

Characterizing invertebrate traits in wadeable streams of the contiguous US: differences among ecoregions and land uses

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Abstract. Much is known about invertebrate community traits in basins across Europe, but no comprehensive description of traits exists for the continental US. Little is known about the trait composition of invertebrates in reference or least-disturbed basins of the US, how trait composition varies among ecoregions, or how consistently traits respond to land use. These elements are essential to development of trait-based tools for conservation and assessment of biological integrity. We compared invertebrate traits of least-disturbed basins among ecoregions of the US. Benthic invertebrate data (presence/absence) from 1987 basins were translated into 56 binary traits (e.g., bivoltine, clinger). Basins were classified as least-disturbed, agricultural, or urban, and grouped into 9 ecoregions. Landuse, climatic, physiographic, and hydrologic data were used to describe ecoregions and to evaluate least-disturbed basin quality. The unique habitat template of each ecoregion selected for trait compositions in least-disturbed basins that differed among ecoregions. Among the traits examined, life-history (e.g., voltinism, development) and ecological traits (e.g., rheophily, thermal preference) differed most among ecoregions. Agricultural and urban land uses selected for trait compositions that differed from least-disturbed, but the extent of the differences depended on ecoregion and quality of the least-disturbed basins. No trait compositions unique to specific land uses were found. However, a *disturbance syndrome* was observed in that the magnitude and direction of trait responses to urban and agricultural land uses were consistent among ecoregions. Each ecoregion had a unique trait composition, but trait compositions could be used to aggregate ecoregions into 3 broad regions: Western Mountains, Plains and Lowlands, and Eastern Highlands. Our results indicate that large-scale trait-based assessment tools for the US will require calibration to account for regional differences in the trait composition of basins and in the quality of least-disturbed basins.

Key words: invertebrate, traits, streams, least-disturbed, ecoregion, land use.

Stream ecologists have used species traits of aquatic organisms to develop ecological theory (Southwood 1977, Townsend and Hildrew 1994), link environmental factors with biological responses (Poff 1997, Richards et al. 1997, Statzner et al. 2004, Pollard and Yuan 2010), develop multimetric indices (Barbour et al. 1999), and predict benthic community vulnerability to large-scale disturbances, such as climate change (Poff et al. 2010). Others have explored the use of trait-based approaches for continental-scale biological assessment (Chevenet et al. 1994, Dolédec et al.

1999, Statzner et al. 2001). In a recent review of trait applications, Menezes et al. (2010) found that a trait-based approach was a promising alternative to taxonomy-based approaches for assessing the condition of freshwater ecosystems. Investigators using a trait-based approach can draw on ecological theory to make specific predictions of trait responses to a change in the environment, whereas investigators using taxonomy-based assessment methods rarely draw from this theory to develop specific predictions, such as mechanistic links between anthropogenic stressors and biological responses (Pollard and Yuan 2010).

Most published large-scale assessments of trait responses to natural and anthropogenic gradients

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were done with benthic invertebrate communities in Europe (Menezes et al. 2010 and citations within). The few trait-based assessments applied in North America show that trait responses to stressors are generally consistent across large spatial scales (Carlisle et al. 2007, Horrigan and Baird 2008, Pollard and Yuan 2010). However, these studies were limited in scope. For example, Horrigan and Baird (2008) investigated trait associations with 3 hydrologic variables, and Pollard and Yuan (2010) investigated how a single trait (clingers) responded to 1 stressor (fine sediment). Bêche and Stutzner (2009) tried to ascertain whether benthic communities in the contiguous US were functionally redundant (the conclusion for European streams). However, their study was limited by geographic distribution (data were primarily from humid mountainous regions). Further testing of trait-based approaches is needed to establish their reliability for assessing benthic invertebrate communities in North America.

Concern about the status and environmental trends of inland freshwaters in the US (Hawkins et al. 2000, Heinz Center 2002, Hawkins 2006, USEPA 2006) has driven development and application of methods (e.g., measures of taxonomic completeness) that provide a consistent measure of biological integrity across various spatial scales. This effort has been based on collection and compilation of reference-site data (hereafter *least-disturbed*, sensu Stoddard et al. 2006) from across the contiguous US. These data have been used to ascertain the existence of general rules of trait composition over different climatic regions, to assess natural variation in trait composition in least-disturbed systems, and to detect specific impacts to stream ecosystems (Menezes et al. 2010). We tested whether conditions in different climatic regions (ecoregions) of the continental US have selected for ecoregion-specific invertebrate trait compositions (functional characteristics) in least-disturbed streams. We also tested whether different land uses (agricultural or urban) alter the trait composition of streams in ways that go beyond selection imposed by natural conditions (e.g., climate). Our study is the first comprehensive examination of traits across the continental US and provides a foundation for developing trait-based approaches for use in large-scale biomonitoring programs.

Methods

Data description

We used invertebrate data (genus-level identification) from wadeable streams in 1987 basins (Fig. 1). These data were compiled for or collected as part of

the US Environmental Protection Agency's (EPA) Wadeable Stream Assessment (WSA; USEPA 2006) or the US Geological Survey's (USGS) National Water-Quality Assessment (NAWQA) Program. Of the 1987 streams, 1257 were previously designated as least disturbed and formally used to develop measures of taxonomic completeness to make large-scale assessments of biological condition of streams in the eastern US (Carlisle and Meador 2007), western US (Carlisle and Hawkins 2008), and the contiguous 48 states (USEPA 2006, Yuan et al. 2008). WSA data from least-disturbed basins were compiled from several sources: NAWQA, EPA, and Utah State University (Herlihy et al. 2008). Samples were collected with various methods described elsewhere (Cuffney et al. 1993, Moulton et al. 2002, USEPA 2004, Herlihy et al. 2008, Hughes and Peck 2008). Herlihy et al. (2008) and Peterson and Zumberge (2006) used these data to investigate the effects of NAWQA and WSA sampling protocols on measures of assemblage composition and found samples comparable. Carlisle and Hawkins (2008) used a subset of these data to investigate bias associated with data source and found only minimal statistical differences in estimates of taxonomic completeness between NAWQA and WSA samples in the western US. Based on these results, we considered samples collected with WSA and NAWQA methods comparable and of good quality. The remaining 731 basins were classified as developed (>25% basin in agricultural or urban land use) based on the upstream land uses observed in the National Land Cover Dataset (www.mrlc.gov/mrlc2k_nlcd.asp; Homer et al. 2004). Data from streams in developed basins were collected with NAWQA richest targeted habitat protocols (Moulton et al. 2002).

All basins were assigned to level-III ecoregions based on Omernik (1987) and were aggregated into 9 ecoregions as described by Herlihy et al. (2008) as previously defined as the WSA regionalization scheme (USEPA 2006, Yuan et al. 2008). These ecoregions are physiographic provinces with similar climate, vegetation, soil type, and geology and have water resources with similar natural characteristics and similar responses to anthropogenic disturbance (USEPA 2006). The 9 ecoregions are Western Mountains (WM), Xeric West (XR), Northern Appalachians (NA), Southern Appalachians (SA), Northern Plains (NP), Southern Plains (SP), Temperate Plains (TP), Upper Midwest (UM), and Coastal Plain (CP). The USEPA (2006) further aggregated these ecoregions into the mountainous regions Western Mountains (WM + XR), Eastern Highlands (NA + SA), and the Plains and Lowlands (NP + SP + TP + CP + UM).

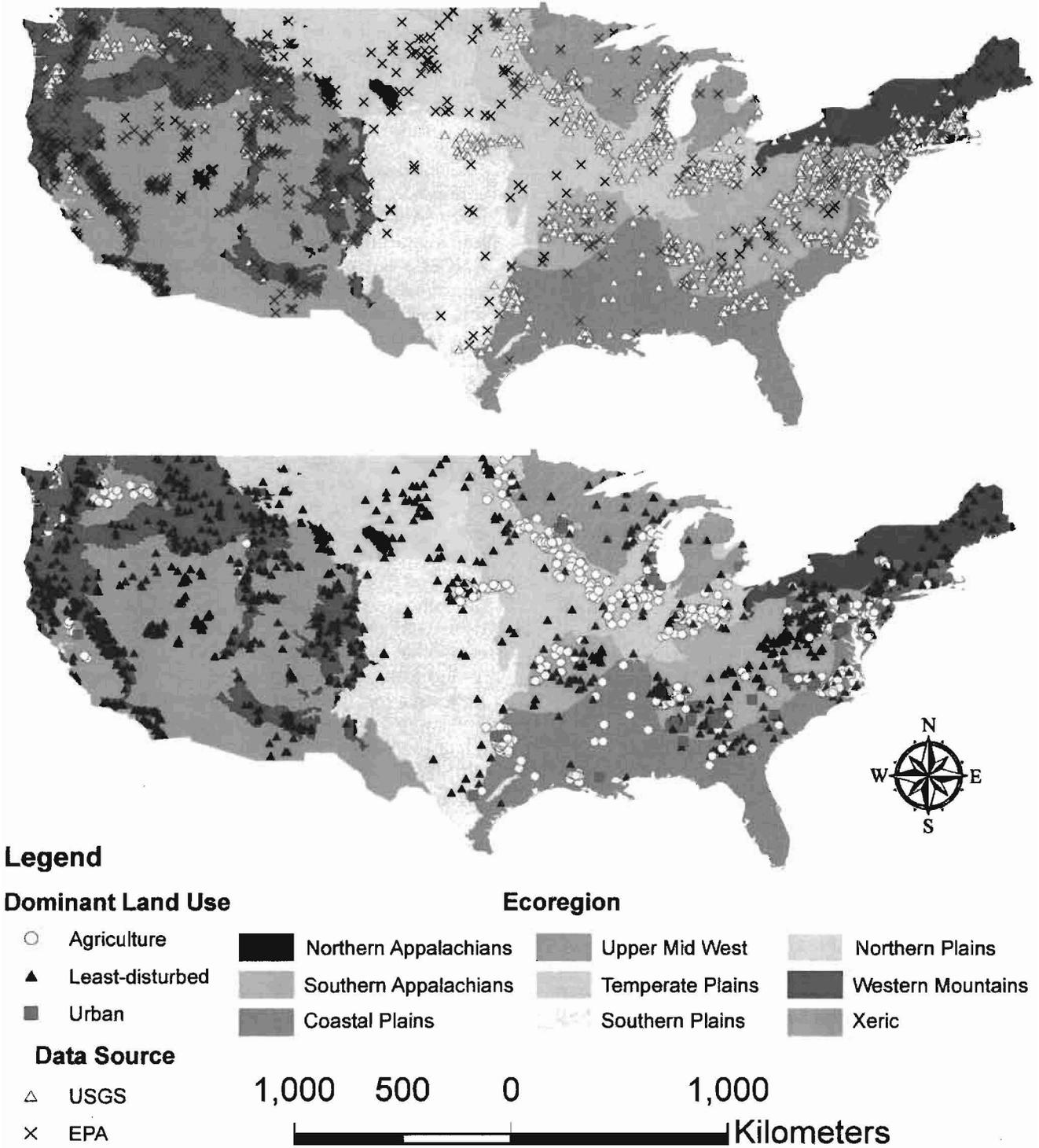


FIG. 1. Map of 1987 sampling sites distributed among 9 ecoregions in the US (modified from USEPA 2006).

We extracted climatic, hydrologic, and landuse data from a geographic information system (GIS) for each sample location. We calculated basin size (km^2), elevation (m), basin slope (%), % agricultural land use, % urban land use, % sand in soil, aquifer permeability (ordinal scale 1–7), mean annual precipitation (cm/y), and mean annual temperature ($^{\circ}\text{C}$) for each basin. Bioclimatic data were derived from the Daymet climate data set (1-km spatial resolution, 1980–1997; www.daymet.org) with methods described by Kumar et al. (2009). We extracted hydrologic data from the USGS hydrologic landscapes data set (<http://water.usgs.gov/GIS/metadata/usgswrd/XML/hlrus.xml>; Wolock et al. 2004).

Invertebrate data preparation

We adjusted the raw data to help account for differences in field and laboratory methods. First, we harmonized taxonomic identifications to WSA operational taxonomic units (OTUs) with methods described by Yuan et al. (2008). Second, we standardized subsampling from the raw counts to 300 by randomly resampling individuals without replacement from samples containing >300 individuals. The goal of assigning OTUs was to ensure individual taxa were consistently counted only once in each sample (Ostermiller and Hawkins 2004, Yuan et al. 2008). After these adjustments, data represented at the OTU level (mostly genus-level, some family, sometimes higher for noninsects) were translated to binary traits listed by Poff et al. (2006) and cross-referenced with the list published by Vieira et al. (2006), expert opinion, and additional literature. Binary traits were represented in 4 trait categories (life history, mobility, morphology, ecology) with a total of 20 traits (e.g., voltinism) and 56 states of these traits (e.g., semivoltine, univoltine, multivoltine) (Table 2). We used binary representation of trait states because of the simplicity of interpretation, but more importantly because national-scale trait information in the US is inconsistent among taxa. When >1 trait state was associated with an OTU by Vieira et al. (2006), the dominant trait state was assigned and cross-referenced with Poff et al. (2006). We checked the list published by Poff et al. (2006) if an OTU was not included by Vieira et al. (2006) and searched the literature in the rare event an OTU was not found in either publication. Last, we calculated the proportion of OTUs having a given trait state for each sample by taking the sum of the occurrence of each trait state and dividing (standardized) by the number of OTUs in the sample and used for all analyses. We avoided closure within a sample (sum

of sample values = 1) by dividing the occurrence of a trait state by the total number of OTUs in the sample because >1 OTU could be assigned the same trait state.

Data analysis

Comparison of invertebrate trait composition among ecoregions and land uses.—We used analysis of similarity (ANOSIM) to test whether trait composition at least-disturbed basins differed among ecoregions. ANOSIM is based on a nonparametric permutation procedure applied to the rank similarity matrix (in this case, Bray–Curtis similarity calculated from $\sqrt{(x)}$ -transformed proportional data) that compares the degree of separation between predefined groups (e.g., ecoregions) with the test statistic, R (Clarke and Warwick 2001). Values of R near 0 indicate no distinguishable separation between groups, whereas values near 1 indicate complete separation. The R test statistic is first calculated as a global test to determine if differences are present between groups. Pairwise comparisons are examined if the global test is significant (<5% of the 999 permuted values > global R). We also used ANOSIM to test for differences in trait composition among least-disturbed, agricultural, and urban basins within each ecoregion. We interpreted significant R -values >0.70 as indicative of strong differences, 0.40 to 0.70 as indicative of moderate differences, and <0.40 as indicative of weak differences. We ran ANOSIM analyses with PRIMER-E software (version 6.1; PRIMER-E, Plymouth Marine Laboratory, UK).

Ecoregional differences in invertebrate traits of least-disturbed basins.—We described the trait composition of least-disturbed basins in each ecoregion relative to the average median value of each trait state by visually examining box plots. We calculated the average median value of each trait state as the median of average trait-state values in the 9 ecoregions. This approach accounted for differences in the number of sites associated with each ecoregion. When the interquartile range of the distribution of a trait state within an ecoregion was above the average median value, then that trait state was considered positively favored (+) (Fig. 2). When the interquartile range was below the average median value, then that trait state was considered negatively favored (–).

Ecoregional differences in invertebrate traits among land-use classifications.—We compared trait composition among least-disturbed, agricultural, and urban basins within ecoregions. In this case, we compared the interquartile range of each trait state from

TABLE 1. Mean (SD) values of environmental characteristics of basins classified by land use (least-disturbed [LD], agricultural [AG], urban [URB]), and grouped by ecoregion. *n* = the number of basins. Aquifer permeability is scored from 1 (lowest) to 7 (highest). WM = Western Mountains, XR = Xeric, NP = Northern Plains, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, NA = Northern Appalachians, SA = Southern Appalachians, CP = Coastal Plain.

Ecoregion	Land use	<i>n</i>	Size (km ²)	Elevation (m)	Slope (%)	% AG	% URB	% sand in soil	Aquifer permeability	Precipitation (cm/y)	Mean annual temperature (°C)
WM	LD	546	238 (976)	1703 (755)	10.58 (4.49)	0.09 (0.61)	0.58 (1.29)	43 (13)	1.72 (1.16)	71.89 (40.78)	9.17 (14.24)
	AG	26	278 (468)	80 (42)	1.42 (1.27)	55.99 (21.25)	10.70 (6.72)	20.46 (12.53)	5.35 (1.05)	133.09 (12.19)	10.88 (17.39)
	URB	15	48 (42)	72 (26)	1.59 (0.67)	4.14 (7.81)	79.99 (15.81)	31.96 (20.55)	5.57 (0.56)	125.64 (7.14)	10.92 (17.39)
XR	LD	173	270 (916)	1616 (811)	7.21 (3.26)	0.65 (3.62)	1.26 (3.33)	39.77 (11.09)	3.11 (1.74)	36.91 (18.50)	10.93 (13.91)
	AG	36	1203 (1432)	417 (401)	2.62 (2.29)	57.76 (21.62)	4.98 (3.31)	24.58 (14.09)	4.85 (0.92)	38.190 (17.44)	10.84 (14.84)
	URB	18	528 (1012)	575 (540)	6.77 (3.24)	2.04 (2.70)	50.40 (23.92)	50.72 (11.88)	4.10 (0.81)	54.30 (10.61)	14.33 (13.59)
NA	LD	92	130 (204)	247 (151)	3.15 (1.88)	7.37 (10.23)	5.57 (5.68)	39.10 (11.35)	1.75 (1.23)	111.07 (7.17)	7.46 (16.14)
	AG	12	1246 (2619)	177 (114)	2.43 (1.09)	36.54 (9.48)	6.49 (3.39)	33.21 (12.46)	2.03 (1.29)	109 (6.95)	8.15 (16.96)
	URB	36	148 (343)	55 (43)	1.28 (0.61)	4.74 (3.72)	46.14 (18.29)	49.14 (9.52)	1.60 (0.88)	117.11 (6.62)	9.62 (16.87)
SA	LD	215	295 (776)	360 (223)	3.20 (2.29)	17.79 (16.43)	4.98 (4.36)	26.00 (7.24)	3.58 (1.95)	117.22 (15.54)	12.32 (15.49)
	AG	129	609 (1740)	180 (93)	1.59 (1.20)	56.72 (17.51)	9.57 (6.24)	24.87 (7.64)	3.19 (2.01)	113.79 (14.27)	12.46 (16.09)
	URB	86	65 (112)	162 (84)	1.20 (0.64)	8.02 (7.78)	60.94 (22.89)	29.90 (6.57)	2.22 (1.59)	123.60 (15.62)	13.99 (15.67)
NP	LD	44	2386 (4581)	862 (347)	2.01 (2.01)	25.14 (25.84)	1.91 (1.64)	30.80 (10.37)	1.91 (0.73)	42.67 (6.22)	6.69 (16.58)
SP	LD	39	2105 (5516)	723 (413)	1.13 (0.73)	15.23 (14.98)	1.88 (1.71)	27.96 (22.75)	4.79 (1.82)	62.03 (15.74)	12.18 (13.75)
	AG	17	576 (539)	532 (118)	0.59 (0.33)	56.51 (21.34)	5.16 (2.79)	25.57 (18.65)	5.69 (1.05)	66.90 (6.73)	10.15 (15.81)
TP	LD	69	1259 (3908)	322 (121)	0.56 (0.24)	52.81 (31.99)	4.49 (2.78)	25.12 (10.15)	2.23 (1.51)	76.04 (22.87)	7.98 (14.53)
	AG	184	2862 (9899)	255 (61)	0.46 (0.21)	78.52 (11.87)	7.91 (4.01)	23.14 (10.21)	3.20 (1.83)	87.92 (14.36)	8.99 (15.81)
	URB	29	121 (297)	206 (20)	0.50 (0.19)	6.52 (7.27)	78.23 (13.98)	17.32 (5.35)	4.32 (1.51)	90.74 (5.51)	9.17 (16.97)
UM	LD	28	520 (1898)	335 (92)	0.77 (0.50)	15.75 (19.05)	4.14 (2.31)	59.39 (15.54)	2.72 (1.44)	78.75 (4.66)	5.66 (16.42)
	AG	12	4016 (12,026)	269 (47)	0.478 (0.30)	67.18 (19.22)	6.75 (5.69)	43.85 (16.93)	2.73 (1.65)	73.56 (7.64)	6.35 (16.64)
	URB	9	181 (258)	249 (27)	0.58 (0.19)	9.30 (8.63)	63.67 (17.01)	50.51 (16.07)	3.10 (1.35)	75.76 (1.50)	7.28 (17.10)
CP	LD	51	256 (986)	50 (40)	0.41 (0.27)	28.20 (14.54)	3.53 (2.27)	52.86 (13.79)	4.04 (1.77)	122.13 (10.83)	16.64 (15.55)
	AG	94	1545 (6826)	54 (56)	0.31 (0.24)	55.02 (19.14)	6.13 (4.38)	36.07 (22.97)	3.49 (1.87)	118.29 (18.43)	16.52 (15.30)
	URB	27	87 (98)	59 (58)	0.56 (0.24)	6.30 (6.64)	68.26 (19.91)	44.67 (25.31)	2.88 (1.60)	110.93 (16.04)	14.59 (14.44)

TABLE 2. Trait categories, traits, and trait-state descriptions applied to macroinvertebrate samples collected from 1987 Wadeable stream basins (modified from Poff et al. 2006).

Category	Trait	Trait state description
Life history	Voltinism	Semivoltine (<1 generation/y)
		Univoltine (1 generation/y)
		Bi- or multivoltine (>1 generation/y)
	Development	Fast seasonal
		Slow seasonal
		Nonseasonal
Synchronization of emergence	Poorly synchronized (wk)	
	Well synchronized (d)	
Adult life span	Very short (<1 wk)	
	Short (<1 mo)	
	Long (>1 mo)	
Adult ability to exit Ability to survive desiccation Female dispersal	Present	
	Present	
	Low (<1 km flight before laying eggs)	
Mobility	Adult flying strength	High (>1 km flight before laying eggs)
		Weak (e.g., cannot fly into light breeze)
	Occurrence in drift	Strong
		Rare (catastrophic only)
		Common (typically observed)
	Maximum crawling rate	Abundant (dominant in drift samples)
Very low (<10 cm/h)		
Swimming ability	Low (<100 cm/h)	
	High (>100 cm/h)	
	None	
Morphology	Attachment	Weak
		Strong
		None (free-ranging)
	Armoring	Some (sessile, sedentary)
		Both
	Shape Respiration	None (soft-bodied forms)
Poor (heavily sclerotized)		
Size at maturity	Good (e.g., some cased caddisflies)	
	Streamlined (flat, fusiform)	
	Tegument	
Rheophily	Gills	
	Plastron, spiracle (aerial)	
	Small (<9 mm)	
Ecology	Thermal preference	Medium (9–16 mm)
		Large (>16 mm)
		Depositional only
Habit	Thermal preference	Depositional and erosional
		Erosional
		Cold stenothermal or cool eurythermal
	Trophic habit	Cool/warm eurythermal
		Warm eurythermal
		Burrow
Trophic habit	Habit	Climb
		Sprawl
		Cling
	Trophic habit	Swim
		Skate
		Collector-gatherer
Trophic habit	Trophic habit	Collector-filterer
		Herbivore (scraper, piercer, and shedder)
		Predator (piercer and engulfer)
		Shredder (detritivore)

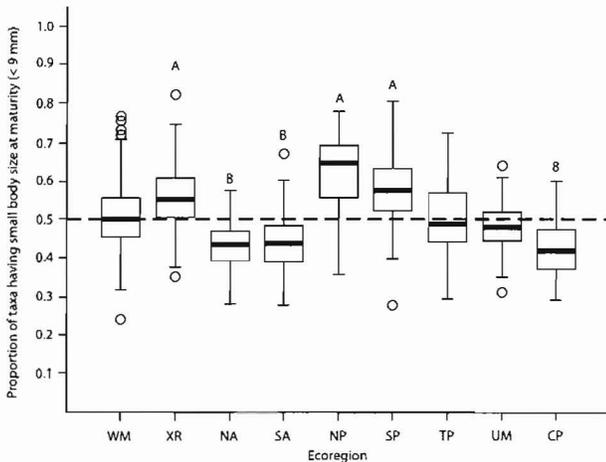


FIG. 2. Example box-and-whisker plot showing the distribution of the proportion of taxa having small body size at maturity (<9 mm) for each ecoregion relative to the average median value (dashed line) for 1257 least-disturbed sites. Boxes represent interquartile range (horizontal line = median), whiskers show values $>1.5\times$ the interquartile range, and open circles indicate outliers. Letters indicate that the trait state was either positively (A) or negatively (B) favored in the associated ecoregion when the interquartile range of the trait state was either above (positively favored) or below (negatively favored) the average median value of all ecoregions (dashed line). We examined similar plots for all trait states to determine which were negatively or positively favored in each ecoregion. WM = Western Mountains, XR = Xeric, NP = Northern Plains, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, NA = Northern Appalachians, SA = Southern Appalachians, CP = Coastal Plain.

agricultural and urban basins to the interquartile range from least-disturbed basins. If the interquartile range from agricultural or urban basins was below the interquartile range of least-disturbed basins, then we considered that trait state as having decreased (–) at agricultural or urban basins in that ecoregion. If the interquartile range was above the interquartile range of least-disturbed basins, then we considered the trait state as having increased (+) at agricultural or urban basins in that ecoregion. No agricultural or urban basins were present in the NP ecoregion, and no urban basins occurred in the SP ecoregion.

Associations between invertebrate trait assemblages and environmental characteristics among land uses.—We used the RELATE (Mantel test equivalent) routine in the PRIMER-E software package to identify relationships between selected environmental characteristics and traits among least-disturbed, agricultural, and urban basins within each ecoregion (Clarke and Warwick

2001). The RELATE routine computes the strength of the relationship between 2 independently derived matrices as the Spearman rank correlation coefficient (Spearman's ρ ; Kendall 1970). In this case, one matrix contained Bray–Curtis similarities calculated from the trait data and the other contained Euclidian distances calculated from selected environmental characteristics. Spearman's ρ will be close to 1 if rank distances from environmental and trait matrices among basins match exactly, whereas ρ will be near 0 when the rank distances do not match. The RELATE routine incorporates a significance test derived by permutation to test the null hypothesis of complete absence of match between the 2 matrices (Clarke and Warwick 2001). We considered ρ values significant when $\leq 5\%$ of the permuted ρ values were greater than the observed ρ value. We interpreted ρ values >0.70 as indicative of a strong match, 0.40 to 0.70 as indicative of a moderate match, and <0.40 as indicative of a weak match. We selected environmental characteristics that typically separate biologically similar groups of least-disturbed basins (e.g., Carlisle and Meador 2007, Carlisle and Hawkins 2008) for this analysis. These variables included latitude and longitude (decimal degrees), basin size (km^2), elevation (m), % basin slope, % sand in the soil, aquifer permeability (ordinal 1–7), mean annual precipitation (cm/y), and mean annual temperature ($^{\circ}\text{C}$). We $\log(x)$ -transformed variables when necessary to meet assumptions of normality, and we normalized all variables to put the different measurement types on the same scale (Clarke and Warwick 2001).

Results

Differences in environmental variables among landuse classes and ecoregions

Evaluation of environmental characteristics among landuse classifications showed some biases associated with sampling locations within and among ecoregions (Table 1). Mean % agricultural land use in least-disturbed basins varied greatly (WM: 0.09%, TP: 52.81%). Mean % urban land use in least-disturbed basins varied much less (WM: 0.58%, NA: 5.57%). In the Eastern Highlands (NA and SA), elevation of least-disturbed basins was higher than elevation of urban and agricultural basins. In the WM ecoregion, agricultural and urban basins had lower elevations, shallower slopes, and more precipitation than least-disturbed basins. Most WM basins occur in the Pacific Northwest Coastal Mountains, a result suggesting a geographic bias among land uses in the WM ecoregion.

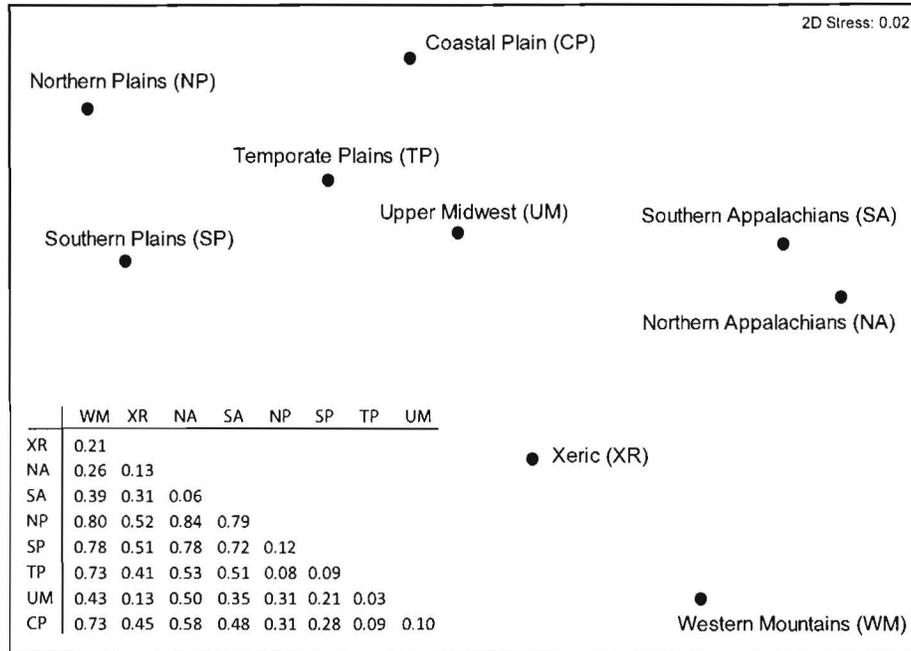


FIG. 3. Nonmetric multidimensional scaling ordination of analysis of similarity (ANOSIM) *R*-values from pairwise comparisons of traits among ecoregions assembled from 1257 least-disturbed basins distributed across the US. Inset shows *R*-values among ecoregions used to construct the ordination. Global ANOSIM *R* = 0.43, *p* ≤ 0.1%. *R*-values >0.70 indicate strong differences, 0.40 to 0.70 indicate moderate differences, and <0.40 indicate weak differences.

Invertebrate trait composition at least-disturbed basins

Throughout the results, trait categories are italicized and traits and trait states are in quotation marks to distinguish them from general descriptors of results. Trait composition differed among ecoregions (ANOSIM, global *R* = 0.43, significance level ≤ 0.1%). The strongest differences (*R* > 0.7) occurred between mountainous regions (Western Mountains and Eastern Highlands) and the Plains and Lowlands (NP + SP + TP + CP + UM) with a few exceptions (Fig. 3). For example, TP streams were strongly different from WM streams (*R* = 0.73) but only moderately different from XR, NA, and SA streams (*R* = 0.41, 0.53, 0.51), whereas UM streams were moderately different from WM (*R* = 0.43) and NA (*R* = 0.50) streams. Trait compositions of least-disturbed streams appeared most similar between WM and XR ecoregions (*R* = 0.21), between NA and SA ecoregions (*R* = 0.06), and among the Plains and Lowlands (*R* range = 0.03–0.31).

The associations of individual traits with ecoregions differed strongly, and most differences were in the *life-history* and *ecology* trait categories, whereas fewer differences were observed in the *mobility* and *morphology* trait categories (Table 3; see Appendix S1, available online from: <http://dx.doi.org/10.1899/11-150.1.s1> for differences in trait states).

WM and XR

Favored *life-history* trait states in least-disturbed WM streams were linked to seasonally timed stream flows and temperatures (e.g., “univoltine” taxa having “fast seasonal development”, “well synchronized emergence”, and “very short” or “short adult life span”; Table 3, Appendix S1). Favored *mobility* trait states included average to weak larval (e.g., “occurrence in drift”, “maximum crawling rate”, “swimming ability”) and adult (e.g., “flying strength”) dispersal ability. Favored *ecological* trait states in the WM basins suggested that taxa in this ecoregion have narrow flow and thermal preferences (e.g., taxa preferring “erosional” habitat and “cold stenothermal” or “cool eurythermal” environments that are “clingers” and “shredders”). In general, these trait states indicate that WM invertebrate assemblages may be susceptible to alterations of flow and temperature regimes. Overall, WM basins had the most trait states that differed from average median trait-state values among ecoregions.

Trait states of XR basins were similar to those of WM basins (e.g., “fast seasonal development”, “well-synchronized emergence”, “rheophily”, and “thermal preference”). However, fewer trait states differed from average median values in XR basins than in WM basins. Taxa with “small body size” of mature

TABLE 3. The ecoregional affinity of each trait category observed from 1257 least-disturbed basins. X = a category that had ≥ 1 trait state where the interquartile range was above or below the average median value for all ecoregions. See Appendix S1 for results for all 56 trait states. WM = Western Mountains, XR = Xeric West, NP = Northern Plains, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, NA = Northern Appalachians, SA = Southern Appalachians, CP = Coastal Plain. – = no differences were observed.

Trait categories	Traits	WM	XR	NA	SA	NP	SP	TP	UM	CP
Life history	Voltinism	X	–	X	X	X	X	–	X	–
	Development	X	X	X	X	X	X	X	X	X
	Synchronization of emergence	X	X	–	–	–	–	X	–	–
	Adult life span	X	X	X	X	X	X	X	–	X
	Adult ability to exit	X	–	X	–	X	–	X	–	–
Mobility	Ability to survive desiccation	X	–	–	–	X	–	X	–	–
	Female dispersal	X	X	X	–	X	X	X	–	X
	Adult flying strength	X	–	X	–	X	X	X	–	X
	Occurrence in drift	X	–	X	–	X	X	–	–	–
	Maximum crawling rate	–	–	X	–	–	–	–	X	X
Morphology	Swimming ability	X	–	–	–	–	–	–	–	–
	Attachment	–	–	X	X	X	–	–	–	–
	Armoring	–	X	X	–	X	X	–	X	X
	Shape	–	–	–	–	–	–	–	–	–
	Respiration	–	–	–	–	–	–	–	–	–
Ecology	Size at maturity	X	X	X	X	X	X	–	–	X
	Rheophily	X	X	X	X	X	X	X	–	X
	Thermal preference	X	X	X	X	–	–	X	–	X
	Habit	X	–	X	X	X	X	–	X	–
	Trophic habit	X	–	X	–	X	X	X	X	X

larvae and more “armoring” were favored in XR basins, a result suggesting that XR communities are adapted to withstand variable flows.

NA and SA

Several similarities in trait states were observed between WM and XR basins and Eastern Highlands (NA and SA) basins (e.g., few taxa with “long adult life spans”, and preferring “erosional” habitats), but many distinct differences were found relative to average median trait-state values (Table 3, Appendix S1). For example, *life-history* traits favored in the Eastern Highlands included “semivoltinism” and “slow seasonal development,” whereas these traits were not favored in the WM and XR. *Morphology* traits favored in Eastern Highlands basins included “attachment” (e.g., sessile, sedentary). *Life-history*, *morphology*, and *ecology* trait-state preferences suggest that assemblages in Eastern Highland basins are adapted for environmentally stable, “erosional” habitats and flows with “cool to warm” thermal regimes.

NP, SP, and TP

Trait states favored in NP basins included “bi- or multivoltinism”, “fast seasonal development”, “small size”, “desiccation tolerant”, and highly mobile larvae

(“dominant in drift samples”, “free-ranging”) and adults (“ability to exit”, “high female dispersal”) and “long adult life spans” (Table 3, Appendix S1). These trait states suggest the need to escape or tolerate changing habitats or otherwise harsh conditions. *Ecology* trait states associated with these basins favored adaptations to slower moving water (e.g., “depositional only”, “collector-gatherer”). Trait states favored in SP basins were similar to those favored in NP basins, except that many of the *life-history* trait states associated with adult mobility (e.g., “adult flying strength” and “female dispersal”) and the “ability to survive desiccation” favored in NP basins were not favored in SP basins. Traits favored in TP basins were similar to those in NP and SP basins except that taxa with “nonseasonal development” and “warm eurythermal” preferences were prominent.

UM and CP

Favored trait states in UM and CP basins suggest that the mix of mountainous and plains habitats in these ecoregions selects for trait states preferred in many other ecoregions. For example, in UM basins, “voltinism” and “trophic habit” traits were preferred as they were in WM basins, “development” and “crawling rate” were preferred as in NA basins, and “armoring” and “thermal preference” were preferred

TABLE 4. Results of Analysis of Similarity testing for differences in invertebrate trait composition among least-disturbed, agricultural, and urban basins within ecoregions. $R > 0.7$ indicates strong differences, 0.4 to 0.7 indicates moderate differences, and <0.4 indicates weak differences. WM = Western Mountain, XR = Xeric West, NA = Northern Appalachian, SA = Southern Appalachian, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, CP = Coastal Plain.

Test	WM	XR	NA	SA	SP	TP	UM	CP
Global R (% significance)	0.80 (0.1)	0.60 (0.1)	0.51 (0.1)	0.28 (0.1)	-0.03 (69.5)	0.13 (0.1)	0.50 (0.1)	-0.01 (66.2)
Pairwise comparison R (% significance)								
Least-disturbed vs agricultural	0.86 (0.1)	0.51 (0.1)	0.29 (0.3)	0.15 (0.1)	-0.04 (72.9)	0.01 (0.2)	0.36 (0.1)	-0.02 (70.5)
Least-disturbed vs urban	0.71 (0.1)	0.80 (0.1)	0.60 (0.1)	0.52 (0.1)	0.02 (45.0)	0.14 (1.5)	0.78 (0.1)	0.13 (0.1)
Agricultural vs urban	0.04 (22.1)	0.21 (0.3)	0.19 (1.5)	0.18 (0.1)	0.41 (22.2)	0.16 (0.4)	0.17 (2.8)	-0.04 (70.5)

as in NP basins (Table 3, Appendix S1). In addition, favored “univoltinism” and “slow seasonal development” trait states suggests that, on average, UM basin habitats are stable.

The traits and trait states of CP basins resembled those in the Eastern Highlands (e.g., “size at maturity” and “thermal preference”) and other plains environments (“synchronization of emergence”, “female dispersal”). However, a few favored trait states were unique to the ecoregion (“predators”, “high maximum crawling rate”, “rheophily”, “depositional”, and “erosional” environments).

Differences in invertebrate trait composition among basin land uses

The ability of trait composition to distinguish among basin land uses (ANOSIM) differed among ecoregions (Table 4). For example, moderate to strong differences in trait composition among landuse classes were observed in WM (global $R = 0.80$), XR (global $R = 0.60$), NA (global $R = 0.51$), and UM (global $R = 0.50$), but only weak differences were detected in the remaining ecoregions (Table 4). Where global differences were observed (WM, XR, NA, UM), trait compositions of agricultural and urban basins were nearly indistinguishable (pairwise comparison R values range = -0.04 – 0.21). In contrast, differences between least-disturbed basins and agricultural or urban basins varied by ecoregion. Overall, the strongest differences were observed between least-disturbed and urban basins except in WM where strong differences also were observed between least-disturbed and agricultural basins.

Trait differences between least-disturbed and agricultural basins.—Relative to least-disturbed basins, favored trait states in WM basins were “bi- or multivoltine” taxa with “nonseasonal development”, “poorly synchronized emergence”, “long adult life span” with the “ability to exit”, “survive desiccation”, “high female dispersal”, and a preference for the habit “burrow” (Table 5, Appendix S2; available

online from: <http://dx.doi.org/10.1899/11-150.1.s1>). Fewer favored trait states were noted in XR and UM, but in most cases, they were subsets of those in WM. Overall, more *life-history* traits than other trait categories differed between least-disturbed and agricultural basins.

Trait differences between least-disturbed and urban basins.—Traits differed more between least-disturbed and urban basins than between least-disturbed and agricultural basins. Differences were similar in most mountainous regions (Table 6; Appendix S3, available online from: <http://dx.doi.org/10.1899/11-150.1.s1>). Relative to least-disturbed basins, urban basins generally selected for below average “slow seasonal development”, “low female dispersal”, “weak adult flying”, “weak ability to swim”, and “climbers”; and above average “adult ability to exit”, “large size at maturity”, and “burrowers.”

Relationships between trait composition and environmental variables among land uses and ecoregions

Environmental variables were weakly associated with differences in trait composition among land uses within ecoregions. The strongest association between environmental variables and traits was observed in WM (RELATE, $\rho = 0.28$, % significance = 0.1) and XR ($\rho = 0.25$, % significance = 0.1). Associations were weak in NA ($\rho = 0.15$, % significance = 0.2), SA ($\rho = 0.18$, % significance = 0.1), UM ($\rho = 0.13$, % significance = 5.6), SP ($\rho = 0.18$, % significance = 0.6), TP ($\rho = 0.15$, % significance = 0.1), and CP ($\rho = 0.17$, % significance = 0.1).

Discussion

Differences in invertebrate trait assemblages among ecoregions

Trait composition of invertebrate communities in least-disturbed basins varies across the contiguous US. Ecoregions, physiographic provinces with similar natural characteristics and similar responses to stress

TABLE 5. Trait categories in which differences in trait states were measured between least-disturbed and agricultural basins. X = a category that had ≥ 1 trait state for which the interquartile range was above or below the average median value for all ecoregions. See Appendix S2 for results for all 56 trait states. WM = Western Mountains, XR = Xeric West, NP = Northern Plains, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, NA = Northern Appalachians, SA = Southern Appalachians, CP = Coastal Plain. - = no differences were observed.

Trait categories	Traits	WM	XR	NA	SA	NP	SP	TP	UM	CP
Life history	Voltinism	X	-	-	-	-	-	-	X	-
	Development	X	X	-	-	-	-	-	X	-
	Synchronization of emergence	X	-	-	-	-	-	-	-	-
	Adult life span	X	X	-	-	-	-	-	X	-
	Adult ability to exit	X	X	X	-	-	-	-	X	-
	Ability to survive desiccation	X	-	-	-	-	-	-	-	-
Mobility	Female dispersal	X	X	-	-	-	X	-	-	-
	Adult flying strength	X	X	-	-	-	-	-	-	-
	Occurrence in drift	-	-	-	-	-	-	-	-	-
	Maximum crawling rate	-	-	-	-	-	-	-	-	-
	Swimming ability	X	-	-	-	-	-	-	-	-
	Attachment	X	-	-	-	-	-	-	-	-
Morphology	Armoring	X	-	-	-	-	-	-	-	-
	Shape	-	-	-	-	-	-	-	X	-
	Respiration	-	-	-	-	-	-	-	-	-
	Size at maturity	X	X	-	-	-	-	-	-	-
	Rheophily	X	X	-	-	-	-	-	-	-
Ecology	Thermal preference	X	-	-	-	-	X	-	-	-
	Habit	X	X	X	X	-	X	-	-	-
	Trophic habit	-	-	-	-	-	-	-	X	-

(e.g., USEPA 2006), are unique habitat templates (Southwood 1977, Townsend and Hildrew 1994, Poff 1997, Statzner et al. 2004) that organize the trait composition of invertebrates in US streams. Therefore, trait-based assessments of biological integrity will require regionalization (sensu Lenat 1993). Recent work limited to the western US supports our findings that trait composition of invertebrate assemblages is region specific (Poff et al. 2010). In contrast, trait assemblages are relatively stable across large spatial scales and natural environmental gradients in Europe (Menezes et al. 2010).

The different conclusions drawn from studies in Europe and the US require explanation. One possibility is that patterns are more similar between the continents than they appear. Investigators in Europe used analytical methods (fuzzy-coded traits weighted by taxon-abundance data) that may be less sensitive to trait differences among ecoregions than our methods. However, other explanations can be offered for the higher degree of functional redundancy in European than in US streams. Physiographic (e.g., elevation), climatic (e.g., precipitation, temperature), and spatial (e.g., latitude and longitude, size of continent) gradients, are more pronounced in the continental US than in Europe. Europe has 2 main climatic regimes (temperate and Mediterranean) and a mix of physiographic provinces (mountains and

lowlands) (Statzner et al. 2001, Bonada et al. 2007), whereas the US has mountainous regions with differing climatic/hydrologic regimes (WM, NA, and SA), deserts (XR), and cool dry plains (SP) vs warm humid plains (CP). Statzner et al. (2001) applied a species-filter paradigm (sensu Poff 1997) to European invertebrate assemblages. They concluded that the pattern of functional redundancy observed in Europe had 2 possible explanations: 1) local-scale factors filtered traits similarly at study streams across large spatial scales or 2) continental-scale factors filtered traits similarly across all study streams. Bêche and Statzner (2009) and Statzner and Bêche (2010) also found high functional redundancy among streams of the US and little change in traits with spatial factors (longitude and latitude). However, their findings were based on different measures of trait composition than ours and were derived from a data set that was geographically biased toward wetter regions of the US.

Traits of stream invertebrate assemblages in the US are organized geographically. Therefore, trait composition characteristic of least-disturbed basins will have to be defined for each ecoregion to be useful for trait-based biomonitoring. We detected differences in trait composition among the 9 ecoregions, but we see opportunities to generalize patterns at a larger spatial scale. For example, patterns detected in our trait-

TABLE 6. Trait categories in which differences in trait states were measured between least-disturbed and urban basins. X = a category that had ≥ 1 trait state for which the interquartile range was above or below the average median value for all ecoregions. See Appendix S3 for results for all 56 trait states. WM = Western Mountains, XR = Xeric West, NP = Northern Plains, SP = Southern Plains, TP = Temperate Plains, UM = Upper Midwest, NA = Northern Appalachians, SA = Southern Appalachians, CP = Coastal Plain. – = no differences were observed.

Trait categories	Traits	WM	XR	NA	SA	NP	SP	TP	UM	CP
Life history	Voltinism	X	X	–	–	–	–	–	X	–
	Development	X	X	X	X	–	–	X	X	–
	Synchronization of emergence	X	X	–	–	–	–	–	–	–
	Adult life span	X	X	X	X	–	–	X	X	–
	Adult ability to exit	X	X	X	X	–	–	X	X	–
	Ability to survive desiccation	X	–	–	–	–	–	–	–	–
Mobility	Female dispersal	X	X	X	X	–	–	X	–	–
	Adult flying strength	X	X	X	X	–	–	X	–	–
	Occurrence in drift	X	X	–	–	–	–	–	X	–
	Maximum crawling rate	X	–	–	–	–	–	X	–	–
	Swimming ability	X	X	X	X	–	–	–	X	–
Morphology	Attachment	X	–	–	–	–	–	–	–	–
	Armoring	–	–	X	–	–	–	–	X	–
	Shape	–	–	–	–	–	–	–	–	–
	Respiration	–	–	X	–	–	–	–	X	–
	Size at maturity	X	X	X	–	–	–	X	X	–
Ecology	Rheophily	X	X	–	–	–	–	–	–	–
	Thermal preference	–	–	–	–	–	–	–	–	–
	Habit	X	X	X	X	–	–	X	X	–
	Trophic habit	–	X	–	–	–	–	–	X	–

based study were similar to patterns found in the EPA's taxonomy-based study (USEPA 2006). In both studies, WM and XR ecoregions could be aggregated as Western Mountains; TP, NP, SP, UM, and CP as Plains and Lowlands; and NA and SA as Eastern Highlands. These generalizations are a first attempt at defining regions by invertebrate trait composition of least-disturbed basins. Further research could focus on a more comprehensive comparison among ecoregions. Such studies might relate trait composition to unique habitat and local-scale environmental variables to further test these generalizations at a smaller scale.

The traits that differentiated least-disturbed basins among ecoregions were generally from 2 trait categories: *life history* and *ecology*. The highly variable (unpredictable or frequent change) environmental setting in the Plains appeared to select for *life-history* strategies that confer an advantage in unpredictable flows and *ecology* traits that confer advantage in soft-bottomed streams. In contrast, traits favored in the Western Mountains were *life-history* traits that confer advantage in temporally predictable environments and *ecology* traits that confer advantage in fast-flowing cold water. The environmental setting in the Eastern Highlands appeared to select for *life-history* traits that confer advantage in streams that are hydrologically predictable on an annual or even longer time scale, as evidenced by the strong presence of semivoltine taxa.

Trait response to land use

The ability to differentiate invertebrate trait composition between least-disturbed basins and agricultural and urban basins depended on ecoregion and possibly on landuse characteristics in least-disturbed basins. Gradients of environmental characteristics (i.e., basin area, elevation, stream order) influence structure and function of stream ecosystems (Southwood 1977, Vannote et al. 1980, Poff 1997, Statzner et al. 2004, Hawkins 2006). The effects of environmental variables can be difficult to separate from the effects of disturbance on stream assemblages. We expected the strong environmental gradients in mountainous regions to confound our ability to identify differences in trait composition among least-disturbed, agricultural, and urban basins. For example, most urban and agricultural basins in WM were in the Pacific Northwest Coastal Mountains. Environmental characteristics and the species pool differ between the Pacific Northwest and the more continental part of the WM. Thus, this geographic bias in basin land use might lead to misinterpretation of natural differences in trait composition as responses to agricultural or urban land use. However, our RELATE analysis suggested that the environmental characteristics we evaluated were only weakly associated with trait composition despite the geographic

bias in some ecoregions. Future investigators might attempt to acquire more data from urban or agricultural basins in the Rocky Mountain region of the WM ecoregion.

Differences among ecoregions in the quality of least-disturbed basins probably influence the ability to detect differences in trait composition within an ecoregion, particularly in the plains. In mountainous ecoregions, only a small percentage of the area of least-disturbed basins was developed (urban or agricultural land uses). However, in the plains, particularly TP, most of the area of least-disturbed basins was used for agriculture. Agricultural land use is often a strong filter for traits (Larsen and Ormerod 2010, Cuffney et al. 2011), so trait composition might not differ between least-disturbed and agricultural basins in regions where least-disturbed basins are influenced by agricultural land uses.

No unique set of traits distinguished agricultural or urban basins from least-disturbed basins. In general, agricultural land use altered *life-history* traits more than other trait categories, whereas urban land use tended to alter traits in all 4 categories. Differences in trait composition among landuse categories (especially for agricultural land use) were markedly stronger in certain ecoregions than in others, but the direction of differences between least-disturbed and urban/agricultural basins was generally similar across ecoregions. Differences between least-disturbed and urban basins were generally similar in magnitude and direction among aggregated regions (Western US, Eastern Highlands, and Plains and Lowlands). This result suggests that certain traits may show a general *disturbance syndrome* in benthic invertebrate assemblages across geographic and physiographic regions.

Implications for bioassessment and future research

Use of traits in assessments of biological integrity has been advocated in recent years (Statzner et al. 2001, Menezes et al. 2010). The advantages of trait-based approaches over taxonomy-based approaches are well known (Poff 1997), but the utility of trait-based approaches across large scales is unknown. We showed that trait-based assessments of biological integrity will require regional development and calibration of metrics to capture regional differences in environmental characteristics and quality of least-disturbed basins. We found differences in trait composition among 9 ecoregions that might be relevant for smaller-scale regional assessments of biological integrity. It might be possible to develop

trait-based tools at aggregated regional scales, such as Western US, Plains and Lowlands, and Eastern Highlands. However, such aggregations should be done with caution and regard to stream type (i.e., xeric vs alpine) because comparisons with least-disturbed trait composition at the very large scale would have limited interpretive value and might not be scientifically defensible. We used a coarse measure of differences between trait composition at least-disturbed, agricultural, and urban basins (separation between interquartile ranges), but our results suggest that the magnitude of change in a trait state might be consistent within large regional areas. If trait based approaches prove to be more powerful than taxonomic-based assessments of biological integrity, then the trends we observed should persist even in smaller scale, more robust analysis.

Data gaps will have to be filled in future evaluations of trait-based approaches at regional or larger scales to tease apart the different effects of agricultural and urban disturbances. For example, new sampling locations could reduce geographic bias in data from agricultural and urban land-use basins in the western US (Western Mountains and Xeric ecoregions) and from urban basins in the Southern Plains ecoregion. The number of urban and agricultural basins sampled in the Plains states could be increased. New basins representing all landuse types (least disturbed, urban, and agricultural) must be identified in the Rocky Mountain area to remove the geographic bias in our database. Filling these data gaps will refine future trait-based assessments of landuse effects on stream assemblages at ecoregional and aggregated regional scales.

We were unable to identify a suite of traits or syndrome that could be used to diagnose biological impairment caused by agricultural or urban land uses across the continental US. These land uses probably do not affect stream communities via a single stressor, but rather via a suite of direct and indirect stressors. As a result, making mechanistic connections between a particular trait response and a specific aspect of land use is challenging. However, we did find that trait states tended to respond to altered land use in a directionally consistent manner across large spatial scales (ecoregions), a result suggesting that certain traits may display a general disturbance syndrome. Future investigators might further evaluate this result by testing whether the magnitude of these changes is also similar across ecoregions. Such a finding would further substantiate a universal response pattern of a specific trait state across large scales.

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