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Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates

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Abstract. A collaborative study among 6 states along the mid-Atlantic seaboard of the USA developed a consistent approach for collecting and interpreting macroinvertebrate data for low-gradient streams of the coastal plain. The study had 3 objectives: 1) to evaluate the validity of aggregating reference site data into a single bioregion, 2) to select biological metrics that best discriminated reference sites from sites impaired by habitat disturbance and organic pollution, and 3) to combine these metrics into an index of biological quality. Macroinvertebrate, physical habitat, and water-quality data were collected in 106 streams during autumn 1995. Fifty-five sites were reference, 34 sites had habitat stresses, and 17 sites had water-quality stresses. Classification of reference sites divided the coastal plain into 3 bioregions, separated north and south by Chesapeake Bay and separated east and west by ecoregion. Five metrics were effective at discriminating impairment: number of taxa, number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, % Ephemeroptera, Hilsenhoff Biotic Index, and % clinger mode of existence. An aggregated index, the Coastal Plain Macroinvertebrate Index (CPMI), was developed using these metrics. The CPMI accurately identified 86% of impaired sites. The precision of CPMI scores was estimated to be ±10% (3 scoring units out of 30) at the 90% confidence interval. The CPMI accurately assigned both habitat disturbance and water-quality impairment indicating a similar degree of ecological impact from these 2 stressors. Guidance is provided for applying the CPMI to other macroinvertebrate data sets in the region.

Key words: benthic macroinvertebrates, metrics, index, ecoregions, coastal plain, streams, low gradient, classification, mid-Atlantic.

The mid-Atlantic coastal plain region of the eastern USA covers ~200,000 km² or 30% of the area of 6 states (New Jersey to South Carolina). A temperate climate, abundant rainfall, flat terrain, and nutrient-rich soil have produced a vast expanse of native forest with extensive nontidal wetlands and low-gradient *swamp streams*. Smock and Gilinsky (1992) summarized the physical, chemical, and biological characteristics of coastal plain streams of the southeastern USA.

Humans have substantially modified the native forest landscape over the last 200 y. Conversion of forests to row crop agriculture initially required only the cutting of trees and the planting of a crop in the fertile soil. However, much of the area was too wet for sustained agriculture. Throughout the 20th century, much of the forested wetlands were converted to agricultural use by the construction of drainage ditches.

This degree of human disturbance in the coastal plain has made it difficult for resource

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scientists to find unimpacted reference sites, prompting this multistate effort. Coordination among neighboring states increases the pool of reference sites available to any 1 state, and ensures that reference conditions and standards reflect the very best conditions available for a particular stream type.

Without this coordination, neighboring states could produce different assessments of similar aquatic resources because of differences in sampling and analytical methods. These differences could hinder the implementation of controls by creating confusion among resource managers, politicians, and the public. The standardization of biological assessment methods is especially important today as the use of aquatic organisms as indicators of environmental health intensifies.

Wadeable streams in the coastal plain have received relatively little attention from research scientists, government agencies, and the public. It has been difficult to draw attention to an aquatic resource with limited direct human use. Swimming and fishing are limited in the small streams that dominate the resource, and nontidal streams are not extensively used as a drinking-water source. The past lack of attention given to coastal plain streams has resulted in a paucity of data. This lack of data provides an opportunity to establish consistency between state monitoring programs without affecting a large amount of historical data.

The purpose of this study was to develop an assessment framework for wadeable coastal plain streams using benthic macroinvertebrates. Biological measures are well suited to the assessment of these streams because 1) chemical criteria do not exist for the major stressors in the coastal plain, including nonpoint source (NPS) pollutants (nutrients and sediment) and habitat disturbance, 2) they are a direct measure of the condition of aquatic life, and 3) they provide a cost-effective way to assess the ecological condition of a large number of streams over large geographic areas.

Wadeable streams in the coastal plain are impacted primarily by NPS discharges and habitat disturbance resulting from agricultural (and to a lesser extent urban) development. Point-source discharges occur less frequently. We wanted to assess the degree to which habitat loss and water-quality stressors affected the macroinvertebrate assemblage. Habitat loss is an important stressor in the coastal plain. For example, 87% of stream length in the coastal plain of Delaware has been degraded through the construction and maintenance of drainage ditches (Delaware DNREC 1994). This activity has resulted in exceedences of temperature and dissolved oxygen (DO) criteria during the summer, and has moved contaminant sources close to streams. We focused attention on rural streams affected by these stressors because they are the dominant land use in the coastal plain.

Major portions of 6 eastern states (New Jersey, Delaware, Maryland, Virginia, North Carolina, and South Carolina) have substantial areas and catchments contained within the Middle Atlantic Coastal Plain and Southeastern Plains ecoregions (Omernik 1987). State and US Environmental Protection Agency (USEPA) biologists formed the Mid-Atlantic Coastal Streams (MACS) workgroup to facilitate the sharing of data and information relevant to the ecological assessment of coastal plain streams. The MACS workgroup modified the USEPA Rapid Bioassessment Protocols for Streams and Rivers (Plafkin et al. 1989) for use in these low-gradient streams (USEPA 1997).

We had 3 objectives. First, we wanted to compare the macroinvertebrate assemblages of reference sites between each state to determine if conditions of each state were different. If not, could they be aggregated into distinct regions? Second, we wanted to identify the metrics that best defined impairment in the coastal plain. Third, we wanted to develop an assessment index following the approach recommended by USEPA (Gibson et al. 1996). Could these metrics be combined into an aggregated index that accurately summarized biological conditions in the coastal plain region?

Methods

Site selection

A total of 106 separate streams was sampled throughout the coastal plain region of the 6 states (Fig. 1). Streams in the Middle Atlantic Coastal Plain Ecoregion (Ecoregion 63) were the primary focus of this study. Streams were also sampled in the Southeastern Plains Ecoregion (Ecoregion 65) to evaluate whether low-gradient streams there should be classified separately or whether the 2 ecoregions could be combined when evaluating macroinvertebrate data.

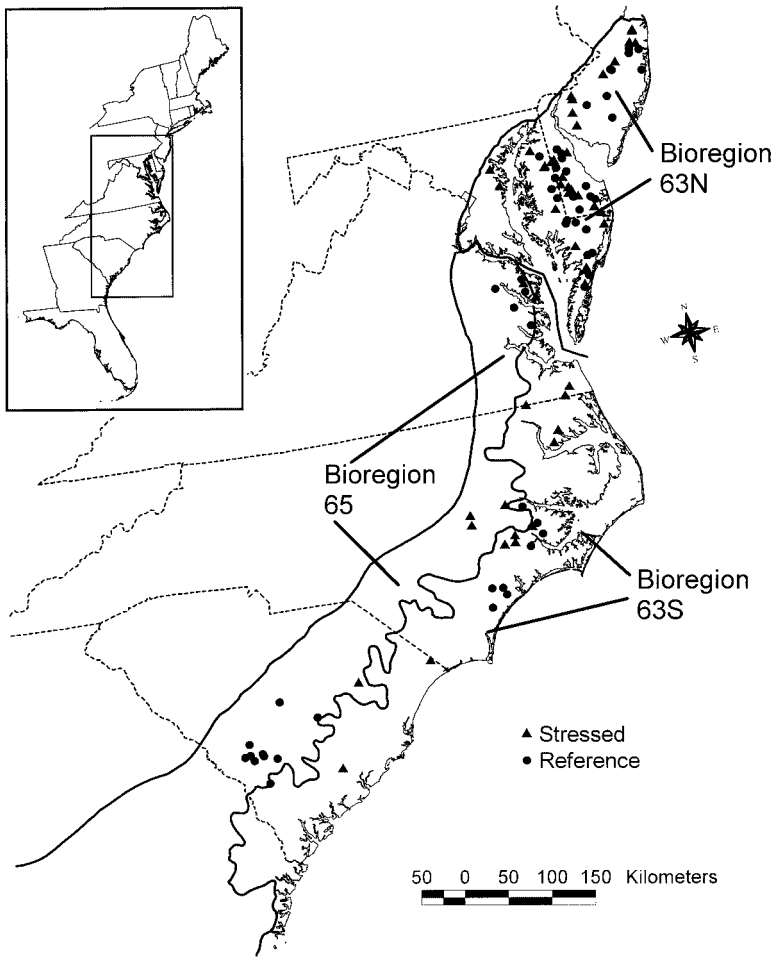

FIG. 1. Location of sampling sites and bioregion boundaries within the mid-Atlantic region. Middle Atlantic Coastal Plain Ecoregion (Bioregions 63N and 63S divided at Chesapeake Bay) and Southeastern Plains Ecoregion (Bioregion 65) according to Omernik (1987).

Flat terrain (slope <10%) and large areas of wooded wetlands characterize Ecoregion 63. Ecoregion 65 is a transitional area between the coastal plain and piedmont regions, and thus contains greater topographic relief and more defined floodplains (White 1997). Both areas are dominated by low-gradient and slow-velocity streams meandering in unconsolidated alluvial sediments, and under natural conditions have riparian corridors consisting of pine and hardwood forests.

Wadeable streams with a defined channel and detectable flow (Table 1) were investigated to minimize the effect that varying hydraulic conditions would have on the macroinvertebrate community; we avoided large rivers and wetlands. The effects of natural acidity and salinity were minimized in all sites sampled. Coastal plain streams are often naturally acidic because of high concentrations of humic and fulvic acids. Streams known to have pH levels <4.5 were not included because they would introduce a considerable degree of variability into the data; for example, many Diptera and Ephemeroptera are sensitive to pH values <5.0 (Johnson et al. 1993). The minimum pH measured was 4.6, and 6 sites had pH values <5.0 (Table 2). Salinity caused by tidal influence was not an important variable because only 4 sites were located near tidal rivers or bays. The maximum conductivity of 2100

TABLE 1. Number of reference (Ref), habitat-stressed (Hab), and water-quality-stressed (WQ) sites, and mean values (and range) for catchment area and stream characteristics by State.

State	No. of sites				Catchment area (km^2)	Stream characteristics		
	Ref	Hab	WQ	Total		Width (m)	Depth (m)	Velocity (m/s)
New Jersey	10	6	4	20	52 (7–200)	6.2 (2.4–10.1)	0.5 (0.2–0.8)	0.3 (0–0.6)
Delaware	10	10	0	20	28 (5–105)	5.0 (2.7–8.5)	0.4 (0.2–0.6)	0.1 (0–0.3)
Maryland	10	6	4	20	33 (1–242)	3.5 (0.9–8.2)	0.3 (0.1–0.8)	0.1 (0–0.3)
North Carolina	8	7	3	18	50 (5–160)	5.5 (2.1–11)	0.6 (0.2–0.9)	0.1 (0–0.2)
South Carolina	10	3	0	13	35 (7–102)	5.3 (2.7–7.9)	0.3 (0.2–0.5)	0.2 (0.1–0.2)
Virginia	7	2	6	15	37 (1–280)	3.2 (1.2–9.1)	0.6 (0.1–2.1)	0.1 (0–0.4)
Entire area	55	34	17	106	39 (1–280)	4.8 (0.9–11)	0.4 (0.1–2.1)	0.2 (0–0.6)

μS/cm (Table 2) was well below the threshold at which salinity adversely affects freshwater biota (Bulger et al. 1990).

Sites were classified as either reference or stressed. Criteria used for selecting reference and stressed sites were based upon non-biological factors (land use, habitat, and water quality) to avoid circular reasoning. The resulting index would provide a living resource measure of ecological condition scaled across the full range of non-biological conditions.

The 55 reference sites showed a minimal degree of human disturbance, and had extensive riparian areas separating the stream from adjacent agricultural and urban land uses. Reference sites covered a range of land-use conditions (Table 3). The Delaware and Maryland reference sites were predominantly agricultural, whereas the catchments in Virginia, North Carolina, and South Carolina were forested. All reference sites except those in New Jersey had <15% of the catchment in urban land use. Best professional judgement and the experience of state biologists were used to select reference sites that reflected the best possible conditions (i.e., minimal human disturbance) in each state.

The 51 stressed sites were affected by habitat disturbance or poor water quality caused by low DO and high temperature. Thirty-four of the stressed sites were channelized streams regularly maintained for drainage. These sites had little or no stable structure in the channel (except macrophytes), had few meanders or pools, and had no native riparian vegetation to shade the channel and buffer the stream from adjacent cultural practices. Most (30 of 34) of the habitat-stressed sites were in agricultural areas, although 4 were in urban areas.

Seventeen of the 51 stressed sites had well-documented, poor, water-quality conditions; yet, the 17 sites had good riparian vegetation that indicated that habitat alteration was not a stressor at these sites. Historical data were used to document DO criteria exceedences at these sites. The sites were located below municipal sewage treatment plants whose effluents did not meet secondary treatment standards; effluents were discharged to streams that provided <1:1 dilution at low flow. Mean conductivity and total suspended solids values measured on the day of sampling were higher than at the other groups of sites (Table 2).

Sample collection and processing

A single sample was collected at each site between 2 October and 22 November 1995. We chose autumn to avoid temperature extremes and because groundwater levels and stream flows are generally lower compared to the spring. This timing made access easier in areas with extensive wetlands and ensured that sampling took place in the main channel at low flow.

A sampling site consisted of a 100-m reach of stream. Benthic macroinvertebrates were sampled in bank margins, woody snags, and submerged macrophytes using a D-frame dip net (0.3 m wide at base, 650–750-μm mesh). These habitats have the highest taxa richness and balance of pollution-sensitive organisms in coastal plain streams, and thus provided the best measure of the overall health of the macroinvertebrate assemblage (USEPA 1997). These habitats were sampled in proportion to their abundance within the 100-m sampling reach. At least 2 of these habitats were found at all sites.

Organisms were collected by aggressively disturbing the target habitat for a distance of 1m

TABLE 2. Mean (and range) for selected measurements at reference and stressed (habitat and water-quality) sites. DO = dissolved oxygen, Cond. = specific conductance, TSS = total suspended solids, *n* = number of sites.

Sites	*n*	Variable			
		pH	DO (mg/L)	Cond. (μS/cm)	TSS (mg/L)
Reference	55	6.4 (4.6–7.5)	7.8 (4.2–10.1)	116 (30–512)	5.7 (1–85)
Habitat	34	6.7 (5.9–7.5)	7.9 (0.5–11.6)	173 (40–423)	12.9 (2–82)
Water quality	17	6.9 (4.7–7.7)	5.9 (0.3–9.7)	668 (60–2100)	87.3 (2–898)

followed by 3 to 4 cleaning sweeps to collect dislodged organisms (USEPA 1997). Twenty of these 1-m collections were composited in a 600-μm mesh sieve bucket to produce a single sample with a total sample area of ~6 m^2. The slightly smaller mesh size than in the net ensured that organisms would not be lost in the sieve bucket. A series of workshops ensured sampling consistency, and all collectors used the same sampling gear.

Samples were preserved in 70–80% alcohol and returned to the laboratory for subsampling, sorting, and taxonomic identification. One hundred organisms were subsampled (USEPA 1997). Chironomidae and Oligochaeta were mounted on slides. Organisms were identified to the lowest practicable taxonomic level (species level for most groups). All samples were identified by the same taxonomist for consistency.

Site classification

We used genus-level composition and abundance data to determine site classes from the reference sites. Use of genus level reduced the variability inherent in species-level data. We used non-metric multidimensional scaling (a distribution-free ordination technique) to evaluate various classification schemes (Kenkel and Orloci 1986). Ordination used Bray-Curtis dissimilarities, which is considered robust for ecological analyses (Ludwig and Reynolds 1988). Regionalization schemes for testing site classes included state boundaries, latitudinal gradient (north to south), catchment area, and ecoregion. Strong correlation of either latitude or catchment area with any of the ordination axes indicated a possible covariate or grouping, and categorical classes were examined graphically in ordination space. The term *bioregion* was used to describe the regions resulting from this biologically driven classification scheme.

Metric screening

Suitable metrics for the coastal plain were selected by comparing reference sites to sites with known poor water quality and physical habitat quality. The species-level composition and abundance data were reduced to the genus level before calculating metrics. The genus level ensured consistency within the data set, and allowed the results to be applied to other data sets from the mid-Atlantic region.

We considered 26 structural or functional

TABLE 3. Mean (and range) % of reference site catchments with 3 major land-use categories; forest category included wooded wetlands. Data not available for 4 of the 55 reference sites.

State	*n*	% of catchment		
		Urban	Agriculture	Forest
New Jersey	6	27 (14–46)	18 (4–50)	54 (36–78)
Delaware	10	6 (1–13)	46 (29–71)	47 (19–65)
Maryland	7	3 (1–9)	60 (25–76)	36 (22–73)
Virginia	8	1 (0–3)	32 (17–62)	64 (30–79)
North Carolina	10	1 (0–5)	17 (0–60)	82 (40–99)
South Carolina	10	2 (0–8)	35 (17–60)	63 (37–80)
Entire area	51	5 (0–46)	35 (0–76)	59 (19–99)

measurements of the benthic assemblages as biological metrics that were ecologically relevant to coastal plain streams, including 6 richness measures, 8 composition measures, 8 tolerance measures, and 4 mode of existence (i.e., habit) measures. Metrics specific to the order Plecoptera, other than EPT, were not considered because of the low occurrence of these organisms.

Most of the metrics were based upon either the relative abundance or the number of taxa within a taxonomic or functional group, and are self-explanatory. The Florida Index (FI) (Barbour et al. 1996) and North Carolina Biotic Index (NCBI) (Lenat 1993) were calculated from published literature. The habit designations (e.g., % clinger mode of existence) for each genus were taken from Merritt and Cummins (1996). Where >1 functional habit was designated for the same genus, the one listed 1st was used.

The Hilsenhoff Biotic Index (HBI, Hilsenhoff 1987), number of intolerant taxa (NIT), and % tolerant organism (%TOL) metrics were determined from genus-level tolerance values published by USEPA (Green 1990). Tolerance values published for the NCBI were used when values were not available for a genus. When values were not available from either source, the average of the species tolerance values from USEPA (Green 1990) was used. A genus was not used in the calculation of a tolerance metric if genus- or species-level tolerance values were not available. Family-level tolerance values from USEPA (Green 1990) were used for organisms that could not be identified to the genus level because of size or condition. The genus-level tolerance values used in this study appear in the appendix. Genera with tolerance values ≤ 3 were considered *intolerant*, whereas those with values ≥ 7 were considered *tolerant*.

A site was correctly assigned as stressed for a particular metric if the site value was $\leq 25^{th}$ percentile of the reference population. We then calculated the % of sites that were correctly assigned for each metric in each bioregion and for all 51 stressed sites combined. Those metrics that had the highest % of sites classified correctly were considered candidates for the aggregated index.

Index development

A Pearson correlation analysis was used to select metrics for the index (SYSTAT, version 5.2 edition, SYSTAT Inc., Evanston, Illinois). Metric combinations that resulted in r values $> \pm 0.75$ were considered highly redundant, which indicated that 1 of the metrics in the pair should not be included in the index. This procedure ensured that each metric contributed independent information to the aggregated index. A high priority was given to selecting at least 1 metric within each of the 4 metric type categories (e.g., richness) to further reduce redundancy. The sensitivity of the recommended index to the number of metrics was then evaluated after the index scoring method was selected.

We used professional judgment guided by metric assessment accuracy and redundancy for final selection of metrics for the index. A single metric in each category was selected that had a high redundancy within the category (i.e., was the best representative of the category). We then considered metrics that had low redundancy between categories. This approach, using a combination of high redundancy within a category and low redundancy between categories, ensured that the index would detect a wide range of biological responses.

Core metrics were combined into an aggregated index following Gibson et al. (1996). Three published methods for establishing scoring thresholds for each metric were considered: 1) the 50^{th} and 10^{th} percentile of the reference distribution (Roth et al. 1997), 2) the 25^{th} percentile of the reference distribution and bisection of the range below the 25^{th} percentile (Barbour et al. 1996), and 3) the 95^{th} percentile of all sites (reference and stressed) and quadrisection of the range (DeShon 1995). The metrics were normalized from different numerical scales (e.g., number of taxa, percentages) into unitless scores (Karr et al. 1986, Gerritsen 1995). Scoring thresholds were determined by calculating population statistics (e.g., 25^{th}, 50^{th}, 95^{th} percentile) and then dividing the range of values for each metric into sections of equal size. Points were assigned to each section using a 6, 3, 0 or a 6, 4, 2, 0 system. A summary score (i.e., index) for each site was then calculated by summing the points from each metric.

The 50^{th} and 10^{th} percentile method used a 6, 3, 0 point system: $>50^{th}$ percentile = 6 points, 50^{th}–10^{th} percentile = 3 points, and $<10^{th}$ percentile = 0 points. The 25^{th} percentile and bisection method also used a 6, 3, 0 point system: $>25^{th}$ percentile = 6 points, upper ½ of the

range below the 25th percentile = 3 points, and the remainder of the range = 0 points. The 95th percentile and quadrisection method used a 6, 4, 2, 0 point system: 1st quarter of the range to the 95th percentile = 6 points, 2nd quarter of the range = 4 points, 3rd quarter of the range = 2 points, and the remainder of the range = 0 points.

The scoring method demonstrating the highest assessment accuracy for all stressed sites and bioregions was selected for the Coastal Plain Macroinvertebrate Index (CPMI). Assessment accuracy was defined by stressed sites receiving CPMI scores <50% of the total possible score. Results were reported separately by scoring method and bioregion.

The sensitivity of the recommended index to habitat and water-quality impairment was determined using box plots to compare the distributions of reference and stressed sites in each bioregion. Separation between interquartile ranges (25th to 75th percentile) was defined as significant for determining impairment because 75% of each group did not overlap with the other group.

The performance of the recommended index was determined by comparing the CPMI score for each site with its nonbiological descriptor (i.e., habitat or water-quality stressed). This procedure avoided evaluating biological thresholds using biological data alone and circular reasoning. A *t*-test was used to compare CPMI scores between the 2 groups of stressed sites (habitat and water quality) to identify differences in biological responses.

Precision estimates for CPMI scores and their component metrics were determined from replicate samples ($n = 6$) collected at 2 sites in Delaware. The 90% confidence interval (CI) was determined as 1.645× the root mean square error. Understanding the precision of the CPMI was necessary for proper interpretation of CPMI scores.

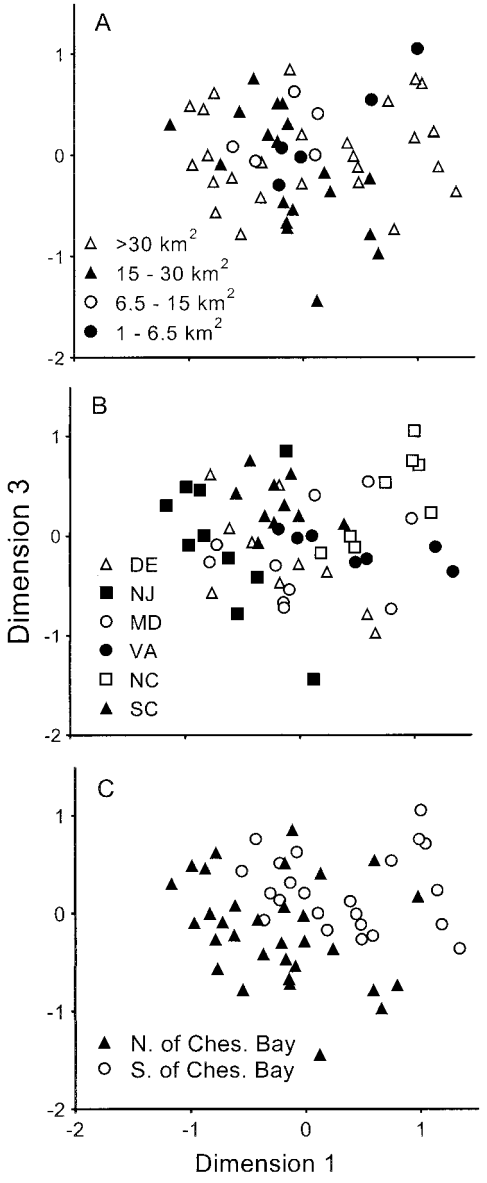

FIG. 2. Ordination plots (non-metric multidimensional scaling) of reference-site data showing spatial patterns by (A) catchment area, (B) state boundary, and (C) latitude (north and south of Chesapeake Bay). Axes 1 and 3 were the most informative for examining potential classes; all 3 axes are shown in the final classification (see Fig. 3).

Results

Site classification

No regional patterns or groupings were found in the ordination analysis using catchment area, indicating that the biological condition of wadeable streams was not affected by stream size (Fig. 2A). However, a regional pattern arranged north to south appeared using state boundaries (Fig. 2B) and latitude (Fig. 2C). The pattern by state boundary appeared to be an artifact of the north/south arrangement of the states. This

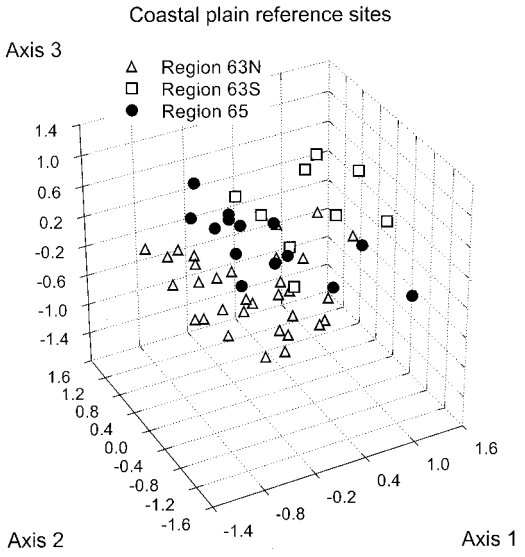

FIG. 3. Ordination plot (non-metric multidimensional scaling) of reference site data showing spatial patterns for 3 bioregions of the mid-Atlantic coastal plain. Stress coefficient = 0.170.

separation was most clearly shown using Chesapeake Bay as the separation line (Fig. 2C). This geographic separation provided the 1st indication that reference conditions were not homogeneous across the 6 states, and that subdividing the region would reduce data variability and improve assessment accuracy.

Further separation was observed when the study area was partitioned by ecoregion. Three regions appeared when the ordination was projected using both north/south and ecoregion divisions (Fig. 3). The use of 3 regions also helped to eliminate some of the overlap resulting from a north/south separation. Therefore, we used Bioregion 63N (Ecoregion 63 north of Chesapeake Bay), Bioregion 63S (Ecoregion 63 south of Chesapeake Bay), and Bioregion 65 (Ecoregion 65) (Fig. 1). NMDS ordination had a stress coefficient of 0.170 with 3 axes. Ordinations are considered acceptable if the stress coefficient is <0.20 (Ludwig and Reynolds 1988).

Metric screening

Twelve metrics were selected as candidates for the index. Metrics were generally eliminated from further consideration if $<50\%$ of the stressed sites were properly assigned. Eleven metrics were selected because they were the most efficient (57–92%) at correctly assigning sites to their a priori designation of stressed (Table 4). The % Diptera and % Chironomidae metrics were eliminated because of the low % of stressed sites properly assigned in Bioregion 65. The Florida Index was eliminated because 4 other tolerance metrics had higher scores. The total taxa richness (TT) metric was selected because of its ecological and societal importance for biological diversity, and because its efficiency approximated 50%. The 12 metrics selected for the aggregated index included 4 richness metrics, 3 composition metrics, 4 tolerance metrics, and 1 habit metric (Table 4).

Index development

The tolerance metrics were evaluated 1st for redundancy because they had the highest assessment accuracies (Table 4). The HBI was selected for the final index because it was strongly correlated with the other tolerance metrics (Table 5) and because experience has shown it to be a reliable metric over a wide geographical range (Hilsenhoff 1987, Stribling et al. 1998).

The richness metrics had the next highest assessment accuracies (Table 4). The EPT metric was selected because its components Ephemeroptera richness (E) and Trichoptera richness (TR) were strongly correlated with EPT (Table 5) and because of its successful record over a wide geographic range (Stribling et al. 1998).

The composition metrics had the next highest assessment accuracies (Table 4). The % Ephemeroptera metric (%E) was selected because it had lower redundancies with the HBI and EPT metrics already selected than the other composition metrics (Table 5). It also had a high redundancy with %EPT, indicating that %E or %EPT should be selected (Table 5). There was low redundancy between %E and % Trichoptera (%TR), indicating that these 2 composition metrics provided very different information and should both be considered for the index (Table 5). The sensitivity of the index to adding a 6th metric and the replacement of 1 metric with another was tested using %TR (see next section). The % clinger (%CL) metric was selected as the sole habit metric because it was not redundant with TT and %E metrics already selected, and only moderately redundant with the HBI and EPT metrics (Table 5).

TABLE 4. Percent of stressed sites correctly assigned as stressed (<25th percentile of the reference site distribution) for 26 metrics. Data are arranged according to bioregion (63N, 63S, 65), and ordered according to the results for all stressed sites (bolded). Metric types included richness (R), composition (C), tolerance (T), and habit (H). EPT = Ephemeroptera, Plecoptera, and Trichoptera; the number of sites appears in parentheses. The 12 metrics selected as candidates for the aggregated index are specified.

		% of stressed sites correctly assigned				
Metric	Type	63N (31)	63S (12)	65 (8)	**All (51)**	Selected
No. intolerant taxa	T	100	67	100	**92**	x
% tolerant	T	100	75	75	**90**	x
N. Carolina Biotic Index	T	100	50	88	**86**	x
No. EPT taxa	R	90	58	100	**84**	x
No. Trichoptera taxa	R	90	50	88	**80**	x
Hilsenhoff Biotic Index	T	74	83	100	**80**	x
No. Ephemeroptera taxa	R	81	50	100	**77**	x
% Trichoptera	C	81	50	100	**77**	x
% clingers	H	61	33	100	**61**	x
% EPT	C	61	33	88	**59**	x
% Ephemeroptera	C	52	50	88	**57**	x
Florida Index	T	42	83	75	**57**	
% Diptera	C	58	67	12	**53**	
% Chironomidae	C	52	67	12	**49**	
Total no. taxa	R	52	17	62	**45**	x
No. Diptera taxa	R	26	67	75	**43**	
No. Chironomidae taxa	R	26	67	75	**43**	
% swimmers	H	42	25	75	**43**	
% Oligochaeta	C	39	8	100	**41**	
% sprawlers	H	45	17	50	**39**	
% 2 dominant taxa	T	39	0	75	**35**	
Hydropsychidae/Trichoptera	T	42	33	12	**35**	
% dominant taxon	T	39	0	62	**33**	
% non-insects	C	26	8	50	**26**	
% tribe Tanytarsini	C	35	17	0	**25**	
% climbers	H	10	17	62	**20**	

The 95th percentile and quadrisection method had the highest overall % (86) of stressed sites correctly assigned as stressed (Table 6). The 25th percentile and bisection method had the lowest % (45) of stressed sites correctly assigned. Only the 95th percentile and quadrisection method had >50% of the stressed sites correctly assigned in all 3 bioregions, so it was selected for the CPMI.

The sensitivity of the CPMI scores to the number and types of metrics was tested. The CPMI was not substantially affected by small changes to either the number of metrics or the composition of metrics. Adding %TR as a 6th metric reduced the accuracy of the CPMI from 86% to 82%. Further, using %TR instead of %E only improved the accuracy of the CPMI from 86% to 87%. Therefore, we used the 5 metrics shown in Table 5. The statistics and metric thresholds used to score the CPMI appear in Table 7.

Separation between interquartile ranges (25th percentile of reference sites <75th percentile of stressed sites) was used to further evaluate assessment accuracy. There was clear separation for 2 metrics (EPT, HBI) in Bioregion 63N and 4 metrics (EPT, %E, HBI, and %CL) in Bioregion 65 (Fig. 4). There was no clear separation for the 5 metrics in Bioregion 63S. The CPMI also showed clear separation in Bioregion 63N and Bioregion 65 (Fig. 5). Variability in the CPMI was high for the reference sites in Bioregion 63N but had little effect on assessment accuracy because of the clear separation between the interquartile ranges. Variability in the CPMI was highest for reference sites in Bioregion 63S.

The CPMI had a margin of error of ±10% (3 out of 30 units) at 90% CI when the specified

TABLE 5. Pearson correlation matrix of *r* values for 12 candidate metrics (genus level) with the correlations between (a) richness (R), (b) composition (C), and (c) tolerance (T) metrics highlighted. H = habit metric, EPT = Ephemeroptera (Ephem.), Plecoptera, and Trichoptera (Trich.), intol. = intolerant, toler. = tolerant.

	Type	TT	EPT	E	TR	%EPT	%E	%TR	NIT	%TOL	HBI	NCBI
[a] Total taxa (TT)	R											
[a] EPT taxa (EPT)	R	0.61			(a)							
Ephem. taxa (E)	R	0.51	0.69									
Trich. taxa (TR)	R	0.54	0.90	0.35								
% EPT (%EPT)	C	0.24	0.71	0.43	0.65							
[a] % Ephem. (%E)	C	0.13	0.44	0.58	0.23	0.72		(b)				
% Trich. (%TR)	C	0.32	0.63	0.10	0.77	0.63	0.07					
No. intol. taxa (NIT)	T	0.50	0.83	0.49	0.74	0.69	0.33	0.60				
% toler. (%TOL)	T	-0.34	0.60	-0.40	-0.55	-0.55	0.28	-0.53	-0.61			(c)
[a] Hilsenhoff (HBI)	T	-0.29	-0.68	-0.39	-0.64	-0.80	-0.47	-0.58	-0.75	0.87		
N. Carolina (NCBI)	T	-0.28	-0.62	-0.40	-0.55	-0.71	-0.53	-0.49	-0.60	0.53	0.75	
[a] % clinger (%CL)	H	0.42	0.70	0.41	0.68	0.67	0.40	0.67	0.65	-0.64	-0.66	-0.62

[a] 5 core metrics selected for the Coastal Plain Macroinvertebrate Index

methods were used. This estimate of precision was determined from replicate samples ($n = 6$) collected at 2 sites. The 90% CI for the 5 core metrics were ±6.0 taxa for TT, ±2.5 taxa for EPT, ±8.9% for %E, ±0.28 units for the HBI, ±13.8% for %CL, and ±3.1 units for the CPMI.

Discussion

Classification of coastal plain streams

The separation of the mid-Atlantic coastal plain into 3 bioregions could be expected because it is such a large geographic area. Both climate and topography likely played important roles. The proposed classification framework was a balance between having too few classes that might miss important regional differences and too many that would complicate the assessment (Barbour et al. 1996, Gibson et al. 1996, Hughes 1995, Karr and Chu 1999). This result confirms the classification of natural systems based upon physiographic characteristics (Barbour et al. 1996, Omernik and Gallant 1990), and suggests that ecoregions covering a wide range in latitude may need to be further subdivided.

TABLE 6. Percentage of stressed sites correctly assigned as stressed (<50% of the total possible points) for 3 methods of scoring, according to bioregion.

		% of stressed sites correctly assigned		
Bioregion	*n*	50th and 10th	25th and bisect	95th and quadrisect
63N	31	77	42	84
63S	12	42	33	83
65	8	88	75	100
All sites	51	70	45	86

None of the alternative classifications we examined was extraordinarily strong statistically. Any 1 classification would have partitioned the data about as well as the others. In addition to partitioning variability, a classification system must also meet the needs of users. The principal users are state agencies that must assess sites in a cost-efficient manner. Therefore, the best classification is one that accounts for natural variability, can be applied rapidly in the field, is easily mapped, and reflects current knowledge.

Large-scale physical differences between the 3 bioregions supported the proposed classification. The southern coastal plain (Bioregion 63S)

TABLE 7. Scoring thresholds used to calculate the Coastal Plain Macroinvertebrate Index (CPMI) for 3 bioregions. The 95th percentile statistic was used to derive the 4 scoring thresholds (DeShon 1995). EPT = Ephemeroptera, Plecoptera, and Trichoptera, HBI = Hilsenhoff Biotic Index.

	Statistics					Scoring thresholds			
	min	5%	50%	95%	max	6	4	2	0
Bioregion 63N (n = 62)									
Total no. taxa	3	12	25	34	35	>25	17–25	9–16	<9
No. EPT taxa	0	1	5	13	22	>9	7–9	4–6	<4
% Ephemeroptera	0	0	8.5	38.8	62.9	>29	20–29	10–19	<10
HBI	2.6	3.5	5.5	7.6	8.5	<4.9	4.9–6.0	6.1–7.3	>7.3
% clingers	0	1.0	30.1	72.0	81.4	>51	34–51	17–33	<17
Bioregion 63S (n = 22)									
Total no. taxa	8	11	20	33	38	>24	17–24	9–16	<9
No. EPT taxa	0	0	2	6	11	>4	3–4	2	<2
% Ephemeroptera	0.0	0.0	2.91	48.1	54.1	>36	24–36	12–23	<12
HBI	2.7	3.7	6.9	8.0	8.3	<5.0	5.0–6.1	6.0–7.3	>7.3
% clingers	0	0	4.7	42.3	44.1	>30	20–31	11–19	<11
Bioregion 65 (n = 22)									
Total no. taxa	8	9	24.5	34	35	>25	17–25	9–16	<9
No. EPT taxa	0	0	5	11	13	>8	6–8	3–5	<3
% Ephemeroptera	0	0	14.3	32.4	33.0	>24	16–24	8–15	<8
HBI	4.5	4.6	5.4	7.9	8.4	<5.7	5.7–6.6	6.7–7.5	>7.5
% clingers	0	0	26.7	48.6	55.6	>36	24–36	12–23	<12

is characterized by extremely low-gradient, sluggish streams that are often tea-colored with naturally low pH and DO. Finding suitable reference sites with neutral pH and detectable flow was the most difficult in this region, and may have contributed to the high variability in reference conditions in Bioregion 63S. The Southeastern Plains Ecoregion is characterized by greater topographic relief than the adjacent Middle Atlantic Coastal Plain Ecoregion. Chesapeake Bay is the largest physical feature dividing the mid-Atlantic region.

Catchment area was not an important variable in the classification. This result differs from other studies of piedmont and montane regions that have shown sensitivity of the macroinvertebrate assemblage to catchment area in the range of 1 to 300 km^2 (DeShon 1995). The relatively homogeneous topography of the coastal plain region may result in more homogeneous geology, water quality, and habitat conditions than other regions. Scientists studying the macroinvertebrate assemblages in wadeable coastal plain streams may not have to factor in catchment area when characterizing biological conditions.

Metrics for the coastal plain

The importance of EPT organisms in coastal plain streams was similar to their importance in rocky bottom streams with 2 exceptions: 1) richness and abundance were generally lower, and 2) Plecoptera were rare. Plecoptera were also rare in a statewide study of low-gradient streams in Florida (Barbour et al. 1996). Approximately 23% of the 11,686 organisms collected in our study were EPTs. The 55 reference sites had an average EPT richness of 7 and an average %EPT abundance of 32. The TT, EPT, %E, and HBI metrics we selected for the CPMI all use the same information on pollution tolerance of EPTs developed for rocky bottom streams. We established thresholds for these metrics customized for the coastal plain region.

Ephemeroptera were an important group, representing 53% of all EPTs collected. Most (6 of 10) of the reference site samples in Bioregion 63S had <10% Ephemeroptera, a level classified by the CPMI as severely degraded (Table 7). This result may explain the wide range in reference distribution in Bioregion 63S, and the poor separation between reference sites and

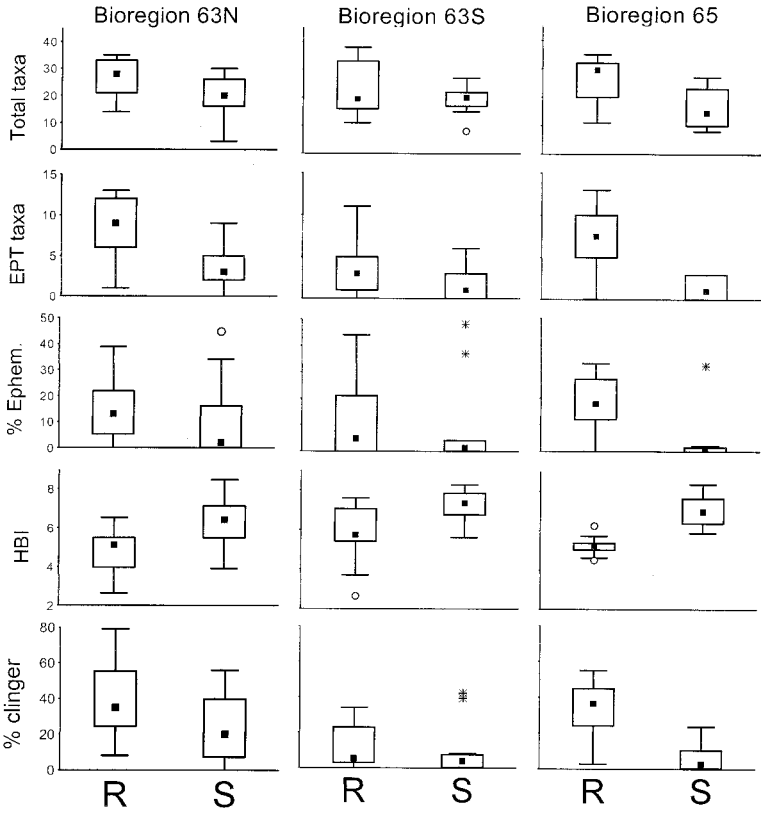

FIG. 4. Distribution of metric values for the 5 metrics selected for the Coastal Plain Macroinvertebrate Index (CPMI) showing variability and sensitivity to impairment for each bioregion. Box represents the 25th and 75th percentiles, whiskers represent the 5th and 95th percentiles, and dots represent the median values. Circles represent outliers and asterisks represent extreme values. EPT = Ephemeroptera, Plecoptera, and Trichoptera, HBI = Hilsenhoff Biotic Index, R = reference sites, S = stressed sites.

stressed sites (Fig. 5). Many Ephemeroptera are highly sensitive to low pH (Johnson et al. 1993). Streams of the southern coastal plain region had a high incidence of natural acidity.

A similar composite index in the nearby Florida coastal plain used 8 metrics (Barbour et al. 1996). The larger number of metrics may have been needed because of the larger geographic area and the greater complexity and heterogeneity of Florida. No testing of the sensitivity of the index to the number of metrics was done in the Florida study. The TT and EPT metrics were used in the Florida composite index and our CPMI. No. of Chironomidae taxa, Florida Index, % dominant taxon, and % Diptera metrics used in the Florida composite index had only a moderate degree of assessment accuracy (33–57% of stressed sites correctly assigned as stressed) in our study, and were not selected for the CPMI.

Application of the CPMI to other data sets

The CPMI for Bioregion 63N had the largest sample size (n = 62) and therefore was the most robust. Although the sample size was smaller for Bioregion 65 (n = 22), this bioregion had the highest % of sites correctly assigned to their a priori designation. Additional research is recommended in Bioregion 63S before using the recommended metrics and thresholds.

The CPMI was designed to apply to other macroinvertebrate data collected in the coastal plain region during the autumn season (1 October to 15 December). Application of the CPMI thresholds to other data sets would require the following: 1) use of the same field methods and gear, 2) use of 100-organism subsamples, 3) taxonomic identifications standardized to the genus level, 4) limiting sample abundance to 100

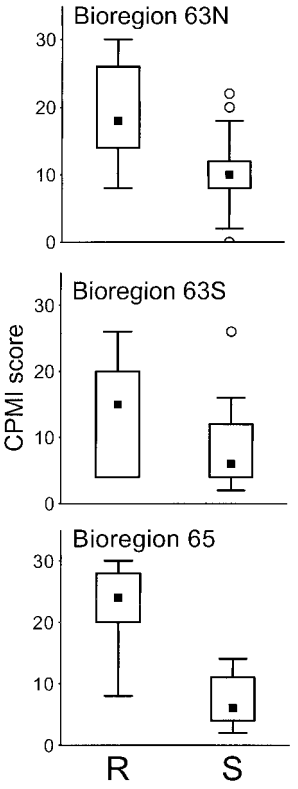

FIG. 5. Distribution of Coastal Plain Macroinvertebrate Index (CPMI) values showing variability and sensitivity to stress, according to bioregion. CPMI scores are out of a possible 30 points. Boxes represent 25th and 75th percentiles, whiskers represent 5th and 95th percentiles, and dots represent the median values. Circles represent outliers. R = reference sites, S = stressed sites.

organisms, 5) use of tolerance values and clinger designations (see appendix), and 6) comparable calculations of metrics and CPMI scores.

In conclusion, our results indicated that 2 groups of states should share their reference site data: the northern region (New Jersey, Delaware, and Maryland) and the southern region (Virginia, North Carolina, and South Carolina). The sharing of reference site data between neighboring states promotes consistency in the interpretation of biological data. This feature is particularly important today because the use of biological data by state agencies has increased in the areas of water-quality standards, assessment, and most recently the listing of waterbodies not meeting state standards (Section 303d of the Clean Water Act).

Aggregating reference site data across state boundaries also increased the sample size and the probability that the CPMI captured least-disturbed reference conditions within each bioregion. Establishing standard methods and assessment thresholds is especially important for states that contain a relatively small proportion of a certain ecoregion and therefore a small pool of potential reference sites.

It might be argued that the use of resident organisms to establish quality classes is circular reasoning because biological data were used to define the biological thresholds. We reject this argument for 3 reasons. First, classification into the 3 bioregions used specific land-use criteria. Second, both metric selection and index development used non-biological measures (habitat and water quality) as decision criteria. Last, resident organisms provide the only practical way to define biological thresholds.

The recommended index provides a practical tool for characterizing ecological health because it is scaled between 2 extremes. Although reference and severely degraded sites are often uncomplicated to assess, moderately degraded sites are more difficult. The proposed index provides a way to assess the full range of biological conditions that is both scientifically defensible and easily understood by non-scientists.

Biological data collected from natural systems are often criticized for being highly variable and insensitive to many pollutants and stressors. The multimetric index we developed was both accurate (correct assessment 86% of the time) and precise ±10% at the 90% CI). Two factors likely contributed to the high degree of accuracy and precision: 1) the standardization of field methods, sampling season, sample sorting, sample size, and taxonomy, and 2) clearly defined criteria for classifying sites as either reference or stressed.

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APPENDIX. Coastal plain macroinvertebrate taxa list, with tolerance values (TV) and clinger habit (CL; identified with X).

Genus	TV	CL
Turbellaria		
Dugesia		
Nemertinea		
Prostoma		
Oligochaeta		
Aulodrilus	8	
Dero	10	
Eclipidrilus	8	
Haemonais	8	
Isochaetides		
Limnodrilus	10	
Lumbriculus	8	
Nais	8	
Quistadrilus	10	
Spirosperma	10	
Stylaria	8	
Hirudinea		
Alboglossiphonia		
Desserobdella		
Placobdella		
Gastropoda		
Amnicola	8	
Campeloma	6	
Elimia	2	
Ferrissia	7	
Gyraulus	7	
Helisoma	7	
Laevapex	6	
Menetus		
Physella		
Pseudosuccinea	6	
Stagnicola	7	
Bivalvia		
Corbicula	4	
Musculium	5	
Pisidium	8	
Quadrula		
Sphaerium	8	
Arachnoidea		
Lebertia		
Piona		
Amphipoda		
Crangonyx	4	
Gammarus	6	
Hyalella	8	
Synurella		
Decapoda		
Orconectes	6	
Palaemonetes	4	
Procambarus	9	

APPENDIX. Continued.

Genus	TV	CL
Isopoda		
Caecidotea	6	
Lirceus	8	
Oniscus		
Ephemeroptera		
Acentrella	4	
Acerpenna	4	
Baetis	6	
Baetisca	4	
Barbaetis	4	
Caenis	7	
Callibaetis	9	
Centroptilum	2	
Eurylophella	4	X
Hexagenia	6	
Isonychia	2	
Labiobaetis		
Paraleptophlebia	1	
Serratella	2	X
Siphloplectron	2	
Stenacron	4	X
Stenonema	3	X
Tricorythodes	4	
Odonata		
Anax	5	
Argia	6	X
Basiaeschna	2	
Boyeria	2	
Calopteryx	6	
Cordulegaster	3	
Didymops	4	
Enallagma	8	
Epitheca	4	
Erythemis	10	
Gomphus	5	
Hagenius	1	
Ischnura	9	
Libellula	8	
Macromia	2	
Nasiaeschna	2	
Pachydiplax	10	
Perithemis	4	
Somatochlora	1	
Sympetrum	4	
Plecoptera		
Acroneuria	0	X
Agnetina	2	X
Allocapnia	3	X
Isoperla	2	X
Leuctra	0	X
Taeniopteryx	2	
Hemiptera		
Belostoma		
Mesovelia		
Microvelia	6	
Notonecta	5	
Palmacorixa		
Pelocoris	7	
Ranatra		
Rhagovelia		
Rheumatobates		
Sigara		
Trepobates		
Trichocorixa		
Megaloptera		
Chauliodes	4	X
Corydalus	5	X
Nigronia	2	X
Sialis	4	
Coleoptera		
Agabus	5	
Anchytarsus		X
Ancyronyx	2	X
Celina	5	
Copelatus	5	
Cyphon	7	
Dineutus	4	
Dubiraphia	6	X
Ectopria	5	X
Gyrinus	4	
Haliplus	5	
Helichus	5	X
Hydroporus	5	
Ilybius		
Laccophilus	5	
Macronychus	2	X
Microcylloepus	2	X
Optioservus	4	X
Oulimnius		X
Peltodytes	5	
Promoresia	2	X
Ptilodactyla		
Sperchopsis		X
Stenelmis	5	X
Tropisternus	10	
Lepidoptera		
Paraponyx	5	
Trichoptera		
Anisocentropus	2	
Brachycentrus	1	X
Ceraclea	3	
Cheumatopsyche	5	X
Chimarra	4	X
Diplectrona	0	X
Heteroplectron	3	
Hydatophylax	2	

APPENDIX. Continued.

Genus	TV	CL
Hydropsyche	4	X
Hydroptila	6	X
Lepidostoma	1	
Lype	2	X
Macrostemum	3	X
Micrasema	2	X
Molanna	6	
Mystacides	4	
Nectopsyche	3	
Neureclipsis	7	X
Nyctiophylax	5	X
Oecetis	8	X
Oxythira	3	
Phylocentropus	5	
Polycentropus	6	X
Psilotreta	0	
Ptilostomis	5	
Pycnopsyche	4	
Triaenodes	6	
Diptera		
Ablabesmyia	7	
Aedes	8	
Anopheles	6	
Apectrotanypus	0	
Atherix	2	
Bezzia	6	
Brillia	5	
Calaparyphus	7	
Ceratopogon	6	
Chironomus	10	
Chrysops	7	
Cladopelma	9	
Cladotanytarsus	7	
Clinotanypus	8	
Conchapelopia	6	
Corynoneura	7	
Cricotopus	7	X
Cryptochironomus	8	
Cryptotendipes	6	
Culex	8	
Culicoides	10	
Dashyhelea		
Demicryptochironomus	8	
Dicrotendipes	8	
Diplocladius	7	
Djalmabatista	3	
Endochironomus	10	X
Geranomyia	3	
Glyptotendipes	10	
Goeldichironomus	8	
Gymnometriocnemus	7	
Helius	4	
Helopelopia	6	
Hemerodromia	6	
Hexatoma	2	

APPENDIX. Continued.

Genus	TV	CL
Hydrobaenus	8	
Kiefferulus	10	
Labrundinia	7	
Larsia	6	
Limnophyes	8	
Meropelopia		
Micropsectra	7	
Microtendipes	6	X
Mycetophila		
Nanocladius	3	
Natarsia	8	
Nilothauma	2	
Odontomyia	7	
Oromosia	3	
Orthocladius	6	
Parachaetocladius	2	
Parachironomus	10	
Paracladopelma	7	
Parakiefferiella	4	
Paralauterborniella	8	X
Paramerina		
Parametriocnemus	5	
Paraphaenocladius	4	
Paratanytarsus	6	
Paratendipes	8	
Pericoma	4	
Phaenopsectra	7	X
Pilaria	7	
Platypeza		
Polypedilum	6	
Potthastia	2	
Probezzia	6	
Procladius	9	
Psectrocladius	8	
Psectrotanypus	10	
Pseudolimnophila	2	
Pseudorthocladius	0	
Rheocricotopus	6	
Rheosmittia		
Rheotanytarsus	6	X
Serromyia		
Simulium	6	X
Stelechomyia	7	
Stempellinella	4	
Stenochironomus	5	
Tabanus	5	
Tanypus	10	
Tanytarsus	6	
Thienemanniella	6	
Tipula	4	
Tribelos	5	
Tvetenia	5	
Unniella		
Xenochironomus	0	
Xylotopus	2	
Zalutschia	7	
Zavrelimyia	8	