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Metformin and Other Pharmaceuticals Widespread in Wadeable Streams of the Southeastern United States

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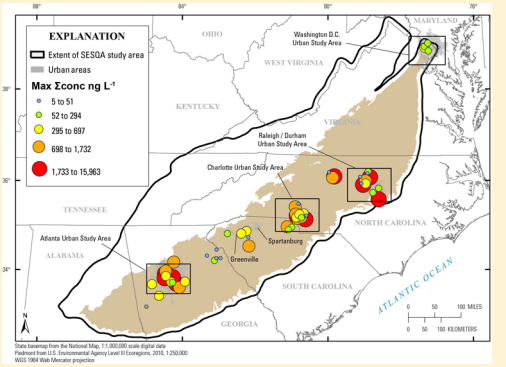
Supporting Information

ABSTRACT: Pharmaceutical contaminants are growing aquatic-health concerns and largely attributed to wastewater treatment facility (WWTF) discharges. Five biweekly water samples from 59 small Piedmont (United States) streams were analyzed for 108 pharmaceuticals and degradates using high-performance liquid chromatography and tandem mass spectrometry. The antidiabetic metformin was detected in 89% of samples and at 97% of sites. At least one pharmaceutical was detected at every site (median of 6, maximum of 45), and several were detected at ≥10% of sites at concentrations reported to affect multiple aquatic end points. Maximal cumulative (all detected compounds) concentrations per site ranged from 17 to 16000 ng L^{-1}. Watershed urbanization, water table depth, soil thickness, and WWTF metrics correlated significantly with in-stream pharmaceutical contamination. Comparable pharmaceutical concentrations and detections at sites with and without permitted wastewater discharges demonstrate the importance of non-WWTF sources and the need for broad-scale mitigation. The results highlight a fundamental biochemical link between global human-health crises like diabetes and aquatic ecosystem health.



INTRODUCTION

Human and environmental health are inextricably linked because infectious and noncommunicable diseases are treated worldwide using biochemical agents and the inevitable environmental release of these bioactive/biocidal compounds threatens aquatic ecosystems.[1−6] Pharmaceuticals are particular concerns because of their general hydrophilicity and aqueous mobility,[7,8] pH-variable activity and sorption profiles,[9] designed bioactivity and biorecalcitrance,[7,8] and characteristic occurrence as complex cocktails.[10] Pharmaceuticals target many highly conserved biological end points,[11−15] resulting in a multitude of potential adverse outcomes, including toxicity, endocrine disruption, immunomodulation, antimicrobial activity, antibiotic resistance selection, cytotoxicity and mutagenesis, and transgenerational effects[1,2,4−6] throughout aquatic foodwebs.[16] Pharmaceutical effects on chemical-information flow[3,17] and predator−prey relations[18−21] are also emerging aquatic ecosystem concerns.

Wastewater treatment facility (WWTF) effluent is generally considered the primary source of pharmaceuticals to stream environments.[2,7,22,23] In contrast to the seasonal/episodic land application of pesticides and manure/biosolid-associated pharmaceuticals, WWTF discharge of pharmaceuticals to aquatic ecosystems is essentially continuous,[1,2] ensuring near-source pseudopersistence and increased chronic-effect risks.[7,8] WWTF outfalls are located, to the extent possible, on higher-order stream reaches to maximize surface water-receptor dilution. Consequently, although recognized sources of surface water pharmaceutical contamination in developed landscapes include various nonpoint sources (failing sewerage infrastructure, septic-field leachate, stormwater runoff, combined sewer overflows, manure-lagoon leakage, and runoff of land-applied manure/biosolid fertilizers), most investigations to date have focused on higher-order stream reaches under direct WWTF-effluent influence.[2,23]

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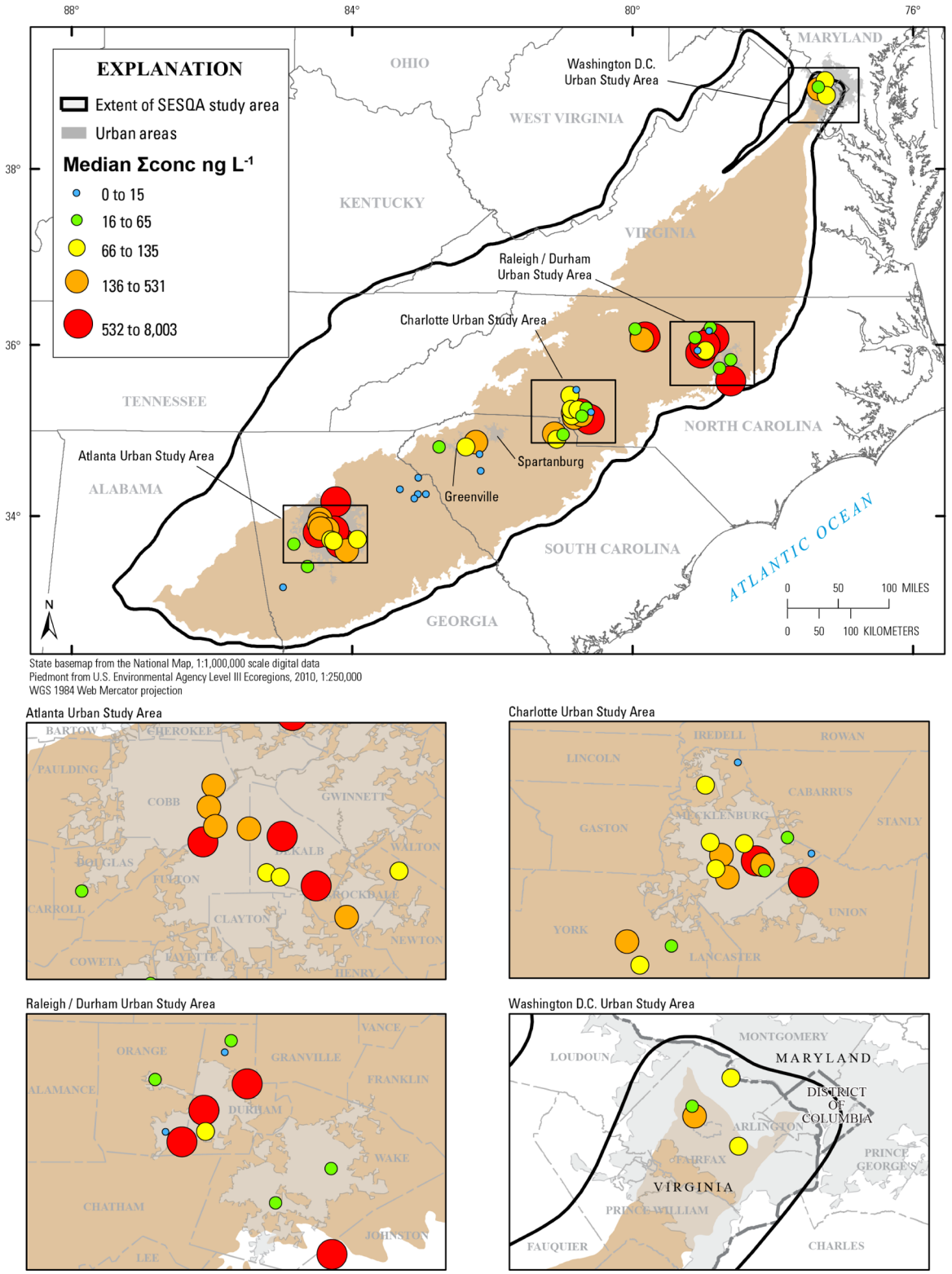

Figure 1. Cumulative median concentrations of human-use pharmaceuticals detected during the June 2014 synoptic sampling of 59 wadeable streams in the Piedmont region. For site details, see Tables S1 and S2.

Information about pharmaceutical occurrence, distribution, primary sources, and spatial and temporal variability is lacking for wadeable headwater stream reaches where WWTF sources are less common, a critical scientific data gap for the management of vital aquatic ecosystem services and environmental health. The U.S. Geological Survey (USGS) assessed pharmaceutical contaminant concentrations in 59 wadeable headwater streams in the highly urbanized Piedmont ecoregion of the southeastern United States during 2014. Primary objectives included addressing the current lack of information about fluvial pharmaceutical contamination in wadeable streams throughout the ecoregion and assessing the relative importance

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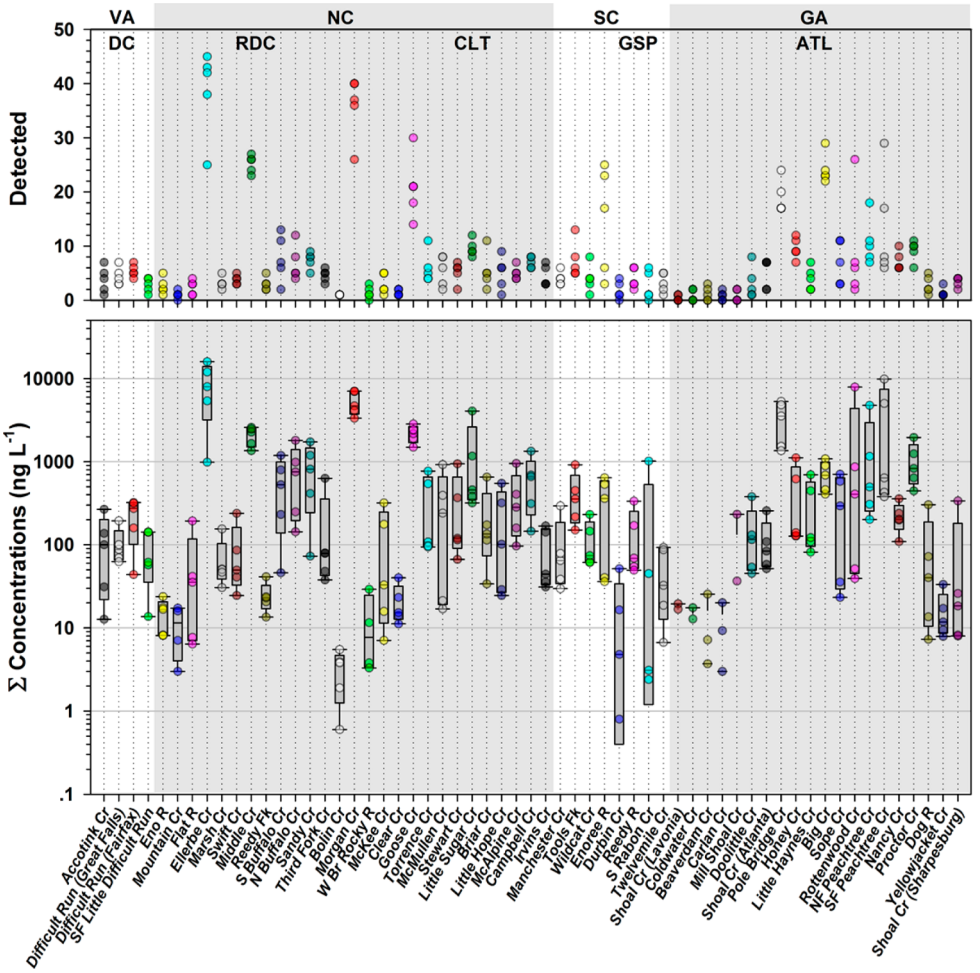

Figure 2. Number of pharmaceuticals detected (top) and cumulative concentrations (bottom) for human-use pharmaceuticals in water samples (n = 5) from 59 wadeable Piedmont streams. Circles are data for individual samples. Boxes, centerlines, and whiskers indicate interquartile range, median, and 5th and 95th percentiles, respectively. Abbreviations indicate states (VA, Virginia; NC, North Carolina; SC, South Carolina; GA, Georgia) and urban centers (DC, Washington, DC; RDC, Raleigh−Durham−Chapel Hill; CLT, Charlotte; GSP, Greenville−Spartanburg; ATL, Atlanta).

of non-WWTF sources of pharmaceutical contamination in these aquatic systems. Identification of potential watershed predictors of in-stream pharmaceutical contamination based on correlations with readily available geographic-information system (GIS) land-use land-cover (LULC) metrics was a secondary objective.

MATERIALS AND METHODS

Site Description. Filtered water samples (10 mL) were collected by the USGS National Water Quality Program (NWQP) Southeastern Stream Quality Assessment (SESQA)[24] from 59 perennial, wadeable (<10 m in width and 1 m in depth at base flow) headwater-stream sites in watersheds with varying degrees of urban land use in four states (Figure 1 and Table S1). Five water samples were collected biweekly at each site from April 14 to June 13, 2014. Site selection and sampling methodologies are described elsewhere.[24] Samples were syringe filtered (0.7 μm pore size glass fiber) into baked (500 C) amber glass vials and shipped on ice for analysis at the USGS National Water Quality Laboratory in Denver, CO.

Water Chemistry Assessment. Direct aqueous injection (100 μL), isotope dilution, high-performance liquid chromatography and tandem mass spectrometry (HPLC−MS/MS) was used to quantify 108 human-use pharmaceutical and pharmaceutical degradate compounds.[25] Analytes, with Chemical Abstracts Services numbers and laboratory reporting limits (LRL), are listed in the Supporting Information (Table S2).

Quality Assurance Quality Control (QAQC). HPLC−MS/MS pharmaceutical analysis included addition of 19 surrogate standards (nominal final concentration of 400 ng L^{-1}) to field-filtered samples to evaluate whole-method recovery [median of 103%, interquartile range of 96−110%, range of 6−196% (Table S3)]. Of the 68 pharmaceutical analytes detected in 295 stream samples, only caffeine and nevirapine were detected (once each) in 14 field blanks at concentrations at or above the LRL (data not shown). Because the detection frequency and concentration for caffeine in blanks above the LRL were 7% and 160 ng L^{-1}, respectively, compared with 49% detection and a median concentration of 227 ng L^{-1} in environmental samples and because nevirapine was only detected once at the LRL, corresponding sample data are presented without censoring. Interpretation of environmental results below blank detection levels, however, warrants caution. The median relative percent difference (RPD) for detected compounds in 12 paired replicate samples was 8% (interquartile range of 4−16%, range of 0−78%).

Statistical Analyses. Laboratory reporting levels were determined for each analyte based on the long-term method-

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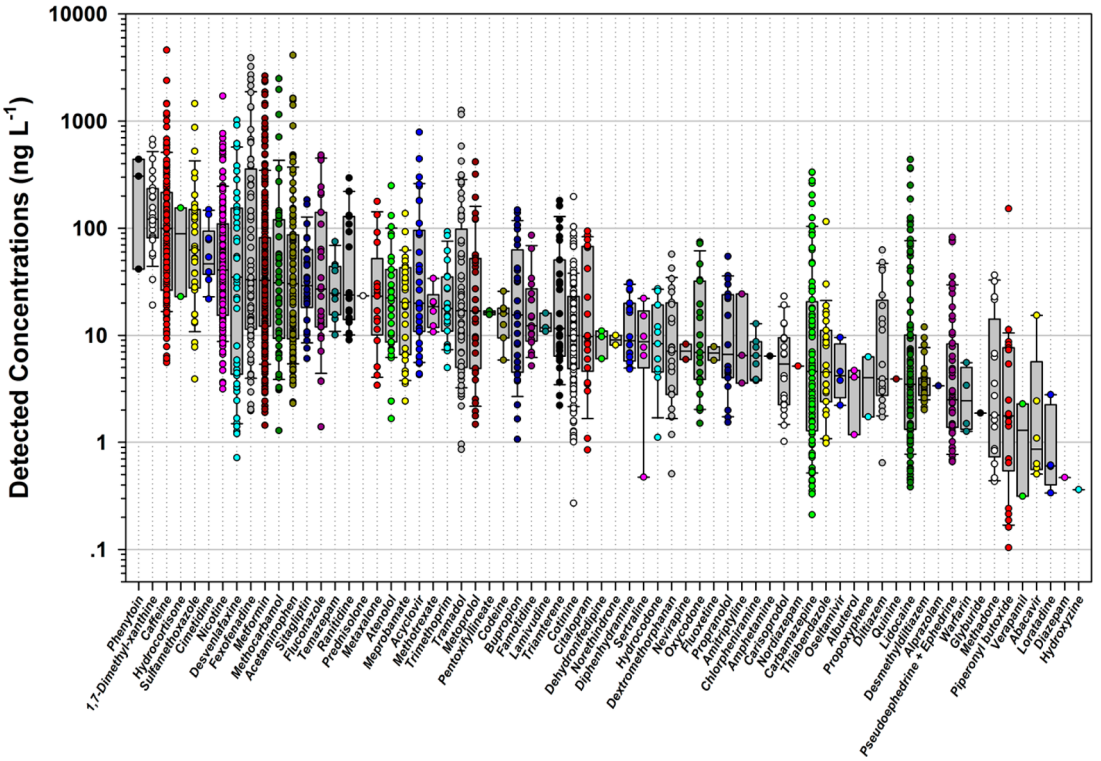

Figure 3. Concentrations of 68 detected human-use pharmaceuticals (in order of decreasing median detected concentration) in water samples (n = 295) from 59 wadeable Piedmont streams. Circles are data for individual samples. Boxes, centerlines, and whiskers indicate interquartile range, median, and 5th and 95th percentiles, respectively.

detection level (MDL)[26,27] (Table S4). Detections between the LRL and MDL are considered semiquantitative; results below the MDL are reported as censored (<LRL).[26] Nonmetric multidimensional scaling (NMDS), one-way analysis of similarity (ANOSIM), and permutation-based cophenetic correlation (RELATE, 999 permutations) routines (Primer 7, PRIMER-E Ltd., Plymouth, U.K.)[28] confirmed statistically significant relations between site/sample/analyte-specific pharmaceutical (log-transformed and normalized) and site-specific LULC data matrices (Tables S5–S9). Correlations between site-specific cumulative (sum of detected analytes) pharmaceutical metrics (maximum/median of concentrations/detections) and individual LULC metrics were assessed by Spearman rank correlation (Table S10); significant differences between WWTF-related site groupings were identified by Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks and Dunn's tests (SigmaPlot 13, Systat Software, San Jose, CA).

RESULTS AND DISCUSSION

Pharmaceutical Contamination Was Prevalent in Sampled Piedmont Streams. Human-use pharmaceuticals were ubiquitous in the 59 wadeable streams sampled throughout the study region (Figures 1–3 and Table S4). Maximal and median cumulative (sum of detected pharmaceuticals) concentrations of five samples at each site ranged from 17 to 16000 ng L^{-1} and from 0 to 8000 ng L^{-1}, respectively (Figures 1 and 2 and Figure S1). At least one pharmaceutical was detected at all 59 streams, with maximal and median cumulative detections ranging from 1 to 45 and from 1 to 42 compounds per site, respectively, and an overall median of 6 (Figures 1 and 2). Pharmaceutical occurrence profiles did not differ significantly among states, urban centers, or sites with or without confined poultry operations (Table S8).

Sixty-eight of the 108 pharmaceutical analytes were detected at least once (Figure 3). The type II diabetes medicine, metformin, was pervasive (89% of samples; 57 sites). Nicotine-related compounds (nicotine, 51%; cotinine, 58%) were detected in 71% of samples (54 sites). Caffeine-related compounds (caffeine, 49%; 1,7-dimethylxanthine, 8%) were detected in 49% of samples (53 sites). Other frequently detected compounds included acetaminophen (oral analgesic, 36%), carbamazepine (antiseizure medication, 28%), fexofenadine (antihistamine, 23%), lidocaine (intravenous/topical analgesic, 38%), methocarbamol (muscle relaxant, 16%), pseudoephedrine/ephedrine (decongestant, 18%), sulfamethoxazole (antibiotic, 12%), and tramadol (opioid analgesic, 22%). These compounds also encompassed the highest maximal and median concentrations measured in the analyzed samples (Figure 3 and Figure S2). Significant correlations between concentrations of co-detected (n > 30) analytes are provided in Table S11.

Importance of Non-WWTF Sources of Pharmaceuticals in Headwater Streams. The ubiquitous occurrence (detected at all sites), multiple detections (median of 6 compounds per site), and elevated fluvial concentrations (up to microgram per liter levels) illustrate the need for identification and mitigation of pharmaceutical sources in wadeable-stream ecosystems. The ubiquity of pharmaceuticals in wastewater effluent and the understandable historical emphasis on wastewater-impacted stream reaches create the perception that wastewater treatment improvements will substantially eliminate in-stream pharmaceutical contamination. Conventional wastewater technologies treat pharmaceuticals only incidentally, resulting in ubiquitous release of pharmaceutical

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contaminants in effluent.[1,2,22,29] Recent studies have demonstrated the utility of WWTF upgrades in reducing the discharge of bioactive anthropogenic contaminants to streams.[30,31] However, the regional impact of WWTF upgrades on stream quality depends on the location and relative spatial importance of WWTF point sources. The six watersheds in this study (Table S7), with major (mean discharge greater than 3.8×10^6 L day^{-1}, as defined in ref 32) U.S. Environmental Protection Agency (USEPA) National Pollutant Discharge Elimination System (NPDES) wastewater discharges, exhibited in-stream cumulative concentrations (median of 1390 ng L^{-1}) and detection frequencies (median of 24%) significantly ($p = 0.012$ and 0.004, respectively) higher than those of the 11 sites with smaller NPDES-wastewater discharges (concentration median of 115 ng L^{-1}, detection median of 4%) and the 42 sites with no NPDES-wastewater discharges (concentration median of 160 ng L^{-1}, detection median of 4%); the latter two groups did not differ significantly ($p = 1.000$). Although the results confirm that WWTFs are important sources of in-stream pharmaceutical contamination,[2,23,33] the concentrations and numbers of pharmaceutical contaminants detected at 71% of sites without NPDES discharges clearly demonstrate that WWTFs are not sole sources and emphasize the need for whole-watershed mitigation approaches.

LULC Predictors of Pharmaceuticals in Headwater Streams. Watershed urbanization, water table depth, soil thickness, and WWTF metrics correlated significantly ($\alpha < 0.05$) with in-stream pharmaceutical contamination (Table S10). For the 17 watersheds with at least one NPDES discharge, only wastewater metrics correlated significantly with in-stream cumulative pharmaceutical contamination; the highest correlation (Spearman rank; $\rho = 0.736$; $p < 0.0001$) was between median cumulative pharmaceutical detections (%) and N_wastewater2002 (2002 estimated total nitrogen in NPDES discharge in kilograms[32]) (Tables S6 and S10). For watersheds with no NPDES discharges, cumulative pharmaceutical metrics (maximum/median of concentrations/detections) were positively correlated with multiple urban development metrics (p values of <0.001, ρ range of 0.678−0.822) and negatively correlated with depth to water table ($p < 0.0001$; ρ range of −0.581 to −0.738) and soil thickness ($p < 0.0001$; ρ range of −0.659 to −0.770) (Table S10), indicating that urban runoff and subsurface hydrologic connectivity may be primary drivers of in-stream pharmaceutical contamination in wadeable urban streams in the absence of NPDES-wastewater discharges. The results indicate LULC metrics are useful predictors of in-stream pharmaceutical contamination but suggest the need for refined metrics, including but not limited to WWTF treatment characterization, WWTF discharge rates and percentage contributions to downstream flow, proximity to and number of watershed NPDES sources, age and integrity of sewer-district infrastructure in WWTF-effected watersheds; septic system density and proximity to the stream in rural watersheds; and improved characterization of stormwater discharge and surface/subsurface hydrologic transport potentials throughout the region.

Implications for Ecosystem Health and Remediation in Wadeable Urban Streams. On the basis of these results, pharmaceutical contaminants are substantial environmental-health concerns in wadeable streams throughout the southeastern Piedmont ecoregion, irrespective of NPDES-wastewater discharge. Although the employed pharmaceutical method[25] is one of the most extensive targeted-analyte approaches currently available, it encompasses a fraction of the pharmaceutical universe, with more than 4000 pharmaceutical parent compounds in current use[2] and an incalculable chemical space[34] of potential daughter products.[10] Thus, actual pharmaceutical occurrence and concentrations undoubtedly substantially exceed current observations. Nevertheless, the nanograms per liter to micrograms per liter concentrations of individual contaminants and multiple pharmaceutical detections per site (median of 6) at cumulative concentrations of up to more than 16 μg L^{-1} are substantial concerns in their own right, because adverse environmental impacts have been documented for single pharmaceutical contaminants at low nanograms per liter concentrations[16] and interactive effects of complex mixtures of bioactive contaminants are global environmental-health priorities.[10,35−37]

Select pharmaceutical results highlight the human-health/aquatic-health connections raised by this study; comprehensive reviews of pharmaceutical aquatic impacts have been addressed elsewhere.[1,4−6] Antibiotic/antibacterial contaminant concentrations of 0.5 μg L^{-1} can affect the structure and composition of aquatic microbial communities, leading to altered carbon and energy flow throughout the aquatic foodweb.[22,38,39] The detection of sulfamethoxazole at 20% of the study sites and at concentrations of up to 1.5 μg L^{-1} strongly suggests the potential for region-wide impacts at the microbial base of the aquatic foodweb, particularly because only two predominantly human-use antibiotics (sulfamethoxazole and trimethoprim) were assessed in the current study and the presence of agricultural-use antibiotics, bioactive metabolites, and environmental transformation products was not measured.[40] Likewise, frequent detections of multiple (diphenhydramine, fexofenadine, and hydroxyzine) antihistamine contaminants at concentrations of up to approximately 4 μg L^{-1} indicate the potential for adverse impacts on aquatic invertebrate communities throughout the region. Histamines are neurotransmitters for many aquatic insects,[1] and exposure to approximately 2 μg L^{-1} fexofenadine has been shown to impair survival behavior (flight response) in damselfly (*Zygoptera*) species and to result in bioconcentration of up to 2000 times the dissolved concentration.[41] Most compelling, however, is the near ubiquity of metformin (89% of samples, 97% of sites) at concentrations of up to micrograms per liter, a finding that cannot be explained by NPDES discharges that occur in only 29% of the study watersheds. Fish are widely used animal models for the development of pharmaceuticals, including antidiabetics.[42,43] Effluent-equivalent metformin exposures (40 μg L^{-1}) have recently been shown to induce upregulation of vitellogenin (Vtg) mRNA[44,45] and other gene targets[45] as well as male intersex[44] in fathead minnow (*Pimephales*). Metformin passes through the human body essentially unchanged, is poorly removed by conventional and many advanced wastewater treatment technologies, and is considered environmentally recalcitrant.[46,47] Metformin is reported widely in wastewater effluent,[47] increasingly in environmental samples,[48] and even in tapwater.[47] Expanded global use is expected as a first-line drug therapy in the global diabetes epidemic[49−51] and a potential treatment for polycystic ovarian syndrome[52] and various cancers.[53]

Considering the probable individual and interactive effects of the 65 other detected pharmaceuticals and the vast majority of pharmaceutical parent compounds, metabolites, and environmental degradates not assessed herein, the results of this study demonstrate a critical need for broad-scale approaches to in-

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stream pharmaceutical contaminant prevention that extend well beyond the current emphasis on WWTF-effluent treatment. The 4-fold global increase in diabetes since 1980,[50] for example, is due overwhelmingly to type II diabetes, a preventable disease attributed to recent changes in food supply and lifestyle.[50,51] Increased emphasis on diabetes prevention simultaneously addresses the costs of diabetes in humans and the presence and effects of antidiabetic drugs in aquatic ecosystems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00170 and the USGS ScienceBase website at DOI: 10.5066/F7V40S99. Complete primary data are accessible at USGS NWIS (http://dx.doi.org/10.5066/F7P55KJN).

Supplemental Data Tables S1−S11 (XLSX)

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Notes

The authors declare no competing financial interest.

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