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Characterizing the Chemical Profile of Biological Decline in Stormwater-Impacted Urban Watersheds

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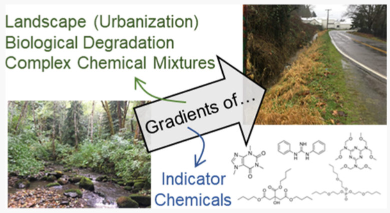

ABSTRACT: Chemical contamination is an increasingly important conservation issue in urban runoff-impacted watersheds. Regulatory and restoration efforts typically evaluate limited conventional parameters and pollutants. However, complex urban chemical mixtures contain hundreds to thousands of organic contaminants that remain unidentified, unregulated, and poorly understood. This study aimed to develop broadly representative metrics of water quality impairment corresponding to previously documented biological degradation along gradients of human impacts. Stream samples (n = 65, baseflow/rainfall conditions, 2017−2018) were collected from 15 regional watersheds (Puget Sound, WA, USA) across an urbanization gradient defined by landscape characteristics. Surface water chemical composition characterized via non-targeted high-resolution mass spectrometry (7068 detections) was highly correlated with landscape-based urbanization gradient ($p < 0.01$) and season ($p < 0.01$). Landscape-scale changes in chemical composition closely aligned with two anchors of biological decline: coho salmon (*Oncorhynchus kisutch*) mortality risk ($p < 0.001$) and loss of stream macroinvertebrate diversity and abundance ($p < 0.001$). We isolated and identified 32 indicators for urban runoff impacts and corresponding receiving water ecological health, including well-known anthropogenic contaminants (e.g., caffeine, organophosphates, vehicle-derived chemicals), two related environmental transformation products, and a novel (methoxymethyl)melamine compound. Outcomes support data-directed selection of next-generation water quality indicators for prioritization and evaluation of watershed management efforts intended to protect aquatic ecosystems.

KEYWORDS: *urban stream syndrome, land cover, index of biological integrity, B-IBI, stormwater, high-resolution mass spectrometry, nontargeted analysis, coho salmon, urban runoff mortality syndrome, macroinvertebrate, nonpoint source pollution*

INTRODUCTION

Globally, urbanization has impaired water quality and degraded ecological function to an extent that scales with the intensity of human development and accompanying landscape changes.[1] Conceptually, this "urban stream syndrome" encompasses the widespread discharge of anthropogenic contaminants and resultant myriad impacts on aquatic systems,[2] including habitat degradation,[3] sublethal effects in fish and macroinvertebrates,[4−7] and even an acute urban runoff mortality syndrome (URMS) in coho salmon (*Oncorhynchus kisutch*), a sentinel species of the Pacific Northwest, USA.[8,9] Most efforts to characterize and resolve urban stream syndrome have focused on physical habitat characteristics and a limited suite of conventional water quality parameters (e.g., suspended solids, nutrients, dissolved oxygen, and metals), evaluated one at a time.[10−14] However, there is growing recognition that these parameters exclude a poorly understood dimension of aquatic habitat quality: the thousands of organic chemicals in routine use[15] and increasingly detected in urban receiving waters.[16−19] The protectiveness of current "one-by-one" approaches to water quality assessment and management that reflect this narrow focus is limited.[20] This approach typically yields receiving waters that meet regulatory standards (e.g., US EPA, Clean Water Act) yet may still demonstrate adverse outcomes for certain aquatic organisms.[21−23]

Urban land use and associated nonpoint source contaminant transport via diffuse stormwater runoff (which enters receiving waters by overland flow and/or stormwater conveyance systems) have been linked to complex mixtures of emerging organic contaminants (e.g., pesticides, pharmaceuticals, flame retardants, and industrial additives) and associated field-based assessments of biological decline.[17,19,20,24−27] However, specific mechanisms driving the link between urbanization and biological degradation remain uncertain[28] and are complicated by the multiple stressors at play.[20,29−31] Thus, identifying water quality metrics or specific contaminants that delineate land-use activities or key chemical sources, and also effectively correspond to diverse ecotoxicological impacts, remains difficult.[32,33] The vast number of unregulated

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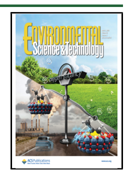

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chemicals and the complexity of chemical mixtures detected in surface waters, sediments, and the tissues of exposed organisms greatly exacerbate the challenge of determining toxicological relevance of individual contaminants.[27,34] Furthermore, mixed chemical exposures often impact toxicity outcomes (i.e., through synergistic, additive, or antagonistic effects), and transformation processes may yield products that present distinct ecotoxicological risks relative to their parent compounds.[20,35]

Accordingly, to effectively protect and restore ecological function in urbanizing watersheds, a more inclusive set of watershed assessment methods and metrics are needed to assess the aggregate impacts of water quality degradation on biological outcomes. Spatially explicit, landscape-based modeling provides a decision-making framework to prioritize specific watersheds, receiving waters, and stream reaches for restoration[24] but can be uncertain in the absence of supporting water quality data. If validated by on-the-ground chemical and biological data, the complex chemical mixtures, or so-called "urban chemical cocktails,"[19] that are predicted by landscape modeling may represent an opportunity to more holistically evaluate the efficacy of remedial actions, relative to single contaminant monitoring.[25,32] However, datasets that support this conceptual approach are not yet fully developed.

To address this gap, we used non-targeted high-resolution mass spectrometry (HRMS) to evaluate chemical habitat quality in surface waters (nontidal creeks) during baseflow conditions and storm events in 15 regional Puget Sound (Seattle, WA, USA) watersheds across a previously defined landscape-based urbanization gradient.[24] We applied two metrics of biological decline[7,24,36] that were previously linked to increasing urbanization. We identified novel chemical indicators that support assessment of aggregate chemical habitat quality (and associated biological impacts) along the urbanization gradient, enabling resolved evaluations of restoration and stormwater treatment efforts. Outcomes support two key approaches for effective direction of limited resources to protect aquatic ecosystems: spatial landscape modeling to prioritize watershed-scale management efforts and development of new chemical indicators to represent the collective ecotoxicological pressure caused by urban water quality degradation.

■ MATERIALS AND METHODS

Chemicals. Chemical standards are listed in Text S1.

Water Samples. Sampling site selection was guided by a previously reported regional (Puget Sound) urbanization gradient across 1481 watersheds, described by a modeled vector of landscape variables that accounted for covariation in specific characteristics (e.g., impervious surfaces, traffic intensity, and land cover),[24] with sites representing a continuous vector also categorized as: low (<50th percentile of all 1481 watersheds), medium (50th–95th), and high (≥95th) urbanization levels (Figure 1). Surface water grab samples (n = 65) were collected (4 L precleaned amber glass bottles without headspace; transported on ice to Center for Urban Waters; stored at 4 °C; and extracted within 24 h) from nontidal creeks during late-summer dry-season baseflow conditions (one event in 10 of 15 creeks; September–October 2017), and eight storm events (one to five storms in each of 15 creeks; October and November 2017, January, June and November 2018; Table S1 and Figure S1). Sample collection targeted the hydrograph rising limb. Precipitation accumulation in each watershed 8 h prior to each sampling event was compiled from the nearest Weather Underground weather station (www.wunderground.com; Table S2) as a metric of relative storm size.

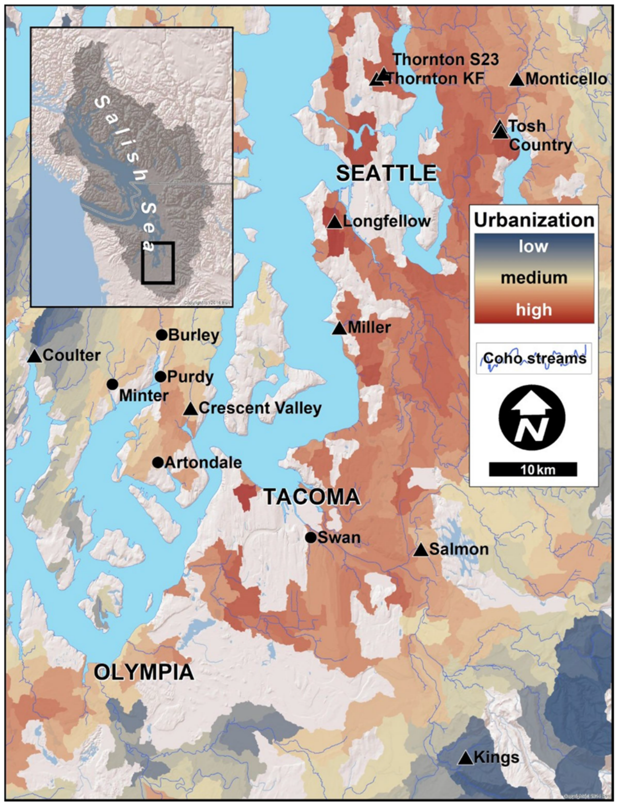

Figure 1. Sampling locations in the Puget Sound (Washington State, USA) region. Watershed coloration (blue, yellow, red gradient) indicates increasing urbanization level (low to medium to high). Circles indicate sites sampled only in November 2018; all other sites (triangles) were sampled during baseflow conditions (2017), October and November 2017, and January, June, and November 2018 (except Thornton Creek, where a June 2018 sample was not collected). "Minter" Creek indicates "Little Minter" Creek due to space limitations. Urbanization gradient and stream data are from the study by Feist et al.[24]

Sample Processing and Analysis. Samples were processed and analyzed by established methods that primarily extract relatively polar, non/semivolatile organic contaminants.[37] Briefly, using a vacuum manifold, Infinity SPE cartridges (3 mL, 100 mg Osorb media; ABS Materials, Wooster, OH, USA) were preconditioned (3 mL of 50% v/v methanol/deionized (DI) water; 25 mL of DI water); 1 L of water samples were loaded (without prefiltration, 5–10 mL/min); cartridges were rinsed (10 mL of DI water), vacuum-dried (15 min), and eluted with methanol (2 × 2.5 mL). Extracts were concentrated with nitrogen to 1 mL and spiked with an internal standard (ISTD) mixture (25 μL, n = 13, 25–500 ng/mL in vial; Table S3). For baseflow samples and all storms except 11/26/2018, four (1 L) field replicates were extracted in parallel, with a single HRMS injection/analysis per final extract (n = 4). For the 11/26/2018 storm, because of sample processing limitations, two (1 L) field replicates were extracted in parallel, with a duplicate HRMS injection/analysis per final extract (n = 4).

Analysis used an Agilent 1290 Infinity ultrahigh performance liquid chromatograph and an Agilent 6530 Quadrupole Time-

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of-Flight HRMS with electrospray Jet Stream Technology (Santa Clara, CA, USA). Detector resolving power was 6000−12,400 within the acquisition range, and mass accuracy was corrected via continuous infusion of purine and HP-921 calibrants (LC/MS tuning mix for ESI; Agilent Technologies, Santa Clara, CA, USA). Chromatography used C18 analytical and guard columns (Agilent ZORBAX Eclipse Plus 2.1 × 100 mm (analytical), 2.1 × 5 mm (guard), 1.8 μm particle size) at 45 °C, an injection volume of 5 μL, a flow rate of 0.4 mL/min, and a 22.5 min binary gradient of 5 mM ammonium acetate plus 0.1% acetic acid in each of DI water (A) and methanol (B) (5% B at 0−1 min, 50% B at 4 min, 100% B at 17−20 min, 5% B at 20.1 min; stop time 22.5 min; post-time 2 min).[37] HRMS spectra were acquired across 100−1700 *m/z* (MS) and 50−1700 *m/z* (MS/MS) in 2 GHz extended dynamic range mode (collision-induced dissociation; data-dependent acquisition, ESI+). This study focused on ESI+, which in our experience typically yields more detections/peak area than ESI−.

For quality assurance and quality control, we checked mass accuracy before each analytical batch and retuned if mass error exceeded 2 ppm. Samples from different storm dates and sampling locations were randomized throughout one analytical batch (~440 injections, run-time > 8 days), with solvent blanks and an ISTD blank (25 μL ISTD mixture in 1 mL of methanol) analyzed every 8−12 samples (no column carryover detected). ISTD mass accuracy was <5 ppm, and relative standard deviation of the peak area and retention time were <19% and <0.6%, respectively. Method and field blanks (DI water through SPE) were analyzed alongside samples, with fold-change analyses (see below) used to exclude background signals.

HRMS Data Reduction. Initial data reduction used published methods.[18] Non-target compounds (exact mass-retention time pairs with grouped adducts/isotopes, representing unique chemical detections) were extracted/aligned in Agilent MassHunter Profinder (B.08.00) and imported into Agilent Mass Profiler Professional (B.13.00) for filtering and analysis (Table S4). For each sample, compounds in ≥75% of replicates ($n = 3/4$) and with peak area both ≥5000 and ≥ 3-fold greater than any blank (method, field, and solvent) peak area were retained.[38−40] Peak areas of zero were reported for all other detections. For detections that met all filtering criteria, the observed peak area in the sample was corrected by subtracting the maximum peak area detected in any blank and then ISTD-adjusted (divided by the median sample-specific ISTD ratio; Text S2) to account for matrix effects.[41]

Statistical Analyses. Permutational multivariate analysis of variance (PERMANOVA) analyses were performed to describe partitioning of covariates associated with the water samples, in relation to peak areas of all HRMS detections (including both identified and unidentified compounds). Analyses examined categories of a landcover-based urbanization gradient (low, medium, and high) defined by Feist et al.[24] and described above. PERMANOVA analyses also included per-storm precipitation accumulation and sampling event season (defined as baseflow conditions [considered a season for model simplicity] and storm events in fall [Oct/Nov], winter [Jan], and spring [June]) (Table S1). Contrast models were run to compare differences between seasons across urbanization categories. Analyses tested November 2017 and 2018 samples as individual and combined categories. A Bonferroni correction was used to adjust resultant *p-values* for multiple comparisons. All PERMANOVA analyses (adonis function, R vegan package) used Bray−Curtis dissimilarity and 9999 permutations, with watershed (delineated by the Washington State Department of Ecology)[42] as a stratum to account for repeat sampling from the same stream.

To evaluate chemical water quality (specifically, the chemical urbanization gradient—NMDS1, described below) in the context of stream health, analyses were conducted using a mixed effects model (lmer function, R lme4 package) to associate HRMS detections with two locally relevant biological metrics: predicted risk of coho salmon URMS (Table S1 and Text S3)[24] and the benthic index of biological integrity (B-IBI—a quantitative index of degraded macroinvertebrate health; Tables S5 and S6, Text S4).[7,42] These evaluations were restricted to storm events and included repeat sampling per watershed as a random effect. Macroinvertebrate analyses were evaluated twice: (1) using only the site with available B-IBI data located nearest to each water quality sampling site and (2) using all sites with B-IBI data located <400 m from each water quality sampling site in the same watershed/stream reach ($n = 2−13$ sites/watershed, $n = 1−15$ data points/site; Figure S1a−j, Table S6).

A multivariate ordination method (non-metric multidimensional scaling; NMDS; metaMDS function, R vegan package) was used to reduce the entire HRMS dataset from multiple correlated variables that were similar in a multidimensional space. The analysis was based on a Bray−Curtis dissimilarity matrix derived from the square root-transformed relative peak area data for each stream sample, standardized by the maximum peak area for each compound. The NMDS analysis inputs were restricted to the HRMS feature data without covariates or identifiers. Water samples were plotted along the resulting axes with markers indicating the urbanization category to visualize patterns and aid interpretation of PERMANOVA analysis results. All analyses were conducted in R (v3.2.2).[43]

Non-target Compound Prioritization. The occurrence and relative abundances of HRMS detections serve as metrics of human impact, associated contaminant loadings, and chemical exposures in aquatic systems. Accordingly, identification and quantification of each individual chemical are not necessary to confirm chemical loadings in receiving water, but, rather, to provide indicator compounds associated with these contaminant profiles. Two complementary methods were used to select indicator compounds associated with a specific trend in chemical water quality (systemic degradation associated with urbanization; discussed further below). All HRMS detections (including again both identified and unidentified compounds) were included, and compounds selected by both approaches were prioritized for identification. First, we selected compounds that were significantly elevated in the high urbanization creek stormwater samples relative to the baseflow samples ($p < 0.05$) in a mixed effect model with the urbanization category of stormwater samples as the predictor (baseflow as the reference) and watershed included as a random effect. Feature selection was further defined by a >2-fold change in feature abundance in the high urbanization samples compared to low urbanization creeks, each relative to baseflow samples. For these analyses, individual features were standardized by the total peak area of all features in a sample, and features with the peak area below minimum filtering criteria were given a value of one. Second, a species indicator analysis (IndVal; multipatt function, R indicspecies package) selected compounds based on their presence and abundance in predefined groups (p < 0.05; 2000 permutations): The "IndVal" groups included baseflow; low, medium, and high urbanization storms; and Salmon Creek. The IndVal analysis was also applied separately to prioritize compounds associated with the uniqueness of Salmon Creek (p < 0.001; discussed further below).

Prioritized detections were identified by screening non-target compounds against in-house stormwater databases (>1000 compounds), the Agilent Forensic/Toxicology database, and the NORMAN Suspect List Exchange Database.[44] Identifications were scored against criteria proposed by Schymanski et al. to communicate confidence (Text S5).[44,45] Expert knowledge was sometimes needed to resolve adducts and validate chemical identities not initially resolved by software-driven workflows.

■ RESULTS AND DISCUSSION

Chemical Water Quality along a Continuous Urbanization Gradient. A total of 7068 unique chemical detections (345−2257 per sample) occurred across 65 water samples (10 during baseflow conditions, 55 during storm events) from 15 Puget Sound creeks representing a previously described landscape-based urbanization gradient, where high urbanization was dominated by developed landcover and low urbanization was dominated by evergreen forest (Figure 1 and Table S1).[24] These HRMS detections included natural products and anthropogenic contaminants,[46] reflecting both the natural background chemistry of each watershed and disparate human inputs. Although method parameters (e.g., sample processing and LC separation) limit the detected chemical space, non-targeted approaches are empirically broader and conceptually less biased than targeted methods that preselect analytes of interest.[47]

Non-targeted chemical composition, represented by both unique clusters of chemical detections and varying relative abundances, was associated with urbanization along a previously defined landscape gradient (p < 0.001; Figures 1 and 2a; Table 1).[24] Together, landscape-based urbanization (low, medium, and high urbanization categories) and season (late-summer baseflow; fall, winter, spring storms; discussed below) explained >40% of the variability in chemical composition (Table 1). Precipitation accumulation explained <2% of the variability in the chemical composition; it was not significant (p > 0.05) and was not included in the final model. This lack of an association for precipitation reflects that many factors impact the relationship between accumulation and runoff, such as watershed hydrology and extent of impervious surfaces.[48] NMDS ordination analysis applied to the chemical detection data, independent of any covariates, yielded four dimensions (vectors; NMDS1−4) that described the water quality data (stress: 0.082; non-metric fit of original and reduced data: R^2 = 0.99, p < 0.01). Plots of the reduced data (Figure 2a−c) communicate that the chemical composition across NMDS1 independently described the gradient from low to high urbanization sites that was also described by landscape characteristics.

The complex chemical urbanization gradient represented by NMDS1 extended beyond well-established metrics of contamination (e.g., targeted analyses of dissolved metals and polycyclic aromatic hydrocarbons). Rather, the chemical urbanization gradient observed here included 100s−1000s of chemical constituents that are neither regulated nor routinely monitored but are present at detectable concentrations and scale with the level of human impact (i.e., increased urbanization), aligning with previous studies that describe significant contributions of emerging organic contaminants to degraded water quality and increased ecological risk.[20,49] The richness and depth of this chemical gradient created

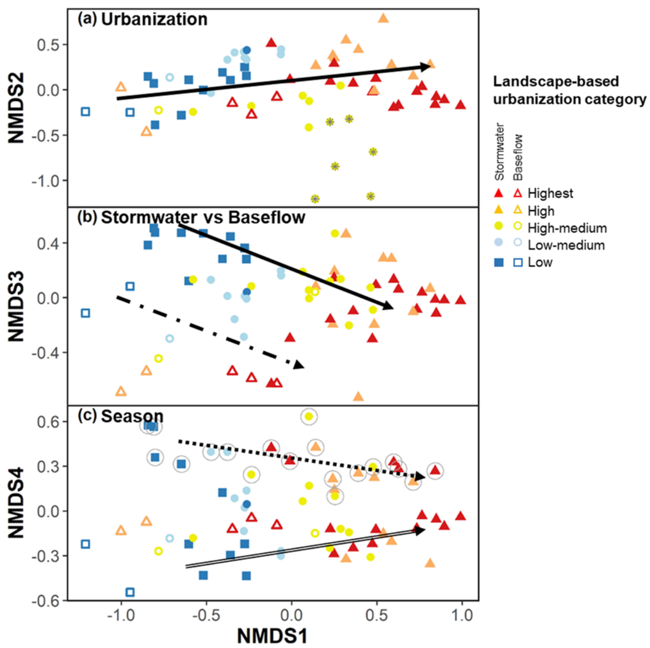

Figure 2. Chemical composition of urbanization shown through the results of NMDS analyses, contrasting NMDS2−4 (a−c, respectively) with NMDS1. Marker color scheme represents landscape-based urbanization previously defined by Feist et al.;[24] these meta-data were not included in the NMDS analysis, rather incorporated into the figure for visualization. All arrows indicate the gradient of increasing urbanization represented by the chemical composition in the water samples. In (a), a single arrow shows the urbanization gradient, and Salmon Creek samples are indicated with a gray asterisk. In (b), the arrows are separated by baseflow samples (dot-dash arrow), and samples collected during a storm event (solid arrow). In (c), the arrows are split by season to show winter/spring water samples at top (dashed arrow; samples circled in gray) and fall water samples at bottom (double-line arrow).

Table 1. PERMANOVA Partitioning and Analysis of Covariates in Water Sample Analysis[a]

covariate	df	SS	MS	F.model	R^2	Pr(>F)
urbanization category	2	2.305	1.152	5.656	0.129	**<0.001**
season	3	2.731	0.910	4.461	0.153	**<0.001**
season: urbanization category (interaction)	6	1.881	0.3134	1.522	0.105	**<0.001**
residuals	52	10.917	0.206	--	0.612	--
total	64	17.834	1	--	1	--

[a]The urbanization gradient is a categorical metric (high, medium, and low) defined by a previously developed landscape characteristics model (Feist et al.).[24] Season represents four groups: late-summer baseflow conditions, and storm events in fall (October/November), winter (January), and spring (June). R^2 describes the proportion of variance explained, and significant p-values (<0.05) are denoted in bold font. Watershed was included as a stratum to account for repeat sampling.

opportunities to further examine specific observations, such as examining the relationship between chemical and biological water quality, and identifying individual chemicals and key contaminant classes/source types that drove water quality differences (examples described below).

Correlating Biological Decline with the Complex Chemical Gradient. The chemical urbanization gradient (NMDS1) was first evaluated with respect to B-IBI, a macroinvertebrate health index[7,50,51] that exemplifies the various (multi-species, multi-endpoint) deleterious impacts of physical and chemical habitat quality. A decrease in B-IBI was significantly correlated with increasing urbanization defined by chemical composition (NMDS1) (mixed effects model slope estimate = −0.009, p = 0.032; watershed included as a random effect to account for repeat sampling) (Figure 3a and Table S7). This analysis was restricted to water quality sampling sites with B-IBI data available in the same watershed and stream reach (n = 10, excluding Artondale, Crescent Valley, Kings, Salmon, and Coulter Creeks; Figure S1). The significance of the association increased (slope estimate = −0.012, p < 0.001) with exclusion of Swan Creek (for which a divergent association with the urban chemical gradient was attributed to collection of a single water quality grab sampling event that likely missed stormwater contaminant flows, instead representing baseflow stream conditions). The observed relationship between water quality and reduced macroinvertebrate diversity/abundance relied on data-driven analyses that did not preselect known organic contaminants (e.g., regulated or priority pollutants with known toxicity endpoints), a cornerstone of previous studies that assessed biological conditions.[20,26,27] Thus, the current association represents the cumulative ecotoxicological pressure exerted by complex contaminant mixtures typical of urban waters. Extending this analysis to all B-IBI sampling sites in the same watershed and stream reach as the water quality sampling site demonstrated a modest correlation of B-IBI and water quality (excluding Swan Creek; slope estimate = −0.002, p = 0.176), with the reduced significance likely explained by the increased variance in the dataset from geographic and temporal differences. Additional sampling specifically designed to examine potential B-IBI—water quality relationships (e.g., co-located, concurrent sampling) may help disentangle the multiple stressors associated with macroinvertebrate decline[29] and isolate subsets of contaminant profiles that are more specific to impacts on B-IBI indices. Co-located concurrent sampling may also provide more opportunities for increased spatial and temporal resolution in efforts to prioritize watersheds for water quality-focused actions to protect and restore biological health.

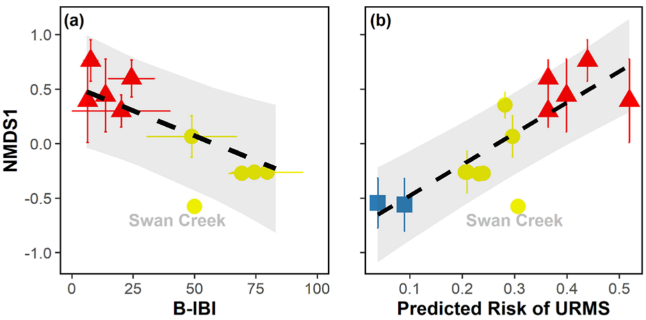

Figure 3. Chemical profile aligned with the urbanization gradient (NMDS1) showed: (a) reduced macroinvertebrate diversity and abundance (B-IBI; p < 0.001) and increased risk of mortality in coho salmon (URMS; p < 0.001), each associated with more urban streams. The analysis was restricted to samples collected during a storm event. Categorically, red triangles, yellow circles, and blue squares represent high, medium, and low urbanization levels, respectively. In the B-IBI plot in (a), each marker represents the average of B-IBI scores (0−100); standard deviation bars represent values across Puget Sound Stream Benthos sampling sites in the same watershed and stream reach. The regression line represents the association of NMDS1 and nearest B-IBI sampling site only (excluding Swan Creek). The B-IBI plot excludes Artondale, Kings, Crescent Valley, Salmon, and Coulter Creeks; no paired Puget Sound Stream Benthos data were available. For both plots, each marker represents the average NMDS1 value; standard deviation bars represent values across all storm event samples collected at each site. For both plots, the gray shaded area represents the 95% confidence interval.

The second evaluated indicator of stream health, a predictive coho salmon URMS risk metric,[9,24] corresponds to an acute species-specific impact. A positive vector for the chemical urbanization gradient (NMDS1) was significantly associated with an increase in URMS risk (mixed effects model slope estimate = 2.86, p < 0.001; separate analyses by season: each p < 0.001; watershed included as a random effect to account for repeat sampling) (Figure 3b; Tables S7 and S8). Although selection of sampling sites for this study was guided by a previous association between landscape-based urbanization and URMS risk,[24] the significant association described here of chemical composition with URMS risk was demonstrated independent of other covariates (e.g., qualifiers of urbanization). Additionally, this significant association was apparent despite inherent variability in the data, such as within-watershed landscape variation (e.g., water quality sampling occurred upstream of most stormwater outfalls in Longfellow Creek), sample timing and quantity (e.g., if grab sampling missed peak contaminant flows; Swan Creek water quality data were limited to a single event), and/or the contribution of impacts not accounted for in this dataset (e.g., undetected chemicals, habitat degradation, other water quality parameters, etc.). Notably, while recent toxicant elucidation efforts ultimately identified a primary causal chemical (and thus an indicator) for URMS,[52] undertaking such efforts for every biological impact is not feasible because of limited time, resources, and the continuous release of new chemicals. However, these regression results (shown in Figure 3) indicated that this complex water quality gradient effectively captured and can be predictive of a representative, yet highly specific, biological impact, in addition to the nonspecific biological effects described by B-IBI.

Current metrics of acceptable water quality, based on analyses of conventional pollutants and existing aquatic life parameters, are intended to preserve ecologic function. However, the associations reported here indicated that existing metrics may not adequately represent anthropogenic drivers of ecological health decline in urban watersheds.[53] Accordingly, our results support implementation of a comprehensive chemical water quality-based metric to augment existing standards that directly scale loss of ecologic function and extent of biological degradation with human impact. Indeed, data presented here indicated that holistic human impacts on chemical water quality are not just quantifiable, but also can exhibit a high degree of resolution and sensitivity at the watershed scale (e.g., ≈5−20 km^2). Additionally, landscape-based predictive metrics can effectively quantify both diffuse human impacts on water quality and associated biological

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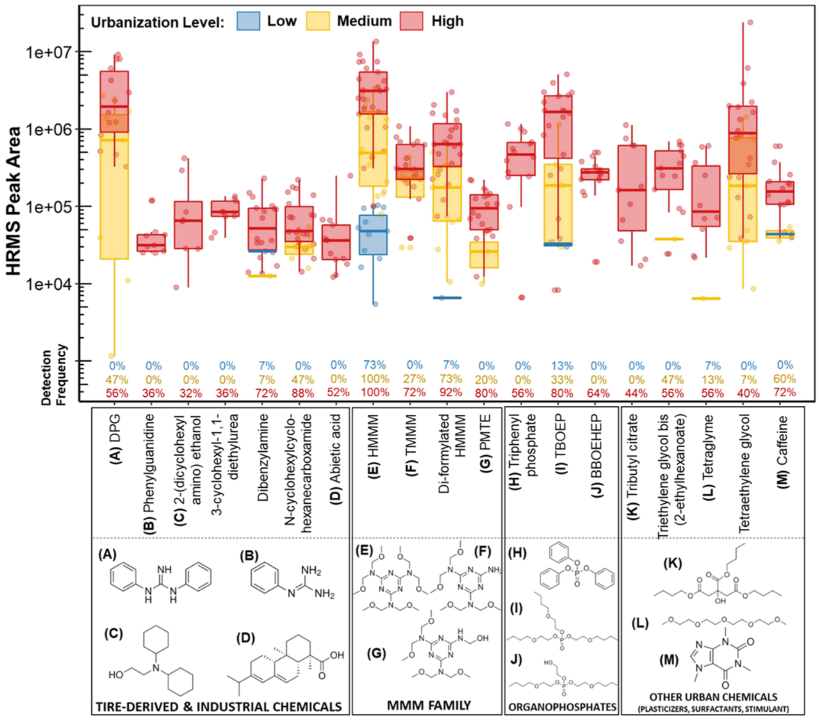

Figure 4. Relative abundance (HRMS peak area) and detection frequency (during storm events) in low (blue), medium (yellow), and highly (red) urbanized watersheds for 19 representative contaminants that were selected statistically and identified as potential chemical indicators whose relative abundance scaled with the degree of water quality degradation during storm events in highly urbanized watersheds. Indicators are grouped by chemical class, and a selection of structures are shown for reference (matched to chemical name by letters A–M). DPG: 1,3-diphenylguanidine; HMMM: hexa(methoxymethyl)melamine; TMMM: tetra(methoxymethyl)melamine; PMTE: pentamethylolmelamine tetramethyl ether; TBOEP: tris(2-butoxyethyl)phosphate, BBOEHEP: bis(2-butoxyethyl)-(2-hydroxyethyl)phosphate.

degradation at similar scales, supporting regional-scale prioritization of restoration and management efforts. Although beyond the scope of this work, future studies should assess whether regional-scale, spatially explicit predictions can be downscaled to assess water quality and management needs at a finer spatial grain within watersheds (e.g., m–km scale) to capture local variability and unique source contributions.

Isolating Chemical Indicators that Predict Urban Water Quality. Comprehensive metrics based on the chemical urbanization gradient represent an opportunity to ascertain the contribution of toxic contaminants (including those not yet identified) to biological degradation, inform source control or restoration actions needed to improve water quality and mitigate biological impairment, and evaluate restoration efficacy more holistically. Use of such metrics is not routine, attributed to the cost and challenge of integrating broader indicator suites into existing monitoring and regulatory frameworks. Selecting effective new indicators is further challenged by limited-to-absent toxicological information for most of the 1000s of chemicals currently discharged to aquatic environments. To address this gap, we extended the statistical analyses used to evaluate the water quality data to isolate a short list of potential chemical indicators using our compound prioritization criteria. These compounds represent systemic receiving water quality degradation associated with urban runoff and are exemplified by biological decline in highly urbanized watersheds, although selection criteria did not include toxicological information (Table S9). At the regional scale (including all watersheds), water quality degradation associated with urbanization and stormwater runoff was indicated by 226 chemical detections. Among these, we identified 32 contaminants, representing relatively well-established chemical families and sources associated with human development, including plasticizers, organophosphates, surfactants, tire rubber- and vehicle fluid-derived chemicals, and the ubiquitous caffeine (Figure 4).[54] Notably, the indicator list included two parent-transformation product (TP) pairs: the tire rubber vulcanization accelerator 1,3-diphenylguanidine (DPG) and its TP phenylguanidine, and the flame retardant/plasticizer tris(2-butoxyethyl)phosphate (TBOEP) and its metabolite bis(2-butoxyethyl)-(2-hydroxyethyl)phosphate (BBOEHEP). Three previously reported members of a (methoxymethyl)melamine (MMM) family of compounds, hexaMMM (HMMM), tetraMMM (TMMM), and di-formylated HMMM were identified.[18] An additional structurally related compound was also detected for the first time in stormwater: pentamethylolmelamine tetramethyl ether (PMTE; 1-[[4,6-bis[bis(methoxymethyl)amino]-1,3,5-triazin-2-yl]amino]methanol).[18,55] Together, these indicators can provide insight regarding key fate outcomes (from parent-TP pairs) and contaminant sources (e.g., vehicles/tire rubber) that drive or reflect chemical water quality degradation.

Seasonal Water Quality Distinctions. Chemical composition varied between baseflow, fall (October/November) storms, and winter (January)/spring (June) storms ($p < 0.001$, Table 1), reflecting Puget Sound seasonal weather patterns (summer dry season, peak rainy season October–January, and intermittent storms through late June). Storm events and baseflow samples were separated along NMDS1 and better visualized by NMDS3 (PERMANOVA test of baseflow versus storm event samples: pseudo-F(1,64) = 5.40, $p < 0.01$; Figure 2b), describing a different chemical composition (i.e., 0.5 ±

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Environ. Sci. Technol. 2022, 56, 3159–3169

Table 2. Pairwise PERMANOVA Analysis by Sampling Season, Separated by Urbanization Category[a]

	all samples (15 creeks, 65 water samples)			low urbanization (2 creeks, 12 water samples)			medium urbanization (8 creeks, 23 water samples)			high urbanization (5 creeks, 30 water samples)		
season	F	R^2	p	F	R^2	p	F	R^2	p	F	R^2	p
base vs fall	5.54	0.11	**0.006**	2.35	0.28	0.276	1.95	0.12	0.084	5.71	0.23	**0.006**
base vs winter	4.04	0.18	**0.006**	2.85	0.59	1.00	1.37	0.26	1.00	4.53	0.36	**0.024**
base vs spring	3.10	0.15	**0.006**	2.35	0.54	1.00	1.23	0.24	1.00	2.83	0.29	**0.042**
fall vs winter	2.96	0.06	**0.006**	2.99	0.33	0.192	1.50	0.09	0.588	2.13	0.10	0.072
fall vs spring	3.21	0.07	**0.006**	2.67	0.31	0.234	1.84	0.11	0.084	3.10	0.15	**0.006**
winter vs spring	2.29	0.12	0.036	2.82	0.59	1.00	1.16	0.22	1.00	3.12	0.31	0.144

[a]Season represents four groups: late-summer baseflow conditions, and fall (October/November), winter (January), and spring (June) storm events. All p-values were adjusted for multiple comparisons, and significant adjusted p-values (<0.05) are denoted in bold font. Watershed was included as a stratum to account for repeat sampling.

0.2-fold fewer unique chemical detections, 0.5 ± 0.3-fold lower average abundance for chemicals detected during baseflow relative to storm conditions) in receiving waters before stormwater mobilized contaminants. However, the chemical urbanization gradient (NMDS1) was correlated with both stormwater and baseflow samples (both $p < 0.01$), reflecting a consistent influence of urban activities on receiving water quality, even without active mobilization by runoff.

The contrast between fall (early in the regional rainy season) and later season winter/spring storm event water quality was visualized along NMDS4. The chemical gradient of urbanization remained evident within each season (Figure 2c). Notably, urbanization predicted seasonal profiles in the storm event receiving water chemical composition (Table 2), indicating distinct contaminant loads and/or transport behavior across landscape regimes. In highly urban watersheds, chemical composition during fall storms was significantly different from dry-season baseflow conditions ($p < 0.01$), and differences remained evident, albeit less pronounced, as the rainy season progressed (winter $p = 0.02$; spring $p = 0.04$). This trend was consistent with "transport limited" behavior for thousands of emerging organic contaminants, where ongoing urban contaminant inputs and/or persistent large contaminant deposits (e.g., in sediments and conveyance pipes) in urbanized watersheds are not quickly depleted but instead continuously mobilized and transported to receiving waters when runoff occurs.[56,57] In contrast, in less urbanized watersheds, differences in the chemical composition during baseflow and fall storm events may reflect early-season contaminant transport events, but did not meet statistical significance thresholds (low urban $p = 0.28$; medium urban $p = 0.08$). However, the low urbanization category in particular had a small sample size, with two creeks and 12 water samples. During later season (winter/spring) storms, receiving water quality was statistically indistinguishable from baseflow conditions (both low and medium urban creeks; $p = 1.00$), pointing to a seasonal depletion of finite reservoirs of contaminant mass and reduced potential for stormwater-derived pollution. Thus, urban runoff impacts on the chemical composition of receiving waters in less urbanized watersheds may often be "mass limited," particularly later in the rainy season (i.e., runoff is occurring, but limited contaminant mass is available in the watershed for transport into receiving waters).[56] Urban stormwater management is often conceptualized by seasonal or within-storm first flushes, favoring treatment of higher contaminant loads in early-season events or initial storm volumes.[58,59] These data suggest that efforts to improve receiving water quality in urbanized watersheds may require both removal of contaminant reservoirs prior to storm events and ongoing source control across much of the rainy season. By contrast, less urbanized systems exhibit more potential for rapid seasonal improvements to receiving water quality, so treatment of initial early-season stormwater volumes may sufficiently mitigate contaminant loads and associated biological risk.

Source Delineation. Given similarities in built environments and urban ecosystems that span geographic differences,[1] we anticipate that landscape-based predictive methodologies can be applied in other metropolitan areas to evaluate geographic gradients of water quality, despite locally unique contaminant sources and fates. In support of this conceptual approach, the reduced chemical composition data isolated a secondary uniqueness in Salmon Creek relative to other watersheds (NMDS2; Figure 2a and Table S10). Notably, the primary water quality composition of Salmon Creek still aligned with NMDS1 according to its landscape-based urbanization assignment. This underscored the potential of chemical profiles and indicators to not only attain the primary objective of representing aggregate urbanization impact on water quality, but also inform a secondary uniqueness that might otherwise remain unexplained.

We hypothesized that a unique local source (an upstream concrete aggregate plant) strongly affected observed Salmon Creek water quality. Among detections unique to Salmon Creek relative to other watersheds, we identified 11 contaminants from two families—nonylphenol ethoxylates (NP_nEO, $n = 2-5, 7-9$) and nonylphenoxyethoxy acetic acids (NP_nEC, $n = 2-5$)—accounting for ≈17% of the total peak area of Salmon Creek-specific detections ($n = 210$ in total) (Figure S2 and Table S9). NPEOs are nonionic surfactants with known aquatic toxicity that are used in commercial/industrial products (e.g., detergents, paints, cosmetics), and NPECs are oxidative transformation products of NPEOs.[60,61] While the selectivity of these compounds is limited by their disparate chemical uses (and site inaccessibility precluded confirmatory sampling), the common use of NPEOs in concrete admixtures and as dust control agents[62,63] supports our local source hypothesis. This approach is directly applicable to situations involving other instances of secondary uniqueness. Via careful inspection, identified indicator chemicals could be used to evaluate the chemical properties and possible sources of causal toxicants, and they represent possible candidates that may be driving, or linked to, an observed biological impact.

Implications. The demonstrated associations between landscape-based predictive metrics, observed biological degra-

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Environ. Sci. Technol. 2022, 56, 3159−3169

dation, and novel chemical indicators exemplify the potential of new capabilities that incorporate increasingly comprehensive water quality assessments to augment existing approaches to protect and restore sustainable, healthy aquatic communities. We report here a complex chemical profile composed of 100s–1000s of unidentified or unmonitored/unregulated organic contaminants that were analytically detectable, repeatable across watersheds and storm events, and scaled with urbanization. These water quality data validated the use of readily accessible geospatial landscape data to predict overall water quality impairment and biological degradation associated with increased urbanization, to effectively prioritize ongoing and planned watershed management and restoration efforts. We anticipate that water quality metrics that scale with urbanization intensity are broadly applicable to metropolitan areas elsewhere because of similarities across built environments and universal sources/classes of chemical pollution (e.g., vehicles and flame retardants).[1] The toxicity for most contaminants will remain uncharacterized for the foreseeable future, emphasizing the utility of establishing associations between biological impairment (B-IBI, coho salmon mortality risk) and complex chemical gradients. In consideration of such uncertainty, a conceptual goal of stormwater treatment and watershed restoration efforts should be to "climb down the ladder" of chemical complexity by shifting overall water quality in highly impacted urban systems toward compositional alignment with that of less urban and less polluted environments.

Furthermore, we extracted and identified chemical indicators associated with stormwater in urban watersheds (thereby also correlated with biological degradation). The compounds identified here represent accessible (e.g., via traditional, targeted analytical techniques) indicators for the chemical composition of urban runoff and corresponding ecological health of receiving waters. Relative to traditional pollutants preselected based on knowledge of contaminant sources, these indicators provide a metric that could support efforts to quantify systemic water quality degradation, provide insight into contaminant sources, inform prioritization of appropriate restoration measures, and enable rapid assessments of remediation or treatment efficacy. Looking ahead, although additional work is needed (e.g., analytical method development, quantitative evaluation of indicator chemicals, focused studies pairing chemical detections with biological outcomes and accounting for other urbanization-related impacts, assessment of transferability across other urbanizing landscapes, etc.), such compounds could be monitored by standard analytical laboratory techniques (e.g., tandem mass spectrometry) and integrated with current water quality standards and decision-making frameworks. Furthermore, relative to biological data, these chemical indicators can support rapid, spatially and temporally resolved prioritization and evaluation of management actions. The comprehensive metrics based on the chemical urbanization gradient presented here represent an opportunity to develop improved tools for evaluating and mitigating water quality degradation in preparation for the coming decades of projected population growth and urban land expansion.[64]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c08274.

Additional method details and statistical results (PDF)

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Notes

The authors declare no competing financial interest.

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