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Responses of benthic macroinvertebrates to environmental changes associated with urbanization in nine metropolitan areas

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Abstract. Responses of benthic macroinvertebrates along gradients of urban intensity were investigated in nine metropolitan areas across the United States. Invertebrate assemblages in metropolitan areas where forests or shrublands were being converted to urban land were strongly related to urban intensity. In metropolitan areas where agriculture and grazing lands were being converted to urban land, invertebrate assemblages showed much weaker or nonsignificant relations with urban intensity because sites with low urban intensity were already degraded by agriculture. Ordination scores, the number of EPT taxa, and the mean pollution-tolerance value of organisms at a site were the best indicators of changes in assemblage condition. Diversity indices, functional groups, behavior, and dominance metrics were not good indicators of urbanization. Richness metrics were better indicators of urban effects than were abundance metrics, and qualitative samples collected from multiple habitats gave similar results to those of single habitat quantitative samples (riffles or woody snags) in all metropolitan areas. Changes in urban intensity were strongly correlated with a set of landscape variables that was consistent across all metropolitan areas. In contrast, the instream environmental variables that were strongly correlated with urbanization and invertebrate responses varied among metropolitan areas. The natural environmental setting determined the biological, chemical, and physical instream conditions upon which urbanization acts and dictated the differences in responses to urbanization among metropolitan areas. Threshold analysis showed little evidence for an initial period of resistance to urbanization. Instead, assemblages were degraded at very low levels of urbanization, and response rates were either similar across the gradient or higher at low levels of urbanization. Levels of impervious cover that have been suggested as protective of streams (5–10%) were associated with significant assemblage degradation and were not protective.

Key words: antecedent agriculture; benthic macroinvertebrates; disturbance; environmental gradients; habitat; land cover; urbanization; water chemistry; water quality; water temperature.

Introduction

Urbanization is widely known to alter the physical and chemical characteristics of streams and to cause significant degradation of invertebrate assemblages wherever urbanization occurs (Klein 1979, Jones and Clark 1987, Walsh et al. 2001, 2005*a*, Roy et al. 2003, Alberti et al. 2007, Gurnell et al. 2007). Most studies that have addressed urbanization and its effects do so within the context of a single city or metropolitan area using objectives, study designs, measures of urban intensity, and sample-collection and processing methods that are unique to each study. Despite these differences, Walsh et al. (2005*b*) has identified a set of environmental changes that are associated with urbanization and that are collectively referred to as the "urban stream syndrome."

While the symptoms of the urban stream syndrome appear to be qualitatively consistent, the differences among studies limit the ability to quantitatively assess similarities and differences among metropolitan areas. To address this issue, the U.S. Geological Survey (USGS) initiated studies of urban streams as part of the National Water-Quality Assessment (NAWQA) Program. These studies were designed to directly compare the effects of urbanization among major metropolitan areas that represent different regions of the continental United States. The nine metropolitan areas that were chosen for study—Boston, Massachusetts (BOS); Raleigh, North Carolina (RAL); Atlanta, Georgia (ATL); Birmingham, Alabama (BIR); Milwaukee-Green Bay, Wisconsin (MGB); Denver, Colorado (DEN); Dallas-Fort Worth, Texas (DFW); Salt Lake City, Utah (SLC); and Portland, Oregon (POR) (Fig. 1)—represent a range of natural environmental features such as potential natural vegetation, temperature, precipitation, basin relief, ele-

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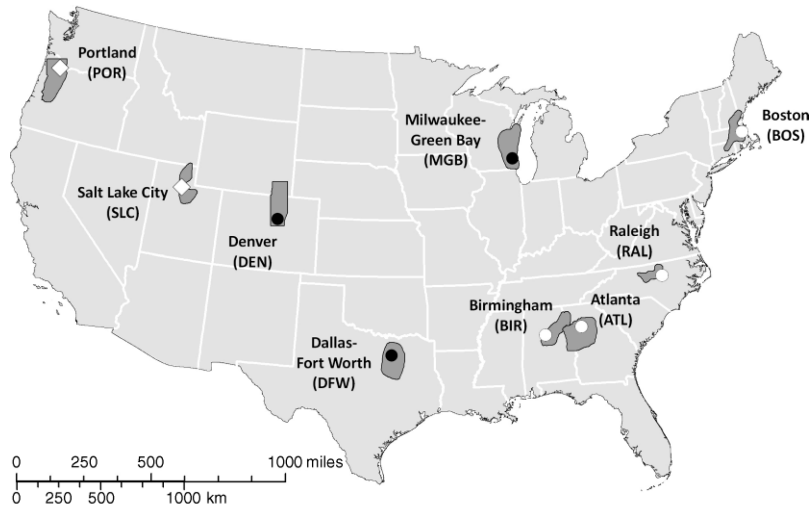

FIG. 1. Locations of the nine metropolitan areas in which urban studies were conducted. The shaded areas show the spatial extent of each metropolitan area. Open circles designate eastern metropolitan areas, solid circles designate central metropolitan areas, and open diamonds designate western metropolitan areas.

vation, and basin slope (Table 1). These studies examined biological, chemical, and physical changes along gradients of urbanization using a consistent experimental design and sample-collection and processing methods (Tate et al. 2005, Giddings et al. 2009). They represent a unique opportunity to begin to address some of the regional- and continental-scale effects of urbanization that have been hypothesized by Grimm et al. (2008).

The NAWQA Program urban stream studies are based on a simple conceptual model (Fig. 2) of region-scale urbanization in which increasing population density causes landscape changes (e.g., increasing housing density, percentage of developed land, impervious surface, and road density) that are associated with providing the goods and services required to support an increasing population. These landscape changes interact with the natural environmental setting (e.g., climate, topography, soils, geology and other natural environmental characteristics) to produce changes in the instream environment that affect the invertebrate assemblages. Since the natural environmental setting varies among metropolitan areas (i.e., regionally); the biological, physical, and chemical responses to urbanization are also expected to change regionally even if the landscape changes are consistent across metropolitan areas. This conceptual model also recognizes that urbanization often occurs in conjunction with other land uses (e.g., conversion of agricultural lands to urban) and that these non-urban land uses can alter the effects of urbanization.

In this paper, we determine if there is a consistent set of landscape variables that are associated with urbanization in all nine metropolitan areas. We test whether the natural environmental template is associated with differences in the invertebrate assemblages that occur in each region (background conditions) and the rates at which urbanization alters these assemblages. We compare the physical and chemical variables that are associated with changes in urbanization and invertebrate responses among metropolitan areas and test whether the natural environmental setting and competing land uses, specifically agriculture, alter the effects of urbanization on macroinvertebrate assemblages. We examine the invertebrate responses to urbanization to determine if responses are consistent with the expectations of the urban stream syndrome and whether they display the response forms (Fig. 3) hypothesized by Booth et al. (2004), King et al. (2005) and Walsh et al. (2005*a*). We also examine invertebrate responses to determine whether the 5–10% criterion for impervious cover that has been suggested as protecting stream integrity (Schueler 1994, Booth and Jackson 1997) actually provides protection.

METHODS

A population of candidate basins (typically basins draining second- to third-order streams) was delineated within each of the nine metropolitan areas based on 1:24 000 digital elevation models expressed as a 30-m raster (U.S. Geological Survey 2003). The number of candidate basins ranged from a few dozen in arid areas (Salt Lake City) to several thousand in wet areas (Raleigh). Landscape and natural environmental features (Table 2) were derived for candidate basins by overlaying basin boundaries with nationally available geographic information system (GIS) variables. National GIS land cover data were also used to estimate the amount of forest (AFOR) and agricultural lands (row crop + grasslands, AAG) that were being converted to urban in each metropolitan area. These antecedent land cover estimates were derived for each metropolitan

TABLE 1. Major environmental characteristics of the nine metropolitan areas.

Metropolitan area	Predominant ecoregion	Natural vegetation	Mean annual: Air temperature (°C)	Mean annual: Precipitation (cm)
Boston, Massachusetts (BOS)	northeast coastal zone	forest	8.7	123.2
Raleigh, North Carolina (RAL)	piedmont	forest	14.9	119.2
Atlanta, Georgia (ATL)	piedmont	forest	16.3	133.5
Birmingham, Alabama (BIR)	southwest Appalachians	forest	16.0	146.8
Milwaukee-Green Bay, Wisconsin (MGB)	southeast Wisconsin till plains	forest	7.6	85.5
Denver, Colorado (DEN)	high plains	grass/shrub	9.2	43.0
Dallas-Fort Worth, Texas (DFW)	Texas Blackland prairies	grass/shrub	18.3	104.2
Salt Lake City, Utah (SLC)	central basin and range	grass/shrub	9.7	68.0
Portland, Oregon (POR)	Willamette Valley	forest	10.8	152.8

Note: Antecedent agriculture is the percentage of basin area in row crop and grasslands for sites with low urban intensity (metropolitan area national urban-intensity index [MA-NUI] $\leq$ 10).

area from the candidate basins that had low urban intensity (MA-NUII $\leq$ 10).

The effects of natural environmental variability were minimized in each metropolitan area by dividing candidate basins into groups with relatively homogeneous natural environmental features (e.g., climate, elevation, stream size, natural vegetation) using hierarchical and K-means cluster analysis (SPSS 2007). Urban intensity was defined for each candidate basin by combining housing density, percentage of basin area in developed land cover, and road density into an index (metropolitan area national urban-intensity index, MA-

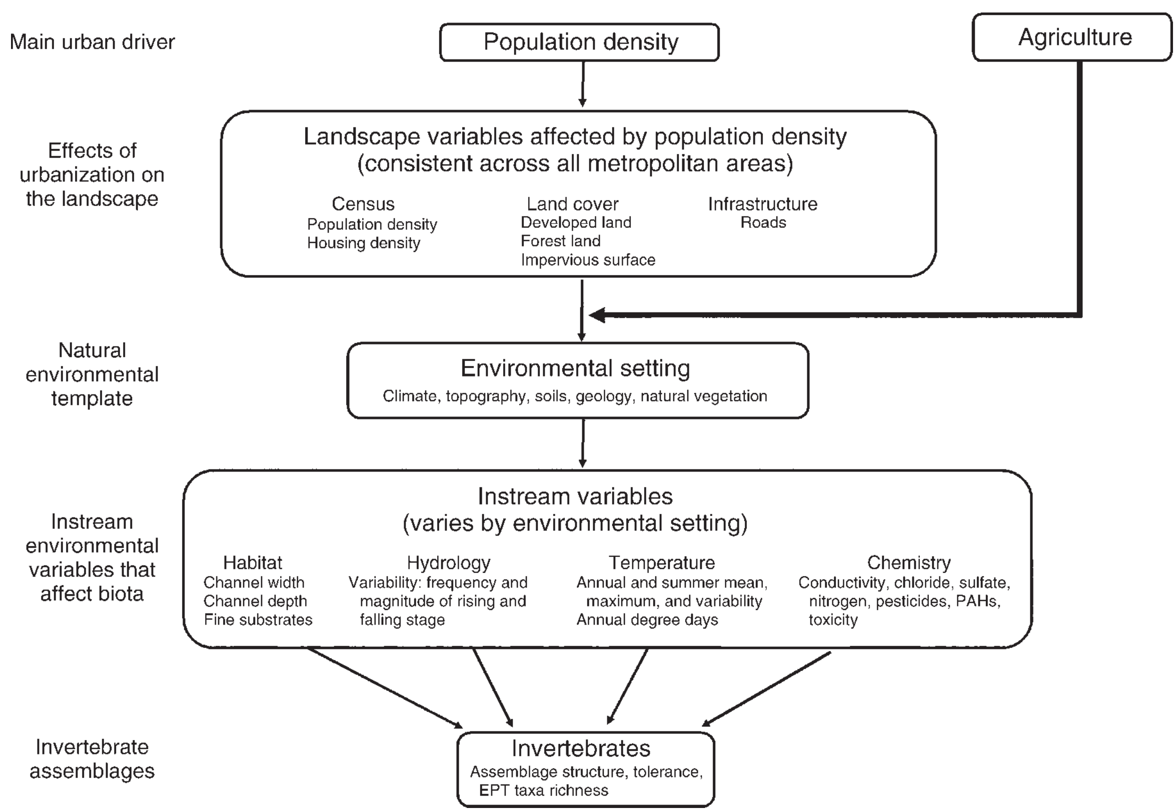

FIG. 2. Conceptual model of the major factors controlling regional patterns of urbanization. Population density drives changes in a consistent set of landscape variables associated with providing the goods and services required by a growing population. The natural environmental setting determines the background biological, chemical, and physical conditions that are modified by the landscape variables associated with changes in population density. The interaction between landscape and natural environmental variables produce changes in instream variables that affect the assemblage characteristics. Abbreviations are: EPT, Ephemeroptera + Plecoptera + Trichoptera; PAHs, polycyclic aromatic hydrocarbons.

TABLE 1. Extended.

Basin			
Relief (elevation range, m)	Mean elevation (m)	Mean slope (%)	Antecedent agriculture (%)
188	113	5.9	10.34
83	180	5.4	24.42
116	278	6.1	17.41
197	234	10.6	15.04
64	236	2.2	79.34
323	1704	6.2	87.97
79	172	2.3	81.65
253	1487	17.8	12.21
418	220	13.4	16.85

NUII) scaled to range from 0 (little or no urban) to 100 (maximum urban) within each metropolitan area (Cuffney and Falcone 2008). Once groups of basins with relatively homogeneous environmental features were defined, 28–30 basins were selected by dividing the gradient into 5–10 equal sections (e.g., 0–10, 10–20, 20–30 MA-NUII) and randomly selecting three to five sites within each section to represent the gradient of urbanization.

Conditions in each basin were verified by field reconnaissance. If conditions in a basin deviated substantially from what was expected, or if a sampling reach (150-m stream section at the base of the basin) was disturbed by local-scale effects (e.g., major point source discharge, channelization, road or building construction), or if land owners denied access, then an alternate basin from the same group or a group with similar natural environmental characteristics was selected to represent the same level of urban intensity. The Boston, Birmingham, and Salt Lake City metropolitan areas were studied during 1999–2000; Atlanta, Denver, and Raleigh were studied during 2002–2003 and Dallas-Fort Worth, Milwaukee-Green Bay, and Portland in 2003–2004. Details of the study designs can be found in Tate et al. (2005) and Giddings et al. (2009).

The Salt Lake City design differed from the other metropolitan areas in that many of the basins were nested, one within another. This modification was necessary because there are only a small number of streams in SLC and many of these are diverted for irrigation and drinking water before they enter urban areas. Nesting was feasible because urban development in the SLC basins has progressed upstream over time, which ensures that urban intensity increases downstream. Landscape characterizations in Salt Lake City were restricted to the portions of the basins that were located in the Central Basin and Range ecoregion (Omernik 1987). Portions in the Wasatch and Uinta Mountains ecoregion were excluded because there is no urban development in this area and the biology and geomorphology of the streams are very different from the Central Basin and Range ecoregion.

Most of the streams in Denver had large reservoirs located in the upper parts of the basins. Since reservoirs constitute major discontinuities in water temperature, sediment transport, water chemistry, and hydrology (Ligon et al. 1995); they effectively disconnect the effects of urbanization in the upper portions of the basin from those in the lower portions of the basins. Consequently, landscape characterizations in Denver were restricted to the portions of the basins that were below the major reservoirs.

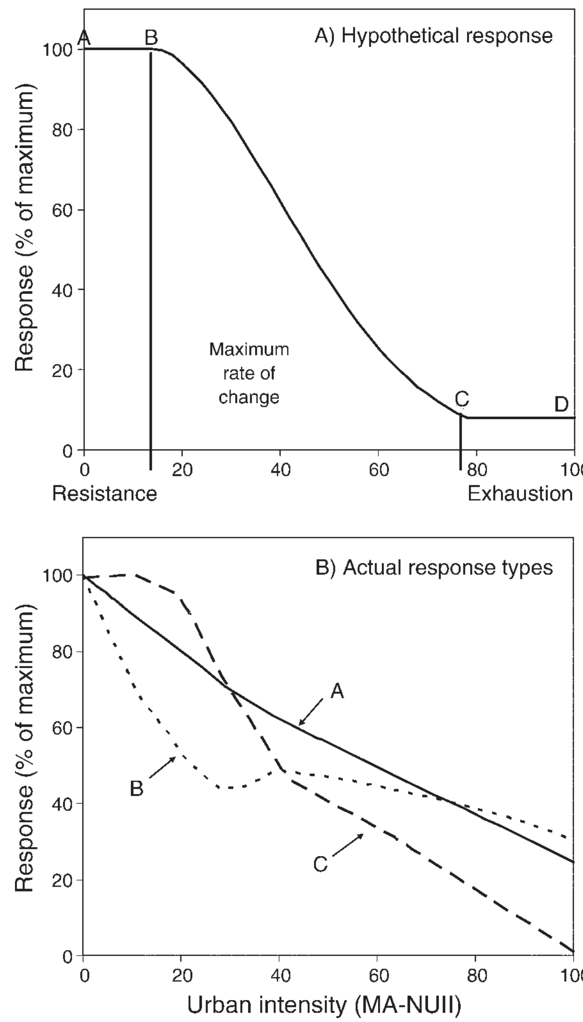

FIG. 3. (A) Hypothetical and (B) actual patterns of response as invertebrate assemblages change along a gradient of urbanization. In the theoretical response, the assemblages initially resist change (resistance, A to B) until reaching a threshold of disturbance (B) beyond which the assemblage changes rapidly (maximum rate of change, B to C) until reaching the exhaustion threshold (C), at which point the assemblage is composed of only the most tolerant taxa and little additional change is possible (C to D). For the actual responses, line A approximates the response hypothesized by Booth et al. (2004); line B approximates the response hypothesized by Walsh et al. (2005*a*); and line C approximates the response hypothesized by King et al. (2005). MA-NUII is the metropolitan area national urban-intensity index.

TABLE 2. Environmental data that were used to characterize urbanization and the physical, chemical, and biological responses to urbanization.

Type of variable	Appendix	References
National GIS coverage		
Natural (environmental template)		
Climate	A: Table A1	Falcone et al. (2007), Giddings et al. (2009)
Soils	A: Table A2	Falcone et al. (2007), Giddings et al. (2009)
Topography	A: Table A3	Falcone et al. (2007), Giddings et al. (2009)
Ecoregions	A: Table A4	Falcone et al. (2007), Giddings et al. (2009)
Landscape (urban indicators)		
Land cover	B: Table B1	Falcone et al. (2007), Giddings et al. (2009)
Infrastructure	B: Table B2	Falcone et al. (2007), Giddings et al. (2009)
Census	B: Table B3	Falcone et al. (2007), Giddings et al. (2009)
Field measurements		
Environmental variables		
Hydrology (continuous stage recorders)	C: Table C1	Giddings et al. (2009)
Water temperature (continuous recorders)	C: Table C2	Cuffney and Brightbill (2008), Giddings et al. (2009)
Water chemistry and pesticide toxicity indices (high and low base-flow samples)	C: Table C3	Munn and Gilliom (2001), Giddings et al. (2009)
SPMD chemistry and toxicity (~30 d deployment)	C: Table C4	Bryant et al. (2007), Giddings et al. (2009)
Habitat	C: Table C5	Giddings et al. (2009)
Invertebrate samples		
Quantitative sample (richest-targeted habitat, RTH)	D	Giddings et al. (2009)
Qualitative multihabitat sample (QMH)	D	Giddings et al. (2009)

Notes: Appendices A–D describe the variables that were examined, and the references provide details on the methods used to collect the data. The data used in these studies are available online at ⟨http://pubs.usgs.gov/ds/423/⟩.

Environmental data

Hourly water temperature and stream-stage data (Table 2) were obtained from pressure transducers. Water-column chemistry data (nitrogen species, phosphorus species, major ions, and pesticides) were collected twice: once during high-base flow (typically spring) and once during low base-flow (typically summer) periods (Table 2). Pesticide concentrations were weighted by toxicity to form an aggregate pesticide toxicity index (PTI; Munn and Gilliom 2001). Dissolved oxygen, pH, and specific conductance were collected every time the sample reach was visited (about once every two to four weeks). High base-flow water-chemistry data were not collected at Birmingham sites because a severe drought kept the streams at low base-flow during the entire study.

Small (9.5×4.5 cm) semipermeable membrane devices (SPMDs; Huckins et al. 1990, 1993) were used to collect hydrophobic organic compounds from water during a four-to-six-week period in early to midsummer in Atlanta, Raleigh, Milwaukee-Green Bay, Denver, Dallas-Fort Worth, and Portland (Table 2). These devices concentrated organic contaminants in neutral lipid triolein placed in low-density polyethylene tubing. Extracts from these SPMDs were analyzed for chemical constituents, gross polycyclic aromatic hydrocarbons, toxicity, and toxic equivalents using a cytochrome P450RGS liver assay (Murk et al. 1996). SPMDs also were deployed in Birmingham, but only measures of toxicity were determined.

Physical habitat structure was characterized using NAWQA Program protocols (Fitzpatrick et al. 1998), generally after invertebrate sampling was completed. Habitat characteristics were measured at 11 equally spaced transects along each sampling reach and included measurements of stream velocity, channel depth and width, aspect of flow, bed substrate, habitat cover, bank morphology, canopy closure, and bank vegetation (Table 2).

TABLE 3. Analysis objectives used to identify and test variables that are associated with urbanization or the response of invertebrates to urbanization.

Objective	Description
I	Identify a subset of landscape (land cover, census, and infrastructure) variables that are strongly associated with changes in population density for all nine metropolitan areas.
II	Identify invertebrate assemblage characteristics that are strongly associated with urban intensity (MA-NUII) with an emphasis on identifying commonality among metropolitan areas.
III	Identify water chemistry, hydrologic, water temperature, and instream habitat variables that are associated with urban intensity (MA-NUII) with an emphasis on identifying commonality among metropolitan areas.
IV	Identify environmental variables that are associated with invertebrate assemblage characteristics (metrics, NMDS) with an emphasis on identifying commonality among metropolitan areas.
V	Determine the influence of environmental settings (ecoregion) on the invertebrate assemblages (RTH, QMH) and habitat.

Note: Key to abbreviations: NMDS, nonmetric multidimensional scaling; RTH, richest targeted habitat; QMH, quantitative multi-habitat.

TABLE 4. Methods for identifying variables strongly associated with urbanization or the response of invertebrates to urbanization.

Step	Description
1	Calculate correlations (Spearman rank, r_s) with predictor variable (X) using SPSS (2007).
2	Discard variables that are not strongly correlated ($\lvert r_s \rvert < Y$) with predictor variable (X) in $\geq Z$ metropolitan areas.
3	Plot remaining variables against predictor variable (X) and analyze using regression and(or) LOWESS smoothing (lm, glm, LOWESS; R Development Core Team 2008). Discard variables that do not have discernible relations (linear, curvilinear) or that are strongly influenced by outliers (analysis of residuals, normal probability plots, Cook's distance for leverage; R Development Core Team 2008).

Note: Values of X, Y, and Z are defined in Table 5.

Macroinvertebrate data

Quantitative and qualitative benthic macroinvertebrate samples were collected over a one-to-four-week period during summer low base flows (Table 2). Quantitative (richest-targeted habitat, RTH) samples were collected from five riffles in each sampling reach using a Slack Sampler (1.25 m^2 total area sampled; Wildlife Supply Company, Yule, Florida, USA) except in Atlanta, Dallas-Fort Worth, and one Salt Lake City basin (Kays Creek at Layton, Utah) where woody snags were sampled (1.4 m^2 mean snag area sampled) because riffles were not available. A qualitative multihabitat (QMH) sample also was collected by using a dip net and hand collection of substrates to obtain taxa from all accessible habitats within the sampling reach. The USGS invertebrate data analysis system (IDAS; Cuffney 2003) was used to resolve taxonomic ambiguities and calculate assemblage metrics and diversity measures. Invertebrate functional group and tolerance values were derived from Barbour et al. (1999) with the southeastern region tolerances supplemented with data from the North Carolina Department of Natural Resources (2006). Tolerance metrics were calculated using regional tolerance values for each metropolitan area as follows: mid-Atlantic (Boston), southeast (Atlanta, Birmingham, Raleigh, and Dallas-Fort Worth); midwest (Milwaukee-Green Bay), and northwest (Denver, Salt Lake City, and Portland). Quantitative data (RTH) were converted to densities (number/m^2) prior to resolving ambiguous taxa and calculating assemblage metrics.

Invertebrate responses were also summarized on the basis of assemblage similarity and ordination sample scores (nonmetric multidimensional scaling, NMDS). Assemblage similarity and ordination site scores were based on fourth-root-transformed data with Bray-Curtis similarity for RTH samples and Jaccard similarity for QMH samples (Clarke and Gorley 2006). NMDS plots were examined for outliers, which were removed prior to the final ordination analysis and calculation of assemblage metrics and similarities. Ordination scores were rescaled to a consistent origin (0) and direction of change (decreasing as urban intensity increases) in order to facilitate the interpretation and comparison of ordination sample scores among metropolitan areas.

Identifying associations with predictor variables

The four major analysis objectives (Table 3) all involved identifying environmental variables that were strongly associated with a predictor variable. The method used to identify these associations was the same for each objective (Table 4) though the selection criteria (Table 5) varied by objective. This approach was intended to facilitate the identification of commonalities among metropolitan areas rather than to identify the best predictor variables within a metropolitan area. Spearman rank correlation (r_s) was used to reduce the large number of candidate variables to a subset that could be examined using regression analysis and LOWESS smoothing to represent coarse- and fine-scale patterns (thresholds) in the responses across the gradient of urbanization. Count (e.g., taxa richness) and percentage data (e.g., percentage of developed land) were modeled using the appropriate transformation (Poisson and logit, respectively) in glm (R Development Core Team 2008). Regression analysis was also used to compare the rates (slopes) at which invertebrate assemblages and metrics responded to urbanization (MA-NUII) for the nine metropolitan areas.

RELATE analyses (Clarke and Gorley 2006) were used to determine the significance of the correlation (r_s) between site similarity matrices (Table 6). Euclidean distance of the normalized variables was used to calculate similarity matrices for urban intensity (MA-NUII) and the landscape variables identified in Objective I (Table 3). The invertebrate similarity matrices used for NMDS (RTH and QMH) were correlated with the similarity matrices derived for urban intensity (MA-NUII) and landscape variables. These

TABLE 5. Parameters used for identifying variables that are strongly associated with urbanization or the response of invertebrates to urbanization.

Objective	X (predictor variable)	Y (criterion for r_s)	Z (number of metropolitan areas)
I	population density	0.65	9
II	MA-NUII	0.65	3
III	MA-NUII	0.50	3
IV	RTH, QMH, metrics and NMDS1	0.65	3

TABLE 6. Method for testing the correlation between similarity matrices (Objective II, RELATE analysis; Clarke and Gorley 2006).

Test	Similarity matrix 1	Similarity matrix 2
A	landscape (objective I)	MA-NUII
B	invertebrates (RTH, QMH)	landscape (objective I)

Note: Tests were conducted individually for each metropolitan area and for all metropolitan areas combined.

analyses supplemented the regression analyses with information derived from the entire assemblage, rather than from a portion of the assemblage or ordination, and do not assume a particular response form (e.g., linear).

ANOSIM analyses (Clarke and Gorley 2006) were used to determine how well environmental settings (ecoregions) accounted for differences in the similarity matrices defined by the invertebrate assemblages (RTH, QMH) and habitat variables (Objective V, Tables 3 and 7). The ANOSIM analysis is an approximate analogue of one-way analysis of variance and tested for the significance of the environmental settings as a treatment effect.

RESULTS

Response of landscape variables to population density

Seventeen landscape variables were strongly associated with changes in population density in each of the nine metropolitan areas (Table 8). Housing unit density (HUDEN), percentage of developed land in the basin (P_NLCD_2), and road density (ROADDEN) showed the most consistent relations with population density for each of the data sources; census, land cover, and infrastructure, respectively. These are the same variables that were combined to form the urban intensity index (MA-NUII; Cuffney and Falcone 2008). Other variables, such as the three measures of impervious cover, showed more variability in responses among metropolitan areas than did the components of the urban intensity index. Consequently, impervious cover was not used in the urban intensity index. The strong correlation (RELATE analysis) between the site resemblance matrices derived from these landscape variables and the urban intensity index (MA-NUII) confirmed that the urban intensity index captured the changes in landscape variables that were associated with changes in population density (Table 9).

Response of assemblage metrics to urban intensity: MA-NUII

Only 23 of the 188 metrics (86 richness, 100 abundance, and two ordination scores) that were examined showed strong associations with the urban intensity index (MA-NUII) in at least three metropolitan areas. Unlike the landscape variables, none of the metrics derived for quantitative or qualitative samples were strongly associated with urbanization in all nine metropolitan areas (Table 10). Metrics based on taxa richness showed a greater number of strong associations with urban intensity than did metrics based on abundance. Richness-based tolerance (RichTol) was the most consistent metric with strong associations in six metropolitan areas for quantitative samples and five for qualitative (QMH) samples. EPT taxa richness (EPTr) and NMDS axis 1 sample scores (NMDS1) showed strong responses in five metropolitan areas for both quantitative and qualitative samples. Abundance of Plecoptera and intolerant taxa (tolerance values ≤ 3) were the only abundance metrics that had strong associations in three or more metropolitan areas. Dominance, behavior, functional group, and diversity metrics had strong associations in just a few metropolitan areas and were not good indicators of urbanization.

Regression analysis revealed statistically significant relationships ($P < 0.05$) between urban intensity (MA-NUII) and richness-based tolerance (RichTol), EPT taxa richness (EPTr), and assemblage ordination scores (NMDS axis 1 sample scores) for eastern and western metropolitan areas based on quantitative (RTH) and qualitative (QMH) samples (Fig. 4). Slopes for the three central metropolitan areas (MGB, DEN, DFW) were much less than for other metropolitan areas and in many cases were not statistically significant (Fig. 4A, C). Intercepts, which estimate conditions in the absence of urbanization (background conditions), varied considerably among metropolitan areas (Fig. 4B, D). The central metropolitan areas had intercept values that were indicative of assemblages that had fewer EPT taxa, lower initial ordination scores (i.e., exhibited less change in the assemblages over the gradient), and contained taxa that were, on average, more tolerant (RichTol) than metropolitan areas in the East and West.

Correlation of the invertebrate assemblage resemblance matrices (RTH and QMH) with resemblance matrices for urban intensity and the landscape indica-

TABLE 7. Method for testing whether invertebrate and habitat data group by environmental setting (objective V) using ANOSIM (Clarke and Gorley 2006).

Test	Similarity matrix	Method for constructing similarity matrix
A	invertebrates (RTH, QMH)	fourth-root transform, Bray-Curtis similarity for RTH, Jaccard similarity for QMH
B	habitat	normalized variables, Euclidean distance

Notes: The dominant Level III ecoregion in each basin was used to represent the environmental setting. Tests were conducted with all metropolitan areas combined.

Table 8. Landscape variables (census, infrastructure, and land cover) that were strongly associated with population density in all nine metropolitan areas (Objective I, Table 3).

Type of landscape variable and variable abbreviation and description
Census (60)
HHDEN: household density (occupied housing units/km^2)
HUDEN: density of housing units (housing units/km^2)
PPURBAN: proportion of population living in urban area
PPRURAL: proportion of population living in rural area
Infrastructure (6)
ROADDEN: road density in basin (km/km^2)
RDARDEN: road area index density, road length multiplied by area factor (km/km^2)
RDTRDEN: road traffic index density, road length multiplied by a traffic factor (km/km^2)
Basin land cover (18)
P_NLCD_2: basin in developed land (%)
P_URBANdw: basin in developed land, weighted for distance from stream (%)
NLCD_IS: mean impervious surface in the basin based on 2001 NLCD data (%)
NOAA_1KM_IS: mean impervious surface in basin derived from NOAA 1990s data (%)
Riparian land cover (9)
P_NLCDB_2: riparian buffer (~200 m) in developed land (%)
NLCD_BIS: mean impervious surface in the riparian buffer, 2001 NLCD data (%)
Basin land cover fragmentation (64)
LPI_C2: largest patch index, largest patch of developed land (% basin area)
PAM_C2: patch area mean, mean patch area for developed land (ha)
PIM_C2: proximity index mean, measure of isolation of patches of developed land (unitless)
PLA_C2: proportion of like adjacencies, patch adjacencies that are developed land (%)

Notes: Values in parentheses indicate the number of variables in each class. Key to abbreviations: NLCD, national land cover data; NOAA, National Oceanic and Atmospheric Administration.

tors of urbanization (RELATE analyses, Table 9) supported the regression results. The eastern and western metropolitan areas had highly significant correlations ($P \leq 0.001$) with urbanization. The metropolitan areas in the central part of the country (MGB, DEN, DFW) were not significantly correlated with urbanization. The ANOSIM analysis established that the similarities among sites based on the RTH and QMH invertebrate samples were significantly correlated with ecoregion ($r_s = 0.848$ for RTH and 0.851 for QMH, $P < 0.001$) establishing that the environmental setting has a strong influence on the invertebrate assemblages and their responses to urbanization.

The differences in responses observed for Milwaukee-Green Bay, Denver, and Dallas-Fort Worth were associated with differences in the land cover that was being converted to urban (antecedent land cover). Antecedent land cover in these three metropolitan areas was dominated by agricultural lands consisting of row crops and grass lands used for grazing (Fig. 5). In contrast, the antecedent land cover in the other metropolitan areas was predominantly forest or shrubland. These two groups show strong differences in background conditions (intercepts, Fig. 6A) and rates of response (slopes, Fig. 6B) for the EPT taxa richness (EPTr), richness-based tolerance (RichTol), and ordination (NMDS1) scores.

The patterns of invertebrate responses suggested by LOWESS smoothing fell into three general types (Fig. 3B). Most of the responses (50 of 54) were linear (Table 11) or had a higher rate of change at lower levels of urbanization than at higher levels (SLC, POR). Only four responses (NMDS1 in BIR and MGB) showed evidence of an initial resistance to change at low levels of urbanization. The urban intensity values for the breakpoints (thresholds) in the LOWESS regressions were consistent within metropolitan areas (42–48 for SLC, 25–29 for POR), but differed substantially across metropolitan areas (12–44 for NMDS1).

Environmental variables associated with urbanization

None of the 225 instream environmental variables (chemistry, hydrology, habitat, temperature) were strongly associated with urban intensity in all nine metropolitan areas (Objective III, Table 3). High base-

Table 9. Spearman rank correlations (r_s) between the site similarity matrix (Euclidean distance) defined by the landscape variables consistently associated with urbanization (Table 8) and the site resemblance matrices defined by the urban intensity index (MA-NUII, Euclidean distance), by the quantitative (RTH, Bray-Curtis similarity) and qualitative (QMH, Jaccard similarity) invertebrate samples based on the RELATE test (Clarke and Gorley 2006).

	MA-NUII		Quantitative (RTH)		Qualitative (QMH)	
Metropolitan area	r_s	P	r_s	P	r_s	P
Boston	0.97	<0.001	0.68	<0.001	0.69	<0.001
Raleigh	0.93	<0.001	0.41	<0.001	0.58	<0.001
Atlanta	0.98	<0.001	0.50	<0.001	0.36	<0.001
Birmingham	0.93	<0.001	0.41	<0.001	0.53	<0.001
Milwaukee-Green Bay	0.98	<0.001	0.02	0.401	0.19	0.0347
Denver	0.99	<0.001	0.03	0.351	0.15	0.0206
Dallas-Fort Worth	0.97	<0.001	0.06	0.277	0.10	0.1204
Salt Lake City	0.87	<0.001	0.35	<0.002	0.38	<0.001
Portland	0.99	<0.001	0.39	<0.001	0.35	<0.001

Note: Probabilities (P) < 0.05 are indicative of statistically significant correlations between similarity matrices.

TABLE 10. Number of assemblage metrics that were strongly associated with urban intensity (MA-NUII) in the nine metropolitan areas based on quantitative (RTH) and qualitative (QMH) invertebrate samples (Objective II, Table 3).

Metropolitan area	Class of assemblage metric								
	NMDS		Taxonomic groupings				Behavior		
			Richness		Abundance	Dominance	Richness		Abundance
	RTH	QMH	RTH	QMH	RTH	RTH	RHT	QMH	RTH
BOS	1	1	16	7	3	5	4	0	3
RAL	1	1	9	10	1	0	3	2	2
ATL	1	1	7	1	1	0	1	0	1
BIR	1	1	8	4	2	0	1	2	1
MGB	0	0	0	17	0	0	0	2	0
DEN	0	0	0	0	0	0	0	0	0
DFW	0	0	0	0	0	0	0	0	0
SLC	0	0	1	0	1	0	0	0	0
POR	1	1	11	14	5	0	2	2	3
Metrics in class	2	2	49	49	49	5	14	14	14
Number of metrics with $\|r_s\| \geq 0.65$	5	5	52	53	13	5	11	8	10
Best metric	Axis 1 (5)	Axis 1 (5)	EPT (5)	EPT (5)	PLEC (4)	DOM5 (1)	CN (4)	CN (3)	SW (3)

Notes: Metrics are grouped by class, and the metric most consistently associated with urbanization is identified (best metric) along with the number of metropolitan areas where $|r_s| \geq 0.65$ for this metric (values in parentheses). Richness and abundance metrics include metrics based on percentages of total richness or abundance. Metrics in class is the number of metrics in the class of metrics (column); No. $|r_s| \geq 0.65$ is the number of metrics in a class of metrics (column) with $|r_s| \geq 0.65$ summed over all nine metropolitan areas. Key to abbreviations: PLEC, Plecoptera; DOM5, percentage abundance based on the five most abundant taxa; CN, clingers; SW, swimmers; PR, predators; SC, scrapers; RichTol, richness-based tolerance (Σ TV_i/N, where TV_i is the tolerance value for taxa i and N is the number of taxa); Intol, intolerant taxa (taxa with TV_i values $\leq$ 3).

flow measurements of conductivity, chloride, sulfate, number of pesticides detected, pesticide toxicity indices, SPMD toxicity equivalents (Murk et al. 1996), and number of compounds detected (SPMD) were the chemical variables that most commonly showed strong associations with urban intensity (Table 12). Strong associations were more common for high base-flow and SPMD measurements than for low base-flow measurements. Measures of hydrologic variability (number of rising and falling hydrographs per day that were five to nine times the daily mean) were the only hydrologic measures that were strongly associated with urban intensity (six of nine metropolitan areas; Table 13). Annual degree days, summer daily mean temperature and annual daily mean temperature were the only temperature measurements that were strongly correlated with urban intensity, and then for only one or two metropolitan areas (Table 13). None of the 89 habitat variables were strongly associated with urban intensity in three or more metropolitan areas (Table 13). The ANOSIM analysis established that the similarity among sites based on habitat variables was significantly correlated with ecoregion (r_s = 0.298, P < 0.001) indicating that environmental settings have a strong influence on habitat and its response to urbanization though this influence was not as strong as observed for the invertebrate assemblages.

Environmental variables associated with invertebrate responses

The water-chemistry variables that were strongly associated with invertebrate responses (EPTr, NMDS1, RichTol) varied widely among metropolitan areas and invertebrate sample types (Table 12). Milwaukee-Green Bay, Denver, Dallas-Fort Worth, and Salt Lake City had very few strong associations with water chemistry. In contrast, Boston had strong associations with 11 chemical variables for both quantitative (RTH) and qualitative (QMH) samples, and these variables were the same ones that were strongly correlated with urban intensity. Portland also had strong associations with water chemistry, mostly pesticide variables measured at high base flow. Raleigh and Atlanta had more variable associations with pesticides and the few strong associations were only observed for quantitative (RTH) samples. As with urban intensity, high base-flow chemistry and SPMD chemistry were more strongly associated with invertebrate response in more metropolitan areas than was low base-flow chemistry. SPMD toxicity equivalents were strongly associated with invertebrate responses in five of the seven metropolitan areas where this parameter was measured. Other SPMD variables were strongly associated with only one or two of the six metropolitan areas where these variables were measured. In general, conductivity, chloride, sulfate, total number of pesticides, and toxicity equivalents were the water-chemistry variables most frequently associated with invertebrate responses. In all cases where strong associations occurred with both invertebrate responses and urban intensity, variables that were positively correlated with increasing urban intensity were negatively correlated with invertebrate responses and vice versa.

Only a few of the temperature, hydrology, and habitat variables were strongly associated with invertebrate responses (Table 13). As with urban intensity, temper-

TABLE 10. Extended.

Class of assemblage metric						
Functional groups			Tolerance			
Richness		Abundance	Richness		Abundance	Diversity and evenness
RTH	QMH	RTH	RTH	QMH	RTH	
5	0	6	4	5	4	9
1	1	0	3	5	3	1
0	0	1	4	0	0	0
1	0	0	3	3	3	0
0	1	0	0	3	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	1	0	0	0
0	1	2	4	4	3	0
16	16	16	7	7	7	9
7	3	9	19	20	13	10
PR (2)	PR (2)	SC (2)	RichTol (6)	RichTol (5)	Intol (4)	Margalef diversity (2)

ature and hydrology were the variables that most frequently had strong associations with invertebrate responses, though never in more than four metropolitan areas. Habitat was again notable by not having any strong associations with invertebrate responses in three or more metropolitan areas.

Discussion

Invertebrate responses to urbanization

Invertebrates showed significant responses to urbanization in most of the metropolitan areas that we studied. EPTr, RichTol, and NMDS1 were good indicators of urban effects. RichTol performed well despite the fact that the underlying tolerance values (Barbour et al. 1999, North Carolina Department of Environment and Natural Resources 2006) were not specifically developed to discern the effects of urbanization. In most cases, the richness versions of metrics (RichTol) were better indicators of urbanization than the abundance versions (AbundTol) in part because of the variability introduced by estimating abundances. Similarly, qualitative samples (QMH) detected urban response in more metropolitan areas (eight) than did quantitative sampling (RTH, 6). Qualitative sampling is advantageous for large regional-studies because it avoids the problem of finding equivalent quantitative habitats in all basins (riffles vs. snags), though it precludes the calculation of some commonly used quantitative metrics (e.g., dominance metrics). NMDS was a very useful measure of invertebrate response in our studies, but NMDS scores depend upon the underlying design gradient and cannot be directly compared with other studies in the way that EPTr or RichTol can. We were able to compare NMDS scores and rates of change in our studies because of the consistency in the study designs, sample collection methods, data processing (e.g., taxonomic consistency) and methods used to extract and transform the NMDS scores.

Conceptual model

The conceptual model (Fig. 2) that was used as a framework to understand similarities and differences in urbanization among metropolitan areas is relatively simple compared to models that describe urbanization within a metropolitan area (Walsh et al. 2001, 2005*b*, Roy et al. 2003). Yet it proved to be a very useful mechanism for structuring our investigations, interpreting our results, and providing a large-scale perspective on the effects of urbanization. Three aspects of the model are particularly important for understanding large-scale patterns of urbanization both as an ecological process and a management problem. First is the consistency in the landscape changes that are associated with urbanization across the country. Second is the importance of the natural environmental template (regional environmental settings) in determining the effects of urbanization on the physical, chemical, and biological components of stream ecosystems. Third is the extent to which antecedent agriculture can mask the urban signal by degrading stream conditions prior to urbanization.

Consistency in landscape changes

The consistency in the landscape variables that change as population density increases confirmed that the manner in which urbanization alters the landscape (e.g., roads and buildings) was similar among metropolitan areas. However, the rates at which landscape variables change as population density increases differed among metropolitan areas (Cuffney and Falcone 2008). Consequently, the process of urbanization is qualitatively similar across the country, but quantitatively different. For example, the percentage of the drainage basin that is developed is strongly related to population density in all metropolitan areas, but the rate at which drainage basins are developed differs among metropolitan areas (e.g., a much higher rate in water-rich Raleigh than in water-poor Denver). These differences reflect

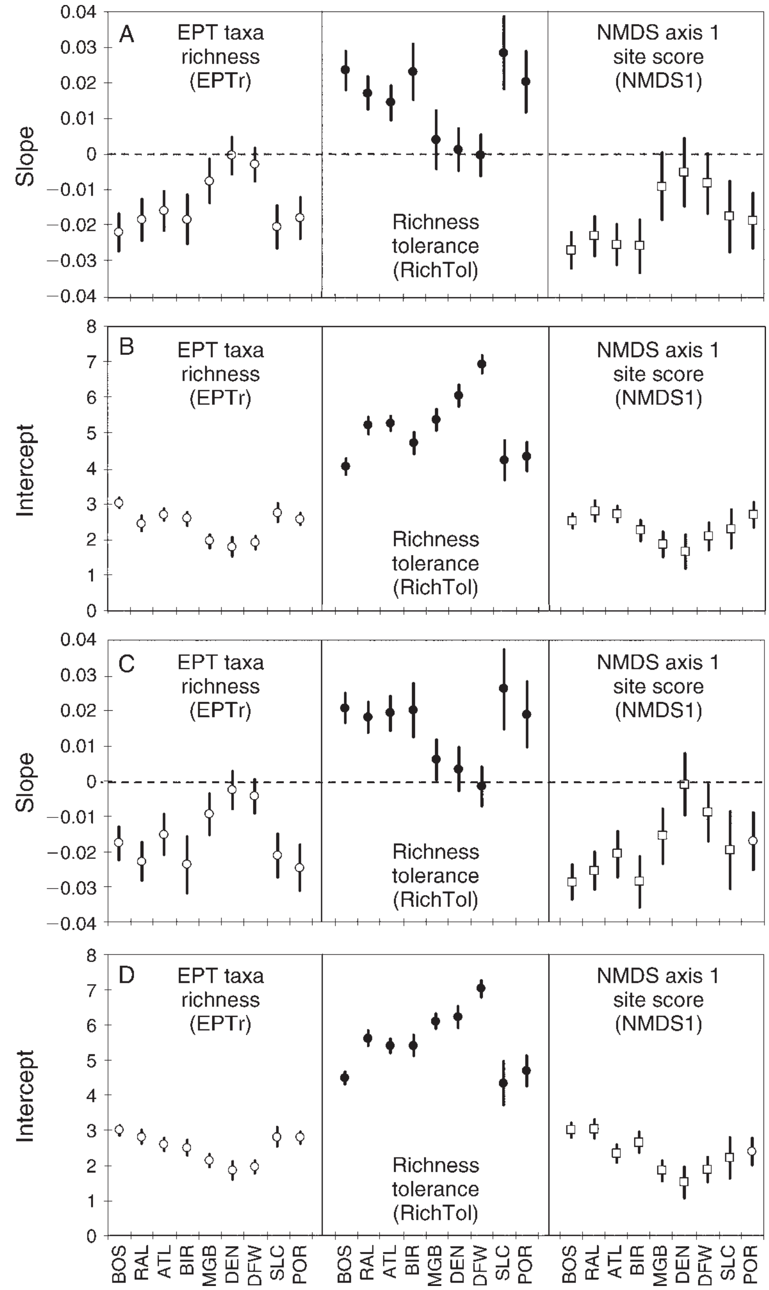

FIG. 4. Slopes and intercepts with 95% confidence intervals for linear regressions relating urban intensity to invertebrate responses for richest targeted habitat (RTH; A slope, B intercept) and quantitative multi-habitat (QMH; C slope, D intercept) samples. EPT richness is expressed as $\ln(X + 1)$.

how the elements of the natural environmental settings, such as topography and precipitation, can affect urban development. Efforts to develop an understanding of urbanization at large spatial-scales (Grimm et al. 2008) or to address commonalities in urbanization (Walsh et al. 2005*b*) must address both the qualitative consistency in the identity of the drivers of urbanization and the quantitative differences in how these drivers interact with the environmental settings.

Importance of the natural environmental setting

Environmental settings were important in determining the physical, chemical, and biological (invertebrate) responses to urbanization. For example, if environmental settings were taken into account by analyzing each metropolitan area separately, then invertebrate responses to urbanization were readily detected in most metropolitan areas as were many of the symptoms (e.g., reduced richness, loss of intolerant taxa) of the

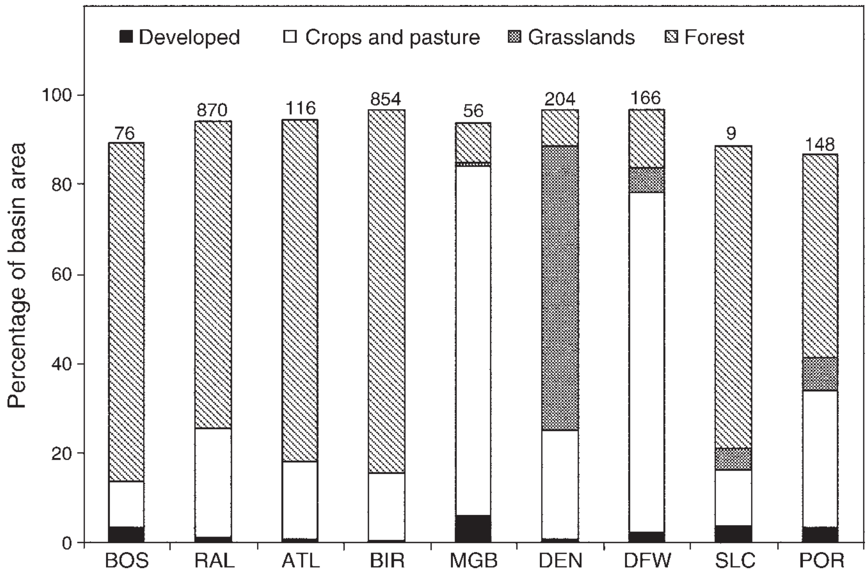

FIG. 5. Antecedent land cover (land cover in basins with MA-NUII $\leq$ 10) in the nine metropolitan areas derived from the population of candidate basins used to characterize the urban gradient. The number above each column indicates the number of basins with MA-NUII $\leq$ 10.

urban syndrome (Walsh et al. 2005*b*). However, if environmental settings were ignored by combining metropolitan areas, relations with urbanization were no longer discernible. The large differences in the assemblages that defined background conditions (MA-NUII $\leq$ 10) in each metropolitan area (Table 14), as well as differences in the rates of response obscured the relation with urbanization when data from metropolitan areas were combined. The ANOSIM analyses established that invertebrate assemblages were associated with the environmental settings, as represented by ecoregions, and that the regional differences in taxonomic composition persisted even in the degraded assemblages.

The physical and chemical variables that were most commonly associated with urbanization and invertebrate responses were increased flashiness, conductivity, sulfate, chloride, pesticides, PAHs, and toxicity indices. These variables have been associated with urbanization and biological degradation of streams in many other urban studies (Poff et al. 1997, Paul and Meyer 2001, Kennen and Ayers 2002, Konrad and Booth 2002, McMahon et al. 2003) and are symptomatic of the urban stream syndrome (Walsh et al. 2005*b*). However, none of these variables were strongly associated with urbanization or invertebrate responses in all metropolitan areas. As with the invertebrate responses, the environmental settings play an important role in determining which physical and chemical variables are associated with urbanization by establishing the background conditions in each metropolitan area (Table 14). For example, changes in conductivity and nitrogen associated with urbanization would not represent as large a change in areas that have high background levels as it would in areas with naturally low levels. Consequently, we would expect that changes in conductivity, nitrogen, and invertebrate assemblages resulting from urbanization would be greater and more easily detected in areas where background levels of conductivity and nitrogen are low.

Our studies showed very limited association between urbanization and water temperature and habitat despite their importance in other studies of urbanization (Sinokrot and Stefan 1993, Waters 1995, LeBlanc et al. 1997, Schueler and Holland 2000, Paul and Meyer 2001, Wang et al. 2001, Nelson and Palmer 2007) and the urban stream syndrome. The lack of association with water temperature may be attributed to the variability in the intensity and duration of precipitation across the large geographic areas encompassed in our studies. This lack of uniformity across the urban gradient made it difficult to detect changes in water-temperature characteristics (e.g., means, variances, rates of change) simply by comparing responses as a function of urban intensity. The lack of association between urbanization and habitat variables was linked to differences in the natural environmental settings that establish many of the characteristics (e.g., channel slope, basin topography, soil types, geology) that determine channel dynamics and sediment transport characteristics (Tables 1 and 14). As with the invertebrate assemblages, ANOSIM showed that environmental settings (ecoregions) have a strong influence on habitat characteristics that persists across the urban gradient.

The natural environmental setting establishes the background conditions upon which urbanization acts and forms the basis for the variability observed among metropolitan areas in the physical, chemical, and

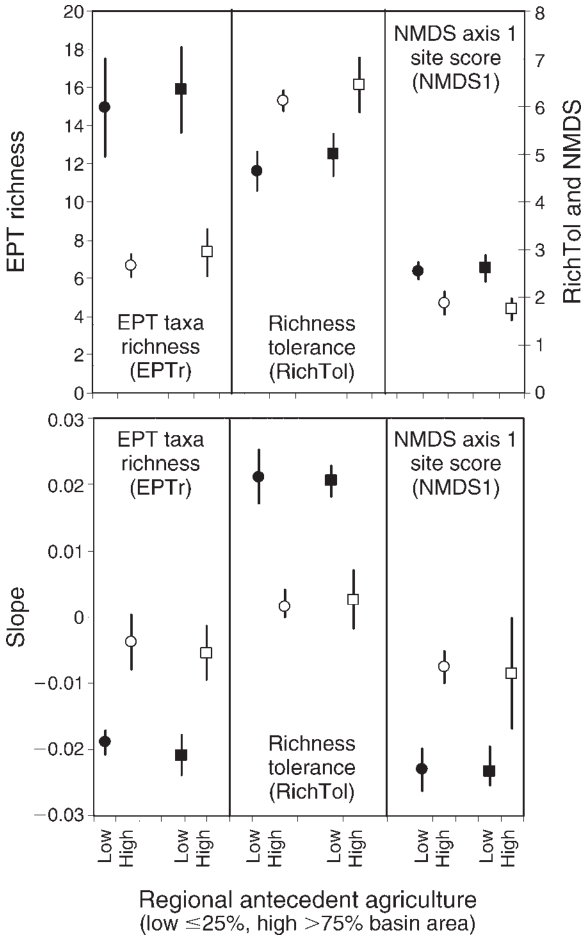

FIG. 6. Mean intercepts and slopes (with 95% confidence intervals) for regressions relating invertebrate responses to urban intensity (MA-NUII) for metropolitan areas with high (≥70%) and low (≤30% basin area) antecedent agriculture.

biological responses to urbanization. The background conditions dictated not only the initial conditions that are modified by urbanization; they can also influence the conditions that exist at the high end of the gradient. For example, the invertebrate assemblages and habitat conditions in different environmental settings did not converge to a common endpoint but maintained some characteristics that were sufficiently different to cause them to group with the background sites rather than with highly impacted sites from other environmental settings. The importance of the environmental settings in determining the physical, chemical, and biological responses to urbanization underscores the necessity of studying large-scale urbanization as a collection of urban-rural gradients that are associated with individual metropolitan areas as suggested by Grimm et al. (2008). It also establishes the importance of properly defining environmental settings in order to maximize the ability to detect urban responses that can be obscured by defining the environmental setting too broadly. The variability in responses to urbanization also suggested that the generalities presented in the urban stream syndrome may not be applicable in all metropolitan areas.

Antecedent agriculture and past land use

The land use that was being converted to urban (antecedent land use) affected the ability to detect invertebrate responses to urbanization. Responses were readily detected in metropolitan areas where forest (BOS, RAL, ATL, BIR, POR) or shrub lands (SLC) were being converted, but areas where agricultural lands (row crop and grasslands) were being converted (MGB, DEN, DFW) showed weak or nonsignificant responses to urbanization because the invertebrate assemblages were already severely degraded by environmental changes associated with agriculture (e.g., high nutrients and pesticides) prior to the onset of urban development (Table 14). Antecedent agriculture obscured the response to urbanization regardless of whether natural vegetation was forest (MGB) or grassland (DEN and DFW) and whether the dominant type of antecedent agriculture was row crop and pasture (MGB and DFW) or grasslands (DEN).

TABLE 11. The pattern of the relation between invertebrate responses (richness tolerance, EPT taxa richness, and NMDS axis 1 site scores) and urbanization as summarized by linear regression and LOWESS smoothing.

Metropolitan area	Richness tolerance		EPT richness		NMDS axis 1	
	RTH	QMH	RTH	QMH	RTH	QMH
BOS	A	A	A	A	A	A
RAL	A	A	A	A	A	A
ATL	A	A	A	A	A	A
BIR	A	A	A	B (40)	C (19)	C (20)
MGB	A	A	A	A	C (15)	C (12)
DEN	A	A	A	A	B (33)	B (33)
DFW	A	A	A	A	B (12)	B (5)
SLC	B (47)	B (48)	B (48)	B (48)	B (42)	B (44)
POR	B (28)	B (28)	B (29)	B (25)	B (27)	B (27)

Notes: The patterns correspond to the lines described in Fig. 3B: A is indicative of a nearly linear response, B shows a rapid initial rate of change followed by a decreased rate of change, and C has an initial period of resistance (no change in slope) followed by rapid change in the rate of response. The urban intensity (MA-NUII) at the possible breakpoints in patterns B and C is shown in parentheses.

Table 12. Chemical variables that were strongly associated ($|r_s| \geq 0.65$) with urban intensity (MA-NUII) or invertebrate responses in at least three metropolitan areas (Objectives III and IV, Table 3).

Variables	BOS	RAL	ATL	BIR	MGB	DEN	DFW	SLC	POR
Nutrients and physical parameters									
High base flow (23)									
Conductivity	rqU	rqU	rqU	nc	U	—	—	—	—
Chloride (mg/L)	rqU	rqU	rqU	nc	U	—	—	—	rq
Sulfate (mg/L)	rqU	rqU	rqU	nc	U	—	—	—	rU
Low base flow (23)									
Conductivity	rq	—	rq	—	—	—	—	rq	—
Sulfate (mg/L)	rqU	rqU	rqU	—	—	—	—	—	—
Pesticides									
High base flow (48)									
Prometon (μg/L)	rqU	—	—	nc	—	—	—	U	rqU
Total herbicide conc.	rqU	U	—	nc	—	—	—	—	rqU
No. pesticides detected	rqU	rU	rU	nc	—	—	—	U	rqU
No. herbicides detected	rqU	—	U	nc	—	—	—	—	rqU
Total pesticide conc.	—	—	q	—	—	—	—	q	q
No. insecticides detected	rqU	rU	—	nc	—	—	—	U	rq
Cladoceran PTI	rqU	—	rU	nc	—	—	—	U	rq
Invertebrate PTI	rqU	r	r	nc	—	—	—	U	rq
Fish toxicity PTI	r	—	r	—	—	—	—	—	r
Low base flow (48)									
No. pesticides detected	—	—	rU	—	—	—	—	rU	rqU
Fish PTI	—	rq	—	—	—	—	—	rq	rq
Semi-permeable membrane devices (29)									
Toxicity equivalents	nc	rqU	rqU	rqU	qU	U	—	nc	rqU
Phenanthrene	nc	—	rU	nc	U	U	—	nc	—
Fluoranthene	nc	—	rU	nc	U	U	—	nc	—
Pyrene	nc	qU	rU	nc	U	U	—	nc	—
Benzophenanthrene	nc	rqU	rU	nc	U	U	—	nc	—
No. compounds detected	nc	U	rqU	nc	qU	U	U	nc	—

Notes: Invertebrate responses are based on richness tolerance (RichTol), EPT taxa richness (EPTr), and NMDS axis-1 sample scores (NMDS1) for quantitative (RTH) and qualitative (QMH) samples. Lowercase letters denote negative correlations, and uppercase letters denote positive correlations with RTH (r,R), QMH (q,Q), and urban intensity (u,U). A dash indicates that no strong correlations were detected. Values in parentheses indicate the number of variables that were evaluated in each category; nc, data not collected. PTI is the pesticide toxicity index.

Table 13. Temperature, hydrology, and habitat variables that were strongly correlated ($|r_s| \geq 0.65$) with urban intensity (MA-NUII) or invertebrate responses in at least three metropolitan areas (Objectives III and IV, Table 3).

Variables	BOS	RAL	ATL	BIR	MGB	DEN	DFW	SLC	POR
Temperature (33)									
Annual degree days	—	—	q	—	rqU	—	—	rqU	q
Summer daily mean	—	—	rq	—	rq	U	—	rq	—
Annual daily mean	—	—	q	—	rqU	—	—	qU	—
Hydrology (65)									
Frequency of rising hydrographs (rises/d)									
>5 times mean	—	rqU	rU	U	U	—	U	—	U
>7 times mean	—	qU	rqU	qU	U	—	U	—	U
Frequency of falling hydrographs (falls/d)									
>5 times mean	—	U	U	rqU	—	—	U	—	qU
>7 times mean	—	rqU	rqU	rqU	—	—	U	—	U
>9 times mean	—	qU	rqU	rqU	—	—	U	—	U
Habitat (89)	—	—	—	—	—	—	—	—	—

Notes: Invertebrate responses are based on richness tolerance (RichTol), EPT taxa richness (EPTr), and ordination scores (NMDS1). Lowercase letters denote negative correlations, and uppercase letters denote positive correlations with RTH (r,R), QMH (q,Q), and urban intensity (u,U). A dash indicates that no strong correlations were detected. Values in parentheses indicate the number of variables that were evaluated in each category.

TABLE 14. Selected physical, chemical, and biological data (means) for background sites (MA-NUII ≤ 10) associated with the nine metropolitan areas.

City	QMH EPTr	QMH RichTol	Conductivity (μS/cm at 25°C)	Total pesticide concentration (μg/L)	Total nitrogen (mg/L)	Median substrate size (mm)
BOS	19	4.6	98	0.003	0.46	148
RAL	15	5.6	95	0.104	0.74	106
ATL	13	5.3	49	0.009	0.33	150
BIR	11	5.6	261	0.019	0.53	95
MGB	8	6.1	769	0.253	1.39	48
DEN	6	6.0	806	0.434	1.60	67
DFW	7	7.1	601	3.916	2.80	64
SLC	17	3.9	129	0.000	0.25	144
POR	16	4.6	115	0.009	0.15	213

Note: Conductivity, total pesticides, and total nitrogen are derived from low base-flow samples.

Past land uses, such as agriculture, have been shown to affect the current diversity of stream invertebrates more than the current land use (Harding et al. 1998). Our results supported the importance of past land use (agriculture), but only if the land use directly proceeds (is antecedent) to urbanization. Many of the forested areas that were being converted to urban in our studies were former agricultural lands (Bürgi et al. 2000) that had reverted back to forest lands. We could not detect the effect of the prior agricultural land use on these forest lands as suggested by Harding et al. (1998). Therefore, we draw a distinction between antecedent land use, which represents the land use prior to the current land use, and historical land use, which represents the history of land use changes.

The effects of competing land uses, such as antecedent agriculture, need to be considered when setting regulatory standards for urbanization, setting expectations for stream restoration that are realistic and feasible (Bernhardt and Palmer 2007), assessing continental-scale patterns of urbanization (Grimm et al. 2008), or defining the symptoms of the urban stream syndrome (Walsh et al. 2005*b*). The background or reference conditions used to measure effects or determine restoration goals for urban streams may not be achievable in areas such as Milwaukee-Green Bay, Denver, and Dallas-Fort Worth unless the effects of agriculture are also addressed. Accounting for the effects of competing land uses may help account for apparent inconsistencies in the symptoms of urbanization and in continental-scale patterns of urbanization.

Thresholds and protective criteria

Our analysis of potential thresholds in the responses of invertebrates to urbanization (Table 11) showed little evidence to support the existence of an initial response threshold (line segment A–B, Fig. 3A) that would have indicated resistance to change at low levels of urbanization (King et al. 2005). Instead, our results show that assemblages begin to change at very low levels of urbanization and most commonly follow the linear response hypothesized by Booth et al. (2004) (Fig. 3B, line A) with some metropolitan areas showing more rapid rates of change at the low end of the urban gradient as hypothesized by Walsh et al. (2005*a*) (Fig. 3B, line B). The lack of a resistance phase indicates that either the assemblages lack the ability to compensate for changes associated with low levels of urbanization or that the basins that we perceive as relatively undisturbed (background) have actually been disturbed beyond the ability of the assemblages to compensate for disturbance, that is, background conditions have been

TABLE 15. Urban intensity (MA-NUII) and change in invertebrate NMDS axis 1 site scores predicted to occur at impervious surface values of 5% and 10% based on linear regressions relating percentage of impervious surface to urban intensity.

Metropolitan area	MA-NUII predicted at 5% and 10% impervious surface				Change in invertebrate assemblage	
	5%	10%	R^2	P	5%	10%
BOS	17.3	32.6	0.97	<0.001	17.3	32.6
RAL	22.5	33.1	0.71	<0.001	22.5	33.1
ATL	20.2	35.8	0.89	<0.001	20.2	35.8
BIR	17.2	28.2	0.82	<0.001	17.2	28.2
MGB	8.6	18.4	0.95	<0.001	8.6†	18.4†
DEN	8.8	19.1	0.94	<0.001	ns	ns
DFW	13.3	25.4	0.99	<0.001	13.3†	25.4†
SLC	15.2	25.2	0.95	<0.001	15.2	25.2
POR	14.0	24.0	0.95	<0.001	14.0	24.0

Notes: Changes in invertebrate assemblages are expressed as a percentage of the estimated background (intercept) value; ns: no significant relation with MA-NUII.

† Significant relation only for qualitative (QMH) samples.

TABLE 14. Extended.

Mean bankfull width-to-depth ratio	Annual degree-days (°C)	Maximum summer (°C)
16.4	3497	27.1
8.2	5260	28.3
6.2	5806	27.0
8.9	5847	27.3
10.6	3524	24.6
6.9	4881	23.7
4.0	6656	30.5
6.7	2674	15.0
10.7	4246	22.4

displaced from some point along line segment A–B to some point along segment B–C in Fig. 3A. If background conditions have been displaced, then this suggests that disturbance is ubiquitous and that true undisturbed conditions no longer exist within the areas of the country represented by these nine metropolitan areas.

Our threshold analyses are also relevant to evaluating criteria that have been suggested as protective of stream ecosystems. For example, limiting impervious surface to a maximum of 5– 10% has been suggested as a criterion for protecting stream ecosystems (Klein 1979, Schueler 1994, Booth and Jackson 1997). Our results indicate that at 10% impervious land cover, the invertebrate assemblages in metropolitan areas with low antecedent agriculture have been degraded by 24–33% compared to estimated background conditions (Table 15). Even a more conservative level of 5% impervious surface corresponds to a change of 13–23% from background conditions. In those cases where a threshold may exist (Table 11, responses B and C), the threshold rarely corresponds to levels of urbanization indicative of 5–10% impervious surface. Clearly, these criteria need to be reevaluated as 5% and 10% impervious surface does not provide a significant safeguard for protection of the invertebrate assemblages.

Large-scale patterns of urbanization and its effects

Our studies provided an opportunity to begin to examine some of the local-, regional- and continental-scale patterns of responses to urbanization that have been hypothesized by Grimm et al. (2008). Our results support the importance of human sociodemographic changes (e.g., increasing population density) as the primary driver of land-use change and the influence of climate and geography (i.e., natural environmental setting) on the pattern of land-use change across the U.S. (hypothesis 1; Grimm et al. 2008). We found that obtaining a realistic understanding of large-scale patterns of urbanization and responses to urbanization are best developed by assembling studies at the scale of the metropolitan areas. Grimm et al. describe this as the concept of viewing "continental gradients as a collection of urban–rural gradients, each associated with individual metropolitan areas."

Legacy human activities (antecedent agriculture) and the environmental template (environmental setting) were also found to interact with pollution gradients (e.g., urbanization) to produce regional variation in ecosystem responses, which is consistent with hypothesis 2 of Grimm et al. 2008. Failure to account for the effects of legacy human activities and the environmental template, which determine background conditions, were found to obscure the effects of urbanization and lead to erroneous conclusions regarding its effects on stream ecosystems, methods of mitigating effects, and expectations for restoration.

The similarity in landscape alteration that we observed across climate zones is consistent with hypothesis 3 of Grimm et al. 2008: urbanization leads to the homogenization of form and function of urban land cover across climate zones. However, while we observed consistency in landscape alteration, we also found that the rates of change varied among metropolitan areas (Cuffney and Falcone 2008). This suggests that there are multiple aspects of land cover homogenization that need to be considered at the regional and continental scales.

Changes in the connectivity of wind, animal, and water vectors have been hypothesized (hypothesis 4; Grimm et al. 2008) as factors that have dramatic consequences for aquatic ecosystems. While our studies did not address wind and animal vectors, we did see hydrologic changes (flashiness) that affected many, but not all, metropolitan areas. Flashiness is symptomatic of changes in the connectivity of surface- and groundwater systems as urbanization increases imperviousness and engineered storm- and wastewater structures modify natural surface- and ground water flow paths.

Collectively, the changes that we observed in the physical, chemical, and biological condition of streams along the urban gradient established the ability of humans to fundamentally change biogeochemical inputs, processing, and flow paths to streams (Grimm et al. 2008: hypothesis 5) in all of our metropolitan areas. As with most generalities dealing with the effects of urbanization, the effects of water chemistry and hydrology varied among metropolitan areas. This variation emphasizes the need to aggregate collections of urban–rural gradients, such as were investigated in our studies, in order to develop an accurate and comprehensive assessment of large-scale patterns of urbanization that can be used to understand the process of urbanization, its effects, and mechanisms for managing and mitigating urbanization.

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APPENDIX A

List of variables and their definitions for natural environmental setting, soils, topography, and ecoregions (*Ecological Archives* A020-048-A1).

APPENDIX B

List of variables and their definitions for land cover, infrastructure, and census (*Ecological Archives* A020-048-A2).

APPENDIX C

List of variables and their definitions for hydrology, water temperature, water chemistry and pesticide indices, SPMD chemistry, and habitat (*Ecological Archives* A020-048-A3).

APPENDIX D

List of variables and their definitions for invertebrate metrics (*Ecological Archives* A020-048-A4).