sampling. In summer 2005, low to normal flows allowed SRBC to initiate the large river sampling project.

SRBC staff collected biological and water quality data at 25 stations on the mainstem Susquehanna River and at the mouth of the major tributaries (Figure 1). The information collected will be used in future years to select and calculate metrics for a benthic macroinvertebrate index of biotic integrity (IBI) to assess the biological conditions in the basin's rivers. The data also will be used in SRBC's Integrated Listing assessments and to complement state assessment efforts.

Benthic macroinvertebrates were used to assess biological conditions for several reasons. They are sensitive to a wide range of stressors, have a large range of documented pollution tolerances, and are found in a variety of habitats throughout lotic systems (Flotemersch and others, 2001a). With two decades of conducting subbasin surveys and interstate streams monitoring, SRBC has compiled significant macroinvertebrate data from various sites on the large rivers. Additionally, SRBC's member states, New York, Pennsylvania, and Maryland, rely heavily on macroinvertebrate data for wadeable streams assessments.

Basin Geography

The Susquehanna River Basin is the largest watershed on the east coast of the United States, draining 27,510 square miles. The Susquehanna River originates at Otsego Lake, N.Y., and flows 444 miles through New York, Pennsylvania, and Maryland to the Chesapeake Bay at Havre de Grace, Md. SRBC has identified six subbasins in the Susquehanna River Basin: the Upper Susquehanna Subbasin, the Chemung Subbasin, the West Branch Susquehanna Subbasin, the Middle Susquehanna Subbasin, the Juniata Subbasin, and the Lower Susquehanna Subbasin (Figure 2).

The study area for this survey stretched from Sidney, N.Y., to Marietta, Pa., and encompassed every subbasin in the Susquehanna River watershed. Seven of the sites were located in the Upper Susquehanna; one site was at the mouth of the Chemung River; nine stations were in the Middle Susquehanna subbasin; one station was at the mouth of the West Branch Susquehanna River; five stations were in the Lower Susquehanna Subbasin; and one station was located at the mouth of the Juniata River. Downstream of Marietta, Pa., the river flows through a series of impoundments created by hydroelectric dams and could not be sampled using the methods in this study.

METHODS

Field and Laboratory Methods

Data collection

During July 5-28, 2005, SRBC staff placed rock basket samplers in the mainstem Susquehanna River from Sidney, N.Y., to Marietta, Pa., and at the mouths of its major tributaries for a six-week colonization period. Staff collected the rock basket samplers as well as traditional RBP kick-net samples from August 29 through October 6, 2005. Field chemistry measurements were taken at five locations across the river channel. Water quality samples also were collected for chemical laboratory analysis.

Samples were labeled with the site number, the date, the type of equipment used (RB for rock basket sampler and KS for kick screen), and the location of the sampler with relation to the site (sampler one at the left bank and sampler five at the right bank). For example, a rock basket sampler collected at JUNR 2 in the middle of the river on August 31, 2005, would be designated as JUNR2 RB3 8/31/05.

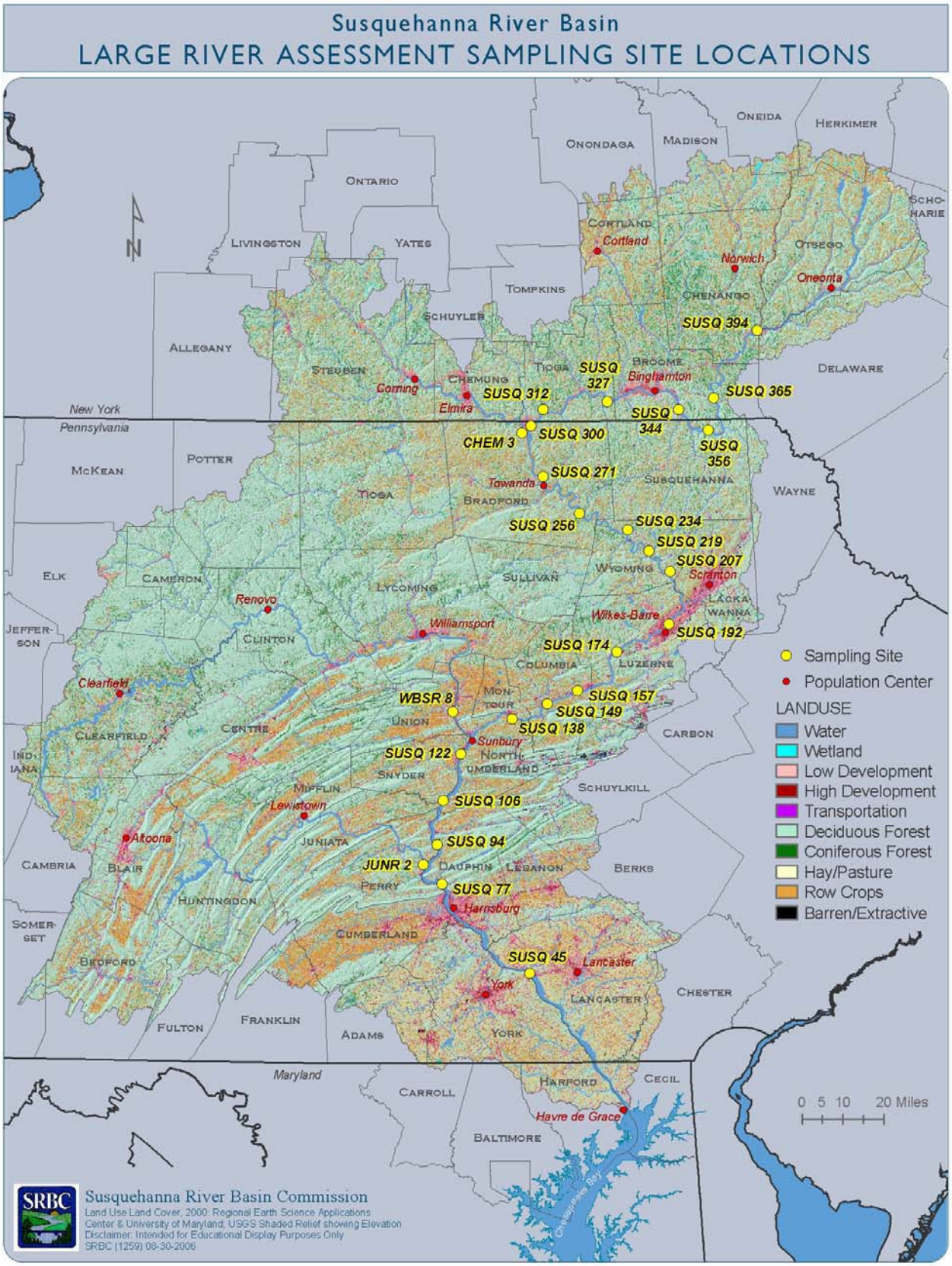

Figure 1. Large River Assessment Sampling Site Locations

Table 1. Susquehanna River Station Locations

Station Number	County/State	USGS Quad	Latitude	Longitude	Site Description
SUSQ 394	Chenango/N.Y.	Sidney, N.Y.	42.3113	-75.4199	Susquehanna River near Sidney, N.Y.
SUSQ 365	Broome/N.Y.	Windsor, N.Y.	42.0747	-75.6351	Susquehanna River at Windsor, N.Y.
SUSQ 356	Susquehanna/Pa.	Great Bend, Pa.	41.9612	-75.6620	Susquehanna River near Oakland, Pa.
SUSQ 344	Broome/N.Y.	Binghamton East, N.Y.	42.0347	-75.8017	Susquehanna River at Kirkwood, N.Y.
SUSQ 327	Tioga/N.Y.	Apalachin, N.Y.	42.0653	-76.1426	Susquehanna River near Apalachin, N.Y.
SUSQ 312	Tioga/N.Y.	Barton, N.Y.	42.0400	-76.4464	Susquehanna River at Barton, N.Y.
SUSQ 300	Bradford/Pa.	Sayre, Pa.	41.9819	-76.5065	Susquehanna River at Sayre, Pa.
SUSQ 271	Bradford/Pa.	Towanda, Pa.	41.7627	-76.4393	Susquehanna River at Towanda, Pa.
SUSQ 256	Bradford/Pa.	Wyalusing, Pa.	41.6705	-76.2786	Susquehanna River near Wyalusing, Pa.
SUSQ 234	Wyoming/Pa.	Meshoppen, Pa.	41.6099	-76.0509	Susquehanna River near Meshoppen, Pa.
SUSQ 219	Wyoming, Pa.	Tunkhannock, Pa.	41.5351	-75.9502	Susquehanna River near Tunkhannock, Pa.
SUSQ 207	Wyoming/Pa.	Ransom, Pa.	41.4594	-75.8524	Susquehanna River near West Falls, Pa.
SUSQ 192	Luzerne/Pa.	Kingston, Pa.	41.2500	-75.8845	Susquehanna River near Wilkes-Barre, Pa.
SUSQ 174	Luzerne/Pa.	Nanticoke, Pa.	41.1774	-76.1085	Susquehanna River near Shickshinny, Pa.
SUSQ 157	Columbia/Pa.	Mifflinville, Pa.	41.0405	-76.2945	Susquehanna River near Berwick, Pa.
SUSQ 149	Columbia/Pa.	Catawissa, Pa.	40.9935	-76.4369	Susquehanna River near Bloomsburg, Pa.
SUSQ 138	Northumberland/Pa.	Danville, Pa.	40.9422	-76.6011	Susquehanna River near Danville, Pa.
SUSQ 122	Snyder/Pa.	Sunbury, Pa.	40.8182	-76.8420	Susquehanna River at Hummels Wharf, Pa.
SUSQ 106	Snyder/Pa.	Dalmatia, Pa.	40.6517	-76.9226	Susquehanna River at McKees Half Falls, Pa.
SUSQ 94	Dauphin/Pa.	Halifax, Pa.	40.4958	-76.9516	Susquehanna River at Montgomery Ferry, Pa.
SUSQ 77	Dauphin/Pa.	Harrisburg West, Pa.	40.3358	-76.9125	Susquehanna River at Fort Hunter, Pa.
SUSQ 45	Lancaster/Pa.	Columbia West, Pa.	40.0365	-76.5239	Susquehanna River at Marietta, Pa.
JUNR 2	Perry/Pa.	Duncannon, Pa.	40.4258	-77.0159	Juniata River at Amity Hall, Pa.
CHEM 3	Bradford/Pa.	Sayre, Pa.	41.9607	-76.5324	Chemung River at Athens, Pa.
WBSR 8	Northumberland/Pa.	Lewisburg, Pa.	40.9679	-76.8797	West Branch Susquehanna River at Lewisburg, Pa.

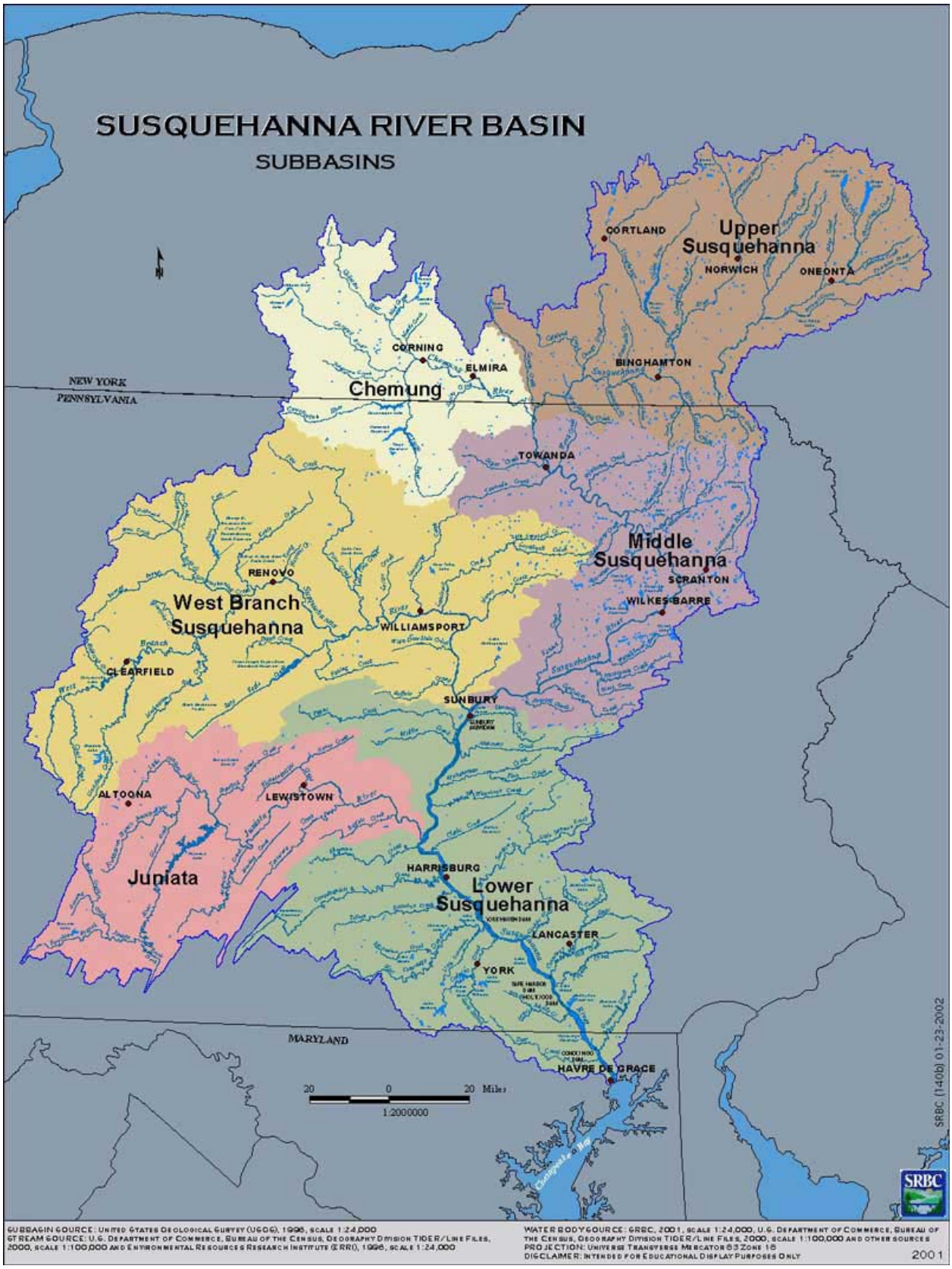

Figure 2. Susquehanna River Subbasins

Chemical water quality

Water samples were collected at each sampling site and analyzed to determine nutrient and metal concentrations in the river. Measurements were made in the field to determine water temperature, dissolved oxygen, conductivity, and pH. Temperature was measured in degrees Celsius with a field thermometer. Dissolved oxygen was measured with a YSI 55 meter that was calibrated at the beginning of every day when samples were collected, and conductivity was measured with a Cole-Parmer Model 1481 meter. A Cole-Parmer Model 5996 meter that was calibrated at the beginning of each sampling day and randomly checked throughout the day was used to measure pH. Field water quality measurements were taken at five points across the river, co-located with the rock basket samplers.

A list of laboratory parameters is located in Table 2. Laboratory samples consisted of one 500-ml bottle of raw water and two 250-ml bottles of acidified water. One of the 250-ml bottles was acidified with nitric acid for metal analyses. The other 250-ml bottle was acidified with sulfuric acid for nutrient analyses. Samples were iced and shipped to Pennsylvania Department of Environmental Protection, Bureau of Laboratories, Harrisburg, Pa., for analysis.

Table 2. Parameters for Laboratory Analysis

Parameter	
Alkalinity, mg/l[a]	Total Suspended Solids, mg/l
Total Nitrogen, mg/l	Total Sodium, mg/l
Total Nitrite, mg/l	Total Chloride, mg/l
Total Nitrate, mg/l	Total Sulfate, mg/l
Total Phosphorus, mg/l	Total Iron, µg/l[b]
Total Orthophosphate, mg/l	Total Manganese, µg/l
Total Organic Carbon, mg/l	Total Aluminum, µg/l
Total Hardness, mg/l	Turbidity, NTU[c]
Total Magnesium, mg/l	Total Calcium, mg/l

[a] mg/l = milligrams per liter
[b] µg/l = micrograms per liter
[c] nephelometric turbidity units

Macroinvertebrates

Benthic macroinvertebrates (organisms that live on the stream bottom, including aquatic insects, crayfish, clams, snails, and worms) were collected for analysis during this survey. Staff collected benthic macroinvertebrate samples using two separate methodologies, rock basket samplers and traditional RBP kick-net methods, based on the results of the 2002 pilot study. Each methodology is described in detail below.

Rock Basket Samplers

Rock basket samplers (Figure 2) are useful in assessing areas that are too deep to sample with traditional RBP methods (Merritt and others, 1996). A wire basket filled with natural river rocks from the sampling area was placed in a riffle/run area, where possible, to ensure a constant flow of water running through the sampler. Before the baskets were placed in the river, they were attached to a concrete block for stabilization and a float for marking the sampler location. Five such baskets were located on a transect across the river and left in place for at least six weeks to allow colonization. Samplers were placed by hand during July 5 - 28, 2005. Sites were chosen across the transect based on depth, velocity,

substrate, and cover within the transect. To retrieve the substrates, the baskets were separated from the concrete blocks and the floats and were placed in a collecting net while still under water. The rock basket and net were placed in a large, plastic bucket and brought to shore, where all macroinvertebrates were rinsed from the substrate and the net and placed in a jar labeled with site information and method of collection. The jar was filled with 95 percent ethanol so that the final concentration was at least 70 percent ethanol. The capped sample bottles were taken back to the laboratory to await analysis.

Figure 3. Rock Baskets Used in Large River Assessment Project

Modified Rapid Bioassessment Protocol (RBP)

SRBC has used this procedure for sampling throughout the basin since 1992. Including this methodology provides a link to past assessments in the river. The USEPA RBP III methodology (Barbour and others, 1999) was used in riffle/run areas, where present. When no riffle/run area was present, this methodology was used along the banks of the river and around the edges of islands. In riffle/run areas, samples were collected at both sides of the river and at three internal sites for a total of five sites across the riffle/run area, where possible.

Sampling was conducted by placing a one meter square kick screen perpendicular to the current and disrupting the substrate so dislodged macroinvertebrates were carried into the screen. All collected specimens were preserved in 95 percent ethanol and returned to SRBC offices for identification and enumeration.

Subsampling and sorting procedures were based on the 1999 RBP document (Barbour and others, 1999). In the laboratory, composite samples were sorted into 200-organism subsamples, when possible, using a gridded pan and a random numbers table. The organisms contained in the subsamples were identified to genus (except Chironomidae and Oligochaeta), when possible, and enumerated. Benthic macroinvertebrates were identified by professional biologists**,** with a minimum of a Master of Science degree in biology, skilled at recognizing most benthos to the family level by sight, and to the genus level with appropriate keys.

After sampling was completed at a given site, all equipment that came in contact with the sample was rinsed thoroughly, examined carefully and picked free of algae or debris before sampling at the next site. Additional organisms that were found on examination were placed into the sample containers.

Data Analysis

Chemical water quality

Chemical water quality was assessed by examining field and laboratory parameters. Limit values were obtained for each parameter based on current state and federal regulations or references for aquatic life tolerances (Table 3 – from LeFevre, 2005).

Table 3. Water Quality Limits and References

Parameter	Limit	Reference Code	Reference Codes and References
Temperature	> 25°C	a,f	a: http://www.pacode.com/secure/data/025/chapter93/s93.7.html
Dissolved oxygen	< 4 mg/l	a,g	b: Hem (1970) – http://water.usgs.gov/pubs/wsp/wsp2254/
Conductivity	> 800 µmhos/cm	d	c: Gagen and Sharpe (1987) and Baker and Schofield (1982)
pH	6 – 9	c,f	d: http://www.uky.edu/WaterResources/Watershed/KRB_AR/wq_standards.htm
Alkalinity	< 20 mg/l	a,g	e: http://www.uky.edu/WaterResources/Watershed/KRB_AR/krww_parameters.htm
Nitrogen	>1.0 mg/l	j	f: http://www.hach.com/h2ou/h2wtrqual.htm
Nitrite	> 0.06 mg/l	f,n,i	g: http://sites.state.pa.us/PA_Exec/Fish_Boat/education/catalog/pondstream.pdf
Nitrate	> 1.0 mg/l	e,j	h: http://www.epa.gov/waterscience/criteria/sediment/appendix3.pdf
Phosphorus	> 0.1 mg/l	e,k	i: http://www.dec.state.ny.us/website/regs/part703.html
Orthophosphate	> 0.05 mg/l	l,f,j,k	j: http://water.usgs.gov/pubs/circ/circ1225/images/table.html
TOC	> 10 mg/l	b	k: http://water.usgs.gov/nawqa/circ-1136/h6.html#NIT
Hardness	> 300 mg/l	e	l: http://www.epa.gov/waterscience/criteria/goldbook.pdf
Magnesium	> 35 mg/l	i	m: based on archived data at SRBC
Calcium	> 100 mg/l	m	n. http://srmwww.gov/bc.ca/risc/pubs/aquatic/interp/
TSS	> 25 mg/l	h	
Sodium	> 20 mg/l	i	
Chloride	> 150 mg/l	a	
Sulfate	> 250 mg/l	a	
Iron	> 1,500 µg/l	a	
Manganese	> 1,000 µg/l	a	
Aluminum	> 200 µg/l	c	
Turbidity	> 150 NTU	h	

Macroinvertebrate analysis

A series of macroinvertebrate metrics was calculated for each sample, and assessments of the sites were performed. Benthic macroinvertebrate samples were assessed using procedures described by Barbour and others (1999), Klemm and others (1990), and Plafkin and others (1989). Using these methods, staff calculated a series of biological indexes for each type of sampler at each station. The metrics used in this survey are summarized in Table 4. Metric 2 (Shannon-Wiener Diversity Index) followed the methods described in Klemm and others (1990), and all other metrics were derived from Barbour and others (1999).

Table 4. Summary of Metrics Used to Evaluate the Overall Biological Integrity of River Benthic Macroinvertebrate Communities

Metric	Description
1. Taxonomic Richness (a)	The total number of taxa present in the 200-organism subsample. Number decreases with increasing disturbance or stress.
2. Shannon-Wiener Diversity Index (b)	A measure of biological community complexity based on number of equally or nearly equally abundant taxa in the community. Index value decreases with increasing stress.
3. Hilsenhoff Biotic Index (a)	A measure of the organic pollution tolerance of a benthic macroinvertebrate community. Index value increases with increasing stress.
4. EPT Index (a)	The total number of Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly) taxa present in the 200-organism subsample. The index decreases with increasing stress.
5. Percent Ephemeroptera (a)	The percentage of Ephemeroptera in a 200-organism subsample. Percentage decreases with increasing stress.
6. Percent Dominant Taxa (a)	A measure of community balance at the lowest positive taxonomic level. Percentage increases with increasing stress.
7. Percent Chironomidae (a)	The percentage of Chironomidae in a 200-organism subsample. Percentage increases with increasing stress.

Sources: (a) Barbour and others, 1999
(b) Klemm and others, 1990

A reference condition approach was used to determine impairment levels for each sample. This protocol entails determining the best score for each metric. The 200-organism subsample data were used to generate scores for each of the seven metrics. Scores for metrics 1-4 were converted to a biological condition score, based on the percent similarity of the metric score, relative to the best possible metric score. Scores for metrics 5-7 were based on set scoring criteria developed for the percentages (Plafkin and others, 1989; Ohio Environmental Protection Agency, 1987). The sum of the biological condition scores constituted the total biological score for the sample, and total biological scores were used to assign each discrete sample to a biological condition category (Table 5). The biological condition scores then were averaged to obtain an overall score and category for each site.

Additionally, descriptive statistics were calculated for each sampler type and metric; F tests were performed to determine if the variances of each metric were equal; and *t* tests were performed for each sampler type and metric to determine if the results were significantly different.

Table 5. Summary of Criteria Used to Classify the Biological Conditions of Sample Sites

SAMPLING AND ANALYSIS

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TOTAL BIOLOGICAL SCORE DETERMINATION				
	Biological Condition Scoring Criteria			
Metric	**6**	**4**	**2**	**0**
1. Taxonomic Richness (a)	> 80%	79-60%	59-40%	<40%
2. Shannon Diversity Index (a)	> 75%	74-50%	49-25%	<25%
3. Hilsenhoff Biotic Index (b)	> 85%	84-70%	69-50%	<50%
4. EPT Index (a)	> 90%	89-80%	79-70%	< 70%
5. Percent Ephemeroptera (c)	> 25%	10-25%	1-9%	< 1%
6. Percent Chironomidae (c)	< 5%	5-20%	21-35%	>35%
7. Percent Dominant Taxa (c)	< 20%	20-30%	31-40%	>40%
Total Biological Score (d)				

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BIOASSESSMENT	
Percent Comparability of Study and Reference Site Total Biological Scores (e)	**Biological Condition Category**
>83%	Nonimpaired
79-54	Slightly Impaired
50-21	Moderately Impaired
<17%	Severely Impaired

(a) Score is study site value/reference site value X 100
(b) Score is reference site value/study site value X 100.
(c) Scoring Criteria evaluate actual percentage contribution, not percent comparability to the reference station.
(d) Total Biological Score = the sum of Biological Condition Scores assigned to each metric
(e) Values obtained that are intermediate to the indicated ranges will require subjective judgment as to the correct placement into a biological condition category.

RESULTS

Water Quality

During late summer 2005, water quality at most of the river sites met water quality standards (Appendices A and B). Limit values were exceeded for 79 out of 950 total water chemistry values (8.3 percent). Most of these exceedances were for total aluminum, sodium, nitrogen, temperature, and conductivity. The exceedances are listed in Table 6 and depicted in Figure 3.

Table 6. Summary of Exceedances of Water Quality Standards

Parameter	Limit Concentration	# of Exceedances	# of Data Points
Temperature	25 degrees Celsius	21	125
Conductivity	800 µmhos/cm	8	125
Total Aluminum	200 µg/l	25	25
Total Sodium	20 mg/l	17	25
Total Nitrogen	1.0 mg/l	6	25
Total Phosphorus	0.1 mg/l	1	25
Total Orthophosphate	0.05 mg/l	1	25