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ASSESSMENT TOOLS FOR URBAN CATCHMENTS: DEVELOPING BIOLOGICAL INDICATORS BASED ON BENTHIC MACROINVERTEBRATES[1]

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ABSTRACT: Biological indicators, particularly benthic macroinvertebrates, are widely used and effective measures of the impact of urbanization on stream ecosystems. A multimetric biological index of urbanization was developed using a large benthic macroinvertebrate dataset (n = 1,835) from the Baltimore, Maryland, metropolitan area and then validated with datasets from Cleveland, Ohio (n = 79); San Jose, California (n = 85); and a different subset of the Baltimore data (n = 85). The biological metrics used to develop the multimetric index were selected using several criteria and were required to represent ecological attributes of macroinvertebrate assemblages including taxonomic composition and richness (number of taxa in the insect orders of Ephemeroptera, Plecoptera, and Trichoptera), functional feeding group (number of taxa designated as filterers), and habit (percent of individuals which cling to the substrate). Quantile regression was used to select metrics and characterize the relationship between the final biological index and an urban gradient (composed of population density, road density, and urban land use). Although more complex biological indices exist, this simplified multimetric index showed a consistent relationship between biological indicators and urban conditions (as measured by quantile regression) in three climatic regions of the United States and can serve as an assessment tool for environmental managers to prioritize urban stream sites for restoration and protection.

(KEY TERMS: multimetric index; quantile regression; NMS; urban gradient; stream; river; management; restoration; Mid-Atlantic; Midwest; Pacific Coast.)

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INTRODUCTION

Biological indicators are widely used to evaluate and characterize changes in the condition of stream systems (Patrick, 1994; Bonada *et al.*, 2006; Carter *et al.*, 2006). Of the organisms used as biological indicators in research and monitoring programs (fish, algae, macrophytes, invertebrates, bacteria, viruses, etc.), benthic macroinvertebrates (e.g., freshwater

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insect larvae, snails, worms, crustaceans) are the most commonly used assemblage worldwide (Rosenberg and Resh, 1993; Resh, 2008). As biological indicators, benthic macroinvertebrates can provide insight into the current and past conditions of a water body and integrate the effects of cumulative stressors (Resh and Jackson, 1993; Barbour *et al.*, 1999; Bonada *et al.*, 2006). Furthermore, benthic macroinvertebrates are ubiquitous in most stream environments, are long-lived compared to algae, possess varying tolerances to perturbations in streams, and are cost-effective to sample – all of which make them an ideal biological indicator (Rosenberg and Resh, 1993; Yoder and Rankin, 1995).

As more and more land area in the United States (U.S.) becomes urbanized, methods that measure the impacts of urbanization on stream systems are needed so as to adequately manage urban catchments. The impacts of urbanization on stream condition have been examined using both stressor and response indicators. Stressor indicators examined in urban systems include hydrologic indicators (Richter *et al.*, 1996; Sheeder *et al.*, 2002; Baker *et al.*, 2004; Booth, 2005), landscape attributes (McMahon and Cuffney, 2000), removal of riparian vegetation (Finkenbine *et al.*, 2000; Groffman *et al.*, 2003), water chemistry (Porcella and Sorensen, 1980), nutrients (Groffman *et al.*, 2004), and contaminants (Neal and Robson, 2000; Paul and Meyer, 2001; Meyer *et al.*, 2005). Response indicators include ecosystem processes (Faulkner *et al.*, 2000; Meyer *et al.*, 2005), microbes (Porcella and Sorensen, 1980; Gibson *et al.*, 1998), algae (Chessman *et al.*, 1999), benthic macroinvertebrates (Steedman, 1988; Suren, 2000), and fish (Fausch *et al.*, 1984; Morgan and Cushman, 2005).

To assess the biological condition in urban ecosystems, it is crucial to explore and define a condition as a target for management. Reference sites are commonly used in bioassessment studies to identify undisturbed or pristine conditions and hence management targets (Hughes, 1995; Prins and Smith, 2007). Yet, the pervasiveness of urban development often results in the absence of reference sites in urban streams (Chessman and Royal, 2004). When these reference sites are unavailable, environmental managers can have difficulty defining a target condition for restoring urban stream sites (Meyer *et al.*, 2005). Restorable benchmarks are generally more complex in urban catchments where multiple stressors are difficult to characterize in a quantitative response-stressor relationship and the effectiveness of different restoration practices are not well understood (Davies and Jackson, 2006). In addition, restorable benchmarks may also vary along a continuum where not all sites or river reaches have the ability to attain a pristine condition. Therefore, a continuum or gradient approach (Carter and Fend, 2005) that sets the realistic minimum condition for biological recovery based on the degree of urbanization provides a framework for evaluating both the current condition and the potential for recovery of impacted waters. This framework can allow for more realistic management targets and prioritization of sites for restoration and protection (Stoddard *et al.*, 2006).

Findings from regions across the globe have shown that benthic macroinvertebrate assemblages are particularly effective response indicators of urbanization because they exhibit a consistent response to urban stressors (Chessman and Williams, 1999; Paul and Meyer, 2001; Roy *et al.*, 2003; Wang and Kanehl, 2003; Miltner *et al.*, 2004; Walsh *et al.*, 2005). Recent studies have called for research to explore the relationships between macroinvertebrate assemblages and urban land use variables in differing climatic regions (e.g., Walsh *et al.*, 2005). Therefore, this study used benthic macroinvertebrate assemblages as biological indicators of urbanization across three climatic regions.

The objective of this study was to develop a broadly applicable biological response indicator that responds to the effects of various levels of urbanization across three distinct climatic regions. The relationship between biological condition (using a biological index based on macroinvertebrate metrics) and urbanization [using an urban gradient developed by Bressler *et al.* (this issue)] is explored with a large dataset from Baltimore, Maryland (hereafter referred to as the Mid-Atlantic region). This model is then validated with datasets from Cleveland, Ohio (n = 79) and San Jose, California (n = 85) (hereafter referred to as the Midwest and Pacific Coast regions, respectively), and a different subset of data from the Mid-Atlantic region (n = 85). This study is the second component of a larger project that is presented in a three-part series: (1) development of a gradient that accurately characterizes urbanization (Bressler *et al.*, this issue), (2) development of an urban biological index (this paper), and (3) definition of observed biological potential that describes the upper boundary of the polygonal distribution as the minimum target level attainable for restoration (Paul *et al.*, this issue). These three steps provide a hypothesized relationship of the biological response to urbanization and the biological potential along an urban gradient (Figure 1). The goal of this study was not to develop new monitoring tools, but rather to demonstrate a process using existing datasets to determine assemblage level indicators that are applicable among regions.

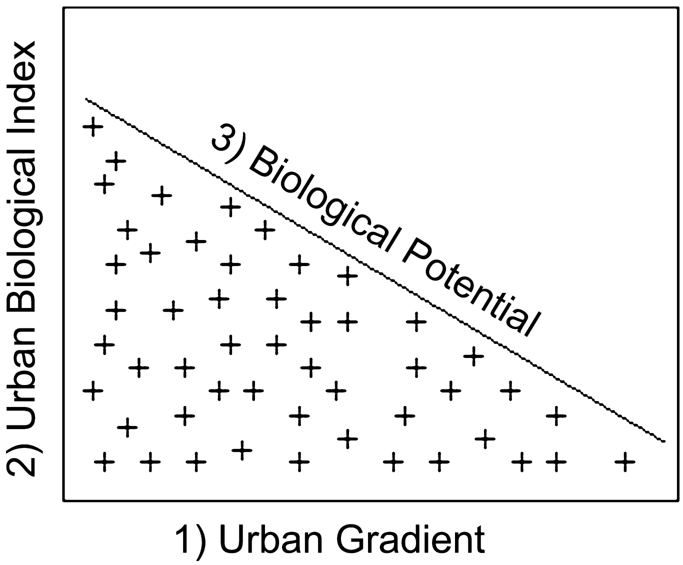

FIGURE 1. Model Showing Components Developed in Three Part Series. (1) The urban gradient along x-axis, (2) the urban biological index along the y-axis, and (3) the biological potential defined by the relationship of the x and y axes.

METHODS

Study Areas

The datasets used to build the model were from stream studies conducted in the catchments surrounding the metropolitan area of Baltimore, Maryland (Mid-Atlantic). This model was validated with datasets from Cleveland, Ohio (Midwest); San Jose, California (Pacific Coast); and a randomly selected different subset of the Mid-Atlantic region dataset. Figure 2 shows the locations of each study region and the sites sampled within each region. Although these urban areas contain similar land use patterns (gradient of nonurban to urban) and biological parameters (benthic macroinvertebrate taxa), their vast differences in climate, geographical location, ecoregion, and history of urbanization provided the opportunity to test whether a common biological response indicator could be constructed and would be related to a single gradient representing urbanization in the three regions (Table 1).

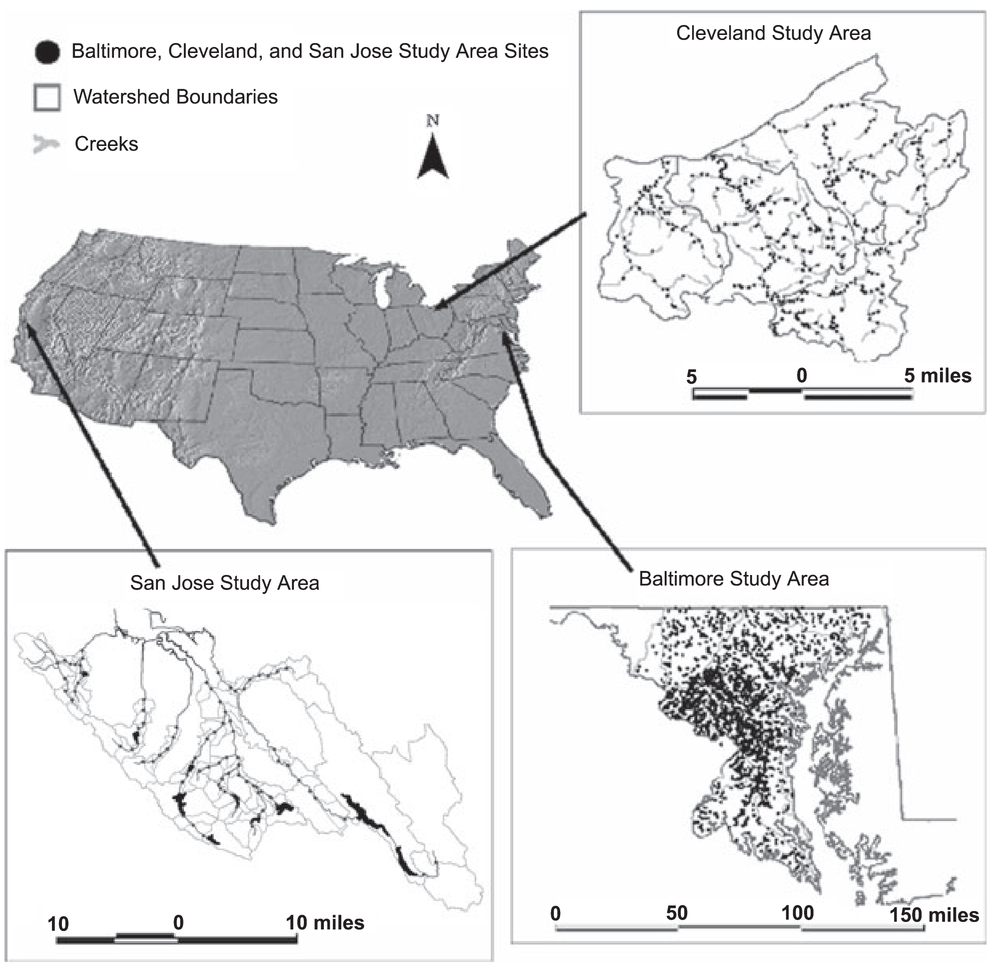

FIGURE 2. A Map of the Three Study Regions and Sampling Sites: Mid-Atlantic (Baltimore, Maryland), Midwest (Cleveland, Ohio), and Pacific Coast (San Jose, California).

TABLE 1. Drainage Basin Characteristics of the Three Study Regions.

Climatic Region	Mid-Atlantic	Midwest	Pacific Coast
Metropolitan area	Baltimore, Maryland	Cleveland, Ohio	San Jose, California
Urban system	Patapsco River and surrounding basins	Cuyahoga River and surrounding basins	Santa Clara Basin (Coyote Creek and surrounding basins)
Provincial description	Mid-Atlantic Piedmont/coastal plains, Chesapeake Bay	Midwestern, Lake Erie	Pacific drainage, Mediterranean climate, South San Francisco Bay
Climate*	Humid subtropical	Humid continental	Mediterranean (warm/dry summers; cool/wet winters)
Human population**	~8,000,000	2,126,318	~1,680,000
Total catchment (km^2)	11,496.3	32.6	1,618.7
Percentage land area above 25% imperviousness	12.30%	13.50%	27.04%
Dominant ecoregions	Northern Piedmont	Erie Drift Plain	Southern and Central California Chaparral and Oak Woodlands
History of urbanization	(Founded in 1729) Initial urban growth 1750-1800, suburban growth 1950-2000	(Founded in 1796) Urban growth between 1832-1920, peaked in 1949	(Founded in 1850) Aggressive urban growth between 1950-2000
Percentage watershed agricultural or urban	<20%	>50%	20-50%

*Köppen climate classification.
**2000 U.S. Census Bureau.

Data Types

Data from sites located in the Mid-Atlantic region (Figure 2) were collected by numerous state, county, and city agencies, although the majority of the data was acquired from the Maryland Biological Stream Survey (MBSS) (http://www.dnr.state.md.us/streams/mbss/). Water chemistry, physical habitat, and biological (benthic macroinvertebrates) data were collected during the spring and summer of the years 1990-2002. Water chemistry data (conductivity, chlorides, acid neutralizing capacity, pH, nitrate, orthophosphate, ammonia, dissolved organic carbon, dissolved oxygen, and sulfate) were collected from approximately 800 sampling sites in the region. Physical habitat parameters were measured using the Environmental Protection Agency's Rapid Bioassessment Protocol (RBP) (Barbour *et al.*, 1999) at >2,000 sites. Biological data were collected using sampling methods adapted from the U.S. Environmental Protection Agency's RBP (Barbour *et al.*, 1999) at >3,000 sites (which included the sites sampled for water chemistry and physical habitat). All biological samples in the three climatic regions were identified to the standard taxonomic level (mostly genus and species) and rarified to 100 organisms per sample to ensure comparability between different sample sizes. Primary identification references included Merritt and Cummins (1996), Thorp and Covich (1991), and Pennak (1989). Lastly, land use data for the Mid-Atlantic region were acquired from the 1997 Maryland Department of Planning.

Macroinvertebrate data from the Midwest region (n = 79 sites) were collected as part of routine biological surveys by the Ohio EPA following their standard protocols (Ohio EPA, 1987, 1989, 1990; DeShon, 1995) (Figure 2). Macroinvertebrates were collected from artificial substrates using a composite sample of five modified Hester-Dendy (HD) multiple-plate samplers colonized for six weeks (June 15 to September 30 between 1990 and 2000). Each HD sampler consisted of eight plates and 12 spacers representing 0.1 m^2 of stream bottom and was located in an average flow of 0.1 m/s over the plates. The HD samplers were retrieved and preserved in 10% formalin and later combined to form a composite sample. Qualitative samples were collected in all available habitats (e.g., snags, wood, submerged vegetation, vegetated banks root wads, and riffles) using a triangular frame 595-μm mesh dip net for approximately 30-60 min. All macroinvertebrates were field-picked in a white pan and preserved in 70% ethanol. Land use data for the Midwest region were acquired from the 1994 National Landcover Dataset (NLCD).

Data from sites located in the Pacific Coast region (n = 85 sites) were collected by federal, state, and local agencies (Figure 2). Biological (macroinvertebrate) data were collected by the U.S. Geological Survey at 85 sites located on 14 streams during May 1997. Five – 0.1 m^2 collections were made systematically using a 0.3 m wide D-frame kicknet fitted with a 500 μm mesh net in a downstream to upstream direction per riffle (=site). All five collections were composited to form one sample per site.

The composited sample was preserved in the field with 10% buffered formalin. All samples were subsampled in the laboratory using a 5 cm^2 gridded tray (Moulton *et al.*, 2000). Physical habitat data were collected at the same 85 sites by researchers at University of California, Berkeley (Kearns, 2003). Land use data for the Pacific Coast region were acquired from the National Oceanic & Atmospheric Administration (NOAA) 2000 Coastal California Land Cover dataset.

Development of Urban Gradients

Several urban gradients were developed within the three study regions to characterize urbanization using different combinations of chemical, hydrological, and landscape parameters (see Bressler *et al.*, this issue). First, a primary urban gradient was constructed to serve as a benchmark of urbanization (based on approaches by Tate *et al.*, 2005; McMahon and Cuffney, 2000). The primary urban gradient consisted of three parameters (population density, road density, and percent urban land use/land cover), which were standardized (using the 1st and 99th percentiles of each parameter's distribution) and combined by taking the average of the standardized values. This urban benchmark was used to test and select additional urban stressor variables with which to construct other urban "alternative" gradients.

To determine additional urban stressor variables suitable for inclusion in the alternative urban gradients, 81 variables in five categories (chemistry, habitat, hydrology, demography, riparian land use/land cover) were tested using simple linear regression to examine the relationship with the primary urban gradient. Stressor variables were retained if they displayed a statistically significant ($p < 0.05$) relationship with the primary urban gradient. Multiple regressions (by category) were used as a final guide for selecting which stressor variables to use in constructing the alternative urban gradients. Stressor variables that were significant in multiple regression analyses with the primary urban gradient were standardized using the 1st and 99th percentiles of each parameter's distribution and assembled into several alternative urban gradients within five categories: stressor based (instream), source based (landscape), comprehensive (mixed stressor-source), cost-effective (built with easy to collect data), and desktop (built without field collected data) for use by agencies with differing data types available. The urban gradients with the highest correlation to the primary urban gradient within each category were selected as the final alternative urban gradients.

Calculating Biological Metrics

Common biological metrics ($n = 56$) were calculated from benthic macroinvertebrate data in each of the three study regions. These biological metrics were required to be widely used by agencies, easily measured, calculated and understood, and to represent ecological attributes of composition and richness, functional feeding group, and habit. Therefore, the following types of metrics were calculated to represent three core ecological attributes of stream systems: (1) composition and richness (e.g., percent Amphipoda, Ephemeroptera richness), (2) functional feeding group (e.g., percentage and richness of scrapers, predators, filterers), and (3) habit (e.g., percentage and richness of clingers, swimmers, climbers) (Merritt and Cummins, 1996; Barbour *et al.*, 1999).

Relationship Between Metrics and Stressor Variables

Nonmetric multidimensional scaling (NMS; Minchin, 1997) was used to determine which biological metrics best represented both sensitivity and tolerance (Sorensen = distance measure). The analysis was conducted using the Mid-Atlantic dataset to examine possible groupings of sensitive and tolerant biological metrics ($n = 56$) based on their arrangement in the ordination plot and correlations to the urban stressor variables used by Bressler *et al.* (this issue). This multivariate analysis was also used to determine which biological metrics would serve as strong candidates for the biological index of urbanization. The two matrices used for the analyses consisted of a site by metric matrix and a site by urban stressor matrix. The NMS analyses were performed using PC-ORD 4.27 software (McCune and Mefford, 1999).

Biological Metric Selection

Prior to the metric selection, 85 randomly selected sites were removed from the Mid-Atlantic dataset. These 85 sites were set aside to later validate the model after its completion. Datasets from the Midwest ($n = 79$) and Pacific Coast ($n = 85$) regions were also used to validate the final model.

Five screening criteria were used to screen each biological metric for use in a biological index (Table 2). These criteria were adapted from several studies that evaluated metric screening and techniques (Hughes *et al.*, 1998; McCormick *et al.*, 2001; Klemm *et al.*, 2003; Ode *et al.*, 2005), which are important for the elimination of both noninformative metrics and those that are redundant to others (Karr *et al.*, 1986;

TABLE 2. Criteria for Biological Metric Selection.

Metric Screening Criteria
1. Range
Percent >10%
Richness >5
2. Area-based effects examined (Vannote *et al.*, 1980)
Determined using linear regression
No clear relationship between metrics and catchment area must be present
3. Quantile regression
Visual inspection for positive or negative trend
Quantile regression criteria to examine spread and shape of upper boundary:
Slope of 95th percentile regression line ~ -0.75
y-intercept between 70-110
Biological index must respond across the entire urban gradient
4. Redundancy
Metrics considered redundant if $r > 0.7$
Metric that best meets quantile regression criteria is retained
5. Ecological category
Two metrics were selected within each ecological category (i.e., composition and metrics, functional feeding group, habit)

Note: Procedure Adapted from Hughes *et al.*, 1998; Klemm *et al.*, 2003; and Ode *et al.*, 2005.

Barbour *et al.*, 1999). First, the range of each metric (from lowest to highest value) was examined to ensure that it was broad enough to discern differences in magnitude. The criteria used for this step were that the range of percentage metrics must be >10 and that the range of richness metrics must be >5 (e.g., Klemm *et al.*, 2003). Second, the relationship of the metrics to catchment area was examined using correlation analysis (as done in Klemm *et al.*, 2003). The step was conducted based on the prediction that faunal shifts occur with increasing catchment size (e.g., The River Continuum Concept, Vannote *et al.*, 1980). Third, the relationship of each biological metric to the primary urban gradient was examined by inspecting each scatterplot for evidence of a directional pattern (positive or negative) and using quantile regression (Koenker and Bassett, 1978; Koenker and Hallock, 2001; Cade and Noon, 2003) criteria to characterize the upper boundary of the polygonal scatterplots. Quantile regression is a way to estimate functional relationships between variables for a designated portion of a response variable's probability distribution. Therefore, quantile regression can yield a more complete view when exploring causal relationships between ecological variables (Cade and Noon, 2003). The quantile regression criteria consisted of the following: (1) the slope of the 95th percentile line using quantile regression must be approximately equal to −0.75 (Blackburn *et al.*, 1992), (2) the y-intercept must fall in the range of 70-110, and (3) the biological index must respond across the entire urban gradient. Fourth, metrics were plotted against each other to test for redundancy. If the Pearson correlation of two metrics was greater than 0.7, the metrics were considered redundant and the metric that best met the quantile regression criteria was retained. Fifth, the final metrics were required to fall within the three ecological categories (composition and richness, functional feeding group, and habit). Percentage and richness of metrics in each ecological category were used in constructing the biological indices.

Development of Biological Index

The metrics remaining after the metric screening procedure ($n = 6$) were scaled (0-100) using the 1st and 99th percentiles of the Mid-Atlantic data distribution (consistent with methods of Klemm *et al.*, 2003). The scaled metrics were then assembled into several candidate multimetric indices by taking the average of the scaled values. Three final metrics (one from each ecological category) were selected for use in the biological index. This process was based on the original concept of multimetric indices (Karr, 1981; Karr *et al.*, 1986).

The candidate indices were tested against the primary urban gradient using quantile regression criteria that characterized the spread and shape of the upper boundary of the data points. Quantile regression was used instead of traditional linear regression because it more effectively characterized the upper boundary of the biological indices and urban gradient plots. Figure 3 shows a comparison of traditional linear regression and quantile regression. The quantile regression criteria used to evaluate the scatterplots were the same as used to select metrics: (1) the slope of the 95th percentile line using quantile regression must be approximately equal to −0.75 (Blackburn *et al.*, 1992), (2) the y-intercept must fall in the range of 70-110, and (3) the biological index must respond across the entire urban gradient. These criteria were designed to select a biological index that responded in a gradual manner across the entire urban gradient. If an index were too sensitive, it would respond quickly and decline close to zero at low urbanization values. The most robust biological index could provide information at all levels of urbanization and therefore be used as a tool along a gradient of low to highly urbanized sites.

Validation of Biological Index

The final biological index, which was constructed with a dataset from the Mid-Atlantic region, was validated using a subset of 85 randomly selected sites from the original Mid-Atlantic dataset, and sites from the Midwest ($n = 79$) and Pacific Coast ($n = 85$)

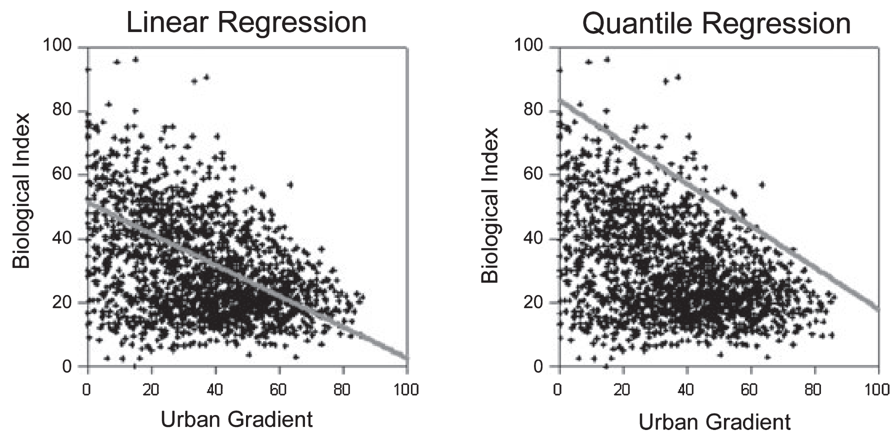

FIGURE 3. Example of Two Scatterplots Showing a Hypothetical Biological Index (y-Axis) Plotted Against an Urban Gradient (x-Axis). The plot on the left shows an example of a linear regression line ($r^2 = 0.19$), while the plot on the right shows an example of quantile regression of the 95th percentile that better characterizes the upper boundary of the wedge-shaped plot.

regions. This final and crucial step in this study was to verify if this model displayed a similar pattern regardless of region. Therefore, all of the datasets were plotted onto a single graph and the quantile regression criteria were used to determine if the regions displayed a pattern similar to the original model developed in the Mid-Atlantic region.

RESULTS

Testing of Urban Gradients

The scatterplots of all biological indices were tested for presence of a gradual relationship along the entire length of the primary and alternative urban gradients. The biological indices did not display a gradual response across the entire extent of any of the alternative gradients (see Bressler *et al.*, this issue). Therefore, the primary urban gradient was the only urban gradient adequate enough to evaluate the biological indicators.

Relationship Between Metrics and Stressor Variables

The results of the NMS used to identify and partition biological metrics based on their relationship to the stressor variables (e.g., population density) illustrated a clear grouping of the sensitive (e.g., Ephemeroptera taxa, percent filterer) and tolerant (e.g., Oligochaeta taxa, percent noninsect taxa) metrics (final stress = 16.24) (Figure 4). Axis 2 was selected as representing the "axis of urbanization" because sensitive and tolerant metrics clustered on opposite ends of

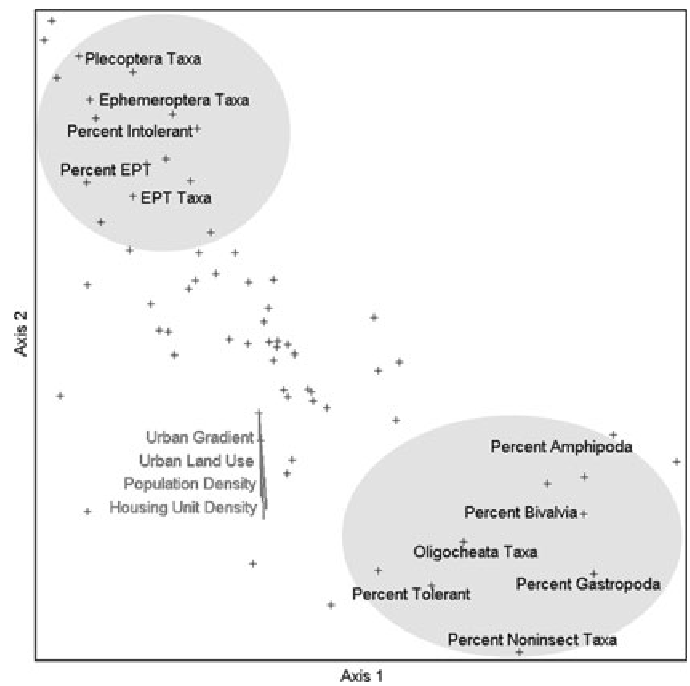

FIGURE 4. Nonmetric Multidimensional Scaling (NMS) Ordination Plot of Biological Metrics. Ellipses show a clear grouping of sensitive (upper left) and tolerant (lower right) metrics (final stress = 16.24). Although all biological metrics are plotted ($n = 56$), only a few representative metrics are labeled (e.g., EPT taxa, percent Bivalvia). The labeled vectors represent the stressor variables most correlated with Axis 2.

Axis 2, the stressor variables related to urbanization were highly correlated with Axis 2, and there were no stressor variables highly correlated with Axis 1. The top 10% of the most highly correlated stressor variables with the axis of urbanization (Axis 2) included: the primary urban gradient developed by Bressler *et al.* (this issue) (population density, percent urban land use, and road density), urban land use, population density, and housing unit density. The lowest 10% of correlations to Axis 2 included: catchment area, water chemistry parameters, and hydrologic indicators. The partitioning of sensitive and tolerant metrics allowed us to proceed with confidence that several metrics would serve as strong candidates for the biological index of urbanization.

Biological Metric Selection

The metric screening procedure resulted in the selection of six candidate metrics for use in constructing a biological index. The first step in the metric screening procedure eliminated four metrics that did not have sufficient range of values for use in the biological index [i.e., percentage of Corbicula individuals, percentage of individuals that were swimmers, percentage of individuals that were in subgroup Tanytarsini (Chironomidae), and Orthocladiinae richness] (Table 3). The second step found that no metrics had a significant relationship (positive or negative) to their catchment area; hence no metrics were eliminated. The third step (quantile regression) eliminated the greatest number of metrics (n = 43). These quantile regressions were used to examine the upper boundary of the relationship between each biological metric and the urban gradient and only nine metrics met the quantile regression criteria (Table 3). The fourth step of the metric screening process (test for redundancy) found that the percent and richness metrics of the individual orders of Ephemeroptera and Plecoptera were redundant ($r > 0.7$) with percent and richness of EPT (the combination of generally sensitive orders Ephemeroptera, Plecoptera, and Trichoptera). These individual order metrics (Ephemeroptera and Plecoptera) did not meet the quantile regression criteria as adequately as percent and richness of EPT and were therefore eliminated in favor of EPT-based metrics. Lastly, two metrics from each ecological category (composition and richness, functional feeding group, and habit) were selected for use in constructing the biological index based on how well they met the quantile regression criteria. Three metrics were added back to the list (% EPT, % filterer, % clinger) because they met the ecological category criteria (paired with EPT richness, filterer richness, clinger richness). Two metrics (shredder and swimmer richness) were deleted in this last step.

Development of Biological Index

The final metrics selected for building the multimetric biological indices included two metrics in each of the three ecological attribute categories: (1) percentage of EPT individuals and EPT richness (composition and richness); (2) percentage of filterer individuals and filterer richness (functional feeding group); and (3) percentage of clinger individuals and clinger richness (habit). These final metrics were standardized and assembled into eight biological indices that contained one metric in each of the three core ecological categories (Table 4).

The biological index that best met the quantile regression criteria was selected as the final multimetric biological index. The final biological index selected was composed of EPT richness, filterer richness, and percent clingers (Table 5 and Figure 5). The response area in this plot showed a similar response when validated with data from the Mid-Atlantic, Midwest, and Pacific Coast regions.

DISCUSSION

The relationship between the biological metrics and stressor variables in the NMS revealed a clear grouping of the sensitive and tolerant metrics and validated the use of these metrics as components in the construction of a biological index for evaluating the complex effects of urbanization. This relationship displayed between the biological metrics and urban parameters is consistent with the findings of other urban ecosystem studies (e.g., Cuffney *et al.*, 2005).

In this study, biological metrics were eliminated in each step of the metric selection process, with the exception of step 2 (area-based effects), in which no metrics were eliminated for having a positive or negative relationship to catchment area (as also found by DeShon, 1995). This lack of relationship to catchment area could be attributed to the fact that all sampling sites were located on first through fourth-order streams. Other studies that have examined fish as biological indicators have found a stronger relationship with catchment area (e.g., Fausch *et al.*, 1984).

Wedge-shaped plots are common in ecological relationships (Thomson *et al.*, 1996; Cade and Noon, 2003) and the considerable scatter on the lower end of both axes of Figure 5 represents the multitude of natural and anthropogenic factors that interact to affect biological communities. Despite the scatter at the lower ends of the plot, there is a clear upper boundary indicating that the current maximum biological condition decreases as urbanization increases.

The candidate metrics selected for the final biological index (EPT richness, filterer richness, and percent clinger) are representative of core ecological attributes that are related to sensitivity to pollutants and

TABLE 3. Results of Biological Metrics Screened for Use in a Biological Index.

Candidate Metrics	Step 1 Range	Step 2 Area-Based Effects	Step 3 Quantile Regression	Step 4 Redundancy	Step 5 Ecological Category
Composition and Richness					
Percent Amphipoda individuals	√	√	X	–	–
Percent Baetidae (Ephemeroptera) individuals	√	√	X	–	–
Percent Bivalvia individuals	√	√	X	–	–
Percent Chironomidae individuals	√	√	X	–	–
Chironomidae richness	√	√	X	–	–
Percent Coleoptera individuals	√	√	X	–	–
Coleoptera richness	√	√	X	–	–
Percent Corbicula individuals	X	–	–	–	–
Percent Cricotopus and Chironomus individuals	√	√	X	–	–
Percent Crustacea and Mollusca individuals	√	√	X	–	–
Crustacea and Mollusca richness	√	√	X	–	–
Percent Diptera individuals	√	√	X	–	–
Diptera richness	√	√	X	–	–
Percent Ephemeroptera individuals	√	√	√	X	–
Ephemeroptera richness	√	√	√	X	–
Percent EPT individuals*	√	√	X	–	EC
EPT richness*	√	√	√	√	√
Percent Gastropoda individuals	√	√	X	–	–
Percent of EPT as Hydropsychidae individuals	√	√	X	–	–
Percent Trichoptera as Hydropsychidae individuals	√	√	X	–	–
Percent Isopoda individuals	√	√	X	–	–
Percent non-insect taxa	√	√	X	–	–
Percent Odonata individuals	√	√	X	–	–
Percent Oligocheata individuals	√	√	X	–	–
Oligochaeta richness	√	√	X	–	–
Percent Chironomidae as Orthocladiinae individuals	√	√	X	–	–
Orthocladiinae richness	X	–	–	–	–
Percent Plecoptera individuals	√	√	√	X	–
Plecoptera richness	√	√	√	X	–
Percent Tanytarsini individuals	√	√	X	–	–
Tanytarsini richness	√	√	X	–	–
Percent Chironomidae as Tanytarsini individuals	X	–	–	–	–
Percent Trichoptera individuals	√	√	X	–	–
Trichoptera richness	√	√	X	–	–
Percent of dominant taxon	√	√	X	–	–
Total taxa richness	√	√	X	–	–
Functional Feeding Group					
Percent collector	√	√	X	–	–
Collector richness	√	√	X	–	–
Percent filterer*	√	√	X	–	EC
Filterer richness*	√	√	√	√	√
Percent predator	√	√	X	–	–
Predator richness	√	√	X	–	–
Percent scraper	√	√	X	–	–
Scraper richness	√	√	X	–	–
Percent shredder	√	√	X	–	–
Shredder richness	√	√	√	√	X
Habit					
Percent burrower	√	√	X	–	–
Burrower richness	√	√	X	–	–
Percent climber	√	√	X	–	–
Climber richness	√	√	X	–	–
Percent clinger*	√	√	X	–	EC
Clinger richness*	√	√	√	√	√
Percent sprawler	√	√	X	–	–
Sprawler richness	√	√	X	–	–
Percent swimmer	X	–	–	–	–
Swimmer richness	√	√	√	√	X

Notes: "X" indicates the step at which a metric was eliminated and a dash indicates that the metric was no longer considered in the selection process. A "√" indicates that the metric met the criteria of that step and was retained. Metrics with a "*" were retained for use in the biological indices. "EC" indicates that the metric was reconsidered because it met the ecological category criteria.

TABLE 4. Candidate Biological Indices Constructed Using Metrics Representing Ecological Attributes of Composition/Richness, Functional Feeding Group, and Habit.

	Composition/ Richness	Functional Feeding Group	Habit
Index 1	% EPT	% Filterer	% Clinger
Index 2	EPT richness	Filterer richness	Clinger richness
Index 3	% EPT	Filterer richness	% Clinger
Index 4	EPT richness	% Filterer	Clinger richness
Index 5	% EPT	% Filterer	Clinger richness
Index 6	EPT richness	Filterer richness	% Clinger
Index 7	% EPT	Filterer richness	Clinger richness
Index 8	EPT richness	% Filterer	% Clinger

Note: Index 6 was selected as the final biological index.

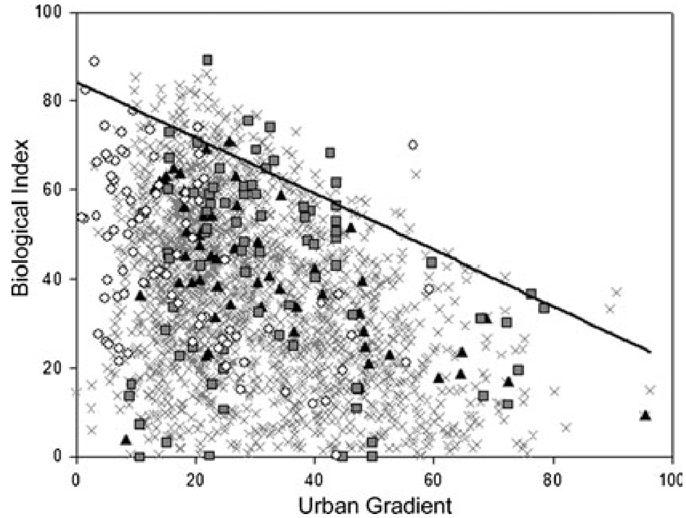

FIGURE 5. A Scatterplot of the Final Biological Index (Index 6) Plotted Against the Urban Gradient. The final biological index was composed of the metrics EPT richness, filterer richness, and percent clingers. Gray "x," Mid-Atlantic sites used to construct the biological index; black triangles, different subset of Mid-Atlantic sites used to validate the model; dark gray squares, Midwest sites used to validate the model; and white circles, Pacific Coast sites used to validate the model. The black line represents the 95th quantile regression line of the entire dataset.

habitat alteration (Karr and Chu, 2000). In addition, the use of three different but complementary metrics provided conceptual simplicity for users of the index. The metrics used in the final biological index were also common constituents of previous multimetric indices (e.g., Klemm *et al.*, 2003; Ode *et al.*, 2005).

In particular, other studies have found the sensitive metrics percentage and richness of EPT (which includes the orders Ephemeroptera, Plecoptera, and Trichoptera) to have strong negative responses to anthropogenic disturbances (Barbour *et al.*, 1992; Hayslip, 1993; DeShon, 1995; Ode *et al.*, 2002, 2005; Carter and Fend, 2005). EPT was selected over the redundant individual order richness metrics Ephemeroptera, Plecoptera, or Trichoptera because EPT had the strongest response to the urban gradient, encompassed a larger range of taxa, and was more broadly applicable (Carter and Fend, 2005).

The use of filterers as a common functional feeding group has been widely cited as a metric that responds to disturbance (Kerans *et al.*, 1992; Hayslip, 1993; Klemm *et al.*, 2003; Ode *et al.*, 2005). Some studies have hypothesized that the proportion of filterers would actually increase with increased human impacts (Kerans *et al.*, 1992), but the results of this study found a consistent decrease in the percentage of filterer individuals and filterer richness with an increase in urbanization. While the proportion of filterer taxa may sometimes increase with increased human impacts, the richness of filterer taxa can be a strong indicator of urbanization.

While the habit characteristic is often overlooked in biological assessments, it can be indicative of the condition and availability of invertebrate habitat at a given site. The general prediction is that clingers will have a negative response to increased urbanization (e.g., May, 1996; Karr, 1998). Clingers prefer stable

TABLE 5. Potential Biological Indices Tested Using Quantile Regression Criteria.

Biological Indices	Quantile Regression Criteria: Slope of 95th Percentile Regression Line ~−0.75	*y*-Intercept Range of 70-110	Biological Index Response Across Entire Urban Gradient	Similar and Overlapping Response Among Regions (Validation)
Index 1	X	–	–	–
Index 2	X	–	–	–
Index 3	X	–	–	–
Index 4	√	√	X	–
Index 5	X	–	–	–
Index 6	√	√	√	√
Index 7	√	√	√	X
Index 8	X	–	–	–

Note: A "√" indicates that the biological index met the criteria and "X" indicates that the biological index did not meet the criteria. A dash indicates that the biological index was not considered further. Biological Index 6 was the only index to meet all of the criteria.

and sediment-free substrate (Merritt and Cummins, 1996), so a decreased substratum particle size in downstream urbanized sites may not provide sufficient habitat for clingers. Furthermore, increased flashiness, a primary stressor of the "urban stream syndrome" (Walsh *et al.*, 2005), in highly urbanized sites can reduce the percentage of clingers by flushing them off benthic substrata (Morley, 2000). However, in some systems such as the arid south west, flashy systems are part of the natural hydrograph and therefore the expectations of biological conditions may differ.

Comparison of Biological Index Across Regions

The large number of Mid-Atlantic data points (n = 1,835) dominated the trend of the final biological index (Figure 5), therefore the Mid-Atlantic data ranges were used for scaling all parameters contained in the urban gradient. As a result, there were little to no Midwest or Pacific Coast data points greater than ~80 on the urban gradient because the metropolitan areas of Cleveland, Ohio, and San Jose, California, have lower values for maximum road density, population density, and percent urban land use compared to Baltimore, Maryland. San Jose has developed in a sprawl-type fashion with fewer, but wider roads (often four lanes or greater) and suburban housing developments that are less dense compared to Baltimore. Even though the data points from the three climatic regions overlap considerably in Figure 5, future research that examines each region separately could reveal if metrics used to construct a regional biological index display a similar pattern.

There were several strengths and weaknesses of using the metropolitan areas of Baltimore, Maryland; Cleveland, Ohio; and San Jose, California as the study sites for this process. Strengths include that these sites had contrasting climates, geology, and topography, yet had a similar land use gradient of rural to urban sites, which was needed so as to construct this model. Also, although the three regions used different biological sampling methods (multihabitat sampling, D-frame kick samples of riffles, and artificial substrate), the data still overlapped in the final plot (Figure 5). This suggests that the biological and urban indices used in this study may be applicable to datasets with differing climatic regions and biological sampling methods. It is important to note that these three study sites did not represent the full range of climatic and geographic conditions in the U.S. and further testing will determine whether other regions, such as the arid southwest, respond in a similar manner as the regions used in this study.

Applications of Biological Index

The final biological index described instream biotic conditions in a manner consistent with the biological response pattern described by the tiered aquatic life use framework (Davies and Jackson, 2006, draft) through the incorporation of ecological attributes for characterizing a response to a stressor gradient. While Maine and Ohio are two states that currently have tiered designated aquatic life uses in their water quality standards (Davies *et al.*, 1999), additional states are currently evaluating application of this framework in their water quality programs (Davies and Jackson, 2006).

The relationships presented in this study can serve as a template for developing management guidelines for urban streams that incorporate consideration of recovery potential (Paul *et al.*, this issue). A gradient approach that defines the observed biological potential at the upper boundary of the data scatter can help managers determine if a site is fully or partially meeting its designated use within a regulatory framework (Barbour *et al.*, 2006). Individual stream reaches can be evaluated based on the biological index score and degree of urbanization across a gradient of natural, rural, exurban, to highly urbanized land use as suggested by Theobald (2001). Importantly, a highly urbanized site may not have the same restoration objectives as a less urbanized site and may have different management goals.

Water quality agencies and managers can use a biological index such as the one developed in this study (EPT richness, filterer richness, and percent clingers) or develop a more region-specific index to guide urban stream protection and restoration efforts. By evaluating the relationship between the lotic assemblages per site, as represented by the biological index, and the urban gradient, managers can prioritize and justify sites for management or restoration. Stream reaches or other water bodies with low biological index values can be reviewed on a site-by-site basis to uncover the specific impairment to determine the restoration feasibility.

Although the consistent response of the biological index to the urban gradient across the three study regions may imply broad geographic applicability, application in other regions may require consideration of other anthropogenic or natural influences besides urbanization. Because this biological index was developed in response to urban land use parameters, the biological index developed in this study may not respond to other land use influences such as mining or agriculture.

In contrast, stream sites with a relatively high biological condition can serve as models for the restoration of sites that are more disturbed but have similar levels of urbanization (see Paul *et al.*, in this issue for more detail). Restoration projects may be unfeasible or cost prohibitive in highly urbanized sites with low biological index scores because of the multitude of urban stressors affecting these sites. Therefore, managers may elect to prioritize restoration activities in catchments that are less impacted by urbanization.

An important aspect of the techniques used for the development of this biological index was that this approach did not require the presence of reference sites *per se*. A 95th percentile relationship between the urban gradient and the biological index was used to represent the current highest quality biological conditions at any given level of urbanization. However, the 95th percentile does not necessarily reflect the upper bounds of restoration potential. The best available biological conditions (i.e., the highest values of the biological index) are variable along the urban gradient and are primarily limited by the level of urbanization. The percentile selected to define the upper boundary of the data can help managers set more practical management targets based on best available conditions. This may be an improvement over setting unrealistic goals of restoring a site to a natural and pristine biological state at all levels of urbanization.

In conclusion, this study outlines a process to use existing datasets provided by federal and state programs, local entities (e.g., regional sewer authorities), and others to develop biological indicators that are responsive to changes in urbanization. If sufficient biological data are available along a gradient of urbanization, the process presented here can be used to develop region-specific biological indicators of urbanization. Local management agencies that have existing regional biological indices can use these methods to further refine their indices in the future. The threshold relationships identified in this study promise more refined management of urban stream systems, increased protection of aquatic life uses, and efficient prioritization of scarce resources.

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