

# METHODS

## DATA COLLECTION

From September 21 through September 23, 2009, SRBC staff collected macroinvertebrate samples using D-frame nets on the mainstem Susquehanna River from Sidney, N.Y., to Towanda, Pa., and at the mouth of the Chemung River. Field chemistry measurements were taken at each site, and chemical water quality samples also were collected for laboratory analysis. Macroinvertebrate samples were labeled with the site number, the date, and the number of bottles used.

### Chemical water quality

Water samples were collected at each sampling site with a depth integrated sampler to measure nutrient and metal concentrations in the river. Field water quality measurements included water temperature, dissolved oxygen, conductivity, and pH. Temperature was measured with a field thermometer in degrees Celsius. Dissolved oxygen was measured with a YSI 55 meter that was calibrated at the beginning of every day when samples were collected. 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.

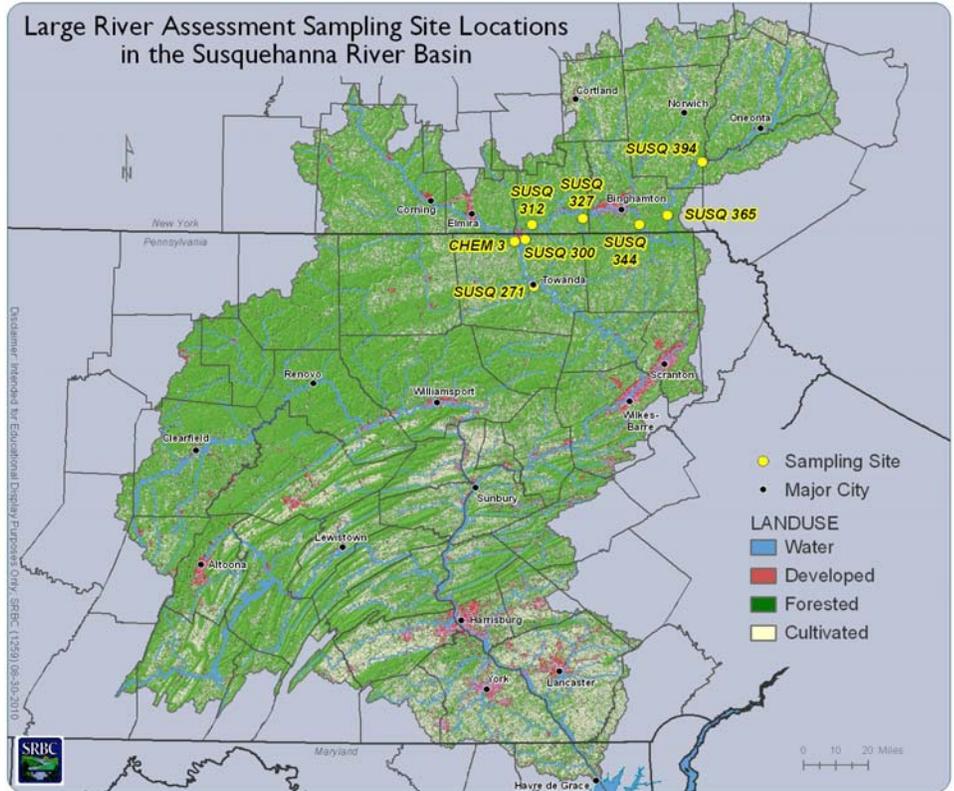


Figure 1. Susquehanna River Site Locations

A list of laboratory parameters is located in Table 2. Laboratory samples consisted of one 500-ml bottle of raw water, one 250-ml bottle preserved with nitric acid for metal analysis, and one 250-ml bottle preserved with H<sub>2</sub>SO<sub>4</sub> for nutrient analysis. Samples were iced and shipped to the Pennsylvania Department of Environmental Protection, Bureau of Laboratories, Harrisburg, Pa., for analysis.

Table 2. Parameters for Laboratory Analysis

Parameter	
Alkalinity, mg/l <sup>a</sup>	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 <sup>b</sup>
Total Orthophosphate, mg/l	Total Manganese, µg/l
Total Organic Carbon, mg/l	Total Aluminum, µg/l
Total Hardness, mg/l	Turbidity, NTU <sup>c</sup>
Total Magnesium, mg/l	Total Calcium, mg/l

<sup>a</sup> mg/l = milligrams per liter    <sup>c</sup> nephelometric turbidity units

<sup>b</sup> µg/l = micrograms per liter

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 at Sidney, N.Y.
SUSQ 365	Broome/N.Y.	Windsor, Pa.	42.0747	-75.6351	Susquehanna River at Windsor, N.Y.
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 at Apalachin, N.Y.
SUSQ 312	Tioga/N.Y.	Barton, N.Y.	42.0400	-76.4464	Susquehanna River at Nichols, 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.
CHEM 3	Bradford/Pa.	Sayre, Pa.	41.9607	-76.5324	Chemung River at Athens, Pa.

## 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 a D-frame kick net with 500 µm mesh. A three-kick composite sample, collected from representative habitat locations, was collected at each of ten equidistant transects along a one-kilometer sampling reach.



*SRBC staff member collects benthic macroinvertebrates at Great Bend, Pa.*

Alternating banks were utilized for transect sampling. For example, transects two, four, six, eight, and ten were sampled on the right bank, while transects one, three, five, seven, and nine were sampled on the left bank. Multiple habitats, including bottom substrate, woody debris, undercut banks, and macrophytes, were included in sample collection. Sampling was conducted in a 10-meter area surrounding each transect, to a depth of 0.5 meters.

Each sample was preserved in the field in 95 percent denatured ethyl alcohol. After sampling was completed at a given site, all equipment that came in contact with the sample was examined carefully, picked free of algae or debris, rinsed thoroughly and sprayed with 10 percent bleach solution before sampling at the next site. Additional organisms that were found on examination were placed into the sample containers.

Subsampling and sorting procedures were based on the 1999 RBP document (Barbour and others, 1999). In the laboratory, composite samples were sorted into 300-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.

## 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, Buda, 2008).

### 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 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 3. Water Quality Limits and References**

Parameter	Limit	Reference Code
Temperature	> 25 degrees	a,f
Dissolved Oxygen	< 4 mg/l	a,g
Conductivity	> 800 µmhos/cm	d
pH	< 5	c,f
Alkalinity	< 20 mg/l	a,g
Total Dissolved Solids		
Total Nitrogen	> 1.0 mg/l	j,k,l
Total Nitrite	> 1.0 mg/l	f
Total Nitrate	> 1.0 mg/l	e
Total Ammonia	based on pH & temperature	a
Total Phosphorus	> 0.1 mg/l	e
Total Organic Carbon	> 10 mg/l	b
Total Hardness	> 300 mg/l	e
Total Magnesium	> 35 mg/l	i
Total Sodium	> 20 mg/l	i
Total Chloride	> 150 mg/l	a
Total Sulfate	> 250 mg/l	a
Total Iron	> 1,500 g/l	a
Total Manganese	> 1,000 g/l	a
Total Aluminum	> 200 g/l	c
Total Orthophosphate	> 0.05 mg/l	l

### Reference Code & References

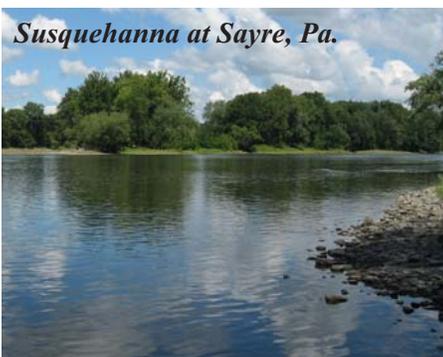
- a. <http://www.pacode.com/secure/data/025/chapter93/s93.7.html>
- b. Hem (1970)
- c. Gagen and Sharpe (1987) and Baker and Schofield (1982)
- d. [http://www.uky.edu/WaterResources/Watershed/KRB\\_AR/wq\\_standards.htm](http://www.uky.edu/WaterResources/Watershed/KRB_AR/wq_standards.htm)
- e. [http://www.uky.edu/WaterResources/Watershed/KRB\\_AR/krww\\_parameters.htm](http://www.uky.edu/WaterResources/Watershed/KRB_AR/krww_parameters.htm)
- f. <http://www.hach.com/h2ou/h2wtrqual.htm>
- g. [http://sites.state.pa.us/PA\\_Exec/Fish\\_Boat/education/catalog/pondstream.pdf](http://sites.state.pa.us/PA_Exec/Fish_Boat/education/catalog/pondstream.pdf)
- h. <http://www.deq.virginia.gov/vpdes/pdf/vpdesregulationfeb02.pdf>
- i. <http://www.dec.state.ny.us/web site/regs/part703.html>
- j. <http://water.usgs.gov/pubs/circ/circ1225/images/table.html>
- k. <http://www.crc.govt.nz/Land/pdf%20files%20sheet13.pdf>
- l. <http://www.water.ncsu.edu/watersheds>

**Table 4. 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 300-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 300-organism subsample. The index decreases with increasing stress.
5. Percent Ephemeroptera (a)	The percentage of Ephemeroptera in a 300-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 300-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 300-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 sample to a biological condition category (Table 5).



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

TOTAL BIOLOGICAL SCORE DETERMINATION				
Metric	Biological Condition Scoring Criteria			
	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 Dominant Taxa (c)	< 20%	20-30%	31-40%	>40%
7. Percent Chironomidae (c)	< 5%	5-20%	21-35%	>35%
<b>Total Biological Score (d)</b>				

BIOASSESSMENT	
Percent Comparability of Study and Reference Condition 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