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BUREAU OF CLEAN WATER

NEONICOTINOID & SULFOXIMINE INSECTICIDES IN FLOWING SURFACE WATER PILOT SURVEY

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INTRODUCTION

Neonicotinoids are a class of insecticides applied as seed treatments, to soil, or sprayed directly on plants. They are applied to seeds and soils to protect seedlings and applied later to protect mature plants (Morrissey et al. 2015). Imidacloprid was one of the first neonicotinoids to be developed in the 1990s and is now widely used in many countries for effective control of sucking insect pests. Neonicotinoids can be classified as *N*-nitroguanidines (imidacloprid, thiamethoxam, clothianidin, and dinotefuran), nitromethylenes (nitenpyram), and *N*-cyanoamidines (acetamiprid and thiacloprid) (Jeschke et al. 2011). Sulfoxaflor is a new insecticide in the class of sulfoximines that has an action similar to neonicotinoids (USEPA 2016). Sulfoximine insecticides function by binding the nicotinic acetylcholine receptors (*n*AChRs), which interferes with the central nervous system (Jeschke et al. 2011). Although data is unavailable on the amount of neonicotinoid and sulfoximine seed treatments used, nearly all corn seeds in North America are coated with these compounds, normally clothianidin or thiamethoxam (Perre et al. 2015). Neonicotinoids are applied in a wide variety of settings including use by homeowners in sprays, dog and cat flea and tick repellents, and forest pest control (John Lake, Pennsylvania Department of Agriculture (PDA), personal communication, 6/15/2016).

Although very beneficial for pest management, neonicotinoids can also persist in soils and leach into surface water (Goulson 2013) and have been shown to be toxic to non-target invertebrates, as well as vertebrates. Studies have been conducted exhibiting toxicity of neonicotinoid insecticides on vertebrates, particularly sub-lethal impacts such as neuro-behavioral and reduced immune response (Gibbons et al. 2015). Non-target terrestrial invertebrates can also be negatively impacted. When exposed to realistic field concentrations of imidacloprid in pollen and sugar water, bumble bees showed a greatly reduced weight growth rate and lower production of new queens than controls (Whitehorn et al. 2012).

Morrissey et al. (2015) described the sensitivity of aquatic macroinvertebrates to concentrations of neonicotinoid insecticides below 1.0 µg/L. Results from this study indicated the most sensitive taxa were Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae species. Sub-lethal concentration endpoints in chronic studies were often an order of magnitude lower than the acute tests. For example, short-term pulsed exposures to ≥1.0 µg/L of imidacloprid caused feeding inhibition in Ephemeroptera, while immobility of Ephemeroptera and Plecoptera was seen at concentrations of 0.1 to 0.2 µg/L over longer exposure periods. Cladocera were substantially more tolerant, with 48-hour immobilization tests showing a half maximal effective concentration (EC_{50}) of imidacloprid ranging from 572 to 45,271 µg/L and an EC_{50} of clothianidin ranging from 1,691 to 67,564 µg/L across five cladoceran species (Hayasaka et al. 2013).

The United States Environmental Protection Agency (USEPA) has published aquatic life benchmarks for many pesticides, including neonicotinoids and sulfoxaflor (USEPA 2018). These benchmarks are based on the most sensitive aquatic toxicity data available and indicate the concentration below which no harm to aquatic life is expected (Table 1).

Table 1. USEPA Aquatic Life Freshwater Benchmarks (µg/L)

Parameter	Fish Acute	Fish Chronic	Invertebrates Acute	Invertebrates Chronic
Acetamiprid	>50,000	19,200	10.5	2.1
Clothianidin	>50,750	9,700	11	0.05
Imidacloprid	114,500	9,000	0.385	0.01
Nitenpyram	N/A	N/A	N/A	N/A
Sulfoxaflor	>181,500	660	>100,000	50,500
Thiacloprid	12,600	918	18.9	0.97
Thiamethoxam	>57,000	20,000	17.5	0.74

Citation: USEPA 2018

The highest concentrations of neonicotinoids in streams due to runoff should be in spring, immediately after coated seeds are planted. Higher concentrations of neonicotinoids in samples of streams draining watersheds with substantial agricultural land use have been seen in rainfall events during crop planting, suggesting that seed treatments are a source (Hladik et al. 2014). In one study, concentrations in water flowing through corn fields showed highest concentrations of neonicotinoids approximately five weeks after planting, and levels returned to pre-planting concentrations approximately seven weeks after planting (Schaafsma et al. 2015). In addition, clothianidin, imidacloprid, and thiamethoxam concentrations between 0.0044 to 0.132 µg/L were detected in streams in agricultural areas in Iowa in late June to early July after most row crops were planted, following heavy rainfall (Hladik & Kolpin 2015). Neonicotinoids can also persist in non-target plants, soils, etc. (Bonmatin et al. 2015).

Neonicotinoid water sampling data is limited in Pennsylvania. The PDA sampled 40 groundwater wells over a period of two years for imidacloprid using United States Geological Survey (USGS) lab schedule 2437 (USGS 2018) and found no detections over the method detection limit (MDL) (Don Gilbert, PDA, personal communication, 6/27/2016). The USGS summarized a nationwide study of neonicotinoid insecticides in streams with a few sites located in Pennsylvania (Hladik & Kolpin 2015). At least one neonicotinoid was detected in 53% of the 38 targeted stream sites sampled nationwide by Hladik & Kolpin (2015), with imidacloprid being the most commonly detected, followed by clothianidin, thiamethoxam, dinotefuran, and acetamiprid. Hladik & Kolpin (2015) found that 92% of the detectable concentrations of neonicotinoids were below 0.1 µg/L with a median of 0.019 µg/L. Other sampling completed by USGS in Adams County in 2003 and 2012, and Franklin, Dauphin, and Montgomery counties in 2010, resulted in low-level detections of neonicotinoids, with detectable concentrations ranging from 0.0057 to 0.0666 µg/L (Joseph Duris, USGS, personal communication, 6/4/2019).

The Pennsylvania Department of Environmental Protection (DEP) does not currently have specific water quality criteria for neonicotinoid insecticides and little data is available on surface water concentrations of these compounds in the state. Aquatic macroinvertebrates are frequently used by DEP to make aquatic life use assessments of streams and rivers (Shull & Lookenbill 2018, Shull & Pulket 2018). Because of their potential to affect non-target invertebrates, a surface water pilot survey of neonicotinoids in Pennsylvania was needed. This study was designed to observe the occurrence and distribution of neonicotinoids in surface waters draining agricultural landscapes in Pennsylvania. Although data were not available on neonicotinoid use in the watersheds sampled for this study, the study was designed under the assumption that neonicotinoid concentrations would be highest in spring after planting of neonicotinoid-coated seeds and lower in late summer.

METHODS AND MATERIALS

Eighteen sites were selected to target streams influenced by agriculture that had corn or other crops nearby that could have neonicotinoids applied (Figure 1, Table 2). The southcentral region of Pennsylvania was chosen for sampling because this region has high amounts of agriculture. Streams were chosen based on proximity to crops likely to have neonicotinoids applied and the potential for accumulation of insecticides as runoff from these crops. Large rivers were excluded from the analysis due to the potential for dilution of runoff. Small, wadable streams were chosen to potentially capture the highest concentrations of neonicotinoids and sulfoxaflor.

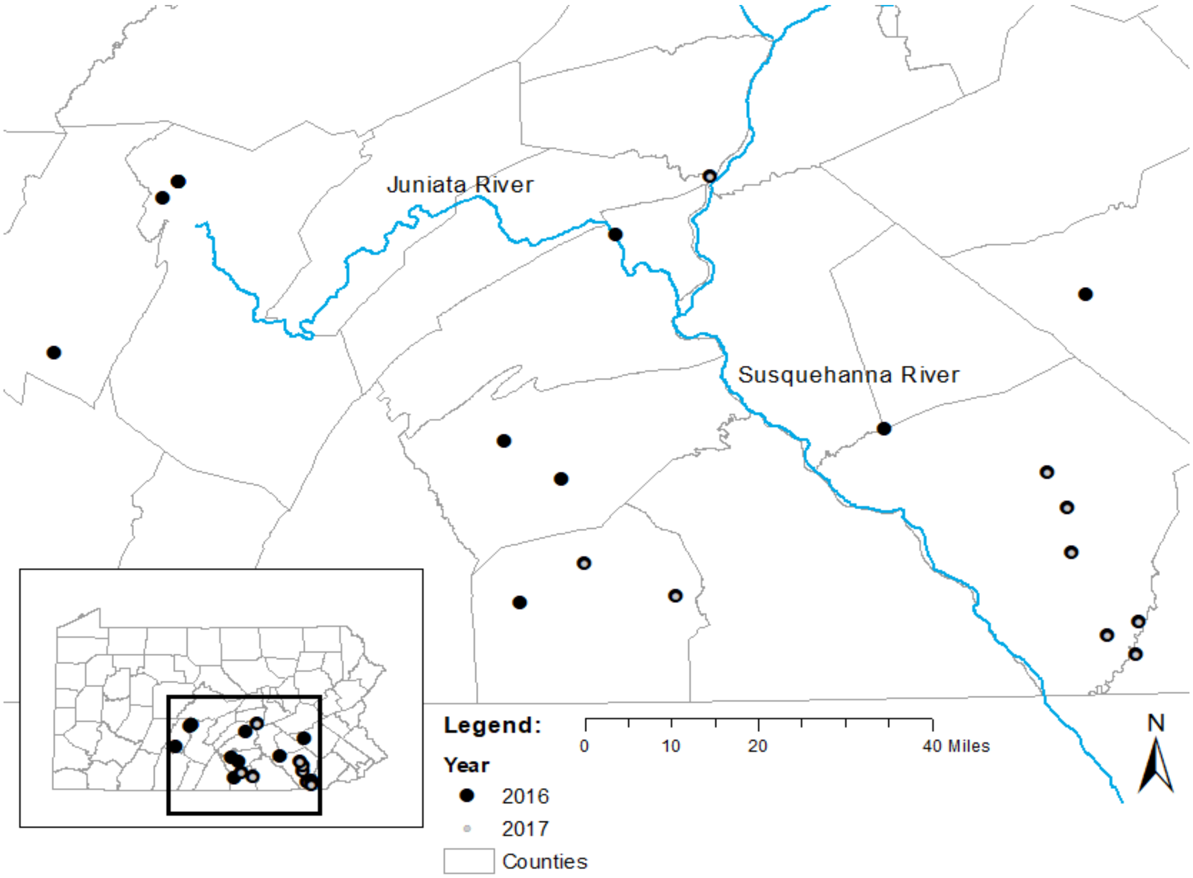

Figure 1. Neonicotinoid and sulfoximine insecticide sample sites, 2016 – 2017

Table 2. Sites sampled for neonicotinoid and sulfoximine insecticides

Site	Latitude	Longitude
Bells Run *	39.84745	-76.02361
Big Spring Creek	40.17977	-77.39225
Cocolamus Creek	40.54050	-77.14550
Conewago Creek (Adams Co.) *	39.90660	-77.02534
Conewago Creek (Lancaster Co.)	40.19528	-76.56770
Groff Creek *	40.11255	-76.21434
Little Beaver Creek *	39.97085	-76.16516
Marsh Creek	39.89667	-77.36064
Muddy Run *	40.05054	-76.17326
Opossum Creek *	39.96471	-77.22159
Plum Creek	40.33567	-78.37425
Spruce Creek	40.60873	-78.13573
Tulpehocken Creek	40.42412	-76.12226
Tweed Creek *	39.79028	-76.02962
W. Br. Mahantango Creek *	40.64220	-76.93960
W. Br. Octoraro Creek *	39.82546	-76.09049
Warriors Mark Run	40.63822	-78.10245
Yellow Breeches Creek	40.11237	-77.26886

* sampled in 2016 and 2017; remainder sampled only in 2016

The last frost in southern Pennsylvania generally occurs between April 11 to April 21 (National Oceanic and Atmospheric Administration (NOAA) FrostFreeze). Since corn can be planted safely about ten to fourteen days before the average date of the last killing frost, May should be an optimal time to sample for neonicotinoids in southern Pennsylvania. Pesticide concentrations should be lowest later in the summer and winter. They should be highest in the spring, after crops are planted that may have insecticidal seed coatings and/or insecticides applied after seed planting (as supported by Hladik and Kolpin 2015). As a result, samples were collected in August 2016 and May 2017 to compare concentrations between seasons. Funding was only available in 2017 for a subset of the 2016 sites. Therefore, after the August 2016 collection, results were analyzed and nine sites with detections or further interest were sampled again in May 2017. August sampling occurred August 2-16, 2016 and May sampling occurred May 16-19, 2017 (Appendix A).

Water samples were collected according to the Discrete Water Chemistry Data Collection Protocol found in Chapter 4 of DEP's Water Quality Monitoring Protocols for Streams and Rivers (Shull and Lookenbill 2018). Water samples were collected at mid-channel, mid-depth using one-liter amber glass jars with tetraflurothethylene (TFE) lined caps. No preservative was required, but samples were immediately refrigerated at ≤6°C until analysis. Samples were mailed on ice immediately following field collection, although holding time is 28 days. Analysis was done at ALS Global in Waterloo, Ontario, Canada. Sample results were sent to DEP (Appendix A).

Samples were analyzed for a suite of six neonicotinoid insecticides and one sulfoximine insecticide (Table 3). The analyses were completed using procedures adapted from Environment Canada. The method was derived from Xiao et al. (2011). Neonicotinoid and sulfoxaflor concentrations are determined by evaporating the sample to near dryness, reconstituting it, then analyzing it on a liquid chromatography-tandem mass spectrometry (LC-MS/MS) (ALS Global, personal communication, July 2016). Further details on the analysis method can be requested from ALS Global in Waterloo, Ontario, Canada.

Table 3. Insecticides analyzed

Parameter	Units	Detection Limit
Acetamiprid	µg/L	0.0050
Clothianidin	µg/L	0.0050
Imidacloprid	µg/L	0.0050
Nitenpyram	µg/L	0.0050
Sulfoxaflor	µg/L	0.0050
Thiacloprid	µg/L	0.0050
Thiamethoxam	µg/L	0.0040

Stream flow data were downloaded for sites sampled in both August 2016 and May 2017 where a USGS stream gauge was located within six miles of the sampling site (Appendix B). Gages were chosen based on proximity rather than location on the stream sampled – no gages were available directly on any of the sampled streams. However, comparing these gage results for both years should provide some indication if any precipitation or high-flow events impacted the areas sampled. The stream flow data were queried by date from the USGS Current Water Data for Pennsylvania website (USGS 2019).

RESULTS

Neonicotinoid concentrations above the associated detection limit were detected at 11 out of 18 sites sampled: Bells Run, Big Spring Creek, Conewago Creek (Adams County), Groff Creek, Little Beaver Creek, Muddy Run, Opossum Creek, Tulpehocken Creek, Tweed Creek, West Branch Mahantango Creek, and West Branch Octoraro Creek (Appendix A).

The only compounds detected above the associated detection limits were clothianidin, imidacloprid, and thiamethoxam (Appendix A, Figure 2). The highest concentrations of clothianidin were observed at the West Branch Octoraro Creek site (August 2016 sample = 0.0317 µg/L, May 2017 sample = 0.300 µg/L). The highest concentration of imidacloprid was observed in the August 2016 sample from Opossum Creek (0.0189 µg/L). The only thiamethoxam result above the detection limit was in May 2017 from the Opossum Creek site (0.0041 µg/L). Concentrations ranged from 0.0053 to 0.0317 µg/L for clothianidin (19 detections), and 0.0052 to 0.0189 µg/L for imidacloprid (16 detections). Of the nine sites sampled in both May 2017 and August 2016, imidacloprid concentrations were higher in the August 2016 samples than in the May 2017 samples at all nine sites, and clothianidin concentrations were higher in the August 2016 samples than in the May 2017 samples at six sites. Out of the nine sites with samples in both August 2016 and May 2017, four sites had USGS stream gages within six miles of the sample point (Appendix B).

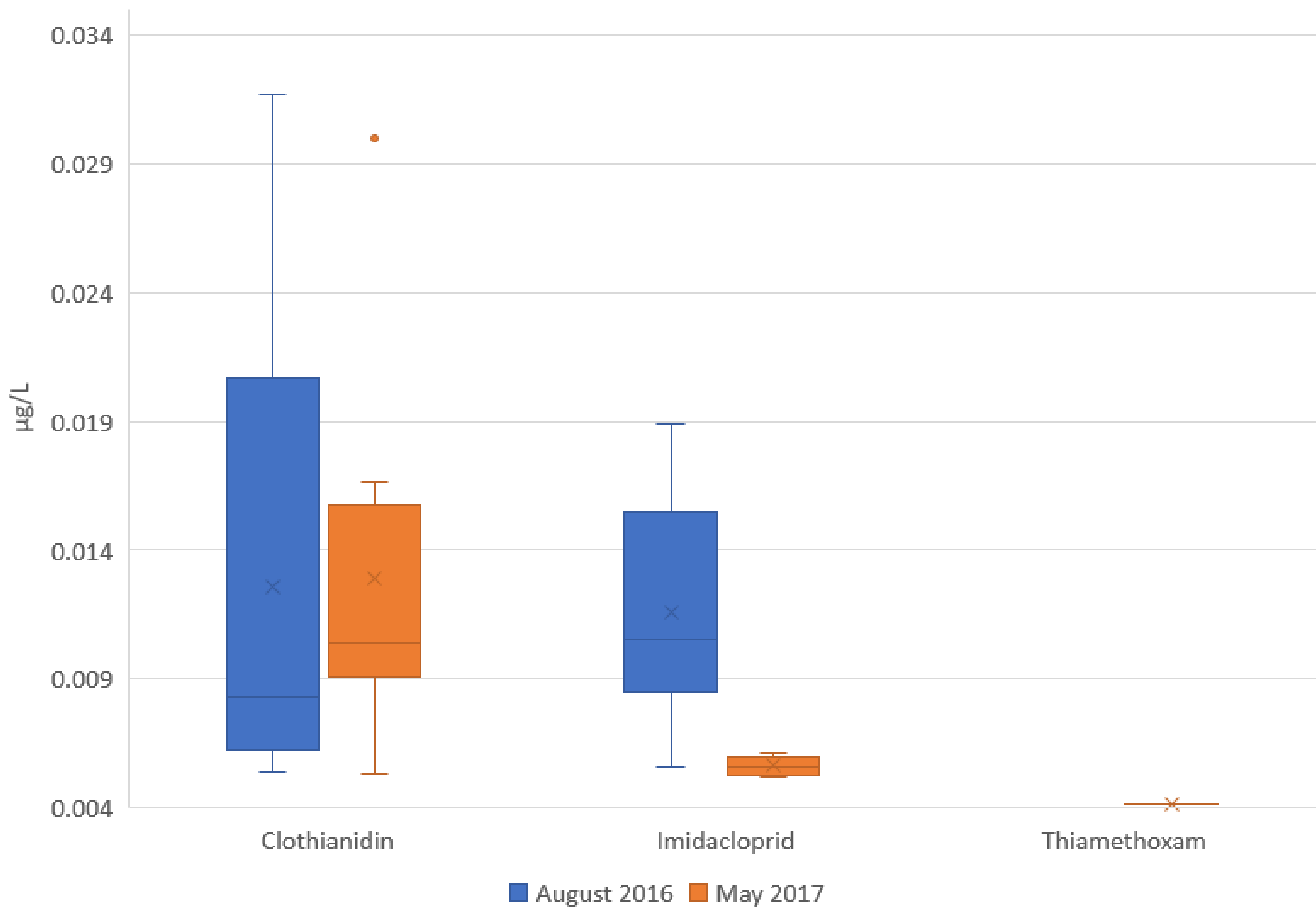

Figure 2. Box-and-whisker plots of clothianidin, imidacloprid, and thiamethoxam concentrations above the associated detection limits.

DISCUSSION

Neonicotinoid insecticides have been detected in soil (Schaafsma et al. 2015), water in agricultural fields (Schaafsma et al. 2015), and surface waters (Hladik & Kolpin 2015) frequently and at varying concentrations. Contrary to the prediction that neonicotinoid concentrations would be higher in spring samples than in summer samples, this pilot study documented higher concentrations of neonicotinoid insecticides in August 2016 samples compared with May 2017 samples from the same sites. This unexpected result may be attributable to some combination of potentially missing the spring peak in concentrations if the 2017 samples were collected too late in the season or dilution of instream concentrations associated with higher stream flows at some sites in May 2017 compared with August 2016 (Appendix B). Homeowner usage of neonicotinoids likely peaks during the summer months, so that may also contribute to the higher summer concentrations in surface waters. In addition, neonicotinoids can be sprayed on growing crops during the summer, which could also contribute to the higher concentrations observed in the August 2016 samples.

All detected concentrations of neonicotinoids observed during this study were relatively low: well below the values literature suggests could impact aquatic macroinvertebrates (<0.1 µg/L), and well below USEPA's acute aquatic life benchmarks (Table 1). Some imidacloprid results were above the chronic freshwater invertebrate benchmark of 0.01 µg/L; however, because only one sample was collected for each year, it is difficult to extrapolate if imidacloprid concentrations are chronically above this level. Imidacloprid, clothianidin, and thiamethoxam were the most frequently detected neonicotinoids in a national study (Hladik & Kolpin 2015), which mirrors the results of this DEP pilot study. In addition, a pilot study of neonicotinoid concentrations in New York and northern Pennsylvania streams had detections of imidacloprid, acetamiprid, and thiamethoxam (Secord & Patnode 2018). In that study, imidacloprid was detected above the EPA chronic benchmark of 0.01 µg/L in several New York samples.

Based on this pilot study and targeted nature of the sites, the likelihood of high levels of neonicotinoids in flowing surface water in agricultural areas in south-central Pennsylvania is low in May and August. However, it has not been determined if the EPA chronic invertebrate benchmark of 0.01 µg/L is consistently exceeded, which means that imidacloprid levels could be impacting aquatic life in these streams. Although the concentrations of neonicotinoids observed in this pilot study were lower than concentrations observed in other studies of surface waters in the United States, the highest concentrations in the targeted stream sites may have been missed. Also, sampling for this pilot study did not target storm flows, when the highest amount of surface runoff likely occurs. Neonicotinoids tend to be retained in soil composed of loam and clay, and do not accumulate in sand (Mörtl et al. 2016). In addition, their half-lives in soil can be highly variable, ranging from 200 to >1000 days (Goulson 2013). This makes it difficult to determine when the highest concentrations in surface water may occur.

The analytical methodology used in this study had very low detection limits (0.004 or 0.005 µg/L), well below the levels thought to impact aquatic life, so this method should be adequate to measure concentrations of the analyzed compounds potentially harmful to aquatic life. Passive water sampling, in which sampling equipment is placed in water for a long period of time, such as a month, could provide information to supplement the findings of this pilot study. Passive sampling is useful for compounds that are typically detected at very low concentrations or occur in bursts, such as during storm flows, and could be missed by traditional grab sampling. For this pilot study, relatively few sites were targeted and only a subset of those sites were sampled on more than one occasion, with a maximum of two samples collected per site. Future studies may help characterize neonicotinoid and sulfoximine insecticide concentrations in Pennsylvania streams by sampling additional sites and by collecting samples more frequently, potentially targeting different runoff and stream flow conditions and/or targeting sampling locations and times based on information about insecticide use. Sampling at sites where stream flow data is available may help future studies better assess the relationship between instream pesticide concentrations and precipitation/streamflow patterns.

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APPENDIX A: SAMPLE RESULTS

May 2017 samples shaded grey

all results in µg/L

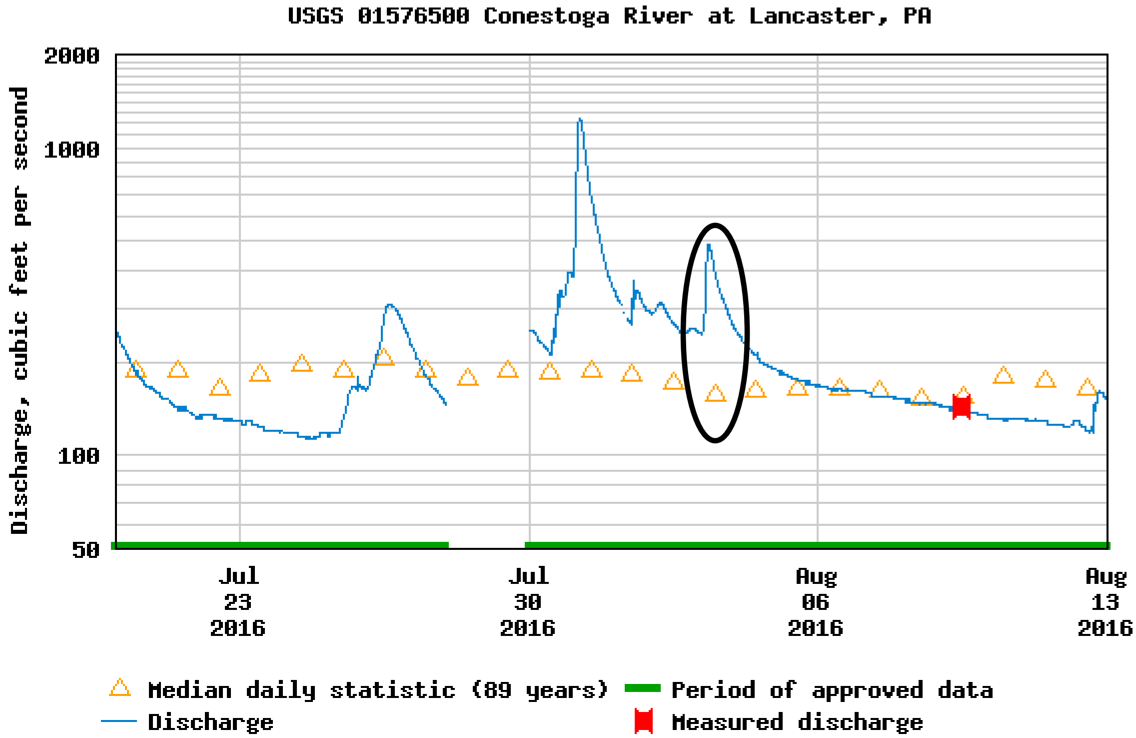
Site	Date	Acetamiprid	Clothianidin	Imidacloprid	Nitenpyram	Sulfoxaflor	Thiacloprid	Thiamethoxam
Bells Run	5/16/2017	<0.0050	**0.0148**	**0.0061**	<0.0050	<0.0050	<0.0050	<0.0040
	8/4/2016	<0.0050	**0.0205**	**0.0116**	<0.0050	<0.0050	<0.0050	<0.0040
Big Spring Creek	8/9/2016	<0.0050	<0.0050	**0.0077**	<0.0050	<0.0050	<0.0050	<0.0040
Cocolamus Creek	8/9/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Conewago Creek	5/19/2017	<0.0050	**0.0096**	**0.0057**	<0.0050	<0.0050	<0.0050	<0.0040
(Adams County)	5/19/2017*	<0.0050	**0.0090**	**0.0055**	<0.0050	<0.0050	<0.0050	<0.0040
	8/2/2016	<0.0050	**0.0072**	**0.0162**	<0.0050	<0.0050	<0.0050	<0.0040
Conewago Creek (Lancaster County)	8/16/2016	<0.0050	<0.0050	**0.0087**	<0.0050	<0.0050	<0.0050	<0.0040
Groff Creek	5/16/2017	<0.0050	**0.0167**	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
	8/4/2016	<0.0050	**0.0212**	**0.0174**	<0.0050	<0.0050	<0.0050	<0.0040
Little Beaver Creek	5/16/2017	<0.0050	**0.0053**	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
	8/4/2016	<0.0050	**0.0065**	**0.0091**	<0.0050	<0.0050	<0.0050	<0.0040
Marsh Creek	8/2/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Muddy Run	5/16/2017	<0.0050	**0.0092**	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
	8/4/2016	<0.0050	**0.0090**	**0.0130**	<0.0050	<0.0050	<0.0050	<0.0040
Opossum Creek	5/19/2017	<0.0050	**0.0104**	<0.0050	<0.0050	<0.0050	<0.0050	**0.0041**
	8/2/2016	<0.0050	**0.0076**	**0.0189**	<0.0050	<0.0050	<0.0050	<0.0040
Plum Creek	8/9/2016	<0.0050	<0.0050	**0.0095**	<0.0050	<0.0050	<0.0050	<0.0040
Spruce Creek	8/9/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Tulpehocken Creek	8/16/2016	<0.0050	**0.0054**	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Tweed Creek	5/16/2017	<0.0050	**0.0110**	**0.0052**	<0.0050	<0.0050	<0.0050	<0.0040
	8/4/2016	<0.0050	**0.0115**	**0.0084**	<0.0050	<0.0050	<0.0050	<0.0040
West Branch	5/19/2017	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Mahantango Creek	8/9/2016	<0.0050	**0.0055**	**0.0056**	<0.0050	<0.0050	<0.0050	<0.0040
West Branch	5/16/2017	<0.0050	**0.0300**	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Octoraro Creek	8/4/2016	<0.0050	**0.0317**	**0.0132**	<0.0050	<0.0050	<0.0050	<0.0040
Warriors Mark Run	8/9/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
	8/9/2016*	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Blank	8/9/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040
Yellow Breeches Creek	8/9/2016	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0040

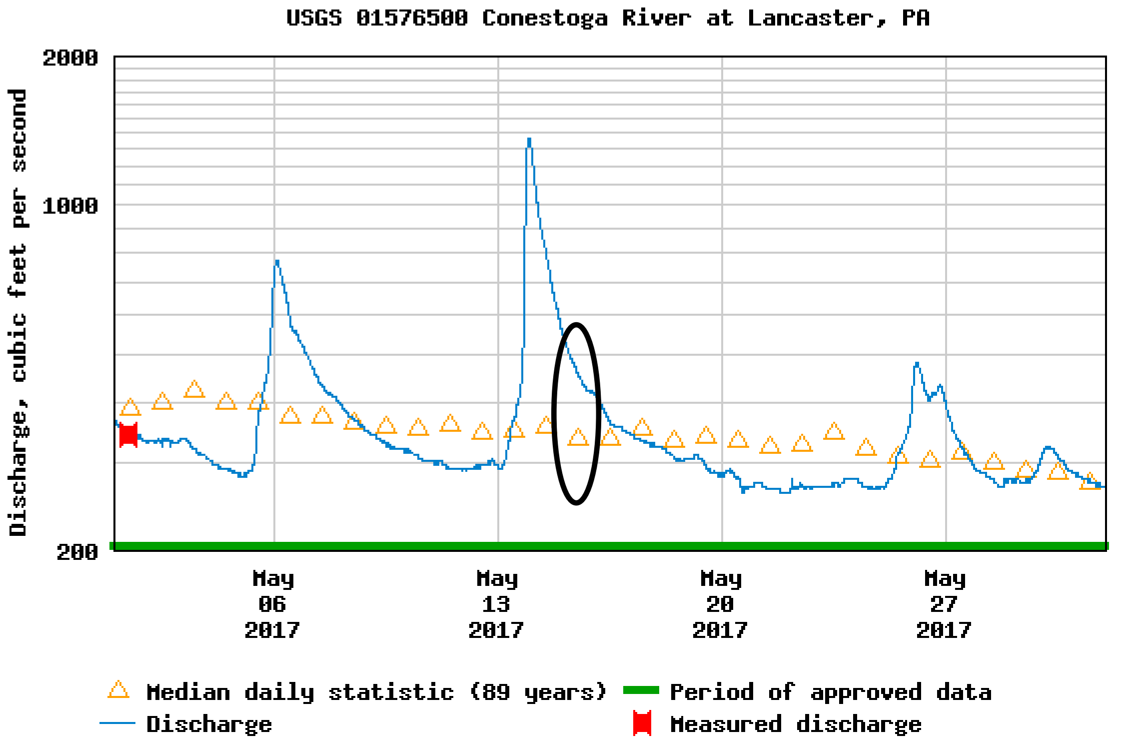
* duplicate

APPENDIX B: USGS STREAMFLOW FIGURES

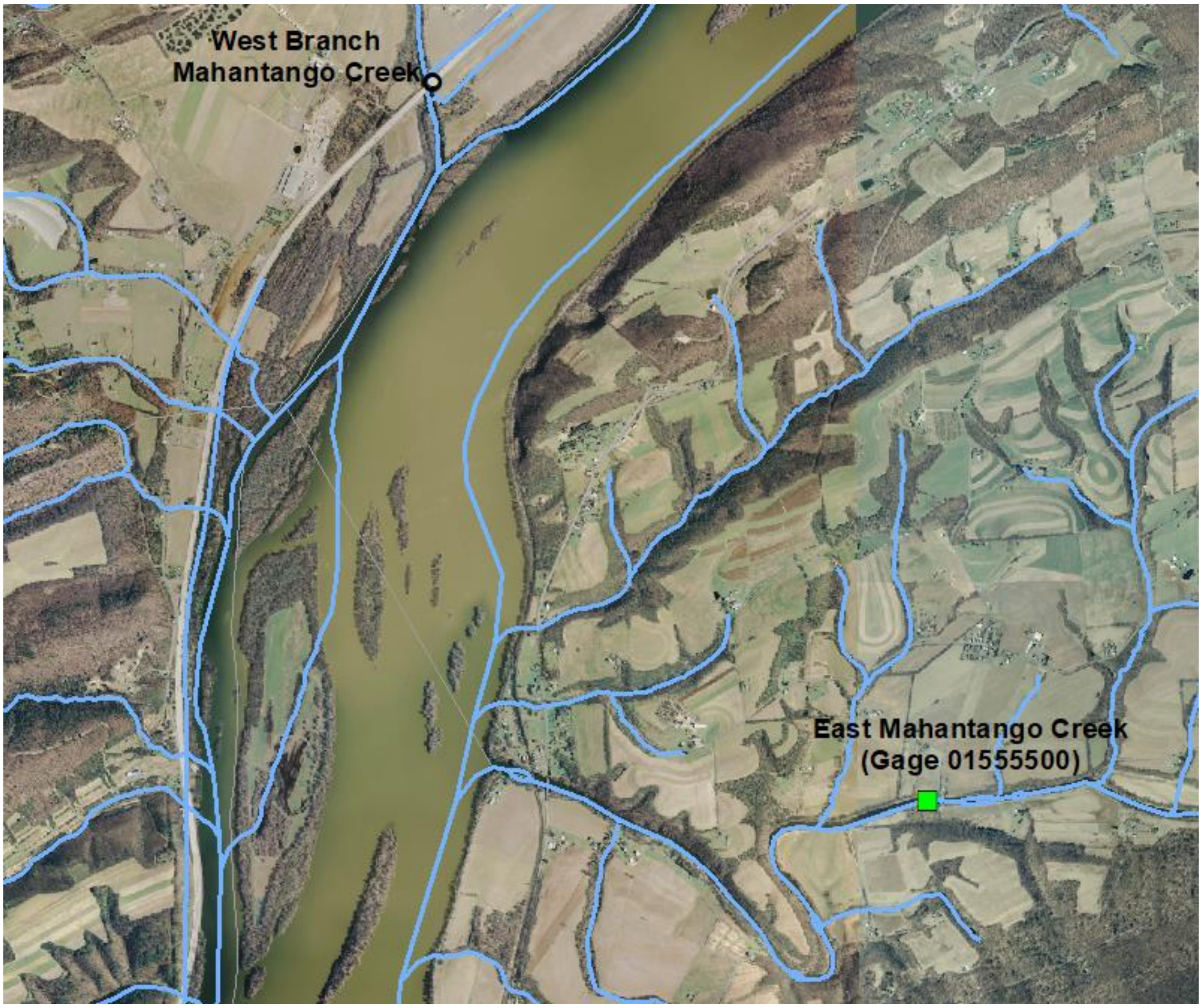
(note varying scales on both axes)

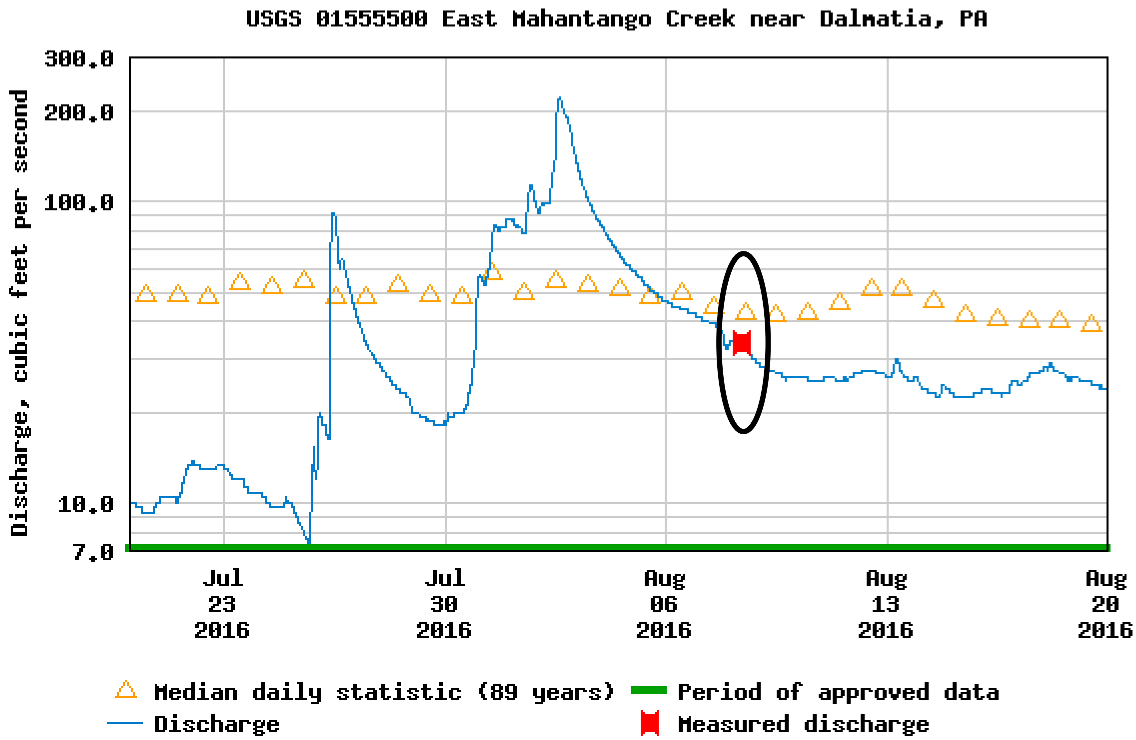
Stream gage within 6 miles of Groff Creek site:

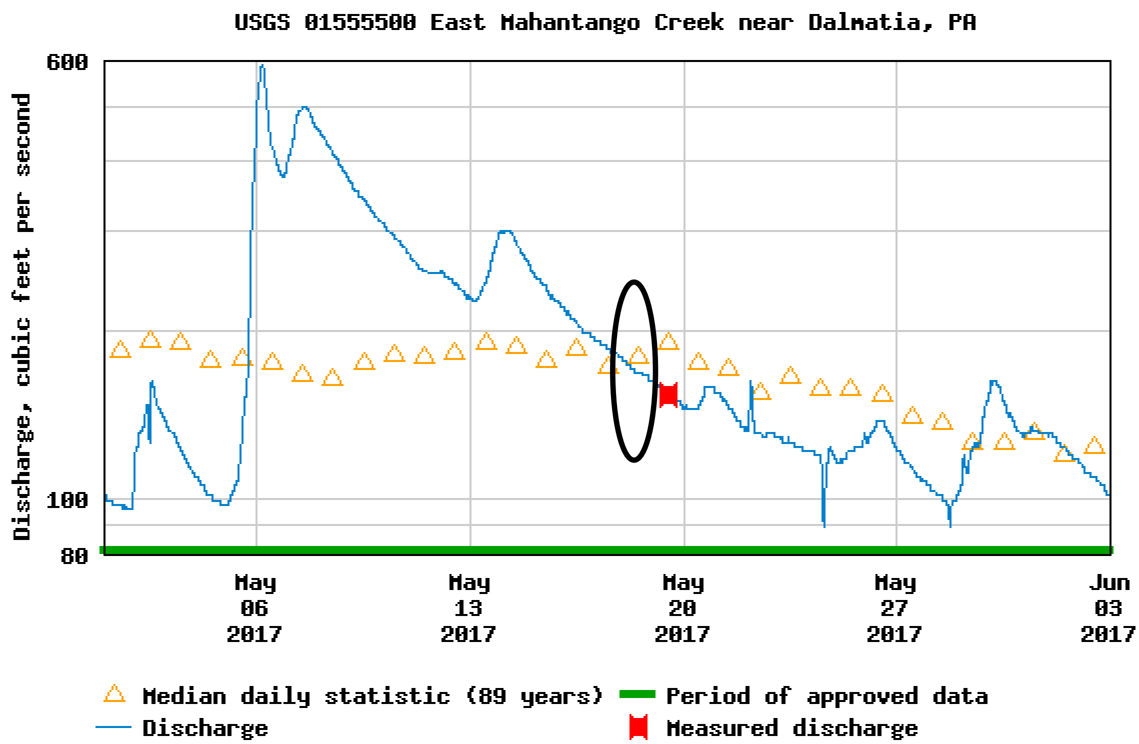

USGS 01576500 Conestoga River at Lancaster, PA
Discharge, cubic feet per second
2000
1000
100
50
Jul 23 2016
Jul 30 2016
Aug 06 2016
Aug 13 2016
Median daily statistic (89 years)
Period of approved data
Discharge
Measured discharge


USGS 01576500 Conestoga River at Lancaster, PA
Discharge, cubic feet per second
2000
1000
200
May 06 2017
May 13 2017
May 20 2017
May 27 2017
Median daily statistic (89 years)
Period of approved data
Discharge
Measured discharge

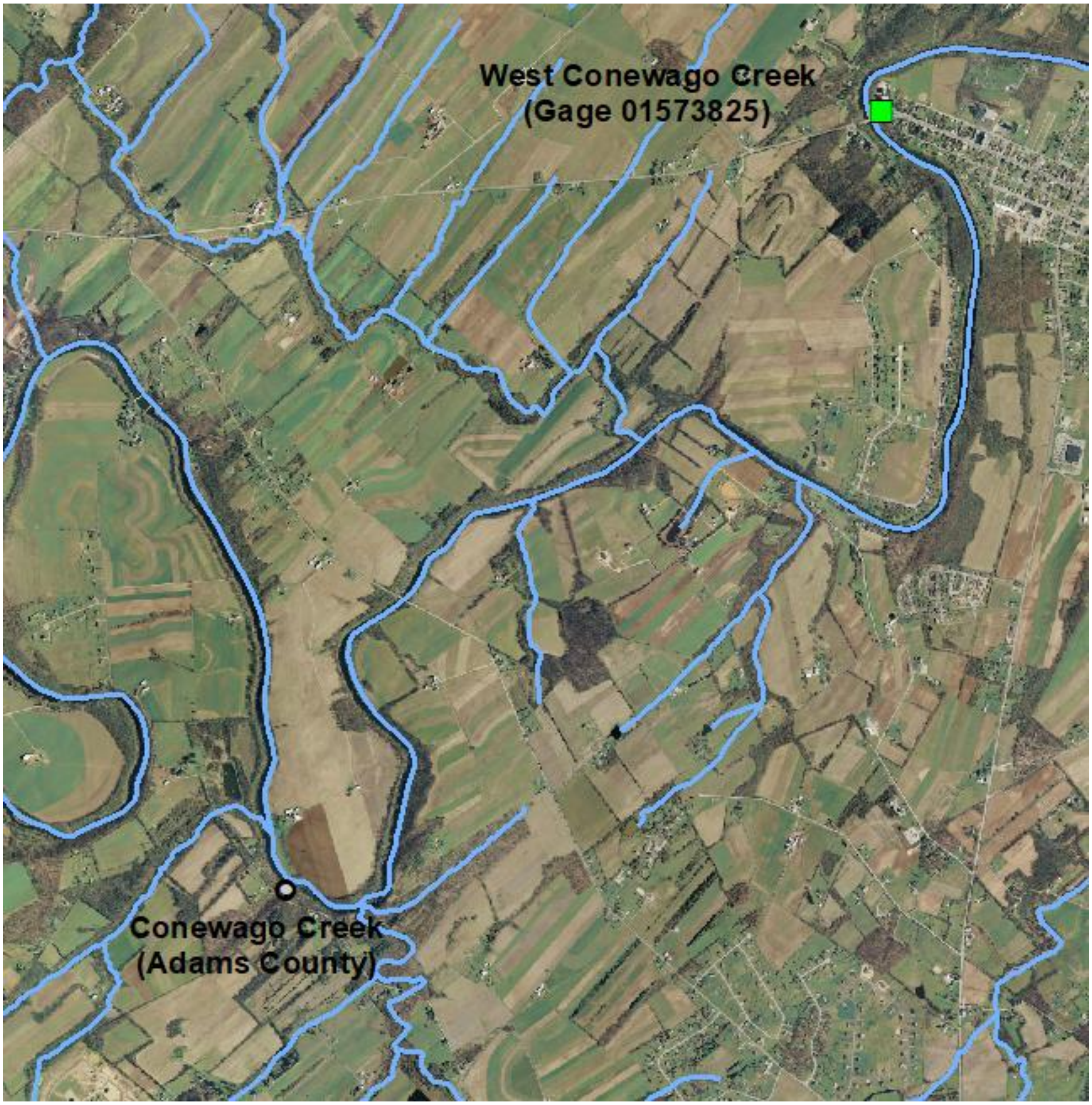
Stream gage within 6 miles of West Branch Mahantango Creek site:

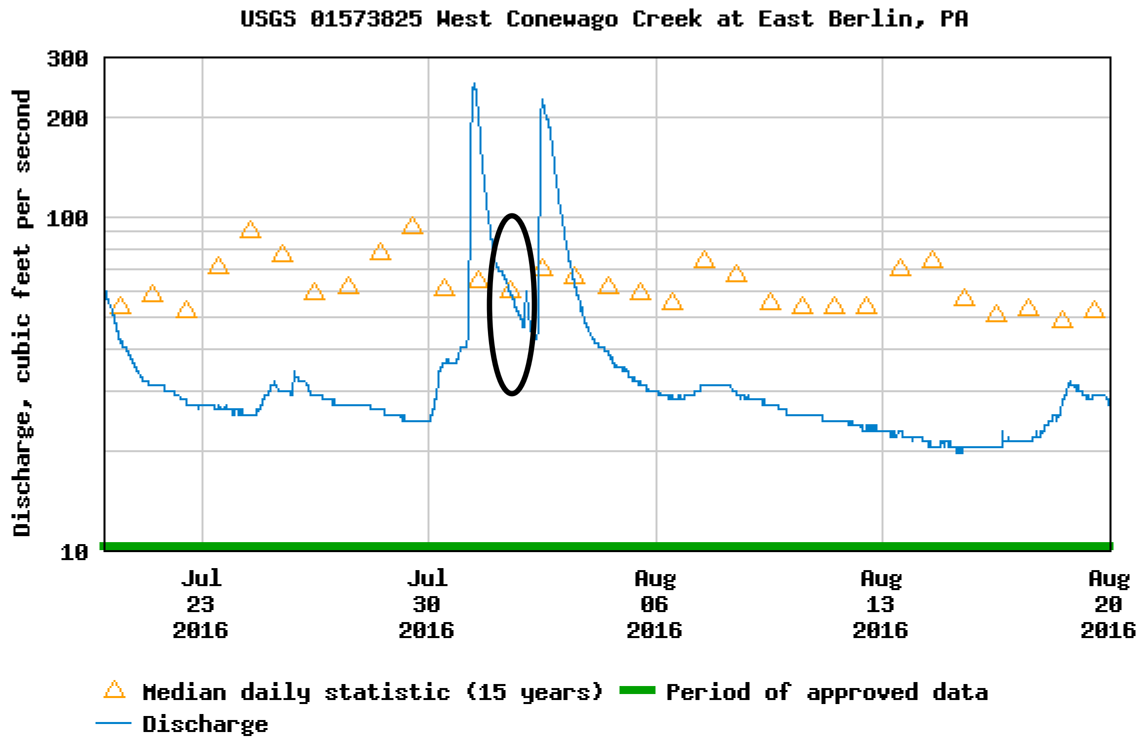


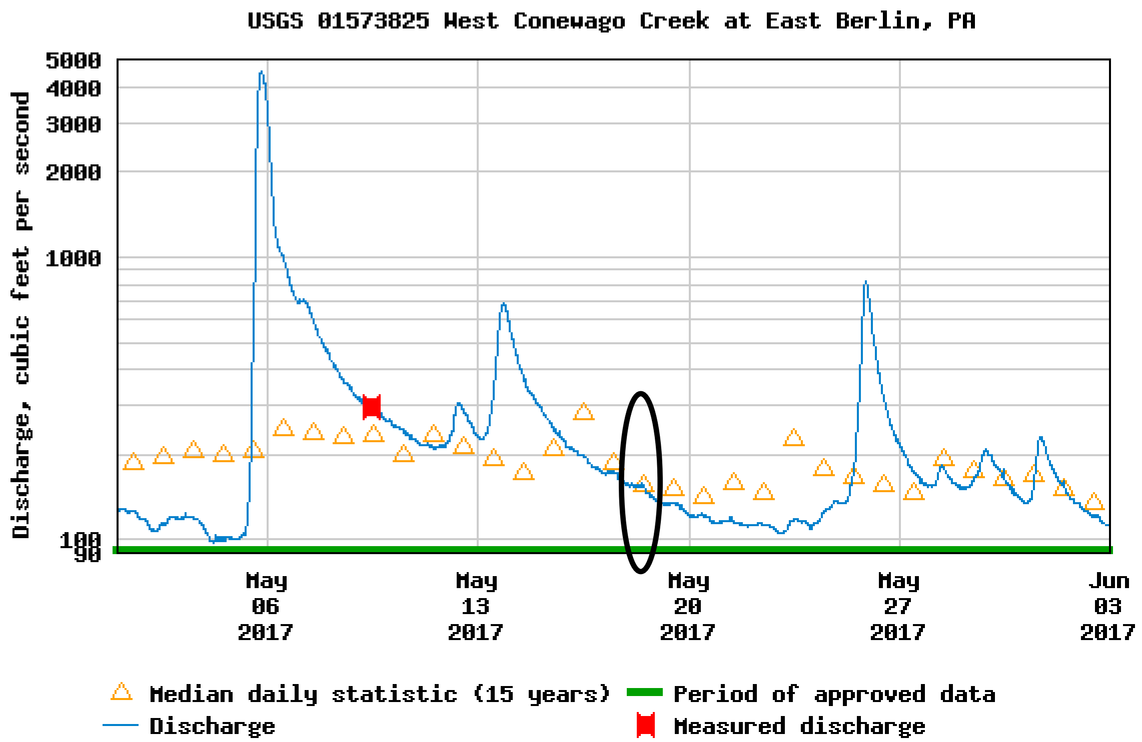

USGS 01555500 East Mahantango Creek near Dalmatia, PA
Discharge, cubic feet per second
300.0
200.0
100.0
10.0
7.0
Jul 23 2016
Jul 30 2016
Aug 06 2016
Aug 13 2016
Aug 20 2016
Median daily statistic (89 years)
Period of approved data
Discharge
Measured discharge


USGS 01555500 East Mahantango Creek near Dalmatia, PA
Discharge, cubic feet per second
600
100
80
May 06 2017
May 13 2017
May 20 2017
May 27 2017
Jun 03 2017
Median daily statistic (89 years)
Period of approved data
Discharge
Measured discharge

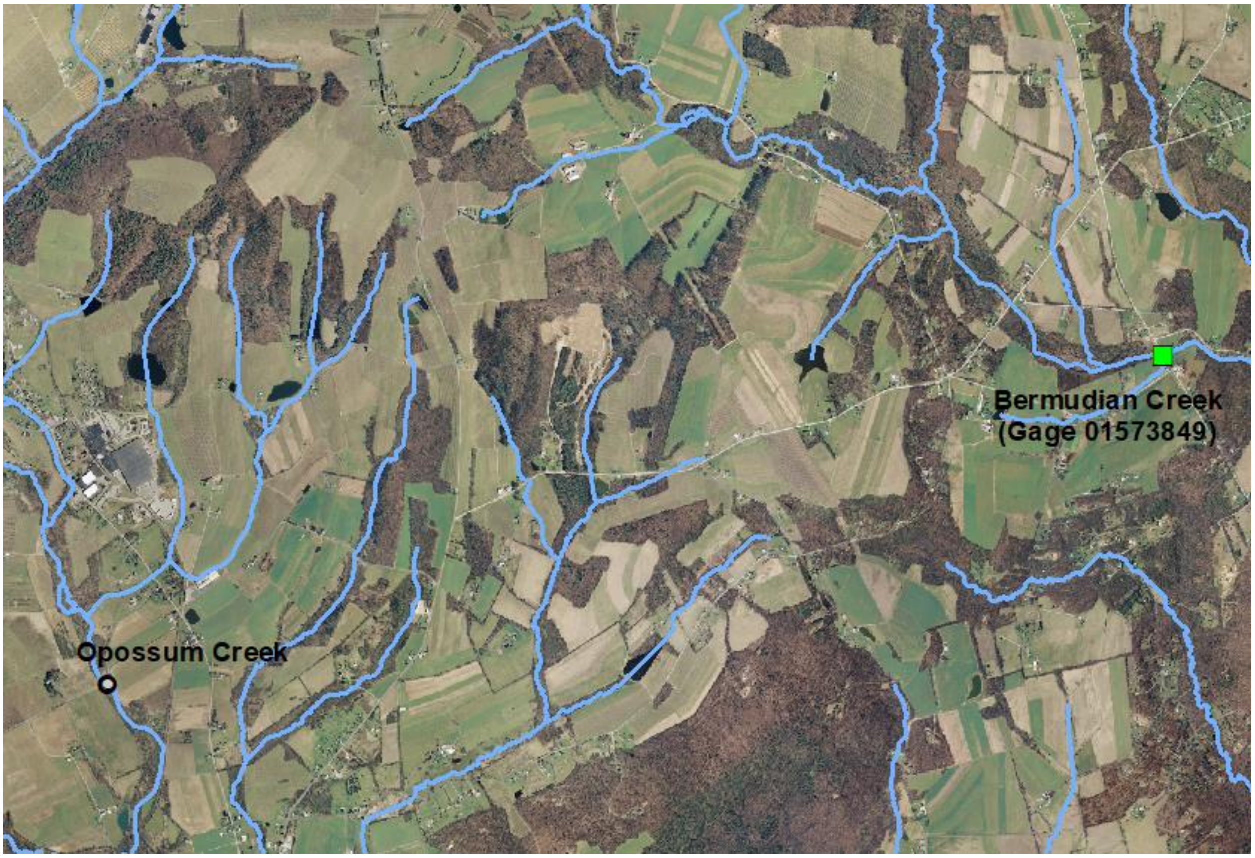
Stream gage within 6 miles of Conewago Creek (Adams County) site:

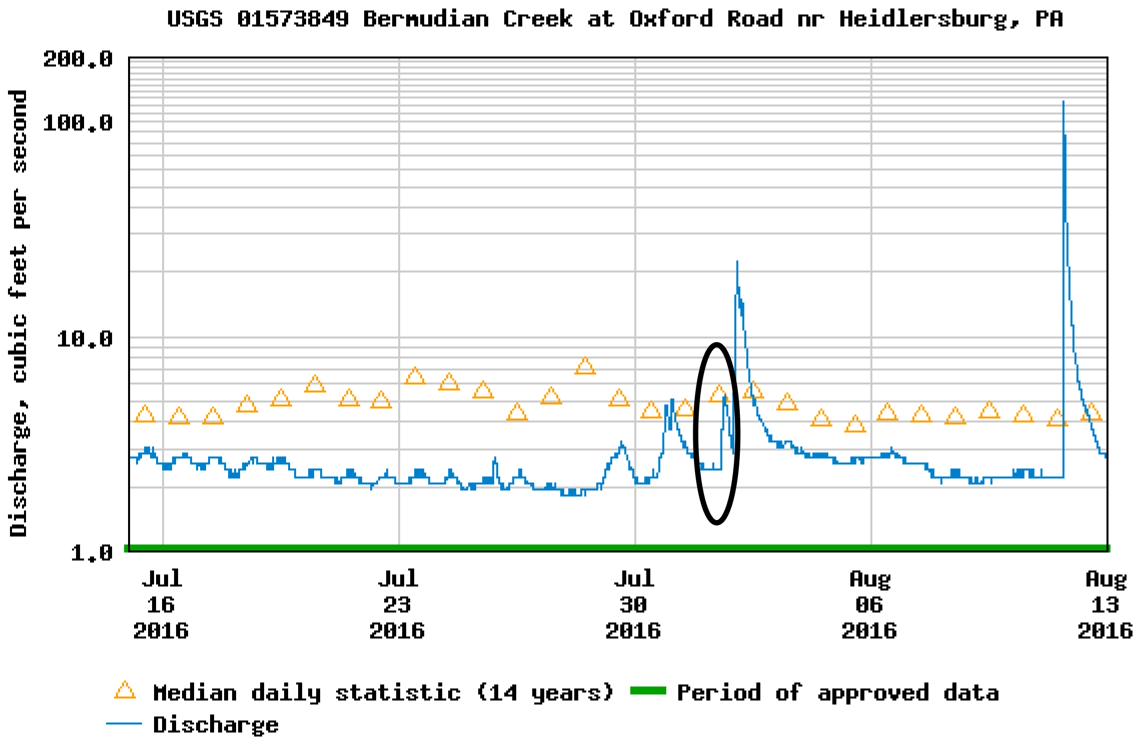


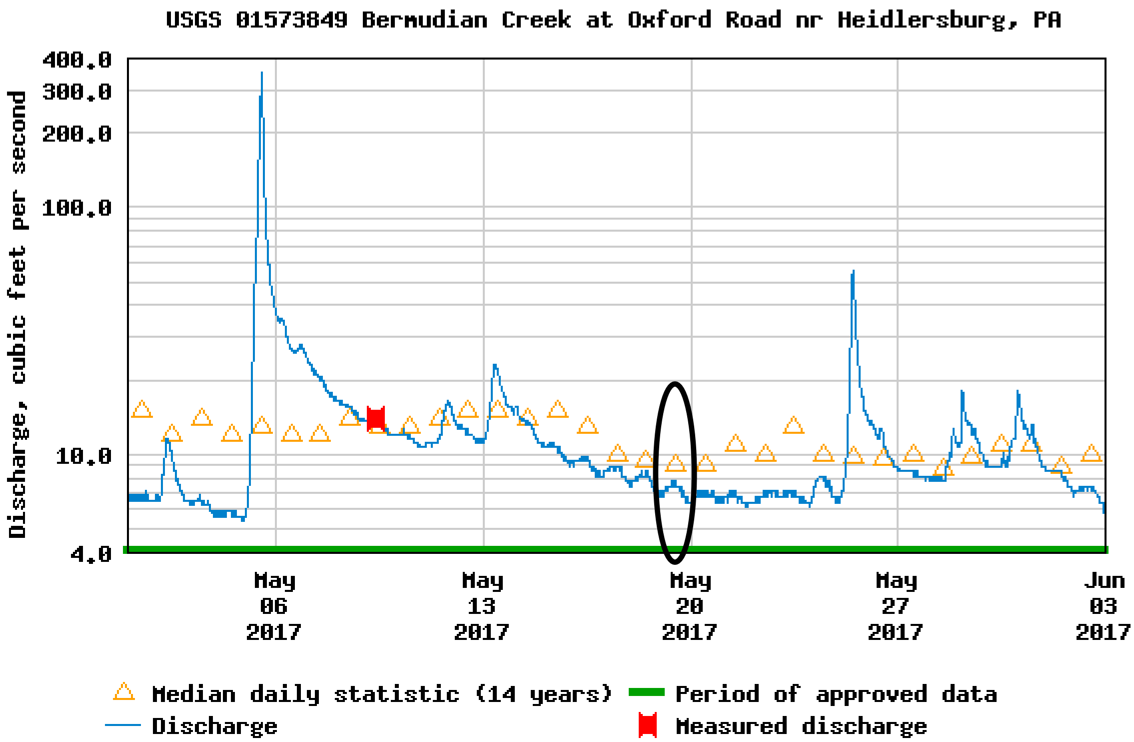

USGS 01573825 West Conewago Creek at East Berlin, PA
Discharge, cubic feet per second
300
200
100
10
Jul 23 2016
Jul 30 2016
Aug 06 2016
Aug 13 2016
Aug 20 2016
Median daily statistic (15 years)
Period of approved data
Discharge


USGS 01573825 West Conewago Creek at East Berlin, PA
Discharge, cubic feet per second
5000
4000
3000
2000
1000
100
90
May 06 2017
May 13 2017
May 20 2017
May 27 2017
Jun 03 2017
Median daily statistic (15 years)
Period of approved data
Discharge
Measured discharge

Stream gage within 6 miles of Opossum Creek site:




USGS 01573849 Bermudian Creek at Oxford Road nr Heidlersburg, PA
Discharge, cubic feet per second
200.0
100.0
10.0
1.0
Jul 16 2016
Jul 23 2016
Jul 30 2016
Aug 06 2016
Aug 13 2016
Median daily statistic (14 years)
Period of approved data
Discharge


USGS 01573849 Bermudian Creek at Oxford Road nr Heidlersburg, PA
Discharge, cubic feet per second
400.0
300.0
200.0
100.0
10.0
4.0
May 06 2017
May 13 2017
May 20 2017
May 27 2017
Jun 03 2017
Median daily statistic (14 years)
Period of approved data
Discharge
Measured discharge