

SUSQUEHANNA RIVER SAMPLING PLAN
WATER QUALITY STANDARDS
BUREAU OF POINT & NON-POINT SOURCE MANAGEMENT
DEPARTMENT OF ENVIRONMENTAL PROTECTION
COMMONWEALTH OF PENNSYLVANIA

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INTRODUCTION

Wide-scale, disease-related mortality of young-of-year (YOY) smallmouth bass was first documented in 2005 and again annually at varying degrees between 2006 and 2011 at the West Branch Susquehanna, Susquehanna, and Juniata rivers. Since 2010, bacterial infections resulting in lesions have also been documented in a number of warm-water streams in the Susquehanna River Basin. Fish pathology studies conducted by USGS indicate there is a high degree of intersex among the smallmouth bass in the river caused by endocrine disruption. Endocrine disruption also compromises the immune system of fish which can lead to the type of infections and lesions seen on the fish. The question is whether the infections and lesions are due to endocrine disruption alone or are there other water quality stressors contributing to the problem. The current hypothesis is that there are other stressors involved.

Excessive nutrients can cause excessive algal growth that in turn leads to depression of oxygen levels. Depressed oxygen levels would be a major stressor on fish. As a result, the proposed study will detail the chemical composition and biological processes associated with nutrient inputs to the Susquehanna River at various locations. Sampling of both the water column and benthic substrate will allow for analyses of both the nutrient inputs and responses to those inputs.

Temperature is another major stressor that may contribute to the problem, especially in the Susquehanna River which is broad and shallow in many segments. During low river flows, the broad area and shallow water results in more solar heating than in other major Pennsylvania rivers. Shallow backwater fish nursery areas are especially susceptible.

Finally, a search for the possible endocrine disrupters and some of the newer agricultural pesticides and herbicides will be conducted in the water column, sediment, and fish organs such as liver and ovaries where the contaminants concentrate.

CRITERIA

Pennsylvania does not currently have numeric criteria for nutrients. Development of numeric criteria for adoption into standards is in progress and being guided for contracted studies looking at the relationships between elevated nutrient concentrations/loadings and aquatic life. Pennsylvania's assessment protocol for identification of nutrient impairments is being revised to include a more robust examination of the adverse impacts from excessive nutrient inputs. Biological indicators such as the macroinvertebrate community, algal biomass, algal indicator species or community structure, along with diurnal oxygen fluctuations will be used in making impairment determinations.

NUTRIENT STUDY PLAN

Phase 1

Sampling Locations

Four sites will be monitored as part of this study. They are:

- Susquehanna River at City Island
- Susquehanna River at Sunbury
- Juniata River at Newport
- Delaware River at Trenton (control)

Phase 1 of this study includes 4 intensively sampled sites. For sites included in this phase, the river will be divided into thirds or halves and sampling will take place at one or more locations within each third or half of the river as discussed below and according to the appropriate attached protocols.

Flow

All sample sites are located at USGS gaging stations where flow is monitored continuously.

Field Chemistry

Continuous field measurements of dissolved oxygen (mg/L), oxygen saturation (%), temperature (°C), specific conductance ($\mu\text{S}/\text{cm}$), and pH (units) will be measured using YSI 6-Series Sondes or Eureka Manta2 Sondes in three separate transects across the Susquehanna River at City Island and the Delaware River at Trenton sites and two separate transects across the Susquehanna River at Sunbury and the Juniata River at Newport sites. Instruments will be calibrated prior to sampling using analytical standards as detailed in the Department's Continuous Instream Monitoring Protocol.

In addition to continuous field measurements, discrete field measurements will be periodically collected along transects in an effort to document mixing processes at each site. Four transects have been established at the Susquehanna River at City Island site. One transect is located downstream of the Rockville Railroad Bridge, a second is located downstream of the Rt. 81 Bridge, a third transects City Island, and the fourth is located downstream of Dock Street Dam. Transects will be established at the Susquehanna River at Sunbury, Juniata River at Newport and Delaware River at Trenton sites.

Benthic Macroinvertebrates

Benthic macroinvertebrates will be collected in three separate transects across the Susquehanna River at City Island and the Delaware River at Trenton sites and two separate transects across the Susquehanna River at Sunbury and the Juniata River at Newport sites. Sample results from within each transect could consequently be composited and rarified to appreciate the community and selected indices across the entire width of the sites. This is a

slight modification from the Department's RBP benthic sampling methodology that would otherwise be a complete width sample composite.

Algal Biomass and Cellular Nutrients

Nutrient and algal sampling will be conducted during the summer low-flow critical condition. Antecedent rainfall and river stage conditions will be monitored throughout the summer and sampling will take place at least two weeks after any potentially scouring flows.

Figure 1 represents a single Phase 1 station of which there are three per sampling location (right near shore, left near shore, center). Continuous data loggers will be placed in each third of the river at each sampling location. Additionally, periphyton will be collected from 27 rocks – three rocks at each of the red triangles in Figure 1 - and composited to provide benthic biomass and cellular nutrient values for each transect (A, B and C).

Epilithic periphyton sampling is conducted using a **Pennsylvania Epilithic Periphyton Sampler (PEP Sampler)**. The PEP sampler provides a fixed circular sample area (17.8 cm², 2" to 1.5" PVC Step Down Fitting) with a foam gasket seal (toilet seal) that is clamped to rock substrates (large gravel, cobble, small boulder) using Trex^R clamping boards that can be tightened with quick release 5-star knobs (Rockler.com) or wing nuts on threaded rods.

A near shore habitat will be selected that is visually representative with regard to substrate size, shading, and periphyton standing crop. Visually stratify the sample area into thirds (upper, middle, lower), and randomly place one flag marker along the bank of the stream in each segment.

Each transect is divided into thirds and three rocks are randomly collected within each third of the transect (n=9 per transect). The periphyton samples from nine rocks along each transect are composited. When retrieving rocks the collector should reach down at the collection location and pick a rock by touching it lightly with a finger while refraining to look at the streambed. If the rock is a suitable size for periphyton sampling (will accept a 2 inch diameter seal) it is placed in an empty dishpan for transport to the PEP sampler. If the substrate is too small to obtain an adequate PEP sampler seal or too large to remove (large boulder, bedrock), the collector should retrieve the closest rock to the selected rock that is of suitable size for sampling. Dishpans can be covered to protect samples from sunlight and desiccation prior to scraping.

Clamp rocks to the PEP sampler. Prior to sampling, check to ensure that water is ponding around the gasket indicating a tight seal. Attached algae is removed by repeated scrubbing with a modified grout brush, rinsing with distilled or deionized water and transferring the slurry to a plastic sample container (approx. 1 liter) with a modified wide bore disposable pipette or turkey baster. Removal of filamentous green algae (eg. *Cladophora*) may require cutting/scraping with an Exacto knife. Scrub the area for a minimum of 60 seconds until the area is visibly devoid of algae. Rinse water will appear clear after sampling unless substrates are easily erodeable (eg. shale, sandstone). Slurries from each transect (9 rocks each) are composited in separate sample containers. Total sample volumes are determined by pouring the algal slurries into a 500 ml volumetric cylinder. A funnel should be used for transfers to minimize spillage. Slurries can be processed in the field or returned to a regional laboratory for processing.

Samples containing filamentous macroalgae require utilizing a blender or food processor to homogenize the sample by breaking apart filaments. Well-mixed subsamples (shaking a wide-

mouth bottle, or blending) from each transect composite are taken for 1) Chlorophyll-a (Chl-a) and Phaeophytin analysis, 2) cellular carbon and nitrogen, and 3) cellular phosphorous. Subsamples for filtration are taken using a Hensen-Stempel pipette (2ml) or a conventional "to deliver" pipette with a modified wide bore tip. Chl-a samples are filtered onto glass fiber filters (Whatman EPM 2000, 0.3 μm , 47mm) using a filter apparatus and vacuum pump (≤ 10 psi). Chl-a filter volumes are dependent upon algal slurry concentrations but should be in the range from 2ml (eutrophic, unshaded) to 10 ml (oligotrophic, shaded). After releasing the vacuum, dry filters are removed from the filter apparatus using flat-ended filter forceps. Filters are quartered and wrapped in aluminum foil. Cellular carbon and nitrogen samples are filtered onto glass fiber filters (Whatman EPM 2000, 0.3 μm , 47mm). Carbon-Nitrogen (CN) Filter volumes are dependent upon algal slurry concentration but should range from 10ml to 30ml. Cellular Phosphorus (P) samples are filtered onto polycarbonate filters (Millipore Polycarbonate, 0.2 μm , 47mm). P Filter volumes are dependent upon algal slurry concentration but should range from 10 ml to 30ml. Cellular P filtration is a time limiting step because of the slow filtration rate. If available, two filter apparatuses will help shorten processing time. As with the Chl-a filters, CN and P filters are quartered and wrapped in aluminum foil. Care should be taken to only handle the cellular nutrient filters with forceps to avoid potential contamination. Filters for each station should be stored on ice (if field filtered) in separate bags and frozen upon returning to the lab.

The remaining transect algal slurries are composited and a well-mixed subsample is taken for algal identification and enumeration. A 100ml subsample is transferred to a 125ml Nalgene bottle and preserved with formaldehyde for algal identification and enumeration.

Labels for all processed samples should include station ID, date, subsample type (Chl-a, CN, P, Algal ID) and subsample volume. Total surface area for each transect (eg. 9 rocks x 18.1 $\text{cm}^2 = 162.9 \text{ cm}^2$), total transect volumes, Chl-a subsample volumes, total composite volume, CN and P subsample volumes and algal ID volumes are recorded on the Periphyton Survey Data Sheet.

QA – One replicate subsample will be collected for Chl-a, CN, P (transect A, B, or C) and Algal ID (composite) for every 10 stations sampled (10%) to examine subsample variability. Replicate information should be on the field form to the right of the routine volume information and marked with an R.

Artificial substrate will also be placed in the river at the time of data logger deployment that will allow for the establishment of algal growth rates with a known starting point. The artificial substrate will consist of three clay tiles will be attached to a cinder block and the periphyton will be harvested from the tiles after 14 and 28 and 42 days.

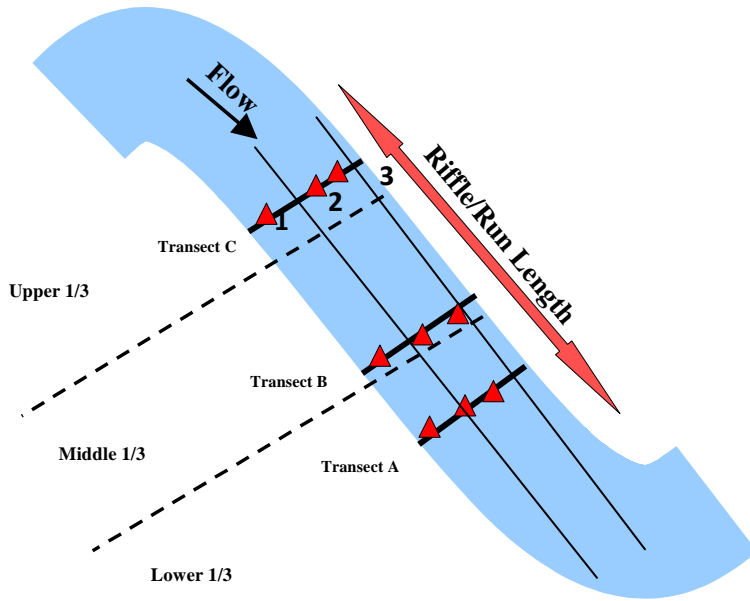


Figure 1. Diagram for intensive Phase 1 sampling that will take place in three sections across the river (only one section is shown in figure).

A series of analyses will be run on the composited slurry at each site to quantify microbial biomass, P-storage, and nutrient content (C, N, P, and Poly-P). Algal biomass will be estimated for each sample by concentrating subsamples onto filter membranes. The chlorophyll concentration in each filter will be determined using an organic extraction procedure (50:50 mixture of 90% Acetone to DMSO); Chl-a concentrations will subsequently be measured using a standard fluorometric technique (Carrick et al. 1993a). Standard analytical techniques will be used to measure nutrients in stream biofilm samples. Total carbon and nitrogen concentrations will be measured via combustion on subsamples concentrated onto Whatman glass-fiber filters (n=90) using a Carlo-Erba CN analyzer (Horneck and Miller 1998). Total P concentrations in the biofilm material will be measured using the persulfate digestion, where liberated soluble reactive P (as PO_4^{3-}) will be measured colorimetrically using a spectrophotometer (method 365.1, USEPA 1997, 2002). Polyphosphate concentrations (Poly-P) in biofilms material will be measured by heating samples at 100°C for 60 minutes, thereby liberating PO_4^{3-} from the condensed, inorganic phosphate compounds that can occur in either cyclic, linear, or cross-linked bonds with oxygen (Fitzgerald and Nelson 1966; Harold 1966); this material will then be analyzed using the spectrophotometric method described above.

Periphyton taxonomic composition will be determined from composite samples taken for each site. The samples will be counted following a double-blind procedure, whereby the counter does not have prior knowledge of the sample identity nor were they involved in the sample collection.

Lab Chemistry

Grab samples for nutrient analyses will be collected as composite samples at each transect. The parameters for analysis include:

- Total Suspended Solids
- Ammonia, Dissolved as Nitrogen

- Ammonia, Total as Nitrogen
- Kjeldahl Nitrogen, Dissolved
- Kjeldahl Nitrogen, Total as Nitrogen
- Nitrate & Nitrite, Total as Nitrogen
- Nitrate & Nitrite, Dissolved as Nitrogen
- Phosphorus, Total as P
- Phosphorus, Dissolved as P
- Phosphorus Ortho Dissolved

Algal Taxonomic Composition

Periphyton community structure will be determined from composite samples taken for each site. The samples will be counted following a double-blind procedure, whereby the counter does not have prior knowledge of the sample identity nor were they involved in the sample collection.

Algal taxonomic composition will be measured using a stratified enumeration technique with a Leica DMR research microscope (Carrick and Schelske 1997; Carrick and Steinman 2001). Periphyton taxonomic composition will be determined from composite samples. The samples will be counted following a double-blind procedure, whereby the counter does not have prior knowledge of the sample identity nor will they be involved in the sample collection.

To enumerate cells, samples will be mixed and injected into Palmer-Maloney counter chambers (0.1 ml), from which >1,000 cells are counted in each sample (<4% counting error assuming Poisson Statistics). The procedure is as follows. First, the entire chamber is scanned for 15 minutes to gain familiarity with the flora present in the sample. Then, the entire chamber is counted at 100x magnification in order to accurately enumerate larger algal species (half chambers are counted for samples with extremely high cell densities). Last, random fields are counted at 400x magnification in order to estimate the abundance of smaller, more numerous algal cells. In most cases, organisms are identified to the species or generic level, but most diatoms are placed into categories based on their general morphology and relative size (e.g., Naviculoid, Cymbelloid, Gomphonemoid). Diatom species are enumerated using a specialized set of techniques as described below. Cyanobacterial filaments are enumerated, and converted to cell numbers using an estimate for the average number of cells per filament as determined for a subset of organisms' encountered (5-10 filaments per sample). The regular geometric figure that best describes the shape of each taxon will be used to calculate its average biovolume in μm^3 (Carrick and Steinman 2001). The biomass of all individuals encountered is calculated by multiplying the average biovolume of each taxon by its cellular abundance; the product is expressed in biomass per unit area of stream bottom assuming a specific density of 1 g/cm^3 . The occurrence (abundance and biomass) of dominant taxa belonging to the five algal phyla present in these samples (Bacillariophyta, Chlorophyta, Cyanobacteria, and Euglenophyta, and Rhodophyta) are summed, and these data are subsequently used to assess nutrient thresholds.

Diatom taxonomic composition will be enumerated from permanent slides prepared using a standard nitric acid digestion and mounting procedure (Patrick and Reimer 1966). Random fields are counted under 1000x magnification until >400 frustules are enumerated (~5% counting error assuming Poisson Statistics). The diatoms are identified to their lowest taxonomic category (species, or variety), and the taxon-specific abundances are tabulated and expressed in cells/cm².

Phase 2

Sampling Locations

Six additional sample locations will be added to the three Phase 1 locations. Two of the three sondes at each Phase 1 site will be moved to the Phase 2 sites with one sonde being left to continue recording at Phase 1 sites. Locations for Phase 2 samples are still being chosen, but the sites will be matched to previously completed YOY studies where diseased fish were found or at sites identified as nutrient enriched sections of the river during flyovers.



Figure 2. Example Clay tiles to be used as artificial substrate in Phase 2. Left photo taken day of deployment, right photo after 14 days growth

Flow

Flow will again be estimated from the USGS gaging stations for the sites that were part of Phase 1. Flow at other sites will be determined by collecting manual discharge measurements.

Field Chemistry, algal biomass and cellular nutrients, algal taxonomic composition and lab chemistry will all be collected the same as Phase 1.

Project Timeline

TASK	T ₀	T ₁₄	T ₂₈	T ₄₂	T ₅₆
Deploy sondes at Phase 1 sites	X				
Deploy artificial substrate at Phase 1 sites	X				
Collect water quality samples at Phase 1 Sites		X			
Collect algae from natural substrate at Phase 1 sites		X			
Collect algae from artificial substrate at Phase 1 sites		X	X	X	
Collect water quality sample at Phase 1 artificial substrate location		X			
Redeploy 2 sondes from each Phase 1 site to Phase 2 sites		X			
Deploy artificial substrate at Phase 2 sites		X			
Collect water quality samples at Phase 2 Sites			X	X	X
Collect algae from artificial substrate at Phase 2 sites			X	X	X

Algal Analysis Budget

Itemized Breakdown*	Time	reps	sites	Total	Unit Cost	Total Cost
CN Periphyton	1	1	63	63	\$50.00	\$3,150.00
Poly-P	1	1	63	63	\$25.00	\$1,575.00
Chlorophyll Biomass	1	1	63	63	\$15.00	\$945.00
Counts Diatoms and Soft algae	1	1	63	63	\$200.00	\$12,600.00
Analysis & Interpretation	1	1	1	1	\$1,000.00	\$1,000.00
					Lab Costs	\$19,270.00
					Overhead	\$8,286.10
					Total	\$27,556.10

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