Filamentous Algae Monitoring Program:

Potomac River Basin

Report to the West Virginia Department of Environmental Protection,

Division of Water and Waste Management

by

A. Griggs, M. Selckmann, and J. Cummins

Interstate Commission on the Potomac River Basin

March 31, 2015

ICPRB Report 15-06

This report can be downloaded from the Publications tab of the Commission's website, www.potomacriver.org. To receive hard copies of the report, please write:

Interstate Commission on the Potomac River Basin
30 West Gude Drive, Suite 450
Rockville, MD 20850
or call 301-984-1908.

Suggested citation for this report
Griggs, A., and M. Selckmann. 2015. Three-year Summary Report on the Filamentous Algae Monitoring Program: West Virginia Rivers of the Potomac River Basin. Report prepared by Interstate Commission on the Potomac River Basin for the West Virginia Department of Environmental Protection, Water Quality Standards Program. ICPRB Report 15-06.

Background

West Virginia Department of Environmental Protection (WVDEP) has been observing and evaluating the breadth and causes of filamentous green algae blooms in rivers across the state since 2007. Blooms of filamentous algae occur in rivers of the Potomac Basin, and the Interstate Commission on the Potomac River Basin (ICPRB) has assisted the WVDEP in documenting algae blooms in the South Branch Potomac, Cacapon, and Shenandoah rivers since 2012. After three years of data collection, a synopsis of results and lessons learned is being provided at the request of WVDEP staff. A summary report of the 2014 season is offered first, followed by the three-year project update.

Field methods

In 2014, ICPRB biologists implemented the WVDEP Filamentous Algae Monitoring Protocol (WVDEP 2013) at 14 fixed locations over 10 rounds between June and October (**Table 1**). Information on the WVDEP filamentous algae monitoring program, including the Standard Operating Procedures for algae sampling and water chemistry, and the program's field data sheet can be found on-line at http://www.dep.wv.gov/WWE/Programs/wqs/Pages/FilamentousAlgaeinWestVirginia.aspx. The protocols consist of routine water chemistry sampling, a rapid assessment style field form, semi-quantitative algae coverage estimates, and longitudinal surveys to document the extent of bloom events. A total of three longitudinal surveys were performed in 2014. The ICPRB field crew consisted of at least two biologists for all sampling rounds and longitudinal surveys. Personnel included ICPRB staff persons Adam Griggs, Mike Selckmann, and/or Jim Cummins.

Sampling Round	Sampling Dates
JUN-1	Jun 11-12
JUN-2	Jun 25-26
JUL-1	Jul 9-10
JUL-2	Jul 23-24
AUG-1	Aug 6-7
AUG-2	Aug 19-20
SEP-1	Sep 3-4
SEP-2	Sep 18-19
OCT-1	Oct 7-8

Table 1. 2014 sample rounds and dates.

Station locations

The fourteen sampling stations were targeted by the WVDEP, based upon past observations, targeted inquiries, and best professional judgment. Eight stations are located in the Cacapon basin, seven on the Cacapon River main-stem between the towns of Largent and Wardensville, and one on North River, the Cacapon's largest tributary. Four historical stations were located on the South Branch Potomac, one above and three below the town of Moorefield, WV (**Table 2 and Figure 1**). An additional two sites were added on the South Branch further upstream, above and below the town of Petersburg. Nine out of fourteen stations were located at or near bridge crossings, while the other five were accessed along parallel roadways. Seven stations had public assess put-ins, and the remainder were accessed from bridge right-aways or through private landowner permission. Stations were generally sampled one river at a time, traveling sequentially either upstream or downstream, depending upon the route. However, overnight accommodations or camping locations influenced sampling routes, if used.

Site Name	Site Location Description	Lat / Long Coordinates	
NO_FRKS	North River at Gaston Rd. / Forks of Cacapon	39.40194	-78.42448
CA_LRGNT	Cacapon River at Rt. 9 in the town of Largent	39.48112	-78.38448
CA_FRKS	Cacapon River at Rt. 127 / Forks of Cacapon	39.40387	-78.41842
CA_D_CPBRG	Cacapon River at farm off Cold Stream Road	39.32716	-78.42336
CA_CPBRG	Cacapon River at Rt. 50 in Capon Bridge	39.29754	-78.43517
CA_RMRCK*	Cacapon River along Capon River Rd.	39.21969	-78.47605
CA_YLWSPR**	Cacapon River at Rt. 259 below Wardensville	39.18281	-78.50597
CA_WRDS	Cacapon River at farm ford in Wardensville	39.07861	-78.61134
SBR_L_TRGH	South Branch at Harmison's Landing	39.22810	-78.85251
SBR_U_TRGH	South Branch at South Branch WMA	39.14630	-78.92519
SBR_L_MRFLD	South Branch at Rt. 220/28 in Moorefield	39.10424	-78.95801
SBR_U_MRFLD	South Branch at Fisher Rd above Moorefield.	39.05006	-78.99316
SB_L_PBRG	South Branch at Weldon Park	38.98815	-79.12126
SB_U_PBRG	South Brnach at Rt. 200 bridge	38.99955	-79.08596

Table 2. Sampling station names and locations. * New site for 2013 **Station location changed from 2012 (Previously CA_LWR_WRDS).

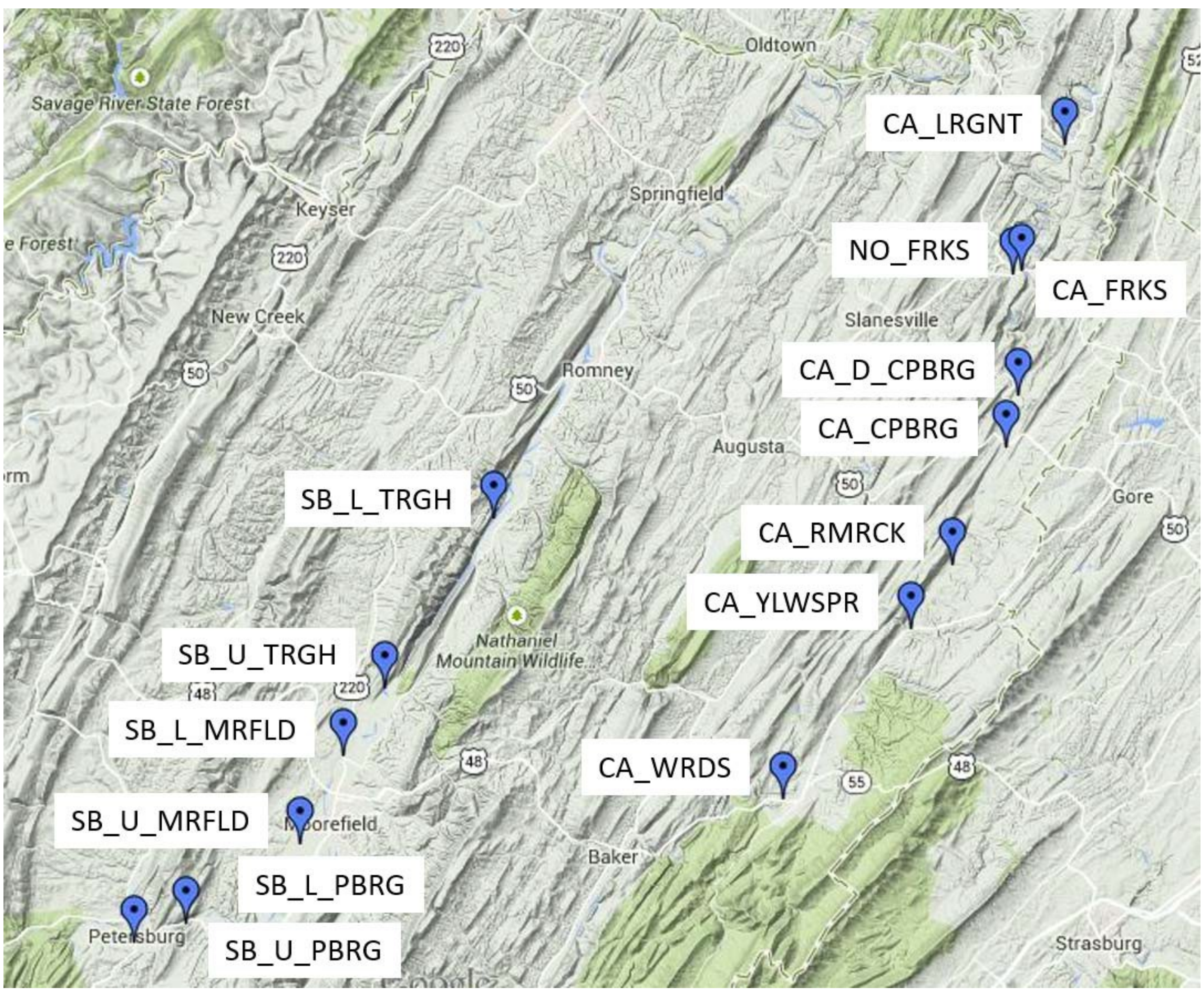


Figure 1 A map of algae monitoring stations on the Cacapon, and South Branch Potomac Rivers.

Site characterization

The WVDEP Filamentous Algae Monitoring Form was generally completed in the field by the crew leader. As the sites are fixed positions, Global Positioning System (GPS) coordinates were taken using a Garmin Etrex20 on the first and occasional subsequent field visits. If for any reason the sampling location was moved, the recorded GPS coordinates reflect that change. GPS coordinates were also taken whenever transect measurements of algae were performed. Relevant USGS gage hydrographs for the study period are included in **Appendix I**. Qualitative observations of periphyton, aquatic moss, aquatic vascular plants, filamentous green algae (FGA) and cyanobacteria/blue-green algae (BGA) abundance were made on each site visit. A site map was drawn on the first visit indicating the water quality sampling location (X-point) and the location of the algae transect, if performed.

Photo documentation

Pictures were taken on each site visit, arranged in folders according to site and sampling date, and stored on a DVD hard copy that was shared with WVDEP staff. Generally, photos were taken at the x-site, one picture each looking upstream, downstream, and across the channel. Photos were also taken of any algae observed or measured, including underwater photos, or anything else of note, including sample collection or processing, in-situ probe placement, etc. Photos were documented on page 4 of the field sheet. A Nikon AW100 was the primary camera used and is capable of attaching GPS coordinates of the pictures as they were taken. This information is in the details of the file properties. GPS coordinates did not always accompany pictures and are generally missing from underwater shots and videos. Backup cameras included personal cameras and camera phones and were used whenever the primary camera wasn’t available. All pictures and videos were arranged by sample location and date and provided on a DVD hard copy to WVDEP.

Filamentous algae abundance measurements

Percent algae coverage measurements were performed according to Standard Operating Procedures (SOP) provided by WVDEP. Measurements were recorded in feet and tenths of a foot. 2014 protocols refinements included guidance on when algae measurements are measured by transect, versus a single visual estimate of the transect. Single visual estimates of the entire transect are sufficient if algae is estimated to be below 10% or above 80%. Moderate amounts of algae require transect-segment based estimate-measures. If algae is measured between 20% and 40%, three separate transect measures are required spanning a length of 3X the average channel width. Lengths and depths of the lateral transects were reported in tenths of a foot using a field tape and surveying rod. Large rivers were measured using a laser range finder. All values were entered on the field form and translated to the percent algae calculation spreadsheet file. The file was modified from that provided by WVDEP to receive the measurements as recorded, in order to calculate the percent coverage of the entire transect. The modified percent algae coverage calculation spreadsheets and associated data are provided separately as a Microsoft Excel© file with each measurement occupying one tab. Algal measurements were taken regularly at the station along Capon River Rd, downstream of Camp Rim Rock (CA-RMRCK), where algae was present throughout most of the field season, and at several other Cacapon stations. South Branch Potomac stations that manifested algae included the upper Moorefield station (SB_U_MRFLD) and the upper Petersburg station (SB_U_PBRG). Other stations experienced slight algae growth but none at levels above 10% which require transect measurements. Algal measurements were also performed during longitudinal surveys when filamentous algae were encountered.

In-situ water quality

In-situ water quality was collected at every site with the same YSI-556 multi-parameter sonde throughout the season. Water temperature (WTEMP), dissolved oxygen (DO), pH, specific conductance (SPCOND) and total dissolved solids (TDS) were measured in-situ and recorded on the field data sheet. The YSI-556 was calibrated at the beginning of each 2-day sampling round using concentration standards. Specific conductance was calibrated using a 447.1 µS/cm standard solution and pH was calibrated using a 2-point (7.01 and 10.00) calibration. Dissolved Oxygen was calibrated using a saturated air calibration method, according to the user manual of the YSI-556.

Water chemistry

Four sample containers were filled at each sampling location for the following parameters: Total phosphorous (TP), dissolved phosphorous (DP), total kjeldahl nitrogen (TKN), nitrate-nitrite-N (NO3-NO2-N), total alkalinity (TALK), calcium (CA), magnesium (MG), and total suspended solids (TSS). Water-chemistry sample containers were provided pre-fixed with acid preservatives by the contracted analysis laboratory Bio-Chem. At each sampling location, a collection container was rinsed 3 times and samples were collected facing upstream. The sampling location within the river was indicated on the monitoring form. Filtering for the dissolved phosphorous sample was performed using a Nalgene© filter funnel cup, Nalgene© vacuum flask, 47 mm 0.45 µm cellulose-nitrate filter papers and a hand-operated vacuum pump. The vacuum flask and filter apparatus were also rinsed 3 times mid-stream prior to filtering. Samples were collected according to WVDEP Standard Operating Procedures for water chemistry sampling. Sample duplicates were collected during each round and were analyzed alongside the 14 station samples.

Toward the end of the sampling season, an investigation by WVDEP staff raised quality assurance concerns about the accuracy of the phosphorous sample analysis performed by the contracted lab, Bio-Chem (Hurricane, WV). Sample blanks revealed that the laboratory's analysis equipment was not being calibrated for low levels of P with a known standard, but rather by means of a calibration curve. Submitted sample blanks turned up false positives and previous results were called into question. WVDEP temporarily suspended the delivery of samples to Bio-Chem while the issue was being resolved. This resulted in a missed round of water chemistry being collected for Round 8. New equipment was ordered by Bio-Chem Testing and follow-up quality assurance investigations eventually resulted in the resumption of sample delivery and analysis to Bio-Chem. The results of the water chemistry will be discussed later in greater detail.

Sample handling

Water chemistry samples were labeled with a permanent marker and immediately stored on ice. All samples were collected on contiguous days and delivered directly to BioChem drivers, typically in

Petersburg at the end of the two-day sampling round. On occasion, when BIoChem drivers could not meet staff at the end of sampling, ICPRB biologists left samples and chain-of-custody forms at the West Virginia Department of Agriculture Moorefield laboratory for later pick-up by BioChem.

Completeness

All 14 stations identified by WVDEP personnel were sampled throughout the study period. Nine sampling rounds were completed during the study period on a roughly bi-weekly schedule. A tenth round was called off after algae was documented to be in rapid decline at the end of its growing season. All sites were monitored within a consecutive 2-day period. Complete sets of water chemistry samples were collected on every round with the exception of Round 8, where no samples were collected due to laboratory issues mentioned earlier. Algae transects were performed whenever algae were observed and estimated to be above 10% coverage. Occasionally, water clarity, or visual surface disturbance due to precipitation, prevented performing the qualitative visual assessments at certain sites. Several in-situ water quality measurements were missed when batteries died in the field, which were always replaced prior to the next station. Photographs were taken during every round with the exception of Round 5 due to mechanical issue with the camera.

Longitudinal surveys

Longitudinal surveys were employed to document the magnitude and extent of filamentous algae blooms in a sequence of targeted areas over the last three years. In order to survey suspected bloom areas that are not visible from roadways, biologists use canoes/boats to travel along a river reach and record observations and measurements in suspected algae occurrence areas. The longitudinal surveys are an informal assessment method, but consist primarily of documenting observations with written accounts, photographs and videos, and associated GPS coordinates at observation points. Three longitudinal surveys were performed during the 2014 season. Two longitudinal surveys were performed on the South Branch Potomac on the same day on contiguous sections between Moorefield and the trough take-out at the SB_L_TRGH site in collaboration with WVDEP. Two ICPRB staff members surveyed the lower reach, and two WVDEP staff members, James Summers and James Peterson, and a third ICPRB staff person teamed up to survey the upper section of the South Branch Potomac River. A third longitudinal survey was performed by ICPRB on the Cacapon River between Capon Bridge, WV and Cacapon Forks, WV. The three sections of river were those that were identified by WVDEP and ICPRB biologists as areas of interest and were sections of river that were not previously surveyed with longitudinal methods in the prior two years. Reports presenting the findings of the three longitudinal surveys are included as separate results sections in this report.

In 2014, ICPRB staff worked separately on a project for the Environmental Protection Agency, Region 3 to develop filamentous algae monitoring methods for the rivers and streams of Virginia, using the Shenandoah Basin as a pilot watershed. The WVDEP filamentous algae program and protocols served as a foundation for this effort. One project task sought to formalize longitudinal methods for documenting algae blooms. A new protocol and datasheet were developed that allow for qualitative description of river segments along a longitudinal route. Early versions of the datasheets and protocol were shared with WVDEP biologists to gain their feedback in an attempt to make the methods useful for future WVDEP assessments.

Data Processing and Laboratory Methods

Data processing

Data were entered into MS Excel for exploratory analyses. Hard-copy datasheets were sent to WVDEP c/o James Peterson. A copy of this electronic dataset is included in the MS Excel spreadsheet appendix accompanying this report. All analyses were performed using R and analysis scripts are provided, preceding the associated analysis or chart in the data file. Four parameters were calculated from the water chemistry data for analysis purposes. Total nitrogen (TN) was calculated by summing the NO3-NO2-N and TKN values for each independent sample. Total hardness (HARDNESS) is represented as molar equivalents of CaCO3 in mg/L, calculated using the equation:

$$[CaCO_3] = 2.5[Ca^+] + 4.1[Mg^{2+}].$$

Two Calcium-Magnesium ratio indices were calculated, following the analysis performed in the 2008 WVDEP Report on filamentous algae assessment report (Summers 2008). A traditional Ca:Mg ratio index with both ratio and additive terms of Ca^{2+} and Mg^{2+} (CA_MG_INDEX):

$$\log[Ca^{2+}/Mg^{2+}] - 0.5 \log[Ca^{2+} + Mg^{2+}],$$

A modified index considering only an additive variable (MOD_CA_MG):

$$-\log[Ca^{2+} + Mg^{2+}].$$

Algal identification

Algae samples were collected during round 5 on August 6th and 7th, preserved in formaldehyde and Lugol's iodine solution and stored on ice in 50 mL graduated sample tubes. Samples were collected from five stations, two on the Cacapon River, one form the North River station, and two from the South Branch Potomac. The samples were transported back to the ICPRB lab where preliminary identifications were made and microscopic pictures were taken. The samples were then shipped to Dr. Todd Egerton at Old Dominion University (ODU) who was kind enough to provide validation identifications for the samples. An additional sample was provided by WVDEP biologists from the Greenbriar (AGB_BC1) that was included in the samples sent to ODU.

Results from the 2014 season

Summary of algal observations and measurements by station

Cacapon River at Rt. 9 in the town of Largent (CA_LRGNT)

Small amounts of FGA were observed persisting on the downstream submerged portion of a gravel bar, just downstream of the Rt. 9 bridge.

North River at Gaston Rd. / Forks of Cacapon (NO_FRKS)

The site at the North River continues to be dominated by a type of benthic riverweed (*Podostemum sp.*). In the last few rounds, much of the river weed was colonized by filamentous algae, but the heterogeneous mixture defied measurements.

Cacapon River at Rt. 127 / Forks of Cacapon (CA_FRKS)

BGA was common and increased in late summer. FGA was observed to increase above trace amounts beginning in August. Measurements were taken on September 3, 2014. The first measurement produced an average of 25.6% prompting the crew to collect additional measurements per the listing criteria. The algae did not extend far enough upstream to make all three measurements, a second measurement was recorded at 14%.

Cacapon River at farm off Cold Stream Road (CA_D_CPBRG)

Filamentous algae was present at low levels through most of the sampling season but never reached a threshold requiring transect measurements to be taken. This level of algae was less than what was observed the previous year.

Cacapon River at Rt. 50 in Capon Bridge (CA_CPBRG)

Filamentous green algae continued to be present at low levels at this site (<5% coverage). Filamentous cyanobacteria was more common and abundant at this site, also a pattern observed in previous years.

Cacapon River along Capon River Rd. and downstream of Camp Rim Rock (CA_RMRCK)

This site continued to manifest filamentous green algae blooms, repeating a pattern observed since before 2012. The magnitude of the blooms did not reach the levels observed last year, peaking at 65% on the July 23, 2014 site visit. Several precipitation and associated scour events worked to reduce the standing crop of algae during this year's sampling season. At one point, scour reduced algae levels to below 10%, only to return to near 30% just two weeks later. In all, algae measurements were recorded on five of the nine sampling rounds.

Cacapon River at Rt. 259 below Wardensville (CA_YLWSPR)

Filamentous green algae was not often observed at this site with non-detects recorded on 6 of the 9 visits. On the 4th round, cyanobacteria was observed in fair abundance and prompted a transect measurement producing a value of near 15% cover.

Cacapon River at farm ford in Wardensville (CA_WRDS)

The most upstream site on the Cacapon has consistently been the Cacapon site where the least amount of algae is observed. Only trace amounts of FGA were observed attached to boulders and at the shore's edge.

South Branch at Harmison's Landing (SB_L_TRGH)

FGA was not typically present in the channel but was restricted to the shallows at the base of the boat ramp.

South Branch at South Branch WMA (SB_U_TRGH)

FGA was not generally observed at this site except what was attached to cobble at the river's banks.

South Branch at Rt. 220/28 in Moorefield (SB_L_MRFLD)

Observed FGA was much reduced over what was detected at the site in previous years.

South Branch at Fisher Rd above Moorefield (SB_U_MRFLD)

FGA was often present in shallow embayments and at the river's edge at this site. During the 7th round in early September, FGA was observed in the channel immediately in front of and just upstream of the boat ramp. This algae was measured at 21% but the bloom did not extend very far up- or downstream.

South Branch at Weldon Park off Rt.220/55 (SB_L_PBRG)

This site was new to the 2014 season and did not manifest much FGA. FGA that was observed was restricted to the cobble at the banks.

South Branch at Rt.220 in Petersburg (SB_U_PBRG)

FGA was often present at this location in small to moderate amounts, though no measurements were taken. The algae at this location did not manifest in the thalweg of the channel but was often present in extensive shallow flats where the river was only a few inches in depth. This habitat extended for more than half the river's width.

Summary algae measurements also are included in **Table 3** below. This table includes actual measurements, and qualitative visual estimates of low abundance algae occurrences.

Table 3. Summary of percent filamentous algae cover measurements made during the 2014 season. Null values indicate when judgment was impaired by poor visibility, "ND" values indicate non-detects, values up to 10% were visually estimated and recorded as "<5" or "<10", all other values are actual algae measurements using the wadeable transect method. *The algae measured and recorded was entirely filamentous cyanobacteria, not green algae.

SITE_NAME	WATERBODY	JUN-1	JUN-2	JUL-1	JUL-2	AUG-1	AUG-2	SEP-1	SEP-2	OCT-1
CA_LRGNT	CACAPON		<5	<5	<5	<5	<5	ND	ND	ND
CA_FRKS	CACAPON	ND	<5	<5	ND	<5	<10	**25.60**	<5	ND
NO_FRKS	NORTH RIVER	<5	<5	ND	ND	<5	ND	<5	<10	<10
CA_D_CPBRG	CACAPON	ND	ND	<5	<10	<10	<5	<10	<5	<10
CA_CPBRG	CACAPON	ND	<5	<5	<5	ND	<5		ND	<5
CA_RMRCK	CACAPON	<5	<5	**20.36**	**64.51**	**49.41**	**16.02**	<10	**29.16**	<10
CA_YLWSPR	CACAPON	ND	<5	ND	**14.71***	ND	ND	ND	ND	<10
CA_WRDS	CACAPON	ND	ND	ND	<5	<5	ND	ND	ND	<5
SB_L_TRGH	SOUTH BRANCH	ND	<5	<5	<5	<5	<5	<5	<5	<5
SB_U_TRGH	SOUTH BRANCH	ND	<5	ND	ND	ND	<5	<5	<5	ND
SB_L_MRFLD	SOUTH BRANCH	<5	ND	<5	<5	<5	<5	<5	ND	ND
SB_U_MRFLD	SOUTH BRANCH	ND	<5	<5	<10	<10	<5	**21.11**	<10	ND
SB_L_PBRG	SOUTH BRANCH	<5	<5	ND	ND	ND	<5	<10	ND	<5
SB_U_PBRG	SOUTH BRANCH	ND	<5	<10	<10	<10	<10	<5	<10	<10

Algae identifications

The following identifications and notes are the product of Dr. Todd Egerton of Old Dominion University. Freshwater filamentous algae are not a type of algae his lab usually studies, and these identifications come with the caveat that they represent his best guess among unfamiliar taxa.

CA_D_CPBRG 8/6/14

Dominant taxa: *Cladophora glomerata* (**Figure 2**).

Other: Very little other phytoplankton (*Meolosira varians* filaments, *Cocconeis spp*. and other epiphytic diatoms).

Figure 2. *Cladophora glomerata* from the CA_D_CPBRG site on the Cacapon River.

CA_RMRCK 8/6/14

Dominant: *Spirogyra cf. setiformis* (cell width 80um, length 60um) (**Figure 3**).

SB_L_MRFLD 8/7/14

Dominant taxa: Cyanobacteria filaments *Jaaginema cf. subtilissimum* (4um wide, 100's um in length), no sheath, cross walls lacking/indistinct (**Figure 4**).

Other: Abundant pennate diatoms (*Cymbella*, *Navicula*, *Cocconeis*, *Pleurosigma*).

SB_U_MRFLD 8/7/14

Dominant: *Spirogyra cf. reticulata* (cells 35-40um width, 130-150um length, 2-3+spiral chloroplasts, 3+ rotations/cell).

NO_FRKS 8/6/14

Dominant: Thin mats of cyanobacteria filaments *Gloeotrichia cf. echinulate* (straight filaments, 3-4um in width, cells round/barrel shape, crosswalls constricted, filaments originating from central locations radially, mats ~3cm, pale blue/green color, basal cells larger and spherical).

AGB_BC1 unknown date

Multiple *Spirogyra spp*. including *Spirogyra cf. reticulata* (cells ~45um width x 250um length, multiple dense chloroplasts), and *Spirogyra cf. borgena.*

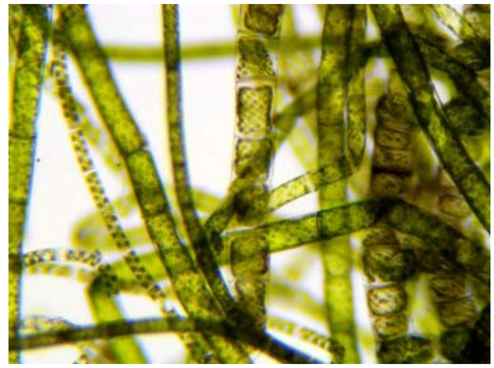


Figure 3. Strands of several *Spirogyra sp*. from the CA_RMRCK site.

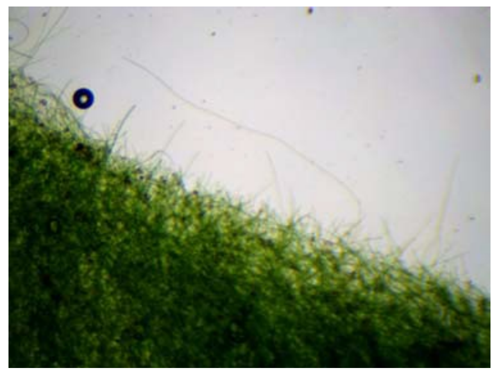


Figure 4. Cyanobacteria filaments (*Jaaginema sp*.). collected on the South Branch Potomac (SB_L_MRFLD) on August 7, 2014.

Longitudinal survey reports

Cacapon River – Capon Bridge, WV to Forks of Cacapon, WV

Figure 5 Map of the longitudinal survey area on the Cacapon River between Rivers Edge Farm and the Rt. 127 Bridge at Cacapon Forks. Segment delineations are represented by letters A-E.

On September 9th, 2014, a longitudinal survey was performed by two ICPRB biologists along a 13.0 Km section of the Cacapon River between Rivers Edge Farm (CA_D_CPBRG, 5.3 Km south of the Cacapon Bridge township) to the Rt. 127 Bridge at Cacapon Forks (CA_FRKS, **Table 4 and Figure 5**). The survey was conducted via canoe for the entirety of the 13.0 Km due to a lack of public roadways and riverside access.

Table 4 GPS coordinates and reach lengths of the assessed longitudinal segments.

	GPS Start/Stop Points		Reach Length	
	Latitude	Longitude		
A	39°19'38.11"N	78°25'24.25"W	Reach 0	0.0 Km
B	39°20'22.52"N	78°25'52.03"W	Reach 1	1.66 Km
C	39°21'56.16"N	78°25'26.98"W	Reach 2	4.56 Km
D	39°22'47.21"N	78°25'41.88"W	Reach 3	3.27 Km
E	39°24'11.11"N	78°25'3.07"W	Reach 4	3.51 Km

The section of river was selected for a longitudinal survey in 2014 for two reasons; 1) filamentous green algae had been observed at both upstream and downstream stations from the reach during previous surveys, and 2) the reach had not been yet been surveyed. Prior to the longitudinal survey in 2014, the Cacapon River sites at River's Edge Farm (CA_D_CPBRG) and Cacapon Forks (CA_FRKS) had not expressed significant blooms, having less than 5% areal coverage of filamentous green algae and cyanobacteria. The C_D_CPBRG station typically had a few isolated long strands of branched algae (± 1m) that originated from freestone cobble bottoms. The Forks site algae abundance was also estimated at less than 5%.

The 13 km longitudinal reach was iteratively divided into four contiguous reaches based on significant changes in river morphology and geology (**Table 4**). Reach 1 extended from the put-in to just downstream from the Cacapon's confluence with Cold Stream. This reach had no major bends, was largely bordered on the left bank by open fields and widely separated homes, and included the input from Edwards Run. Reach 2 extended from Cold Stream to near the terminus of old WV state road 15/1 on the left bank and a rock ledge on the right bank. Reach 2 was a series of six quick bends of fair gradient which ran through mostly forested terrain. Reach 3 consisted of two large gentle bends which also ran through mostly forested terrain and ended at the prominent rock feature called Caudy's Castle (**Figure 6**). Reach 4 extended from Caudy's Castle to the bridge at Rt. 127, consisted of two straight sections with one 90˚ left bend, and was mostly forested but had two large farm fields near the river left side.

Figure 6 Caudy's Castle, at end of third assessment segment on the Cacapon River.

The first three reaches did not manifest filamentous algae greater than 5% coverage. All reaches frequently had large and healthy beds of *Vallisneria sp., Potomageton sp., a*nd *Hydrilla sp.* Infrequently, *Hydrilla sp.* fronds provided a rigid structure for attachment of cyanobacteria filaments. Within reach 3 and into reach 4, infrequent *Spirogyra* colonies were observed forming in shallow, slow-flowing environments.

The only reach where substantial filamentous green algae was observed was the final reach (reach 4), which extended from Caudy's Castle rocks to the Rt. 127 bridge. A transect was performed at 39.390838, -78.414890. The transect revealed 41% coverage of filamentous green algae which exceeded the 20%-40% criteria threshold to run an additional two transects. The entirety of reach 4, the final reach, was estimated at 15% filamentous green algae. Reach 4 also had the largest, and densest *Vallisneria sp., a*nd *Hydrilla sp*. beds observed within the surveyed 13 Km section. The surveyed section as a whole had low periphyton, low attached cyanobacteria and low filamentous green algae (FGA) loads while having high densities of submerged aquatic vegetation. Filamentous green algae and blue green algae were infrequent, therefore the section did not violate the West Virginia recreational use criteria.

South Branch Potomac River- South Branch Wildlife Management Area – Harmison's Landing

Figure 7 Map of the lower longitudinal survey route performed on the South Branch Potomac River on August,

A 11.34 Km longitudinal survey of the trough region of the South Branch Potomac river was conducted via canoe on August 27, 2014 between 10:55 and 16:30 (**Figure 7**). The most convenient put-in to the trough region of the South Branch was at the South Branch Wildlife Management Area (McNeil) located off of Local Rt 6, Trough Road. The 11.34 Km survey reach was broken into 5 predetermined intervals defined by stream morphology (**Table 5**). The survey reach terminated at Harmison's Landing, a boat ramp frequented by many recreational boaters and outfitters. ICPRB biologists Adam Griggs and Mike Selckmann took in-situ water quality measurements (temperature, pH, DO, and specific conductance) and qualitatively assessed filamentous green algae (FGA), blue green algae (BGA), moss, periphyton, emergent vegetation, floating aquatic vegetation, submerged aquatic vegetation (SAV) at the start and end of each reach. The trough reach was targeted for a longitudinal survey for several reasons. A new WWTP came on-line in this section over the winter, and the SB_L_TRGH often had elevated dissolved oxygen measures, indicative of increased photosynthetic activity upstream of water sample.

The longitudinal survey began (Reach 1) at a shallow riffle comprised of mostly barren cobble. Trace amounts of FGA and BGA were observed in near-shore eddies. Beyond the initial riffle, we observed a broad glide with single 1-4ft long stargrass colonies every 2-3 meter radii. The boulder substrate was largely barren of periphytic growth. Reach 2 began at the railway bridge downstream from Falling Springs Gap. Reach 2 is often considered the entrance to "the trough" and is the start of the low velocity region that begins as a deep channel that slowly widens to a slow shallow section. Within reach 2 we observed an increasing abundance of periphyton and freshwater sponges. Filamentous green algae was common but not abundant near-shore. We could identify near-shore algae from greater than 100m away due to its bright green coloration contrasting with the boulder substrate. Closer investigation revealed floating FGA mats were often roughly 70% of the total FGA present, as benthic mats radiated from the floating masses towards the channel. The FGA mats were always relatively small (spanning no greater than 40m) and were extremely localized. Similar patterns of near-shore FGA were observed for the entirety of the third reach (**Figure 8**). Morphometrically, the third reach was very similar to Reach 2 in that it was primarily slow glides and pools with cobble and boulder substrata. The continued patchy distribution of floating filamentous algae along the rivers banks and shallow bars prompted discussion of an additional algal descriptor in which channel location is defined. Reach 4 river morphology was morphometrically similar to the previous two reaches, in that, it was defined by mostly slow shallow moving water with the occasional shallow riffle. A single transect was conducted on a near-shore patch of floating FGA that appeared on a river left boulder bar. Additional transects were not conducted as the algal mass ended abruptly due to water velocity. Reach 4 had an extremely high abundance of SAV, more so than had been observed on any other section of the South Branch in the 2014 sampling season (**Figure 9**). Reach 5

Figure 8 Reach 3; Near-shore filamentous green algae (FGA) floating mat with benthic FGA spanning into main channel.

Figure 9 Reach 4; Highly to extremely abundant SAV colonies were observed up to the start of Reach 5.

morphology was morphometrically similar to the previous three reaches, in that, it was defined by mostly slow shallow moving water with the occasional shallow riffle. Interestingly, a single riffle that occurred at the start of the reach was the terminus of the SAV abundant region that occurred between reaches 2 and 5. There did not appear to be any shifts in stream morphology or water chemistry to explain the abrupt stop in SAV presence. Reach 5 had very little photosynthetic growth and was primarily cobble and boulder substrate with light sedimentation on top.

Table 5 Coordinates of longitudinal assessment segments and accompanying written observations.

Points	Start Coordinates	End Coordinates	Observations made
Segment 1	39.14647, -78.92494	39.15239, -78.91439	Independent stargrass growths.
Segment 2	39.15239, -78.91439	39.15239, -78.90207	Dominated by a long deep run resulting in reduced visability. High quantity of freshwater sponges, Floating algae mat observed at shoreline. Increased periphyton from first reach.
Segment 3	39.15239, -78.90207	39.18434, -78.89020	Near starting point FGA is abundant near the shore. Lots of FGA on shore, none in main channel. Consider channel/shore proximity as an additional descriptor.
Segment 4	39.18434, -78.89020	39.21674, -78.86253	Run partial transect (FGA mat too short). Abundant SAV. Shoreline FGA. Highest SAV abundance observed in 2014.
Segment 5	39.21674, -78.86253	39.22384, -78.85580	Post final riffle, no SAV or FGA observed.

Three-year summary (2012-2014 field seasons)

The last two annual reports for the filamentous algae monitoring program's Potomac drainage included a summary descriptive analysis of each individual season's data. By request of WVDEP, the 2014 work plan included a summary analysis of the three years, to be performed after the close of the 2014 season. This summary analysis includes descriptive and comparative analyses between three years (2012-2014), and three Potomac drainages (Shenandoah, South Branch Potomac, and Cacapon). An attempt was also made to explore the collected physical and chemical data to look for factors related to the growth of filamentous algae.

Since 2012, continuous revisions and improvements have been made to the filamentous algae monitoring protocol. Changes have included better capture of the variety of plants and algae encountered, when and how algae measurements take place, and additional supplemental parameters including transect canopy cover, channel orientation, and additional chemistry parameters. Additionally, certain sites have been dropped and added as information has been gathered on where filamentous algae blooms occur. The following descriptive analyses will make note of gaps, changes, and additions apparent in the data as they occur.

Stations

Fourteen stations were targeted during each year of the survey (**See Table 6**). The 2012 strategy targeted two sites on the Shenandoah River. Seven sites were targeted in the Cacapon River watershed, with one on the North River and the other six on the Cacapon River. Four sites were targeted on the South Branch of the Potomac River, above and below Moorefield, WV. In 2013, a single site was added to the Cacapon below Camp Rim Rock (CA_RMRCK) after algae was observed there in 2012. Another site (CA_L_WRDS) was relocated downstream after no significant increases of nutrients or algae were detected immediately below the Wardensville Waste Water Treatment Plant (WWTP). The site was relocated to the area of Yellow Spring, WV (CA_YLWSPR) for the 2013 and 2014 seasons. In 2014, two sites on the Shenandoah River were dropped after two years of monitoring failed to detect significant algae at the two locations, and monitoring resources were reallocated to the South Branch of the Potomac River in an area above and below the town of Petersburg, WV. Also in 2014, an attempt was made to find additional algae-impacted areas by means of windshield surveys during routine

Table 6 Site names of the stations sampled under this project from 2012-2014. An "X" indicates the site was regularly sampled in that year. The "1" indicates a single incidental observation while performing windshield surveys of the basin.

Year	SHEN_LWR	SHEN_UPR	CA_LRGNT	NO_FRKS	CA_FRKS	CA_D_CPBRG	CA_CPBRG	CA_RMRCK	CA_YLWSPR	CA_L_WRDS	CA_WRDS	SB_L_TRGH	SB_U_TRGH	SB_L_MRFLD	SB_U_MRFLD	SB_L_PTBRG	SB_U_PTBRG	SB_NF_CBNS
2012	X	X	X	X	X	X	X	1		X	X	X	X	X	X			
2013	X	X	X	X	X	X	X	X	X		X	X	X	X	X			
2014			X	X	X	X	X	X	X		X	X	X	X	X	X	X	1

monitoring. An area upstream of the current monitoring area detected a moderate algae bloom on the North Fork of the South Branch of the Potomac River in the area of Cabins, WV.

Sampling dates

In each of the three sampling seasons, stations were generally sampled every two weeks, unless interrupted by weather or other conflicts. The sampling seasons ran roughly from early June to early October, with a target of 10 sampling rounds, depending upon mobilization and algae occurrence. Sampling generally ended when algae was observed to be in decline. **Table 7** provides the sampling dates where rounds were performed, typically requiring two days per round, sometimes three or one. Alternating rounds are shaded grey for distinction. The 2012 season required more initial time for project design and mobilization and only seven rounds were completed. Ten rounds were completed in 2013, and nine rounds were completed in 2014.

Table 7 Sampling dates and round through the three initial years of study (2012-2014).

2012	2013	2014
7/18/2012	6/4/2013	6/11/2014
7/19/2012	6/5/2013	6/12/2014
7/20/2012	6/18/2013	6/25/2014
8/4/2012	6/19/2013	6/26/2014
8/5/2012	6/20/2013	7/9/2014
8/16/2012	7/1/2013	7/10/2014
8/17/2012	7/2/2013	7/23/2014
8/29/2012	7/18/2013	7/24/2014
8/30/2012	7/19/2013	8/6/2014
9/12/2012	7/30/2013	8/7/2014
9/13/2012	7/31/2013	8/20/2014
9/25/2012	8/14/2013	8/21/2014
9/26/2012	8/15/2013	9/3/2014
10/10/2012	8/27/2013	9/4/2014
10/11/2012	8/28/2013	9/18/2014
	9/12/2013	10/7/2014
	9/13/2013	10/8/2014
	10/2/2013	
	10/3/2013	
	10/17/2013	
	10/18/2013	

Qualitative vegetation and algae data

The filamentous algae monitoring program includes a site characterization and a rapid visual qualitative assessment of different plant and algae types in the site reach. In 2012, parameters included: Foam (FOAM), periphyton (PERI), filamentous algae (FA), moss (MOSS), and aquatic vegetation (AV). In 2013, aquatic vegetation was divided into submerged (SAV), emergent (EAV), floating (FAV), and a total category (TAV), while filamentous algae was divided into filamentous green algae (FGA) and cyanobacteria/blue-green algae (BGA). In comparative analyses, the relic 2012 parameters are equated as (FA)=(FGA) and (AV)=(SAV). Each parameter of the qualitative assessment was rated either 0 (absent), 1 (low), 2 (moderate), 3 (high), or 4 (extreme). Null values in the descriptive statistic tables indicate reflect changes in the program over time. Null values in the raw data are indicative of sampling events where visibility was obscured by either turbidity or surface disturbance due to falling rain/wind.

Comparison of physical habitat data across stations and river basins

Table 8 provides calculated means for the qualitative (0-4) algae and plant type parameters collected during the three years of study. Each mean is averaged over the number of samples and stations in each of the four waterbodies. For example, the Cacapon mean is calculated across seven stations while the North River mean refers to the average from a single station observed over the course of a season. Median values were also calculated and are included in the MS Excel Appendix file.

Table 8 Mean values of the qualitative vegetative parameters across waterbodies and years sampled (2012-2014).

Years	2012				2013				2014		
PARAMETER	**Cacapon**	**North**	**Shen.**	**So. Br.**	**Cacapon**	**North**	**Shen.**	**So. Br.**	**Cacapon**	**North**	**So. Br.**
FOAM	0.8	0.8	1.3	1.0	0.9	1.2	1.2	0.7	0.9	1.1	1.1
PERI	2.2	1.7	1.9	2.0	1.8	1.7	2.3	2.1	2.0	1.4	2.2
FGA	0.8	0.2	0.7	0.9	0.5	0.4	0.2	0.3	1.0	0.9	1.0
BGA	NaN	NaN	NaN	NaN	0.6	0.3	0.1	0.1	0.8	0.1	0.3
MOSS	0.0	1.5	0.0	0.0	0.0	0.6	0.1	0.0	0.0	0.9	0.0
SAV	1.0	2.0	0.4	2.2	1.0	3.0	0.0	0.7	1.1	2.1	0.9
EAV	NaN	NaN	NaN	NaN	1.1	2.9	1.2	1.3	1.6	3.0	1.4
FAV	NaN	NaN	NaN	NaN	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAV	NaN	NaN	NaN	NaN	1.4	3.0	0.9	1.1	1.3	2.6	1.2

Most qualitative factors had averages between the scores of "0" and "1" either being non-detects or present at low levels at most sites. Periphyton and vegetation factors such as SAV and EAV were somewhat higher at certain sites and waterbodies, usually scoring a "2" or "3" (**Table 10**).

Non-parametric Kruskal-Wallis Analysis of Variance tests were used to test for significant differences in the qualitative assessment parameters between the four waterbodies, and between stations within waterbodies (**Table 9**). The four waterbodies differed significantly among most qualitative parameters with the exception of filamentous green algae (FGA) and floating aquatic vegetation (FAV). Filamentous algae was generally present in trace amounts at most locations, earning a frequent "1" value. Floating vegetation was not documented during this project, making the comparison between the waterbodies or stations impossible. Moss had the greatest distinction among the waterbodies, where it was absent from all sites save for the sole North River station. Cyanobacteria (BGA) was generally more abundant in the Cacapon River system.

Table 9 Kruskal-Wallis test for differences between waterbodies across all three years of data.

PARAMETER	**Chi-x^2**	**df**	**P-value**		**Sign.**
FOAM	9.90	3	=	0.01945	*
PERI	16.99	3	=	0.00071	***
FGA	6.71	3	=	0.08183	
BGA	33.47	3	=	2.57E-07	***
MOSS	119.83	3	<	2.20E-16	***
SAV	55.81	3	=	4.61E-12	***
EAV	40.12	3	=	1.01E-08	***
FAV	NaN	3	=	NA	
TAV	40.67	3	=	7.67E-09	***

Mean values from each of the 17 total stations monitored over the three years are included in **Table 10** and included MS Excel appendix. The table displays the means of all values collected at each site that were monitored in the year indicated. Kruskal-Wallis tests between stations were performed within waterbody datasets, to eliminate inter-basin variability. These tests were performed on the Cacapon and South Branch sites, as they had a sufficient number of sites (groups) to test.

Table 10 Calculated means for the nine qualitative parameters across the 17 routine stations over the three year period (2012-2014). * Represents a single incidental sample collected in that season.

	SHEN_LWR	SHEN_UPR	CA_LRGNT	NO_FRKS	CA_FRKS	CA_D_CPBRG	CA_CPBRG	CA_RMRCK	CA_YLWSPR	CA_L_WRDS	CA_WRDS	SB_L_TRGH	SB_U_TRGH	SB_L_MRFLD	SB_U_MRFLD	SB_L_PTBRG	SB_U_PTBRG
2012								*									
FOAM	1.2	1.3	0.8	0.8	0.3	1.3	1.0	1.0		0.4	0.9	0.9	1.4	0.7	1.2		
PERI	2.3	1.5	2.6	1.7	2.0	1.4	2.1	1.0		2.9	2.3	2.0	2.4	1.5	2.3		
FGA	1.2	0.2	0.6	0.2	0.9	1.0	0.1	4.0		0.6	0.9	1.1	0.2	1.0	1.2		
BGA	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA		
MOSS	0.0	0.0	0.0	1.5	0.1	0.0	0.0	0.0		0.1	0.0	0.0	0.0	0.0	0.0		
SAV	0.8	0.0	1.2	2.0	1.1	1.7	1.1	3.0		0.4	0.0	2.4	1.8	3.3	1.2		
EAV	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA		
FAV	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA		
TAV	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA	NA	NA	NA	NA		
2013																	
FOAM	1.1	1.2	0.8	1.2	0.5	1.0	0.9	0.6	1.5		0.9	0.1	1.1	1.0	0.6		
PERI	2.4	2.2	1.8	1.7	2.2	1.6	1.6	1.3	1.3		2.4	2.6	2.0	1.9	2.1		
FGA	0.4	0.0	0.2	0.4	0.2	0.7	0.1	1.8	0.3		0.4	0.4	0.0	0.2	0.3		
BGA	0.0	0.1	0.1	0.3	0.8	0.3	0.7	0.9	1.0		0.9	0.0	0.0	0.1	0.3		
MOSS	0.1	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0		
SAV	0.0	0.0	0.1	3.0	1.1	1.3	1.3	2.3	1.1		0.1	0.4	0.9	1.5	0.1		
EAV	2.0	0.4	2.0	2.9	1.9	0.9	1.7	0.1	0.9		0.0	1.1	1.0	1.3	1.6		
FAV	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0		
TAV	1.7	0.1	1.6	3.0	1.6	1.3	1.4	2.3	1.4		0.1	1.0	1.0	1.4	1.0		
2014																	
FOAM			0.9	1.1	0.2	1.4	1.1	0.6	0.7		1.1	0.1	1.4	1.0	1.1	0.8	2.1
PERI			1.6	1.4	1.9	1.8	1.6	1.9	1.9		2.9	2.4	1.9	1.7	2.4	2.6	2.0
FGA			0.6	0.9	0.9	1.2	0.6	2.4	0.4		0.3	1.3	0.4	0.7	1.2	0.7	1.6
BGA			0.4	0.1	0.7	0.3	1.4	1.6	1.0		0.4	0.4	0.0	0.9	0.3	0.2	0.1
MOSS			0.0	0.9	0.0	0.1	0.0	0.0	0.0		0.1	0.0	0.0	0.0	0.0	0.0	0.0
SAV			0.6	2.1	1.2	1.2	1.4	3.0	0.2		0.0	1.3	0.8	1.4	0.1	0.6	0.9
EAV			2.8	3.0	2.0	1.1	2.4	1.3	0.9		0.5	0.9	1.3	1.1	2.0	1.4	1.7
FAV			0.0	0.0	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
TAV			1.6	2.6	1.4	1.1	1.5	2.4	0.7		0.5	1.2	1.2	1.3	1.2	1.1	1.3

Kruskal-Wallis tests indicated that the plant and algae communities varied between stations within each river system. **Tables 11 & 12** provide the chi-square statistics and significance of differences among the nine qualitative parameters between the South Branch Potomac River Stations and Cacapon River stations, respectively. The Cacapon stations were significantly different in most of their measured vegetative aspects, except for moss and floating aquatic vegetation. South Branch stations were more similarly rated overall, and were not significantly different in their relative abundance of cyanobacteria (BGA) and overall vegetation (TAV). BGA was overall less common and abundant in the South Branch of the Potomac River than it was in the Cacapon River.

Table 11 Kruskal-Wallis test for differences among the nine qualitative parameters across the seven South Branch stations.

Parameter	Chi- x^2	df		P-value	Sign.
FOAM	47.40	6	=	1.6E-08	***
PERI	20.17	6	=	2.6E-03	**
FGA	27.27	6	=	1.3E-04	***
BGA	9.29	6	=	1.6E-01	
MOSS	NaN	6	=	NA	
SAV	40.53	6	=	3.6E-07	***
EAV	21.53	6	=	1.5E-03	**
FAV	NaN	6	=	NA	
TAV	5.87	6	=	4.4E-01	

Table 12 Kruskal-Wallis test for differences among the nine qualitative parameters across the seven/eight Cacapon River stations.

Parameter	Chi-x^2	df		P-value	Sign.
FOAM	34.93	7	=	1.15E-05	***
PERI	48.60	7	=	2.72E-08	***
FGA	39.90	7	=	1.32E-06	***
BGA	22.00	6	=	0.00121	**
MOSS	6.71	7	=	0.4601	
SAV	90.41	7	<	2.20E-16	***
EAV	69.40	6	=	5.44E-13	***
FAV	NaN	6	=	NA	
TAV	51.41	6	=	2.45E-09	***

Evaluation of physical habitat data over time

One-way Analysis of Variance (ANOVA) tests were used to test for differences between years among stations within the same waterbody (**Tables 13 and 14**). Cacapon River stations and South Branch Potomac River stations were analyzed. Among the qualitative parameters, only emergent vegetation (EAV) was significantly different between years.

Table 13 One-way Analysis of Variance (ANOVA) of the qualitative vegetative parameters over time among the South Branch Potomac stations.

Parameter	df	Sum Sq	F	P value	Sign.
FOAM	1	0.67	1.27	0.262	
PERI	1	0.31	0.71	0.401	
FGA	1	1.57	2.89	0.092	.
BGA	1	0.779	3.03	0.085	.
MOSS	1	0			
SAV	1	22.61	32.20	0.000	***
EAV	1	0.285	0.82	0.369	
FAV	1	0			
TAV	1	0.251	1.10	0.298	

Table 14 One-way Analysis of Variance (ANOVA) of the qualitative vegetative parameters over time among the Cacapon River stations.

Parameter	df	Sum Sq	F	P value	Sign.
FOAM	1	0.17	0.391	0.533	
PERI	1	0.77	1.643	0.202	
FGA	1	1.47	1.839	0.177	
BGA	1	0.83	1.315	0.254	
MOSS	1	0.002	0.101	0.751	
SAV	1	0.39	0.413	0.521	
EAV	1	6.79	7.816	0.006	**
FAV	1	0			
TAV	1	0.09	0.116	0.735	

Water chemistry and quality

Each round of filamentous algae monitoring has included the collection of water chemistry samples and in-situ water quality data. The parameters collected under this program have been determined by WVDEP and designed to collect information about the potential drivers and symptoms of filamentous algae blooms. Constituents have included routine water chemistry parameters using multi-parameter sondes (dissolved oxygen, specific conductance, pH, and water temperature), nutrients including species of Nitrogen and Phosphorous, and certain measures of dissolved metals and salts that are predicted to be associated with nutrient availability and osmotic regulation of algae.

Unfortunately, this project has not had access to consistent and reliable water chemistry analysis for certain parameters throughout the three years of data collection. Phosphorous samples, including both Total Phosphorous (TP) and Dissolved Phosphorous (DP), have been the parameters that have had the most issues. In 2012, the contracted laboratory (Reliance Labs) reported many samples throughout the season at detection limit levels. These results were found to be incorrect in the latest rounds when duplicate samples were delivered to a second laboratory. In 2013, BioChem was contracted to analyze the collected water chemistry samples and water chemistry results were greatly improved, allowing for more investigation of phosphorous levels and algae-chemistry relationships. New issues with Phosphorous laboratory techniques arose in 2014 when it was discovered that a calibration curve method being employed to calibrate the lab equipment resulted in measured concentrations being reported higher than actual concentrations. This error was not discovered until late in the sampling season, and resulted in a missed water chemistry collection during the eighth round, and questionable reliability of the previous seven rounds of data. More thorough data quality assurance investigations should be performed on the 2014 water chemistry data before it is accepted or finalized.

Comparison of water chemistry data among the four river basins

Table 15 provides median values of the water chemistry (lab) and water quality (in-situ) samples collected from each river, as a product of multiple stations and sampling events over the three years. Blank values in the table occur where parameters were added to the program beginning in the second year. ANOVA models were used to test for differences in the water chemistry and quality data between the four waterbodies over the three years of the study.

Table 15 Median water chemistry and quality values by waterbody for the three years of the study (2012-2014).

	2012				2013				2014		
Parameter	**Cacapon**	**North**	**So. Br.**	**Shen.**	**Cacapon**	**North**	**So. Br.**	**Shen.**	**Cacapon**	**North**	**So. Br.**
CA (mg/L)	29.3	18.6	44.4	38.4	24.5	15.7	40.4	39.4	28.3	24.3	43.1
MG (mg/L)	6.0	5.7	7.0	16.5	4.6	4.6	6.0	12.7	5.2	6.1	6.3
ALK (mg/L)	74.2	52.7	93.9	119.5	67.0	47.0	96.0	130.0	77.5	72.0	103.0
HARD (mg/L)	98.2	69.3	142.5	161.8	80.3	57.3	125.0	157.5	91.4	85.2	134.9
CA_MG_INDEX	2.33	2.27	2.35	1.95	2.04	1.94	2.07	1.72	2.40	2.29	2.41
MOD_CA_MG	2.87	3.04	2.69	2.70	3.10	3.24	2.90	2.80	2.89	2.94	2.71
DP (mg/L)	0.01	0.01	0.01	0.01	0.03	0.03	0.07	0.04	0.03	0.03	0.04
TP (mg/L)	0.01	0.01	0.01	0.01	0.03	0.03	0.09	0.05	0.04	0.04	0.04
NO3-NO2-N (mg/L)	0.45	0.56	0.91	1.18	0.21	0.23	0.45	1.04	0.17	0.03	0.25
TKN (mg/L)					0.2	0.2	0.2	0.3	0.2	0.2	0.2
TN (mg/L)					0.40	0.46	0.62	1.31	0.31	0.27	0.45
SPCOND (uS/cm)	188	154	260	332	175	139	264	324	189	190	268
WTEMP (▫C)	22.6	22.6	27.0	23.7	22.4	21.9	23.5	24.9	23.9	22.7	24.5
DO (mg/L)	12.31	11.39	12.15	10.91	8.37	8.11	9.17	7.60	8.65	8.56	8.78
PH	8.61	8.01	8.94	8.85	8.06	7.64	8.34	8.21	7.89	7.66	8.00
TDS (mg/L)					116	91	175	224	120	123	173
TSS (mg/L)	3	4	3	5.5	2	4	2	6	2.5	2.5	2

Table 16 provides the results of the ANOVA model. Nearly every water chemistry and quality constituent was significantly different between waterbodies. Only dissolved oxygen (DO) and total suspended solids (TSS) were not significantly different.

Table 16 ANOVA model results testing for differences among water chemistry variables between waterbodies.

Parameter	df	Sum Sq	F	P value	Sign.
DO (mg/L)	3	17	2.1	0.104	
SPCOND (µS/cm)	3	857218	331.1	<2e-16	***
PH (SU)	3	7	13.3	0.000	***
WTEMP (▫C)	3	194	4.1	0.007	**
NO3-NO2 (mg/L)	3	16	63.2	<2e-16	***
TKN (mg/L)	3	0	7.7	0.000	***
TN (mg/L)	3	15	55.0	<2e-16	***
TP (mg/L)	3	0	16.3	0.000	***
DP (mg/L)	3	0	17.5	0.000	***
TSS (mg/L)	3	533	1.7	0.161	
ALK (mg/L)	3	107670	220.3	<2e-16	***
CA (mg/L)	3	18242	211.6	<2e-16	***
MG (mg/L)	3	2198	390.7	<2e-16	***
CA_MG_RAT	3	0	361.7	<2e-16	***
HARDNESS (mg/L)	3	211132	232.8	<2e-16	***
CA_MG_INDEX	3	4	381.1	<2e-16	***
MOD_CA_MG	3	3	170.7	<2e-16	***

Median values across stations (2012-2014)

Means and median values for all water chemistry and quality parameters were calculated for each combination of station and year. The combined tables were too large to be included in this document, but can be found in the packaged MS Excel spreadsheet appendix file. **Table 17** includes the median values for each station on the Shenandoah River and South Branch Potomac River calculated over the three years of study (2012-2014).

Table 17 Median values of all water chemistry and quality data across time for the stations of the Shenandoah River and South Branch Potomac River.

Parameters	SHEN_LWR	SHEN_UPR	SB_L_TRGH	SB_U_TRGH	SB_L_MRFLD	SB_U_MRFLD	SB_L_PTBRG	SB_U_PTBRG	SB_NF_CBNS
DO (mg/L)	8.50	8.08	8.82	9.21	8.63	9.30	9.82	9.12	10.03
SPCOND (µS/cm)	326	309	268	276	264	243	260	247	262
PH (SU)	8.45	8.40	8.43	8.39	8.14	8.31	8.12	8.15	8.36
WTEMP (▫C)	23.66	23.57	23.69	24.45	23.14	23.55	22.97	24.18	22.59
NO3-NO2 (mg/L)	1.16	1.01	0.55	0.59	0.55	0.36	0.28	0.13	0.03
TKN (mg/L)	0.35	0.32	0.28	0.24	0.20	0.16	0.18	0.11	0.11
TN (mg/L)	1.46	1.29	0.68	0.68	0.61	0.43	0.46	0.24	0.14
TP (mg/L)	0.05	0.04	0.11	0.15	0.10	0.03	0.04	0.03	0.04
DP (mg/L)	0.04	0.03	0.10	0.13	0.09	0.02	0.03	0.03	0.04
TSS (mg/L)	7.8	8.3	9.1	3.0	2.7	2.6	3.5	2.0	NaN
ALK (mg/L)	125.19	124.21	93.20	95.13	95.06	98.32	105.88	102.38	95.00
CA (mg/L)	40.04	38.64	41.34	41.06	40.28	41.35	44.58	42.59	45.30
MG (mg/L)	14.44	13.90	6.72	6.53	6.28	5.97	6.20	5.59	5.70
CA_MG_RAT	0.22	0.22	0.10	0.10	0.10	0.09	0.08	0.08	0.08
HARDNESS (mg/L)	159.30	153.61	130.89	129.43	126.44	127.82	136.86	129.38	136.62
CA_MG_INDEX	2.02	2.03	2.37	2.38	2.40	2.43	2.43	2.46	2.47
MOD_CA_MG	2.70	2.72	2.73	2.74	2.75	2.74	2.70	2.72	2.70

Table 18 includes the median values for each station in the Cacapon River system (2012-2014). Within each waterbody, ANOVA models were used to test for differences among the water chemistry and

Table 18 Median values of all water chemistry and quality data across time for the stations of the Cacapon River and its tributary the North River.

Parameters	CA_LRGNT	NO_FRKS	CA_FRKS	CA_D_CPBRG	CA_CPBRG	CA_RMRCK	CA_YLWSPR	CA_L_WRDS	CA_WRDS
DO (mg/L)	8.03	8.54	8.86	8.56	8.86	10.88	8.69	NaN	9.65
SPCOND (µS/cm)	170	158	186	178	170	170	178	NaN	197
PH (SU)	8.01	7.77	8.14	7.80	8.21	8.39	8.01	NaN	8.43
WTEMP (▫C)	23.26	21.63	22.03	21.52	22.67	22.61	22.22	NaN	21.88
NO3-NO2 (mg/L)	0.24	0.28	0.26	0.30	0.31	0.30	0.29	0.59	0.52
TKN (mg/L)	0.23	0.25	0.18	0.21	0.19	0.19	0.22	NaN	0.17
TN (mg/L)	0.37	0.41	0.34	0.43	0.44	0.48	0.50	NaN	0.64
TP (mg/L)	0.03	0.04	0.03	0.03	0.03	0.04	0.04	0.02	0.03
DP (mg/L)	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.01	0.02
TSS (mg/L)	4.6	7.8	3.8	3.9	3.8	5.3	6.5	3.6	3.8
ALK (mg/L)	66.53	58.87	75.93	70.68	68.53	70.04	71.13	79.94	79.00
CA (mg/L)	24.57	20.27	28.30	26.54	25.38	26.18	27.05	32.81	30.25
MG (mg/L)	5.14	5.36	5.29	5.04	4.87	4.86	4.84	6.30	5.71
CA_MG_RAT	0.13	0.16	0.11	0.12	0.12	0.11	0.11	0.12	0.12
HARDNESS (mg/L)	82.49	72.67	92.43	87.04	83.43	85.37	87.46	107.87	99.04
CA_MG_INDEX	2.37	2.30	2.39	2.40	2.40	2.41	2.42	2.35	2.37
MOD_CA_MG	2.95	3.03	2.89	2.92	2.94	2.93	2.92	2.82	2.87

quality parameters between stations of the same waterbody.

Variability of water chemistry and quality within river basin

ANOVA models were used to test for differences in water chemistry between stations of the same waterbody. South Branch Potomac River stations differed among the four measured and one calculated (TN) nutrient parameters with nutrients generally increasing moving downstream. (**Table 19**). Magnesium and its related measures were also significantly different between South Branch sites, also generally increasing moving downstream.

Cacapon River stations differed significantly in most of the measured parameters, with the

Table 19 ANOVA test for among water chemistry parameters between South Branch Potomac stations.

Parameter	df	Sum Sq	F	P value	Sign.
DO (mg/L)	6	12	1.144	0.344	
SPCOND (µS/cm)	6	15649	2.541	0.024	*
PH (SU)	6	2	1.691	0.13	
WTEMP (▫C)	6	28	0.267	0.951	
NO3-NO2 (mg/L)	6	2	5.9	2.53E-05	***
TKN (mg/L)	6	0	2.471	0.031	*
TN (mg/L)	6	2	7.7	1.57E-06	***
TP (mg/L)	6	0	4.444	0.000	***
DP (mg/L)	6	0	4.091	0.001	**
TSS (mg/L)	5	573	0.521	0.759	
ALK (mg/L)	6	1417	2.03	0.068	.
CA (mg/L)	6	140	0.775	0.591	
MG (mg/L)	6	13	2.261	0.043	*
CA_MG_RAT	6	0	5.735	3.53E-05	***
HARDNESS (mg/L)	6	822	0.471	0.828	
CA_MG_INDEX	6	0	5.152	0.000	***
MOD_CA_MG	6	0	0.588	0.739	

major exception of most nutrient parameters. Only Nitrate-Nitrite displayed significant differences between Cacapon River stations (**Table 20**). Nitrate levels are likely highest upstream because the Cacapon River emerges from the limestone caverns of the Lost River Valley's karst geology just above the most upstream site (CA_WRDS). The elevated Nitrate-Nitrite levels are likely due to elevated groundwater levels in the area. Dissolved Oxygen and pH were highest at the CA_RMRCK site, where excess productivity contributed to diel swings in dissolved oxygen and carbon dioxide. Magnesium, Calcium, Hardness, and Alkalinity concentrations all decrease moving downstream, reaching their lowest levels in the area including the CA_RMRCK site, between CA_CPBRG and CA_YLWSPR. Concentrations begin to increase again at CA_D_CPBRG and CA_FRKS until the North River mixes with the Cacapon.

Table 20 ANOVA test for differences among water chemistry parameters between the mainstem Cacapon River stations.

Parameter	df	Sum Sq	F	P value	Sign.
DO (mg/L)	7	122	5.855	7.02E-06	***
SPCOND (µS/cm)	7	18094	5.467	1.21E-05	***
PH (SU)	7	9	7.718	5.06E-08	***
WTEMP (▫C)	7	68	0.645	0.718	
NO3-NO2 (mg/L)	7	2	3.502	0.002	**
TKN (mg/L)	6	0	0.779	0.588	
TN (mg/L)	6	1	1.834	0.099	.
TP (mg/L)	7	0	1.051	0.398	
DP (mg/L)	7	0	1.338	0.237	
TSS (mg/L)	7	92	0.291	0.956	
ALK (mg/L)	7	3145	2.946	0.006	**
CA (mg/L)	7	752	4.342	0.000	***
MG (mg/L)	7	23	4.369	0.000	***
CA_MG_RAT	7	0	4.403	0.000	***
HARDNESS (mg/L)	7	7382	4.368	0.000	***
CA_MG_INDEX	7	0	4.066	0.000	***
MOD_CA_MG	7	0	3.567	0.001	**

Variability of water chemistry and quality over time

The purpose of this analysis is to determine if water chemistry and quality varies annually among stations in the same waterbody. Three years of data is not yet adequate to look for trends in time or season, however, a recent report produced by ICPRB staff for WVDEP provides trend analyses for fixed stations throughout the state of West Virginia (ICPRB Report 14-6).

Water chemistry differed significantly between years among the South Branch Potomac River stations among certain parameters, including Nitrate-Nitrite, Total Nitrogen, Alkalinity, Calcium, Magnesium, and in-situ parameters (**Table 21**). Changes in Phosphorous concentrations were not significant, though

Table 21 ANOVA test for differences of South Branch Potomac River water chemistry parameters between years (2012-2014).

Parameter	df	Sum Sq	F	P value	Sign
DO (mg/L)	1	33	22.85	6.61E-06	***
SPCOND (µS/cm)	1	5443	5.079	0.0262	*
PH (SU)	1	13	241.4	<2e-16	***
WTEMP (▫C)	1	0	0.004	0.948	
NO3-NO2 (mg/L)	1	5	154.1	<2e-16	***
TKN (mg/L)	1	0	2.478	0.119	
TN (mg/L)	1	1	16.39	0.000114	***
TP (mg/L)	1	0	0.626	0.431	
DP (mg/L)	1	0	0.469	0.495	
TSS (mg/L)	1	3	0.016	0.9	
ALK (mg/L)	1	728	6.21	0.0142	*
CA (mg/L)	1	13	0.445	0.506	
MG (mg/L)	1	9	9.677	0.00239	**
CA_MG_RAT	1	0	4.999	0.0274	*
HARDNESS (mg/L)	1	456	1.623	0.205	
CA_MG_INDEX	1	0	9.829	0.00221	**
MOD_CA_MG	1	0	0.975	0.326	

aforementioned quality assurance issues may be affecting results. **Table 13** demonstrates an overall decrease in TP concentrations from 2013 to 2014, expected after the 2013 Moorefield WWTP upgrades, however, the incorrect 2012 detection limit values, and potential exaggerated 2014 TP values likely confound the observed relationship. Also, only two of six total stations now occur below the WWTP as opposed to 3 of 4 in 2013. **Figure 11** also demonstrates how the magnitude of variability in Phosphorous concentrations might mask annual influences, as compared to the Cacapon where ranges were more confined.

Table 22 ANOVA test of water chemistry parameters among Cacapon River stations between years.

Parameter	df	Sum Sq	F	P value	Sign
DO (mg/L)	1	34	9.62	0.002	**
SPCOND (µS/cm)	1	98	0.174	0.677	
PH (SU)	1	12	85	<2e-16	***
WTEMP (▫C)	1	74	5.062	0.0258	*
NO3-NO2 (mg/L)	1	2	22.80	4.0E-06	***
TKN (mg/L)	1	0	1.62	2.1E-01	
TN (mg/L)	1	0	0.20	6.6E-01	
TP (mg/L)	1	0	33.51	3.8E-08	***
DP (mg/L)	1	0	50.62	4.3E-11	***
TSS (mg/L)	1	2	0.04	8.4E-01	
ALK (mg/L)	1	262	1.59	2.1E-01	
CA (mg/L)	1	0	0.00	9.8E-01	
MG (mg/L)	1	14	18.16	3.4E-05	***
CA_MG_RAT	1	0	60.67	7.8E-13	***
HARDNESS (mg/L)	1	246	0.89	3.5E-01	
CA_MG_INDEX	1	0	54.99	6.5E-12	***
MOD_CA_MG	1	0	0.22	6.4E-01	

Cacapon River stations also differed among certain parameters between years (**Table 22**). Dissolved Oxygen, pH and Specific Conductance differed between both river groups and results are likely due to an issue with the Hydrolab used during the first half of the 2012 season. A loaner Hydrolab replaced the unit in 2012, and a new YSI multiprobe (556) was employed in years 2013-2014. The Cacapon data also showed differences among most Phosphorous measures and Nitrate-Nitrate. As no significant changes occurred in this watershed likely to affect nutrient concentrations, the significant results may be further evidence of data quality issues in the analyzed nutrient samples. Magnesium concentrations also differed between years in the Cacapon River.

Boxplots of individual water chemistry parameters for all three years are included in the MS Excel appendix and help to visualize differences between waterbody, station, and year. Below is a sample boxplot of Total Alkalinity, plotted across station and sample year (**Figure 10**). The two Shenandoah stations appear at left, followed to the right by stations moving upstream along the Cacapon River, and finally the six routine and one incidental South Branch Potomac stations. All stations are in order of ascending river mile. In **Figure 10**, the Shenandoah sites stand out for having higher Total Alkalinity compared to the other river systems, where concentrations are lower but generally increase moving upstream. Total Hardness followed a very similar pattern to Alkalinity. In the Cacapon, the input of the North River adjacent to the NO_FRKS station greatly influences the downstream CA_LRGNT, as compared to the sites of CA_FRKS and above.

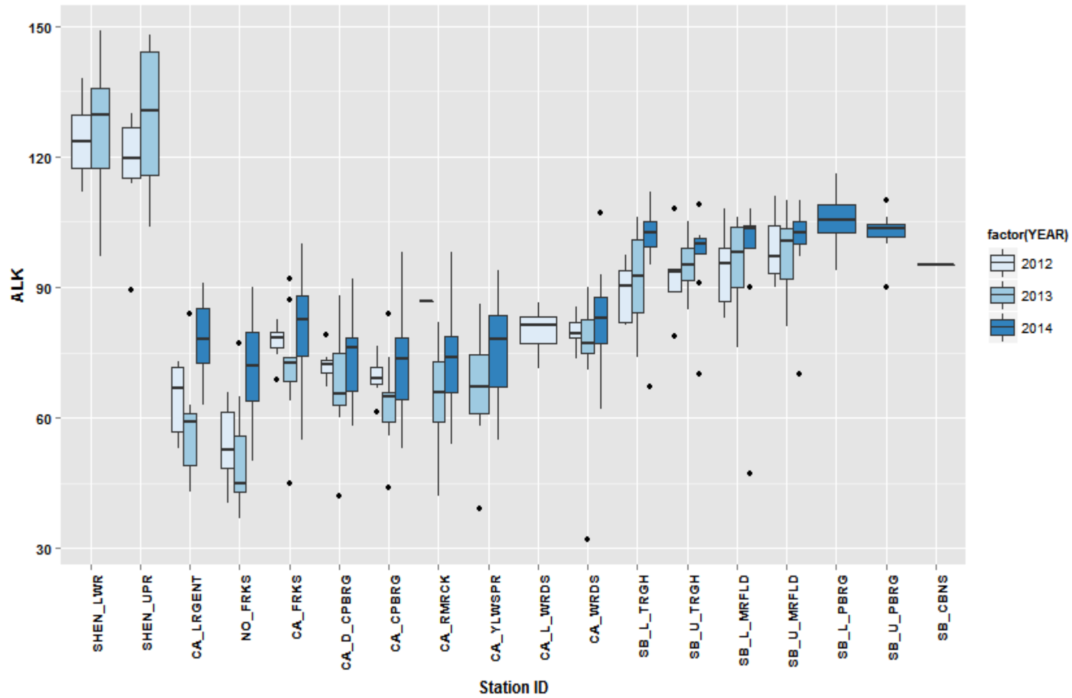


Figure 10 Sample boxplot of all Total Alkalinity data gathered during the project, arranged by station and year (2012-2014).

Figure 11 is a sample plot of Total Phosphorous across all stations and time. In this plot one can see the effect of moving the Moorefield WWTP discharge location between the 2013 and 2014 seasons.

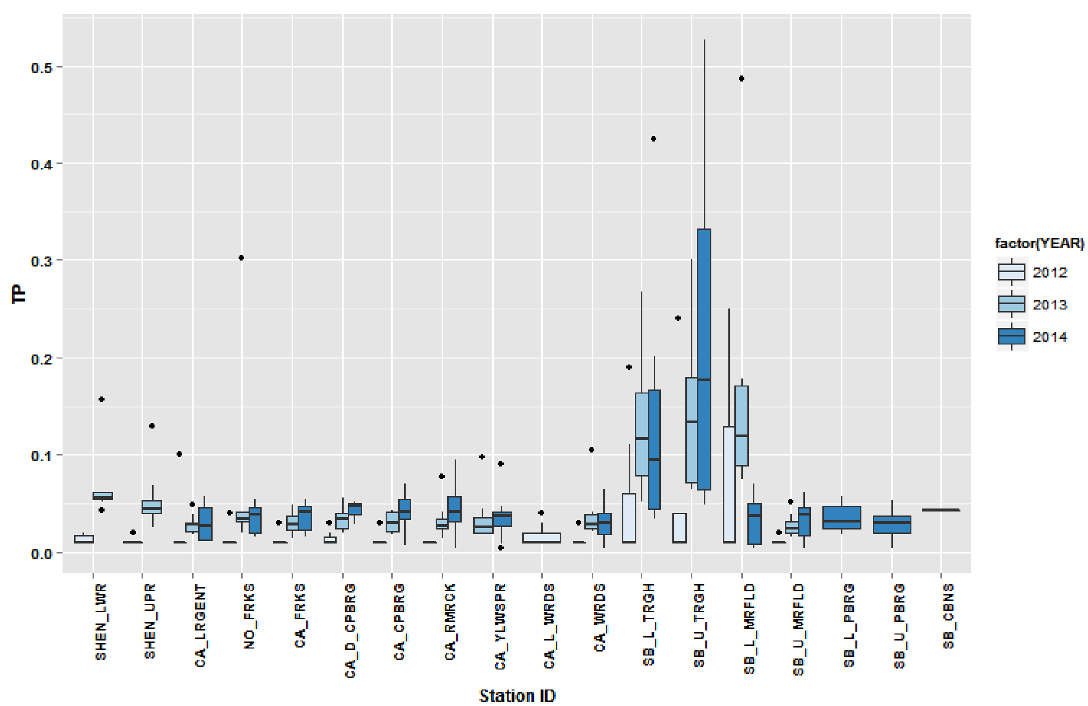
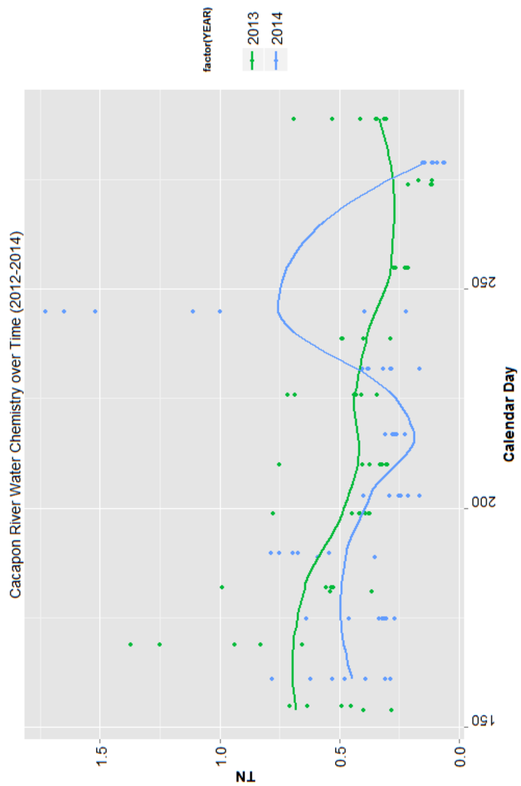


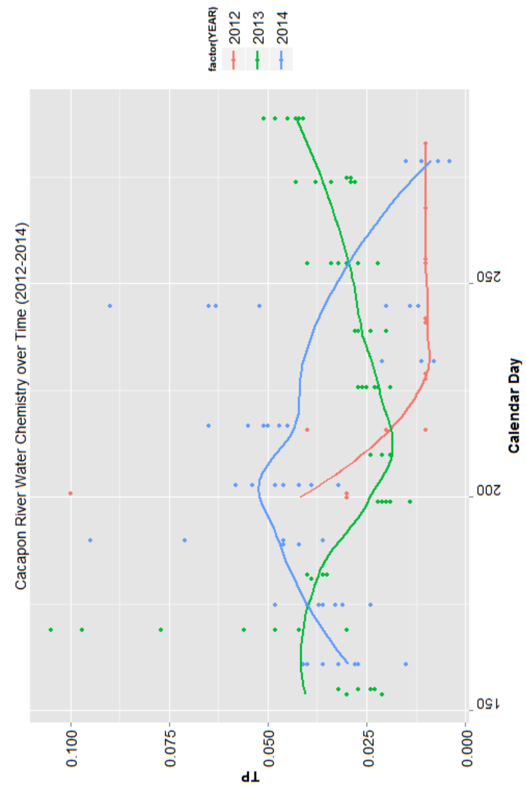
Figure 11 Sample boxplot of all Total Phosphorous data gathered during the project, arranged by station and year (2012-2014).

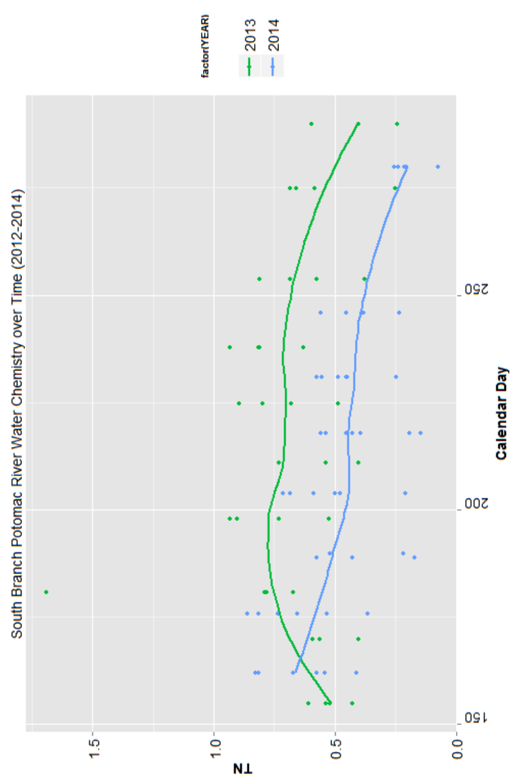
To visualize seasonal trends, scatter plots of each water chemistry parameter were produced with data by river system, plotting chemical concentrations and in-situ parameters against calendar day. Data are again factored, and color-coded by year to help observe annual differences. LOESS curves were added to help visualize the moving average amongst year-river groups. Four plots are provided (**Figure 12**) for example, and the remainder are included in the included spreadsheet appendix. Beginning with the Cacapon River stations, Nitrate and TN concentrations and patterns were similar between 2013 and 2014. 2012 Nitrate results were overall higher and likely led to the significant difference between years. A storm sample was caught in the 2014 season that detected Total Nitrogen concentrations about three-fold more than baseline and is the cause of the bend in the LOESS curve. Total Phosphorous data in the Cacapon reflects the situation with the contracted laboratory, where concentrations were being reported higher than would be expected. When the change in laboratory equipment was made, the reported concentrations were suddenly much lower. Indeed, the final round of concentrations were lower than the 2013 round.

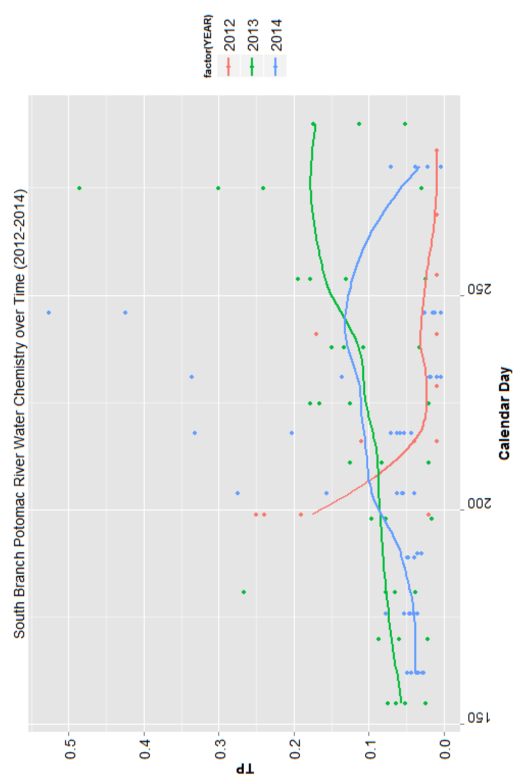
Among the South Branch Potomac River stations, TN concentrations were significantly different between 2013 and 2014 via a paired t-test, with 2014 concentrations being overall lower. Phosphorous data for the 2014 season are interesting, and raise questions that should be investigated further. Overall, most stations reported concentrations ranging between 0.01 and 0.07 mg/L, with four of the six stations having median values of 0.04 mg/L or less. The station at the Trough put-in (SB_U_TRGH) however, frequently had increased TP and DP concentrations, with a median TP value of 0.18 mg/L. The site just downstream (SB_L_TRGH) had a median value of 0.09 mg/L. These data seem to reflect the new Moorefield WWTP discharge location, now just upstream of SB_U_TRGH. It is not immediately evident from reviewing the South Branch Potomac River TP data that the calibration curve method employed by the laboratory affected the relatively higher South Branch Potomac River nutrient data as it may have among the Cacapon River samples. It should be noted, however, that the outlier South Branch TP values did not occur in the last two samples analyzed with the replacement TP laboratory method.

Figures 12. Scatter plots of Total Phosphorous (mg/L) and Total Nitrogen (mg/L) samples collected in the Cacapon River by calendar day. Total Nitrogen was only calculated for years 2013 and 2014.









Summary of algae observations and measured abundance data

Four algae variables were tested under this analysis. The first variable “PCT_ALGAE” are all the transect measurements that were recorded. This data does not occur for all sites and eventually was only collected where algae was present. This means many stations have null values and their water chemistry isn’t considered when investigating chemistry-algae relationships. In the future, all sites should have a percent algae recorded in the field, whether the data is 0%, 1%, or 65%. The second variable is “PCT_ALGAE3” which is an attempt to account for missing algae measurements by using proxy values determined by qualitative ratings. Where algae measurements existed, they were maintained, but null values were updated with surrogate percentage values. Qualitative FGA scores of “0” translated to “0”, a “1” was replaced with a value of 1.5% and a “2” was replaced with a value of “10%. The last two variables being tested are the qualitative scores of filamentous green algae (FGA) and cyanobacteria (BGA).

Pearson correlations were performed across each of the four algae variables and all of the water chemistry variables. Only Nitrate-Nitrite was identified as having a significant relationship with algae among the explanatory variables. Algae was associated with dissolved oxygen and pH values, reinforcing the linkage between those two variables as being influenced by respiring algae blooms. No significant algae-water chemistry relationships were found amongst the South Branch Potomac data.

Identifying drivers of algae abundance

Simple Regression and Classification trees were used to test the four algae variables. The package “rpart” was used to perform the recursive partitioning, or tree-analysis, and the package “partykit” was used to plot the trees. Data from the two main river systems were analyzed separately due to their differing water chemistry profiles. Also, nutrients, specifically TN and TP were considered in one series of tests, while the dissolved metals and alkalinity measures of Hardness, Total Alkalinity, Calcium, Magnesium, Ca:Mg Ratio, Ca-Mg Index, and modified Ca:Mg Index were tested in a second series.

In the Cacapon River, Total Nitrogen was chosen as the top explanatory nutrient variable over Total Phosphorous in all four tests of the algae variables. Interestingly, every primary split occurred between 0.22 and 0.30 mg/L TN, with higher algae events occurring in lower TN conditions. This seems to indicate that algae may be taking up Nitrogen where it occurs, lowering TN concentrations in those algae areas. It should also be noted that in each of those same four tests, secondary splits chose Total Phosphorous as an explanatory variable of algae abundance, continually choosing a threshold where algae was more abundant above about 0.025 mg/L (**Figure 13**).

Alkalinity was the predominantly chosen factor for explaining algae abundance amongst the tested dissolved metals and salts. Thresholds between 64 and 73 mg/L Total Alkalinity often divided samples into higher and lower algae abundance, with higher algae found with higher alkalinity (**Figure 14**).

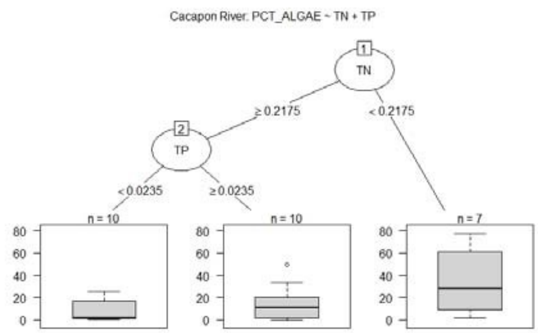


Figure 13 Regression tree for Percent Algae Cover and nutrient variables in the Cacapon River.

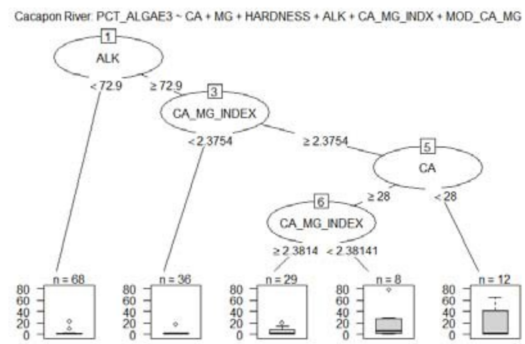


Figure 14 Regression tree for percent algae cover and dissolved salt and metal variables in the Cacapon River.

Calcium, Magnesium, and Hardness associations were less evident and less often chosen as primary explanatory variables in the Cacapon.

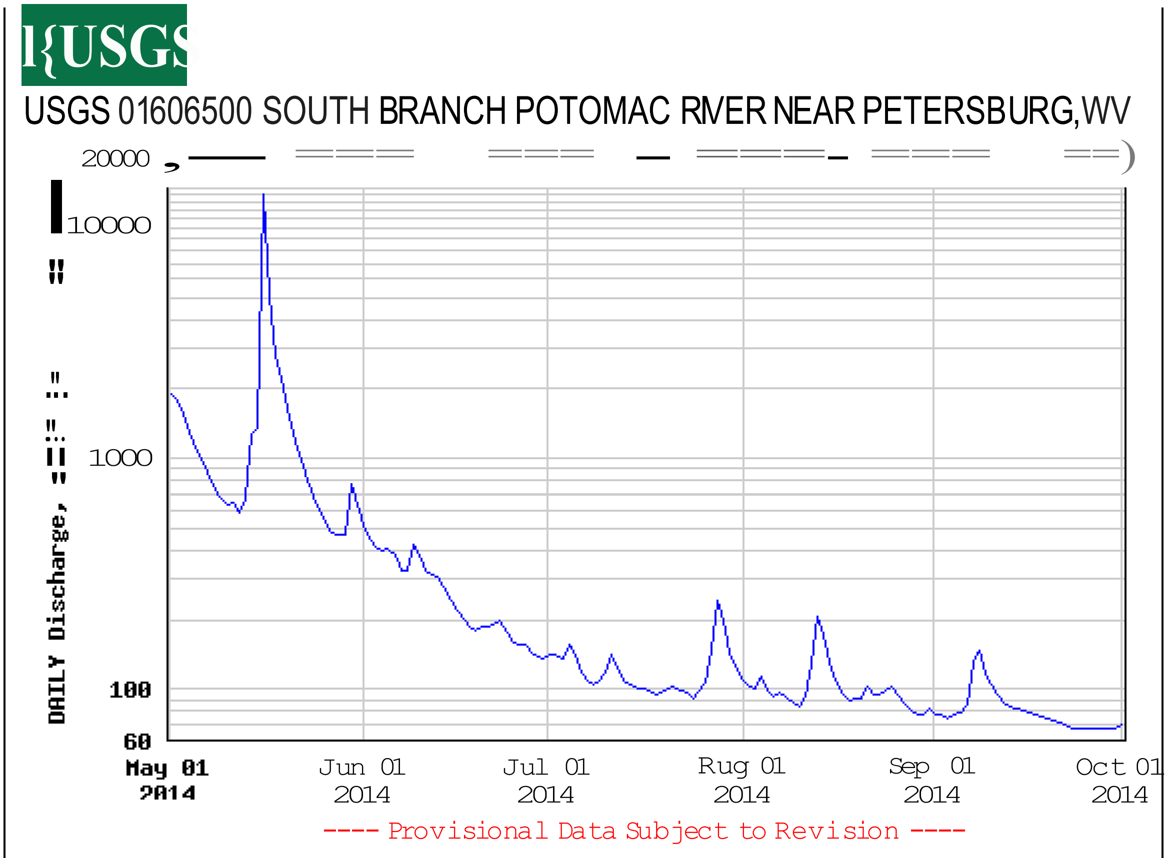
Similar analyses in the South Branch Potomac did not produce very meaningful results as there have not been many occurrences of high algae abundance, greatly limiting the variability in the response variable.

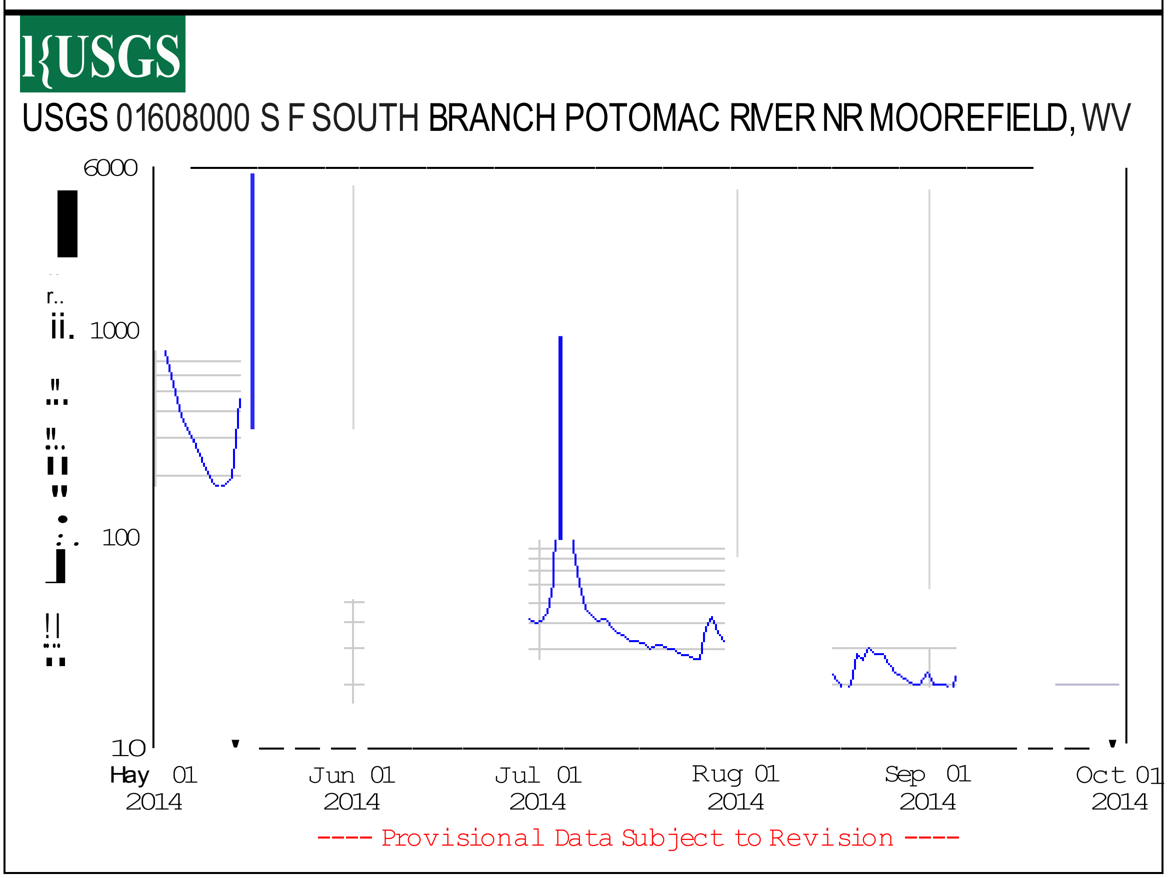
Suggestions for future

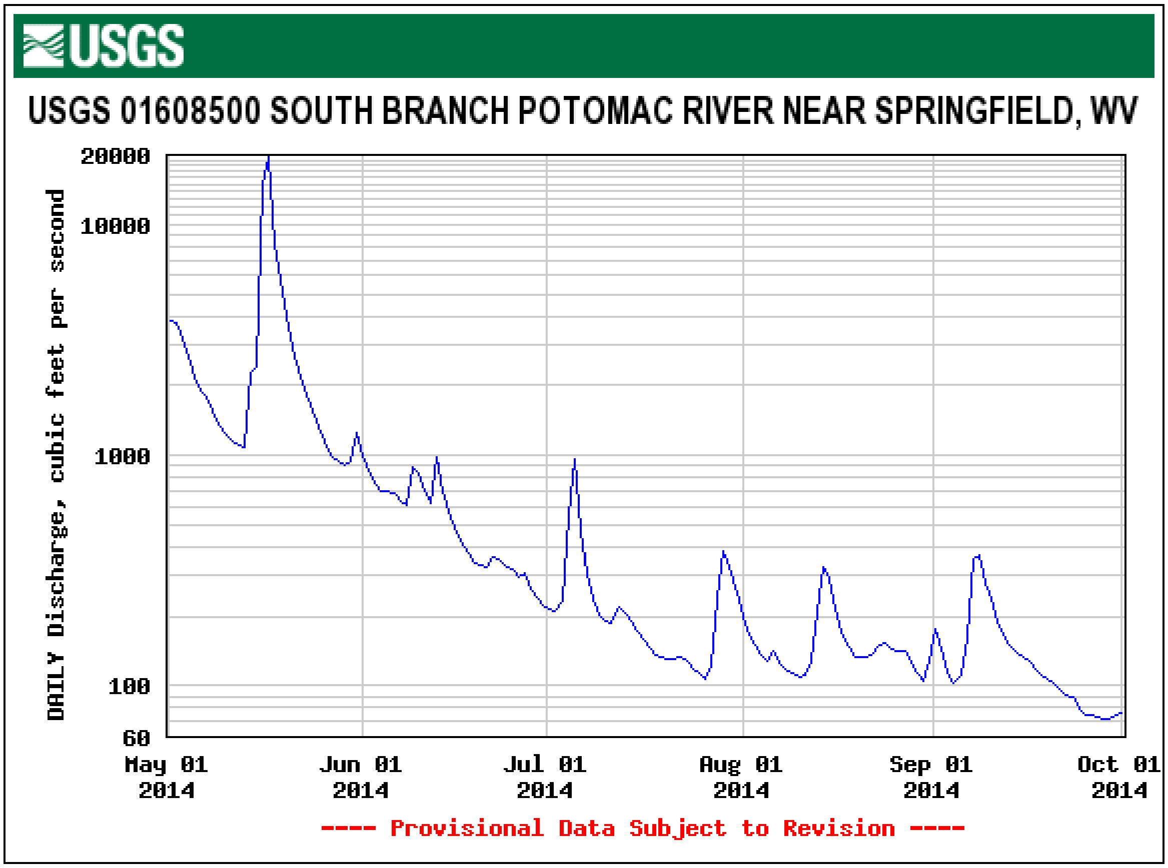
Collect numerical algae coverage values on every site visit in order to increase the sample size of ecological algae analyses. Consider adding a place on the form where an estimated numerical value can be entered (<10%, >80%), or indicated that a cross-sectional measurement was taken.

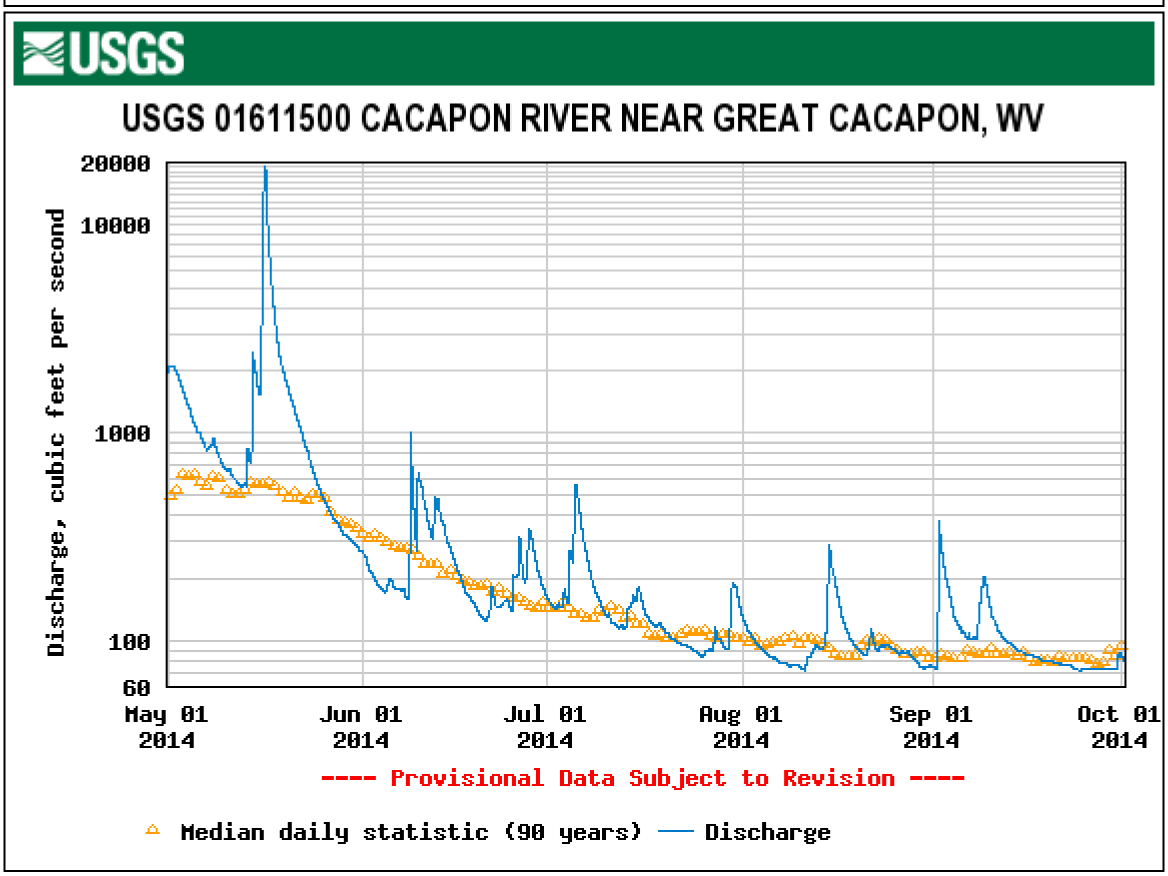
Investigate the additional algae methods developed by ICPRB for use in the Shenandoah watershed.

Appendix 1 Relevant hydrographs from USGS gages for the 2014 algae monitoring season.






USGS
USGS 01608500 SOUTH BRANCH POTOMAC RIVER NEAR SPRINGFIELD, WV
DAILY Discharge, cubic feet per second
20000
10000
1000
100
60
May 01 2014
Jun 01 2014
Jul 01 2014
Aug 01 2014
Sep 01 2014
Oct 01 2014
---- Provisional Data Subject to Revision ----


USGS
USGS 01611500 CACAPON RIVER NEAR GREAT CACAPON, WV
Discharge, cubic feet per second
20000
10000
1000
100
60
May 01 2014
Jun 01 2014
Jul 01 2014
Aug 01 2014
Sep 01 2014
Oct 01 2014
---- Provisional Data Subject to Revision ----
Median daily statistic (90 years)
Discharge