes at 60 °C, the derivatized extract was evaporated to dryness under a gentle stream of nitrogen and reconstituted in a final volume of 100 µL hexane. Twenty µL 10 ng/µL internal standards (5α-cholestane and d_8-anthracene) were added to the final sample before GC-MS analysis.

GC-MS analysis was conducted with a Varian Saturn 2000 GC/MS instrument. GC separation of analytes was performed with a fused silica column (DB-5MS, 30 m × 0.32 mm, 0.25-µm film; Agilent, Palo Alto, CA). Initial oven temperature was 120 °C for 1 minute, increased to 190°C for 8 minutes and 290°C for 38 minutes. Splitless injection, 2 µL, was at 280°C. Ion-trap MS was operated with electron-impact ionization at 70 eV. Quantification of the analytes was performed by external standard method and adjusted for the recovery of surrogates and internal standards. After qualitative identification criteria were met, compound concentrations were calculated from 4- to 5-point calibration curves generated from standards concentrations ranging from 0.2 to 5 ng/µL.

Precipitation and Historic Data

Daily precipitation data were obtained from the National Climatic Data Center (NCDC 2003, 2004). The closest precipitation station with a sufficiently large data set for comparison with water quality was Dora, MO, which is approximately 15 miles from the North Fork of the White River site and 60 miles from the Eleven Point River site. Data were available for 8 of the 16 sampling dates. Historic water-quality data were obtained from published sources and personal communication. M. Barr (United States Geological Survey [USGS]) provided data for the Eleven Point River from November 1993 to January 1997 (20 sampling dates) and for the North Fork of the White River from January 1993 to August 1994 (6 sampling dates). The USGS Water Resources Investigation Report 96-4003 (Davis et al. 1996) reports median nutrient concentrations from 1980 to 1990 for sites throughout the Ozark Plateau Study Unit. Median concentrations were available for specific stations and also summarized by physiographic and land-use categories. Two water stations were along the Eleven Point River (Bradley, MO, and Pocahontas, AR), and two were along the North Fork of the White River (Tecumseh, MO, and Norfork, AR). The two rivers of interest are in the Salem Plateau physiographic region.

Statistical Analysis

Correlations among organic chemicals and between organic chemicals and precipitation, turbidity, total phosphorus, total nitrogen, total organic carbon, and dissolved organic carbon were assessed by calculating Spearman correlation coefficients using SYSTAT 9 (Statistical Product and Service Solutions, Chicago, IL). We used the false discovery rate (FDR) approach to adjust our cutoff for statistical significance given the multiple correlations that we made (Benjamini & Hochberg 1995; McBride 2005). Given the correlations we list in Table 3 and their p values, the corrected p value for significance (with an α of 0.05) was 0.005. A quantile test (Conover 1999) was used to test the hypothesis that the median concentration of nutrients in the present study was different from the historic median.

Results

Physicochemical Monitoring

Between August 2003 and November 2004, 16 water samples were collected from the Eleven Point River and 15 from the North Fork of the White River. The median water temperatures of the Eleven Point River and the North Fork of the White Rivers were 16.2 °C and 15.9 °C, respectively, whereas the median pH values were 7.9 and 8.2 and median turbidity was 3.2 and 2.0 NTU. Median concentrations of total nitrogen were 0.53 and 0.68 mg/L, and median concentrations of total phosphorus were 0.02 mg/L for both rivers (Table 1). These values were not significantly different from those reported historically, with the exception of mixed (pasture and grassland plus forest) reaches of the Salem Plateau, which had greater total phosphorus concentrations than those measured in the present study ($p < 0.01$; Table 2). Median total organic carbon concentrations were 2.2 and 1.2 mg/L for the Eleven Point River and the North Fork of the White River, respectively.

Organic Chemical Monitoring

Mean recoveries of all analytes in the laboratory spike generally exceeded 70% at all spike levels. The linearity of calibration curve (R^2) was >0.97 for all compounds, with the exception of simazine at 0.87. Detection limits for all

Table 1 Physiochemical properties of the Eleven Point River ($n = 16$) and North Fork of the White River ($n = 15$) from August 2003 through November 2004

Physiochemical properties	Eleven Point River			North Fork of the white River		
	Minimum	Median	Maximum	Minimum	Median	Maximum
Temperature (°C)	9	16.2	22.8	7.4	15.9	23.3
pH	7.6	7.9	8.3	5.7	8.2	8.6
Turbidity (NTU)	0.83	3.2	70.1	0.68	2	300
Dissolved oxygen (mg/L)	6.7	10.3	15.1	6.5	10.0	21.7
Conductivity (mg/L)	218	348	391	97.8	378	406
Total nitrogen (mg/L N)	<0.5	0.53	10.3	<0.5	0.68	1.7
Total phosphorus (mg/L)	<0.02	0.02	0.83	<0.02	0.02	0.5
Total organic carbon (mg/L C)	<0.3	2.2	17.4	<0.3	1.6	15.6
Dissolve organic carbon (mg/L C)	<0.3	1.2	16.6	<0.3	0.9	13.6
Alkalinity (mg/L as $CaCO_3$)	87	181	231	38	197	258
Hardness (mg/L as $CaCO_3$)	116	226	265	62	225	314
Bromine (mg/L)	<0.05	<0.05	3.7	<0.05	<0.05	0.66
Free chlorine (mg/L)	<0.02	<0.02	0.05	<0.02	0.02	0.24
Total chlorine (mg/L)	<0.02	<0.02	0.5	<0.02	0.01	1.4
Chloride (mg/L)	<0.1	2.5	4.1	<0.1	3.2	17.8
Total iron (mg/L)	<0.02	0.05	0.19	<0.02	0.04	0.3
Manganese (mg/L)	<0.02	0.1	0.8	<0.02	<0.02	0.3
Silica (mg/L)	<1.0	11.0	22.2	<1.0	10.2	26.7
Sulfide (ug/L)	<5	<5	63	<5	<5	320
Sulfate (mg/L)	<2	<2	6	<2	<2	18

Table 2 Historic median concentrations of total nitrogen and total phosphorus at the Eleven Point River, the North Fork of the White River, and Salem Plateau Region compared with concentrations from the present study[a]

Eleven Point River				
Nutrients (mg/L)	Aug 2003 to Nov 2004	Nov 1993 to Jan 1997	1980 to 1990, Bradley, MO	1980 to 1990, Pocohontas, AR
Total nitrogen	0.55 (16)	0.5 (20)	–	–
Total phosphorus	0.02 (16)	0.02 (20)	0.01 (43)	0.02 (122)
North Fork of the White River	Aug 2003 to Nov 2004	Jan. 1993 to Aug 1994	1980 to 1990, Tecumseh, MO	1980 to 1990, Norfork, AR
Total nitrogen	0.68 (16)	0.5 (3)	–	0.7 (14)
Total phosphorus	0.02 (16)	0.02 (6)	0.01 (43)	0.02 (33)
Regional comparisons	Aug 2003 to Nov 2004	1980 to 1990 Salem Plateau Forested	1980 to 1990 Salem Plateau Mixed	
Total nitrogen	0.6 (32)	0.7 (155)	0.72 (86)	
Total phosphorus	0.02 (32)	0.02 (651)	0.03 (872)[a]	

[a] Data from both study sites were combined for the regional comparison ($p < 0.01$, medians compared using a quantile test, value in parentheses = n)

chemicals ranged between 0.1 and 3.4 ng/L (Table 3). Dibutyl phthalate and benzyl butyl phthalate were detected in the laboratory blank samples because plastic components (*e.g.*, sampling tube, SPE cartridge) used during the analysis. Measured dibutyl phthalate and benzyl butyl phthalate concentrations in the stream samples were corrected for the contaminants found in the laboratory blank samples.

The most frequently detected chemicals were benzyl butyl phthalate and dibutyl phthalate (Table 3). Concentrations of bisphenol A, nonylphenol, 4-octylphenol, β-sitosterol, di-p-tolyl sulfone, metolachlor, and tebuthiu-

Table 3 Detection frequency and median concentrations of organic chemicals at the Eleven Point River (n = 16) and the North Fork of the White River (n = 15) from August 2003 through November 2004

Chemical	DL (ng/L)	Eleven Point River			North Fork of the White River		
		Detection frequency (%)	Median (ng/L)	Maximum (ng/L)	Detection frequency (%)	Median (ng/L)	Maximum (ng/L)
Benzyl butyl phthalate	0.8	100	67	144	93	44	351
Dibutyl phthalate	0.3	94	164	1364	100	143	4141
Bisphenol A	0.1	94	18	198	80	24	72
β-Sitosterol	1.0	89	146	826	88	234	414
Di-p-tolyl sulfone	2.5	56	9	36	25	<DL	20
Nonylphenol	1.7	44	<DL	4055	47	<DL	2317
Metolachlor	0.4	44	<DL	978	27	<DL	846
Tebuthiuron	3.4	19	<DL	83	40	<DL	323
17α-Ethinylestradiol	0.8	ND	–	–	ND	–	–
4-Octylphenol	0.3	ND	–	–	20	<DL	102
17α-Estradiol	1.3	ND	–	–	ND	–	–
17β-Estradiol	1.3	ND	–	–	ND	–	–
Atrazine	0.9	ND	–	–	ND	–	–
Bioallethrin	0.8	ND	–	–	ND	–	–
Diethylstilbestrol	0.6	ND	–	–	ND	–	–
Estriol	0.8	ND	–	–	ND	–	–
Estrone TMS	0.9	ND	–	–	ND	–	–
p,p'-DDE	0.6	ND	–	–	ND	–	–
Permethrin	0.9	ND	–	–	ND	–	–
Simazine	2.4	ND	–	–	ND	–	–
Tamoxifen	1.1	ND	–	–	ND	–	–

DL = Detection limit

ND = Not detected

ron varied seasonally and between years, with no detection of nonylphenol and metolachlor occurring after March of 2004 (Fig. 1). Several of the detected chemicals were found to be highly correlated to each other, including dibutyl phthalate and nonylphenol, metolachlor and nonylphenol, dibutyl phthalate and benzyl butyl phthalate, and dibuytl phthalate and metolachlor (Table 4). Rainfall was also correlated with the concentrations of several organic chemicals, particularly the plant sterol β-sitosterol (Table 5). There were no significant correlations between organic chemicals and turbidity, total nitrogen, total phosphorus, total organic carbon, or dissolved organic carbon.

Discussion

Ozark Hellbenders prefer habitats with moderately shallow, fast-moving, cold water and sufficient rocks for cover (Nickerson & Mays 1973a). Destruction of this habitat is one possible explanation for the recent decrease in Hellbender populations. Along with habitat destruction, changes in water quality may also explain the observed lack of recruitment of young animals.

Nutrients

Increased nutrient concentrations from farming activities are considered pervasive throughout the world and have been offered as an explanation for some population decreases (Guillette & Edwards 2005). Our comparison with historic data indicated that nutrient concentrations at the study sites have not changed drastically in the last 20 to 30 years. In addition, median pH, temperature, alkalinity, and dissolved oxygen were all within the ranges measured by Nickerson and Mays (1973a) at a site with thriving Hellbender populations. Turbidity was low in both rivers, with measurements <10 NTU during all site visits, except April of 2004, when turbidity was 70 and 300 NTU at the Eleven Point River and the North Fork of the White River, respectively. However, concentrations of total phosphorus and total nitrogen exceeded the Environmental Protection

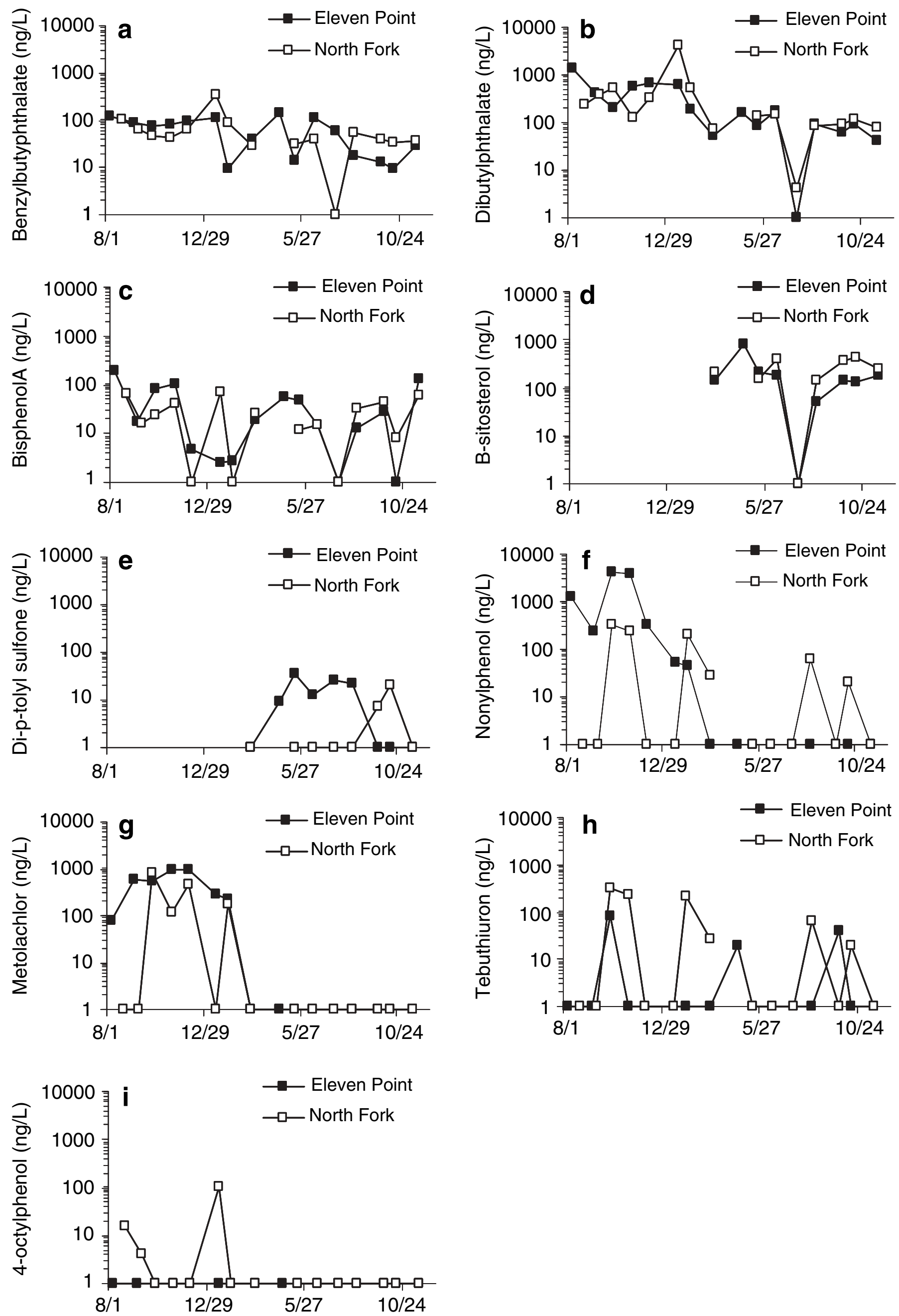

Fig. 1 Temporal variation in the concentrations of the 10 detected organic chemicals at the Eleven Point River and the North Fork of the White River, August 2003 to November 2004. (a) benzyl butyl phthalate. (b) dibutyl phthalate. (c) bisphenol A. (d) β-sitosterol. (e) Di-p-tolyl sulfone. (f) Nonylphenol. (g) Metolachlor. (h) Tebuthiuron. (i) 4-Octylphenol. Values displayed at 1 on the y-axis are less than or approximately at detection limits

Agency (EPA)–recommended criteria of 10 and 0.31 mg/L, respectively, for Ecoregion XI (EPA 2000) two thirds of the time. On three occasions, measured concentrations of total nitrogen were >2 mg/L (all at the Eleven Point River site), and concentrations of total phosphorus were >0.5 mg/L on two occasions (both at the Eleven Point River Site). The acute effects of these concentrations of total nitrogen and total phosphorus on Ozark Hellbenders are not known, although comparison with historic data suggests that concentrations have not recently changed.

Organic Chemicals

The presence of estrogenic chemicals in the environment has been proposed to be associated with decreased wildlife species (Tyler et al. 1998). Data for this study reveal that Hellbenders are exposed to several industrial chemicals, plant sterols, and an herbicide. Hellbender-specific responses to these sources have not been determined, although studies with other amphibians indicated that many of these chemicals have estrogenic effects.

Table 4 Spearman correlation matrix of the nine detected organic chemicals (n = 31 for all chemicals except β-sitosterol and di-p-totyl sulfone (*n* = 17)[a]

Chemical	Dibutyl phthalate	Benzyl butyl phthalate	Bisphenol A	β-sitosterol	Di-p-tolyl sulfone	Nonylphenol	Metolachlor	Tebuthiuron
Benzyl butyl phthalate	**0.73**							
Bisphenol A	0.08	0.26						
β-Sitosterol	0.54	0.37	0.54					
Di-p-tolyl sulfone	0.18	0.24	–0.09	0.07				
Nonylphenol	**0.80**	**0.56**	0.26	NA[a]	NA			
Metolachlor	**0.67**	0.37	–0.05	NA	NA	**0.76**		
Tebuthiuron	–0.02	0.00	0.11	0.24	-0.15	0.13	0.18	
4-octylphenol	0.34	0.36	0.24	NA	NA	0.37	–0.24	–0.21

[a] Values in bold are significant per the FDR approach; see Methods)

[b] NA = Not applicable because the two chemicals were never detected on the same day

Table 5 Spearman correlation coefficient (*p*) between the nine organic chemicals and the sum of precipitation from to days before sample collection[a]

Chemical	Days	p
Benzyl butyl phthalate	Two	0.30
Dibutyl phthalate	Two	**0.51**
Bisphenol A	Four	**–0.46**
β-Sitosterol	Two	**0.60**
Di-p-tolyl sulfone	Seven	**0.66**
Nonylphenol	Two	0.17
Metolachlor	Two	0.26
Tebuthiuron	Three	0.21
4-Octylphenol	One	0.17

[a] (n = 15 for all chemicals except di-p-totyl sulfone and β-sitosterol, where n = 9. Only the most significant correlation for each chemical is given values in bold are significant at p = 0.05)

The industrial chemicals benzyl butyl phthalate, dibutyl phthalate, and bisphenol A were all detected in the majority of samples, with median concentrations ranging from 18 to 164 ng/L. These chemicals are ubiquitous in the environment, and despite the fact that these rivers are not near any industrial source, it is possible that they are contaminated with very low concentrations of industrial chemicals, as our data indicate. Occurrences of dibutyl phthalate, benzyl butyl phthalate, and nonylphenol were all highly correlated (Table 4). This correlation may indicate a common source or process controlling their concentrations in the rivers, but further study would be needed to determine such mechanisms. In addition, the correlation between nonylphenol and metolachlor is consistent with nonylphenol being used as an inert ingredient in herbicides (EPA 2006). Dibutyl phthalate (at concentrations of 100 to 10,000 μg/L) and bisphenol A (at concentrations of 2.3 to 228 μg/L) have both been shown to be estrogenic in amphibians, causing deformities and impaired spermatogenesis (Lee & Veeramachaneni 2005) and altered sex ratios (Levy et al. 2004), respectively. Nonylphenol has been shown to alter sex ratios in *Rana pipiens* and *R. sylvatica* tadpoles at concentrations as low as 10 μg/L (MacKenzie et al. 2003). Concentrations of industrial chemicals in the Eleven Point River and the North Fork of the White River are much lower than those shown to have estrogenic effects in amphibians, although the effects of these chemicals in combination, as well as hellbender-specific responses, remain to be determined.

The plant sterol β-sitosterol was detected in the Eleven Point River and the North Fork of the White River. Kolpin et al. (2004) measured β-sitosterol in urban streams in Iowa during varying flow conditions and reported detection frequencies ranging from 44% to 82% and a maximum concentration of 2.9 μg/L. In the present study, β-sitosterol was detected during 88% to 89% of the sampling dates, and the maximum concentration was 826 ng/L. We are not sure whether the current concentrations of β-sitosterol are different than those occurring when hellbender populations were thriving because it is a naturally occurring plant sterol. β-sitosterol was correlated with recent rainfall events (Table 5), as would be expected if plant-derived compounds are flushed from soils to rivers with precipitation.

Metolachlor, an herbicide generally used in soybean fields and in mixtures with atrazine on corn fields, was the most commonly detected herbicide. Tebuthiuron, an herbicide typically applied to roadsides and other noncropland areas, was also detected. Atrazine, an herbicide commonly applied to corn fields, was not detected. Metolachlor, tebuthiuron, and atrazine were previously detected in Ozark Plateau rivers, with median concentration lower than the detection limit and maximum concentrations of 5, 29, and 22 ng/L, respectively (Bell et al. 1997). Metolachlor is considered toxic (Osano et al. 2002), but little evidence is available related to its estrogenicity. Atrazine is estrogenic; however, its effects and potency on wildlife are still under vigorous investigations (EPA 2003).

Conclusion

Median and maximum concentrations of all nutrient parameters measured at the Eleven Point River and the North Fork of the White River. All of them were lower than EPA water-quality criteria, except total phosphorus and total nitrogen, which exceeded the EPA-recommended criteria for Ecoregion XI. Nine organic chemicals were detected at least once. Benzyl butyl phthalate, dibutyl phthalate, bisphenol A, and β-sitosterol were all detected in >85% of the samples, with median concentrations ranging from 18 to 234 ng/L. Maximum concentrations of dibutyl phthalate and nonylphenol were >1 µg/L. The total effects of these identified chemicals on Ozark Hellbenders remains to be elucidated, although it is clear from this monitoring study that Hellbenders are exposed to a variety of organic chemicals with potential estrogenic activity.

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