Appendix B: Analysis Worksheets

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Candidate Cause 1: High Flows

Worksheets: 1, 2, 4, and 6

Title: Temporal Co-occurrence of Flow (WS #1)

Agency: Pennsylvania Department of Environmental Protection

Temporal Co-occurrence using Data from the Case

Candidate Cause: Increased frequency of high spring discharge and low summer discharge.

Introduction:

Stressful discharge conditions for smallmouth bass (SMB) young of year (YOY) can be two fold. High discharges during spring can remove fry from their habitat and summer low discharges can cause stress to the point where YOY survival is decreased or YOY are more likely to become prey. Both situations can increase mortality and decrease recruitment to age class 1+. If spring high discharges or summer low discharges were the cause of decreased recruitment to age class 1+ observed post-2002, then we would expect to see higher discharges in spring or lower discharges in summer.

Data:

The Pennsylvania Department of Environmental Protection (PA DEP) obtained hourly discharge data from the Harrisburg USGS gage station for water-years 1990 to 2014.

Analysis and results:

Discharge data were summarized into seasonal daily durations that were ≥50,000 CFS (for spring) and ≤5,000 CFS (for summer) using AQUARIUS software. These thresholds characterized the upper 75th percentile and lower 25th percentile of the entire dataset, respectively. The spring time period was defined as discharge from the beginning of March to the end of June, and the summer time period was defined as discharge from the beginning of July to the end of September. Datasets were then qualified as either pre-2002 or post-2002 for comparison purposes. The resulting seasonal datasets were analyzed using R software.

Daily spring high discharges were normally distributed; therefore, time period differences were evaluated using ANOVA. However, daily summer low discharges did not exhibit acceptable normal distribution; consequently, Kruskal-Wallis test was used. There was no significant difference between pre and post-2002 spring high discharges ($p = 0.98$). Pre and post-2002 spring high discharges were surprisingly similar, averaging 26.5 days per year and 26.6 days per year, respectively (Figure 1). There was also no significant difference between pre and post-2002 summer low discharges ($p = 0.06$). Although summer low discharges were not statically different, it was interesting to see that pre-2002 summer low discharges had longer durations and were more frequent (year by year) than post-2002 (Figure 2).

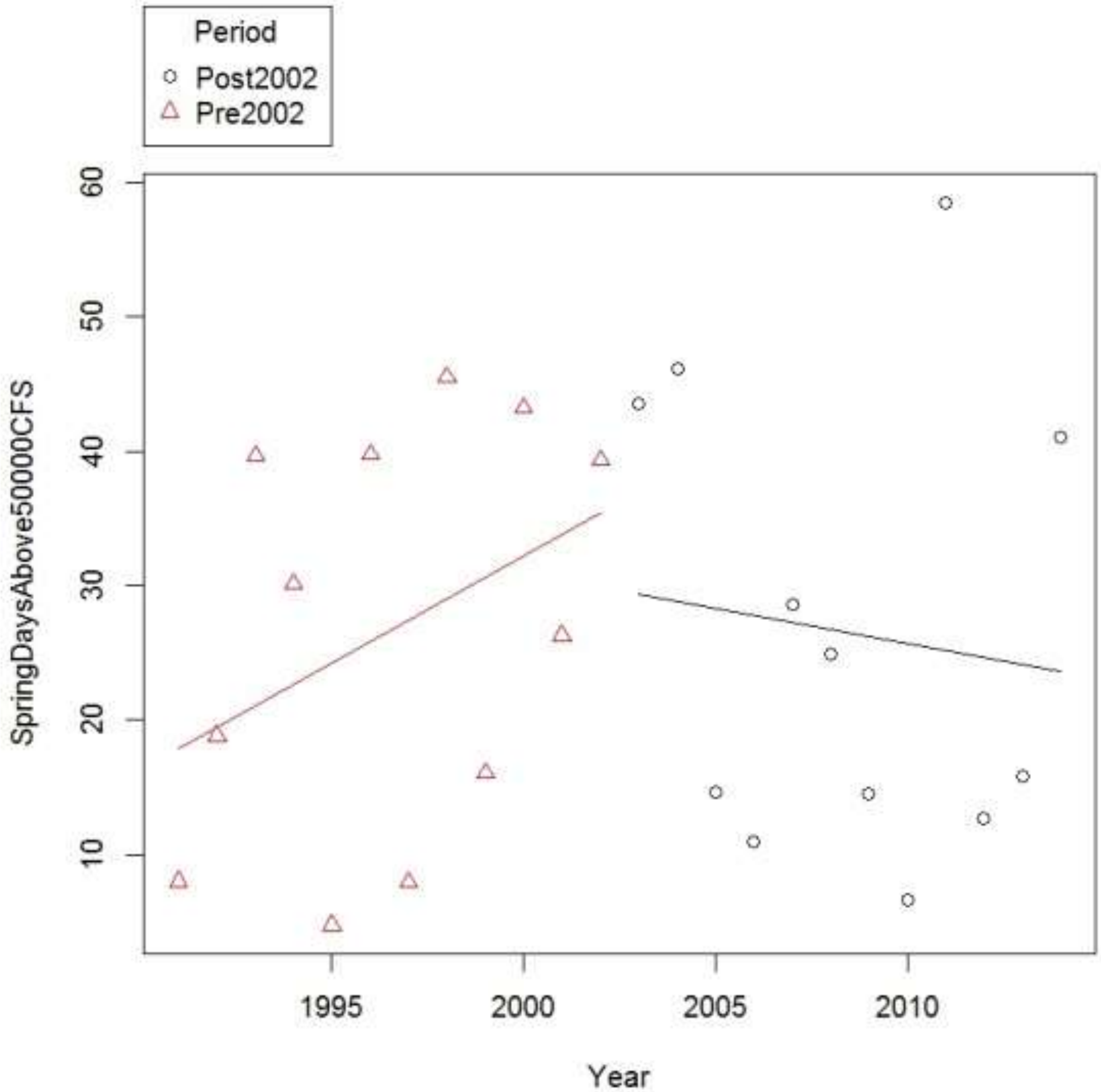


Figure 1. The number of spring days per year where discharge exceeded 50,000 CFS. Trend lines were added for qualitative purposes only.

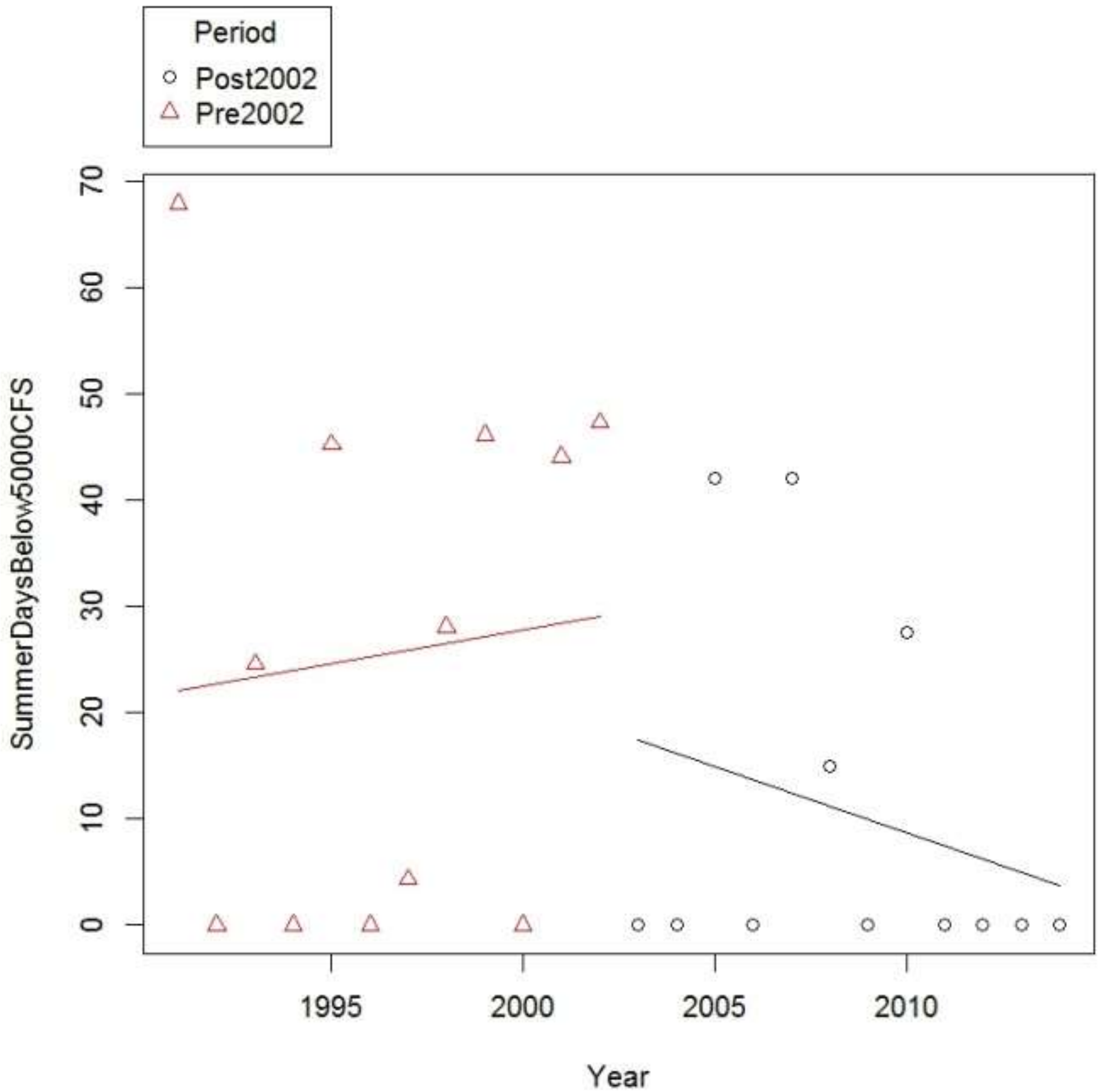


Figure 2. The number of summer days per year where discharge was at or below 5,000 CFS. Trend lines were added for qualitative purposes only.

Conclusion: The evidence weakens the argument for increased frequency of high spring flow and low summer discharge as the cause of the low recruitment of Age1+ SMB.

Title: Smallmouth Bass Abundance and Stream Discharge (Q) (WS #2)

Agency: Environmental Protection Agency

Stressor-Response Relationships

Candidate Cause: Decreased abundance of YOY SMB because of stream discharge

Introduction:

The temporal pattern of streamflow fluctuation appears to be the most important abiotic factor determining nesting success and failure of SMB in perennial streams (Lukas and Orth 1995). Increased stream discharge increases water velocity which can result in mechanical nest failure or dislodge young fry from their habitat, increasing mortality and decreasing survival and subsequent recruitment. Nesting success during a stable low flow year was 73% whereas nesting success was only 35% during a year with heavy precipitation in a Tennessee Stream (Reynolds and O'Bara 1991). Similarly, Buynak and Mitchell (2002) found that year-class strength of SMB in Elkhorn Creek (KY) was inversely related to April-July rainfall totals. In the South Anna River (VA), most nest failures were directly attributable to flows (Lukas and Orth 1995). In three other Virginia rivers, Smith et al. (2005) found that mean June stream discharge most consistently related to age-0 CPUE. Similarly, in Pennsylvania rivers, low river discharge during select spring periods tends to lead to above average YOY catch rates (Lorantas and Kristine 2004). However, below average catch rates of YOY SMB in recent years and reductions in catch rates of all sizes of SMB and SMB ≥ 300mm in the Susquehanna River relative to reference periods have been uncharacteristic of changes evident in other rivers across the state (Lorantas et al. 2012).

The intent of this comparison is to assess the relationship between YOY SMB abundance and stream discharge and determine whether there have been temporal and/or spatial changes in the relationship. Specifically, If stream discharge were influencing abundance, we would expect to see (1) a strong negative relationship between the two variables; (2) the negative relationship would be as strong or stronger at the subject sites and time periods than at the comparison sites and time periods; and (3) recent YOY CPUE (i.e., from 2005 to present) would fall within the prediction limits calculated based on the historical (i.e., comparison time period) relationship between YOY CPUE and stream discharge.

Data:

Back-pack electrofishing catch per unit effort (CPUE; fish/ 50 m) data for YOY SMB from PFBC's state-wide monitoring network was included in the analysis. Site-specific CPUE data were aggregated by river reach and by year.

Mean June stream discharge data (Q) was obtained from U.S. Geological Survey, USGS Surface-Water Monthly Statistics for the Nation application. Values were generated for representative stream gaging locations for each reach of river used in the analysis. These included Susquehanna River at Harrisburg (01570500) and Danville (01540500), Delaware River at Trenton (01463500), Juniata River at Newport (0156700) and Mapleton Depot (01563500), West Branch Susquehanna River at Lewisburg (01553500), and Allegheny River at Franklin (03025500).

Analysis and results:

Spearman Rank correlation were conducted for YOY CPUE and mean June Q (suggest by Smith et al. 2005) values for each of the subject and comparison locations for the 1990-2003 and 2005-2013 time periods to assess the relationship between the two variables for the two time periods. Relationships between YOY CPUE weakened during the 2005-2012 time period compared to the 1990-2004 time period for all subject reaches while the same relationships at comparison site strengthened (Table 1).

The relationship between June Mean flows and YOY CPUE was plotted for the comparison period from 1990 to 2003. The relationship between mean June flow (on the X-Axis) and YOY CPUE (on the Y-Axis) was modelled using linear regression after log- transforming flow. The regression line and prediction limits were back-transformed to produce the plots shown (Figures 1-8). YOY CPUE and flow data from the pre-2005 and post-2005 periods are plotted on the same figures.

Post-2005 data for both case reaches (lower Juniata River and middle Susquehanna River) largely fall near or below the prediction limits based on discharge (Figures 2 and 4) while post-2005 data from comparison reaches (upper Juniata River, upper Susquehanna River, lower Susquehanna River, West Branch Susquehanna River, upper Allegheny River, and lower Delaware River) fall near or within prediction limits based on discharge (Figures 1,3,5-8).

Conclusion:

For the subject sites, the relationship between YOY SMB abundance and flow has weakened for the post-2005 period while this relationship has strengthened in comparison reaches for the post-2005 period. Further, reach-specific linear regression models of YOY SMB catch rates based stream discharge demonstrated that post-2005 catch rates of YOY SMB frequently fell outside of prediction bounds set by the models. Previous analysis by Lorantas et al. (2012) to develop recruitment models to predict future age class abundance also identified discrepancies in predicted results for portions of the Susquehanna Basin.

These analyses suggest the decrease YOY SMB catch rates in subject reaches is not being completely driven by natural factors that typically control YOY SMB mortality. Regression analysis suggests that while high stream discharges in June still negatively affects YOY SMB abundance, other factors are affecting abundance within subject reaches during years with low stream discharge in June.

Literature Cited:

Buynak, G.L., & B. Mitchell. 2002. Response of Smallmouth Bass to regulatory and environmental changes in Elkhorn Creek, Kentucky. North American Journal of Fisheries Management 22:500-508.

Lorantas, R.M., & D.P. Kristine. 2004. Annual monitoring of young-of-year abundanceof Smallmouth Bass in rivers in Pennsylvania; relationships between abundance, river characteristics, and spring fishing. Pennsylvania Fish and Boat Commission, Bureau of Fisheries, Fisheries Management Division, Bellefonte, Pennsylvania.

Lorantas, R., T. Wagner, D. Arnold, J. Detar, M. Kaufmann, K. Kuhn, R. Lorson, R. Wnuk, & A. Woomer. 2012. Smallmouth Bass electrofishing catch rate in large Pennsylvania rivers and examination of Smallmouth Bass recruitment indices. Pennsylvania Fish and Boat Commission, Bureau of Fisheries, Fisheries Management Division, Bellefonte, Pennsylvania.

Lukas, J.A., & D.J. Orth. 1995. Factors affecting nesting success of Smallmouth Bass in a regulated Virginia stream. Transactions of the American Fisheries Society 124:726-735.

Reynolds, C.R., & C.J. O’Bara. 1991. Reproductive ecology and spawning habitat of Smallmouth Bass in two small streams of the Tennessee River system. Pages 61-65 *in* D.C. Jackson, editor. Proceedings of the First International Smallmouth Bass Symposium. Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Starkville, Mississippi.

Table 1: Spearman Rank correlation of reach-wide YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q) at large river reaches in Pennsylvania. Results are broken into pre-2005 and post-2005 time periods. Direction values (weakening (-), substantial weakening (--), and strengthing (+)) comment on the changes in the relationship between the values from the pre-2005 period to the post-2005 period.

Spearman's rank correlation rho (YOY SMB cpue: mean June Q)

Reach	x	y	S	p	rho	status	direction
upper Juniata River	uJpreC	uJpreQ	538	9.61E-05	-0.88112	comparison	
	uJpostC	uJpostQ	285.3653	0.008323	-0.72949	comparison	-
lower Juniata River	lJpreC	lJpreQ	606	0.007998	-0.66484	subject	
	lJpostC	lJpostQ	172.0864	0.45351	-0.04295	subject	--
upper Susquehanna River	uSpreC	uSpreQ	1543.177	0.06872	-0.35366	comparison	
	uSpostC	uSpostQ	230	0.000656	-0.91667	comparison	+
middle Susquehanna River	mSpreC	mSpreQ	864	<2.2e-16	-0.8989	subject	
	mSpostC	mSpostQ	280	0.01557	-0.69697	subject	-
lower Susquehanna River	lSpreC	lSpreQ	1608	0.001851	-0.65944	subject	
	lSpostC	lSpostQ	252.2648	0.058	-0.52888	subject	-
West Branch Susquehanna River	WBpreC	WBpreQ	640.3799	0.001305	-0.75929	comparison	
	WBpostC	WBpostQ	223.4319	0.001403	-0.86193	comparison	+
upper Allegheny River	uApreC	uApreQ	482	0.4219	-0.05934	comparison	
	uApostC	uApostQ	211.5386	0.008407	-0.76282	comparison	+
lower Delaware River	LDpreC	LDpreQ	860	0.0422	-0.53571	comparison	
	LDpostC	LDpostQ	1414	0.05759	-0.71429	comparison	+

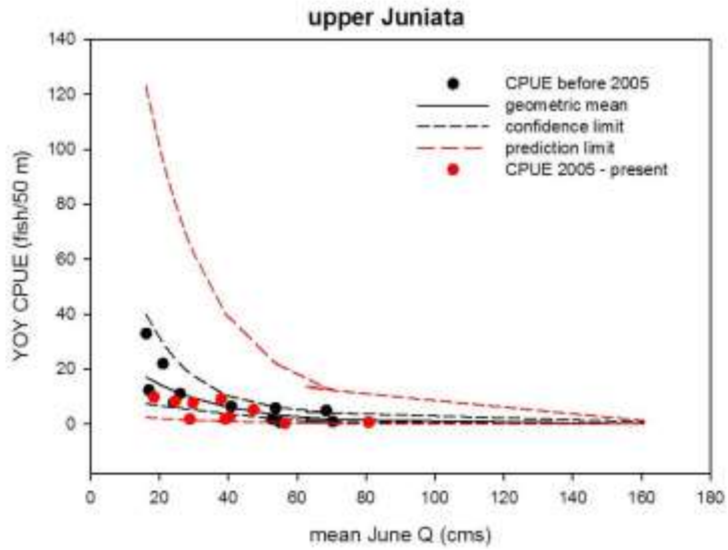


Figure 1. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the upper Juniata River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

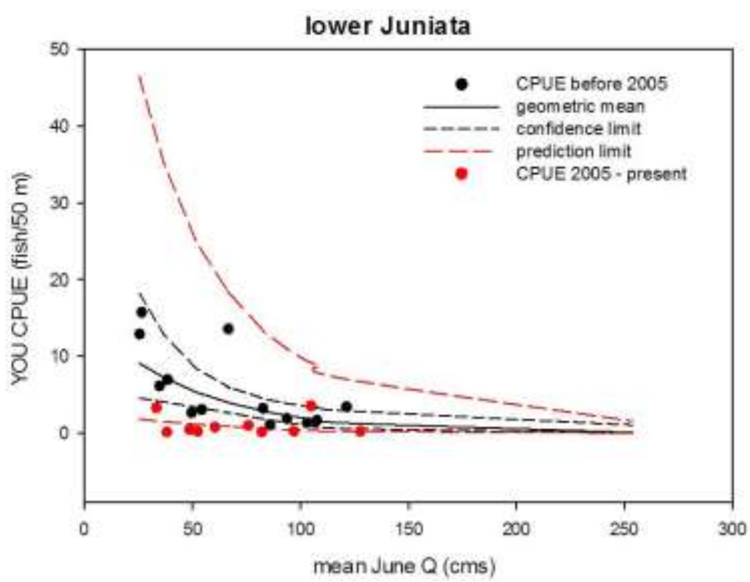


Figure 2. Linear regression model of young-of-year YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the lower Juniata River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

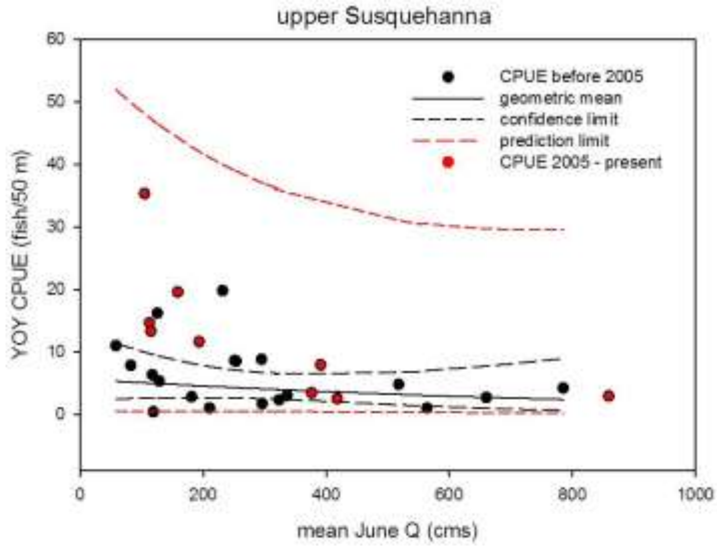


Figure 3. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the upper Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

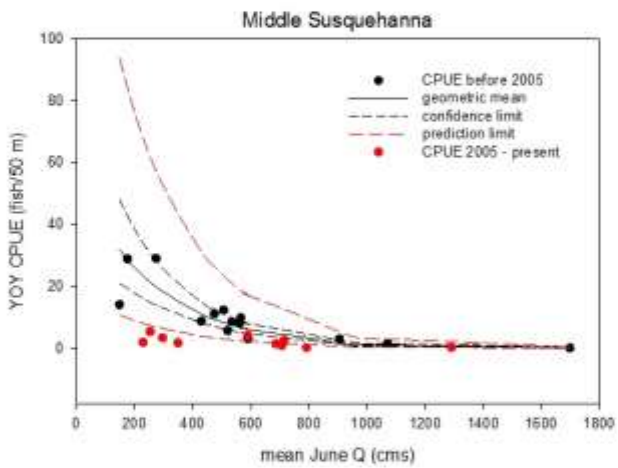


Figure 4. Linear regression model of young-of-year YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the middle Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

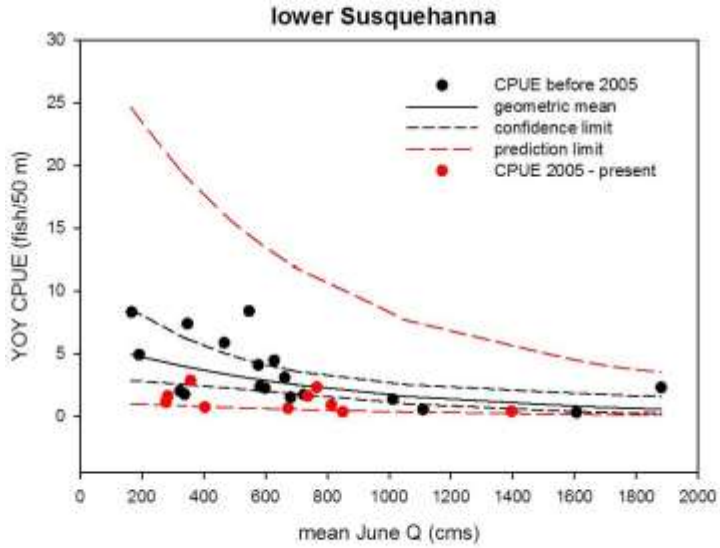


Figure 5. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the lower Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

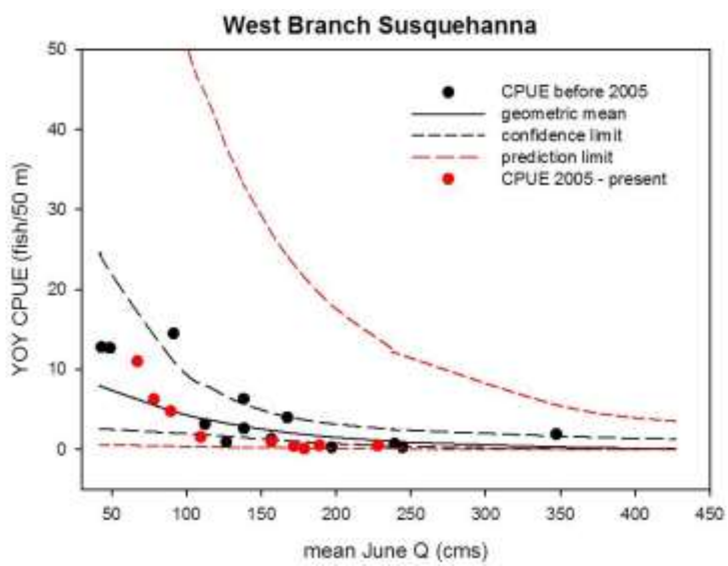


Figure 6. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the West Branch Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

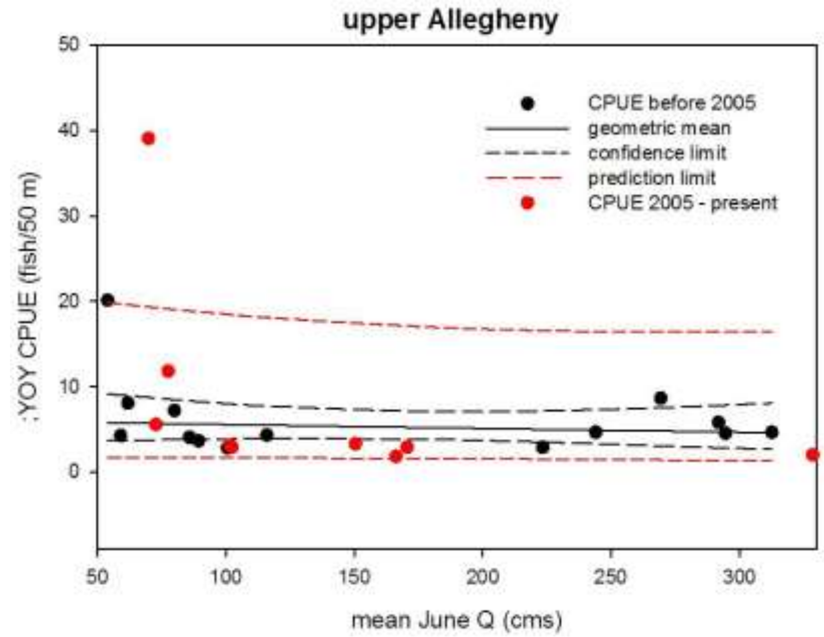


Figure 7. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the upper Allegheny River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

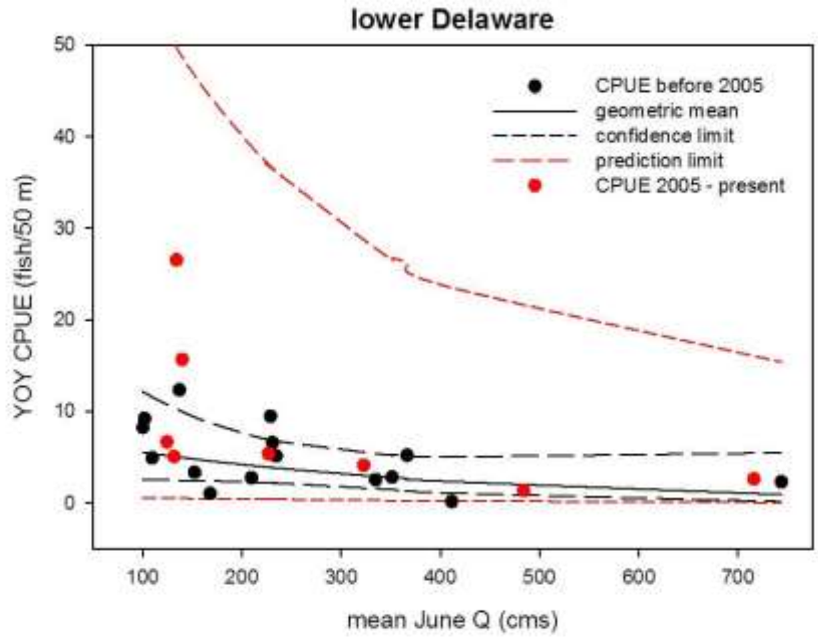


Figure 8. Linear regression model of YOY SMB catch per unit effort (CPUE; fish/ 50 m) and mean June discharge (Q; cubic meters per second) at the lower Delaware River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period.

Title: **Effects of previous year mean June discharge and previous year YOY CPUE on SMB age-1 CPUE (WS #4)**

Agency: Pennsylvania Fish and Boat Commission

Stressor-Response Relationships

Candidate Cause: Decreased abundance of YOY SMB because of stream discharge.

Introduction: Fish recruitment is typically defined as the number of fish that survive to a specific age or size in a given year. Although recruitment can be defined in a number of ways, it is most commonly specified as either the number of fish that reach age 1 each year or the number of fish that survive to the first age they can be captured in the fishery. In most cases fish are considered recruited to the population after they reach a size or age at which very high larval mortality rates have already occurred and the fish can be considered part of the adult population (Allen and Hightower 2010).
This analysis will focus on two associated variables (previous year mean June discharge and previous year YOY CPUE) and their influence on age-1 CPUE during adult SMB surveys. The intent of the analysis is to evaluate whether there have been changes in relationship of river flow the previous year, which strongly drives YOY SMB abundance, and age-1 CPUE. Also, whether there have been changes in the relationship between YOY SMB CPUE and age-1 CPUE. Comparison of these variables with age-1 CPUE would help to account for changes in survival between age-0 (YOY) and age-1 SMB during the pre-2005 and post-2005 period. Varying levels of mortality from age 0 to age 1 would have occurred in these SMB populations historically (e.g., predation, over-winter mortality); however, more recent events may have changed this dynamic. Temporal changes in these relationships would suggest that factors dictating recruitment of SMB from age 0 to age 1 (and the adult population) have changed between the two periods of time.

As with YOY SMB CPUE and mean June discharge, you would expect a negative relationship when comparing age-1 CPUE and mean June discharge the previous year. Several other factors will contribute to the survival of SMB from age 0 to age 1 so the relationship should be weaker than the YOY SMB CPUE and mean June discharge relationship; however, the directionality should be similar. The relationship between age-1 CPUE and the previous year YOY CPUE should be positive (i.e., the higher abundance YOY SMB leads to the higher abundance of age-1 SMB the following year).

Data:

Boat electrofishing catch per unit effort (CPUE; fish/h) data of age-1 SMB from Pennsylvania Fish and Boat Commission (PFBC) state-wide monitoring network was included in the analysis. Age-1 catch was estimated using an age-length key based on a stratified, random sample of aged adult SMB for each site. Cumulative age-1 catch and cumulative effort were calculated for each reach for each year and applied to generate age-1 CPUE values. Backpack electrofishing CPUE (fish/ 50m) data for YOY SMB data from PFBC state-wide monitoring network was also included in the analysis. Site-specific CPUE data were aggregated by river reach and by year.

Mean June stream discharge data (Q) was obtained from U.S. Geological Survey, USGS Surface-Water Monthly Statistics for the Nation application. Values were generated for representative stream gaging locations for each reach of river used in the analysis. These included Susquehanna River at Harrisburg (01570500) and Danville (01540500), Delaware River at Trenton (01463500), Juniata River at Newport (0156700) and Mapleton Depot (01563500), West Branch Susquehanna River at Lewisburg (01553500), and Allegheny River at Franklin (03025500). Discharge values used were for the year the fish would have been spawned (i.e., 2008 CPUE would relate to mean June Q during 2007).

Analysis and results:

Spearman Rank correlation were conducted for age-1 SMB CPUE and mean June Q values for the previous year for each of the case and comparison locations for the 1990-2003 and 2005-2013 time periods to assess relationship between the two variables for the two time periods. Relationships between age-1 CPUE and previous year's mean June discharge substantially weakened during the 2005-2012 time period compared to the 1990-2004 time period for the upper Juniata River (comparison), lower Juniata River (case), and middle Susquehanna River (case); weakened at the upper Susquehanna River (comparison); and substantially strengthened at the upper Allegheny River (comparison) (Table 1).

Spearman Rank correlation were conducted for age-1 SMB CPUE and previous year YOY SMB CPUE for each of the subject and comparison locations for the 1990-2003 and 2005-2013 time periods to assess relationship between the two variables for the two time periods. Relationships between age-1 CPUE and previous year's YOY SMB CPUE substantially weakened during the 2005-2012 time period compared to the 1990-2004 time period for the upper Juniata River (comparison), lower Juniata River (case), and middle Susquehanna River (case); weakened at the upper Susquehanna River (comparison); and substantially strengthened at the upper Allegheny River (comparison) (Table 1).

The relationship between previous year's June Mean flows and age-1 SMB CPUE was plotted for the comparison period from 1990 to 2003. The relationship between mean June flow (on the X-Axis) and YOY CPUE (on the Y-Axis) was modelled using linear regression after log- transforming flow. The regression line and prediction limits were back-transformed to produce the plot shown to the right. YOY CPUE and flow data from the post 2005 period were then plotted on the same figure.

The data for the subject reaches(Figures 2 and 4) plotted largely at or near the lower prediction limit while comparison reaches were more widely distributed within the prediction limits (Figures 1, 3, and 5). The only exception being the West Branch Susquehanna River (Figure 6) where the predicted distribution of the post-2005 results is similar to those of the subject reaches.

Conclusion:

For the case sites, the relationship between age-1 YOY SMB CPUE and previous year mean June flow and previous year YOY SMB CPUE has weakened in the near term for subject sites. For comparison sites, the relationship between YOY abundance and flow has strengthened for the post-2005 period. It is likely that flow is not the major factor in the subject areas. A decrease in abundance is not being completely driven by natural factors controlling YOY mortality, although there may be some component of it that is.

Literature Cited:

Allen, M.S., & J.E. Hightower. 2010. Fish population dynamics: mortality, growth, and recruitment. Pages 43-80 in W.A. Hubert and M.C. Quist, editors . Inland fisheries management in North America, 3rd edition. American Fisheries Society, Bethesda, Maryland.

Table 1: Spearman Rank correlation of reach-wide Age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year's mean June discharge (Q) at large river reaches in Pennsylvania. Results are broken into pre-2005 and post-2005 time periods. Direction values (weakening (-), substantial weakening (--), and substantial strengthening (++)) comment on the changes in the relationship between the values from the pre-2005 period to the post-2005 period.

Reach	x	y	S	p	rho	status	direction
upper Juniata River	uJpreC	uJpreQ	52	0.1778	-0.48571	comparison	
	uJpostC	uJpostQ	78	0.5799	0.071429	comparison	--
lower Juniata River	lJpreC	lJpreQ	90	0.083333	-0.60714	subject	
	lJpostC	lJpostQ	18	0.9977	0.85	subject	--
upper Susquehanna River	uSpreC	uSpreQ	494	0.005	-0.72727	comparison	
	uSpostC	uSpostQ	192	0.0484	-0.6	comparison	-
middle Susquehanna River	mSpreC	mSpreQ	58	0.0875	-0.65714	subject	
	mSpostC	mSpostQ	66	0.7089	0.214286	subject	--
upper Allegheny River	UApreC	UApreQ	84	1	0	comparison	
	UApostC	UApostQ	216	0.01383	-0.8	comparison	++

Table 2: Spearman Rank correlation of reach-wide Age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year's YOY Smallmouth Bass catch per unit effort (CPUE; fish/ 50 m) at large river reaches in Pennsylvania. Results are broken into pre-2005 and post-2005 time periods. Direction values (weakening (-), substantial weakening (--), and strengthening (+)) comment on the changes in the relationship between the values from the pre-2005 period to the post-2005 period.

Reach	x	y	S	p	rho	status	direction
upper Juniata River	uJpreC	uJpreQ	4	0.1333	0.8	comparison	
	uJpostC	uJpostQ	47.7838	0.2862	0.431146	comparison	-
lower Juniata River	lJpreC	lJpreQ	16	0.881	0.714286	subject	
	lJpostC	lJpostQ	90.036	0.8657	-0.07186	subject	--
upper Susquehanna River	uSpreC	uSpreQ	362	0.9928	0.005495	comparison	
	uSpostC	uSpostQ	38	0.171	0.547619	comparison	+
middle Susquehanna River	mSpreC	mSpreQ	16	0.2972	0.542857	subject	
	mSpostC	mSpostQ	98	0.7033	-0.16667	subject	--
lower Susquehanna River	lSpreC	lSpreQ	220	0.4709	0.230769	comparison	
	lSpostC	lSpostQ	40.073	0.7841	-0.14494	comparison	-
West Branch Susquehanna River	WBpreC	WBpreQ	32	0.115	0.619048	comparison	
	WBpostC	WBpostQ	110.9623	0.8473	0.075314	comparison	-
upper Allegheny River	uApreC	uApreQ	41.8733	0.5852	0.252263	comparison	
	uApostC	uApostQ	12	0.002028	0.9	comparison	+

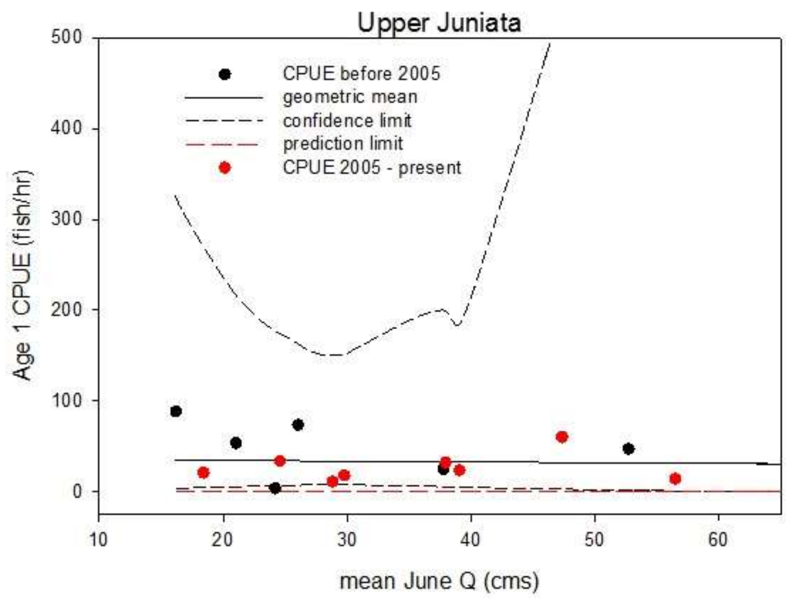


Figure 1: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the upper Juniata River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

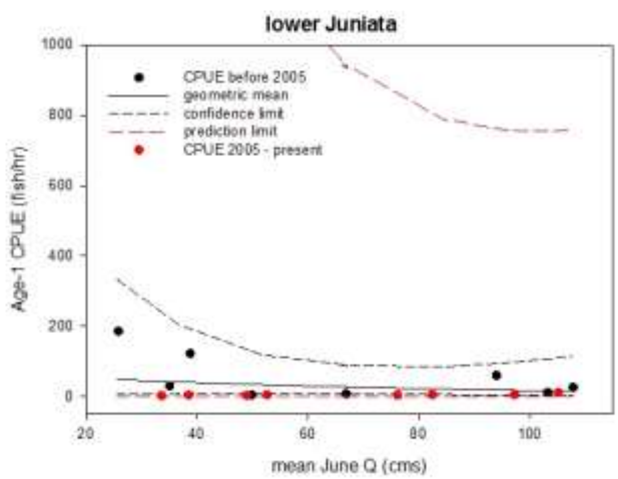


Figure 2: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the lower Juniata River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

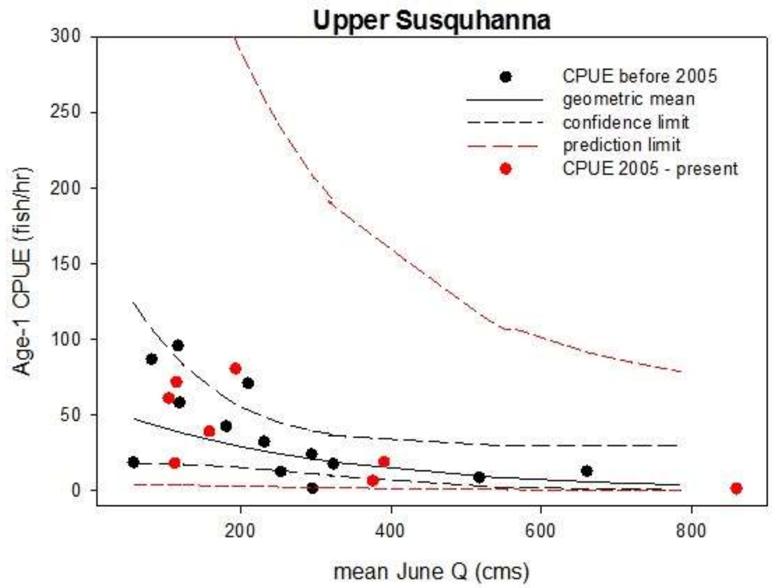


Figure 3: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the upper Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

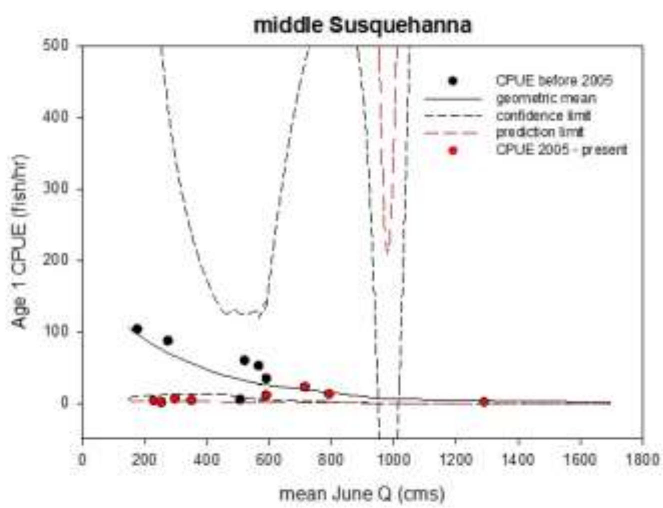


Figure 4: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the middle Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

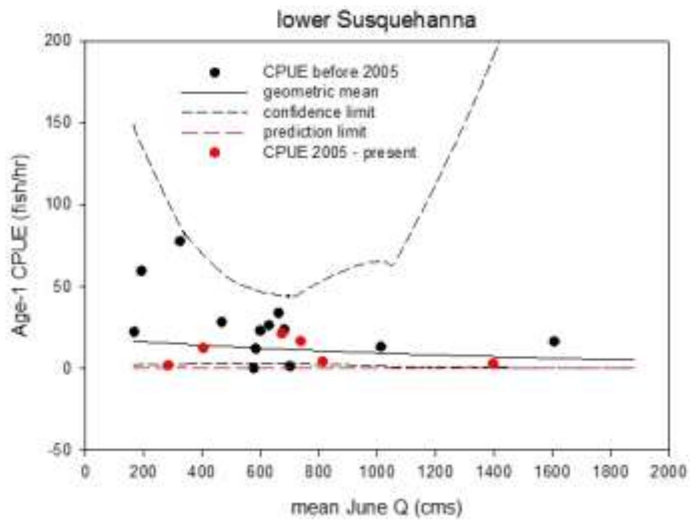


Figure 5: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the lower Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

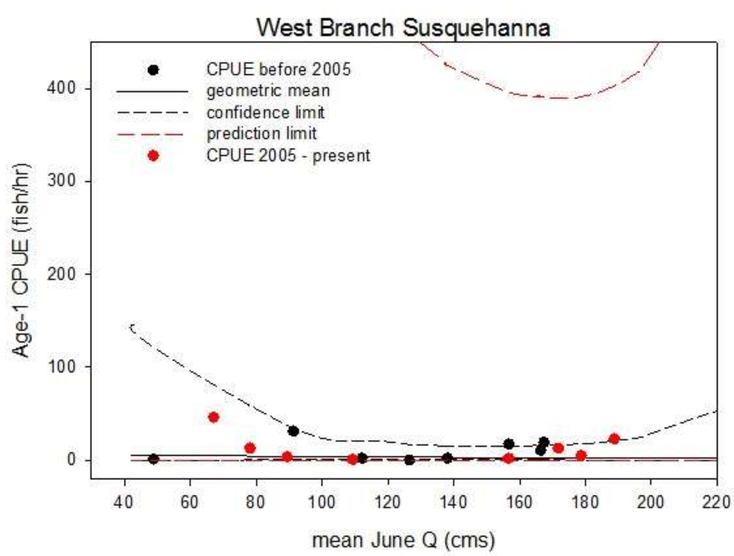


Figure 6: Linear regression model of age-1 SMB catch per unit effort (CPUE; fish/ 50 m) and previous year mean June discharge (Q; cubic meters per second) at the West Branch Susquehanna River for the period 1990 – 2014. Regression lines, confidence limits, prediction limits are from back transformed log discharge values. Black dots indicate YOY CPUE values for pre-2005 period and red dots indicate CPUE values for post-2005 period

Title: Stream Discharge (Q) and Smallmouth Bass Spawning (WS #6)

Agency: Pennsylvania Department of Environmental Protection

Temporal Co-occurrence using Data from the Case

Candidate Cause: Limiting water quality conditions, elevated contaminant loads or other candidate causes that may be impacting YOY SMB vs. temporal development of SMB.

Introduction:

The candidate cause analyses characterized to specifically affect SMB YOY should consider when the data was collected in any given year in order to validate that SMB were obligated to a specific habitat from which the data may have been collected. SMB YOY may only be vulnerable to specific constituents or conditions in near-shore habitats for a short period each spring. And on any given year that period could shift due to warmer/colder than normal temperatures or could be interrupted due to, among other conditions, elevated flow events.

Data:

DEP Water Quality Standards staff performed a literature review of the early life history of SMB in an attempt to create a hypothetical timeline that could promote temporal context of data analyses. This temporal context could be used to determine the potential stage of YOY development when particular conditions or exposure(s) and if YOY were physically obligated to a particular habitat.

In addition continuous instream monitoring data collected by USGS New Cumberland for Juniata River at Newport (main channel) and Howe Twp. (micro habitat) and the Susquehanna River at Clemson Island (main channel and micro habitat) for 2008 & 2009 were plotted to compare to life history information.

Analysis and Results:

When the littoral water temperature approaches 13 C - 15 C, male SMB migrate to the littoral zone of lakes and rivers to build nests. Once nests are built, spawning may occur at temperatures ranging from 12.8 C - 23.5 C but typically occurs at 16 C (Ridgway 1988, Armour 1993, Scott 1993). Sudden drops in temperature below 14 C are lethal to the embryos and may also result in the male abandoning the nest (MacLean et al. 1981, Armour 1983).

Eggs hatch 2-10 days after deposition and the hatchlings or wrigglers are at first clear in color and absorb nutrients from their yolk sacs. They remain on the bottom of the nest and within days develop a black pigmentation. The black-fry are 5.6-5.9 mm in length during this time and remain on the nest for 3-11 days before rising (Beeman 1924; Shuter et al. 1980; Scott and Crossman 1973). Larvae are 8.1-10.1 mm total length at the time of swim-up from a nest (Vogel 1981, Shuter et al. 1980), and grow about 1 mm/d over the following 30 d (Scott and Crossman 1973, Carlander 1977, Becker 1983).

After rising they remain in a dense mass close to the bottom of the nest and within two days they begin to disband. Seven to nine days after rising from the nest, they metamorphose into typical SMB (Ridgway 1988). During this time habitat use is dictated by the movement of the male. The male prevents the young from straying too far and patrols the area for predators (Ridgway 1988, Sabo and Orth 1994). As the YOY grow, they begin feeding on larger invertebrate prey (Sabo and Orth 1994), male defense declines (Ridgway 1988) and they begin to disperse further and further from the nest site during the day. They continue to aggregate into dense schools above the nest at night (Wales 1981).

Once the fry are 20-30 mm in length, the male reduces parental care and eventually abandons the nest (Ridgway and Friesen 1992). Desertion coincides with the dispersal of the fry from the nest site and the infiltration of new microhabitats. Two to four weeks after dispersal the YOY move in small schools to shallower waters than their nest sites but remain within 200 m of the nest. They show a preference for boulder and rock habitats, possibly to avoid predation (Serns 1982, Houpt 1991, Sabo and Orth 1994).

Temperatures during the first summer growing season are positively correlated with year class strength. This relationship exists because energy reserves which the young use to survive their first winter are produced in the summer when food is abundant and the water temperature is most suitable for growth (Horning and Pearson 1973, Clady 1975, Shuter et al. 1980).

Analysis/Continuous Instream Monitoring (CIM) Data:

Continuous temperature, pH and dissolved oxygen data from near-shore/micro-habitats were plotted together on charts to determine the potential sequence of events for smallmouth bass pre- through post-spawn and YOY dispersal.

Results:

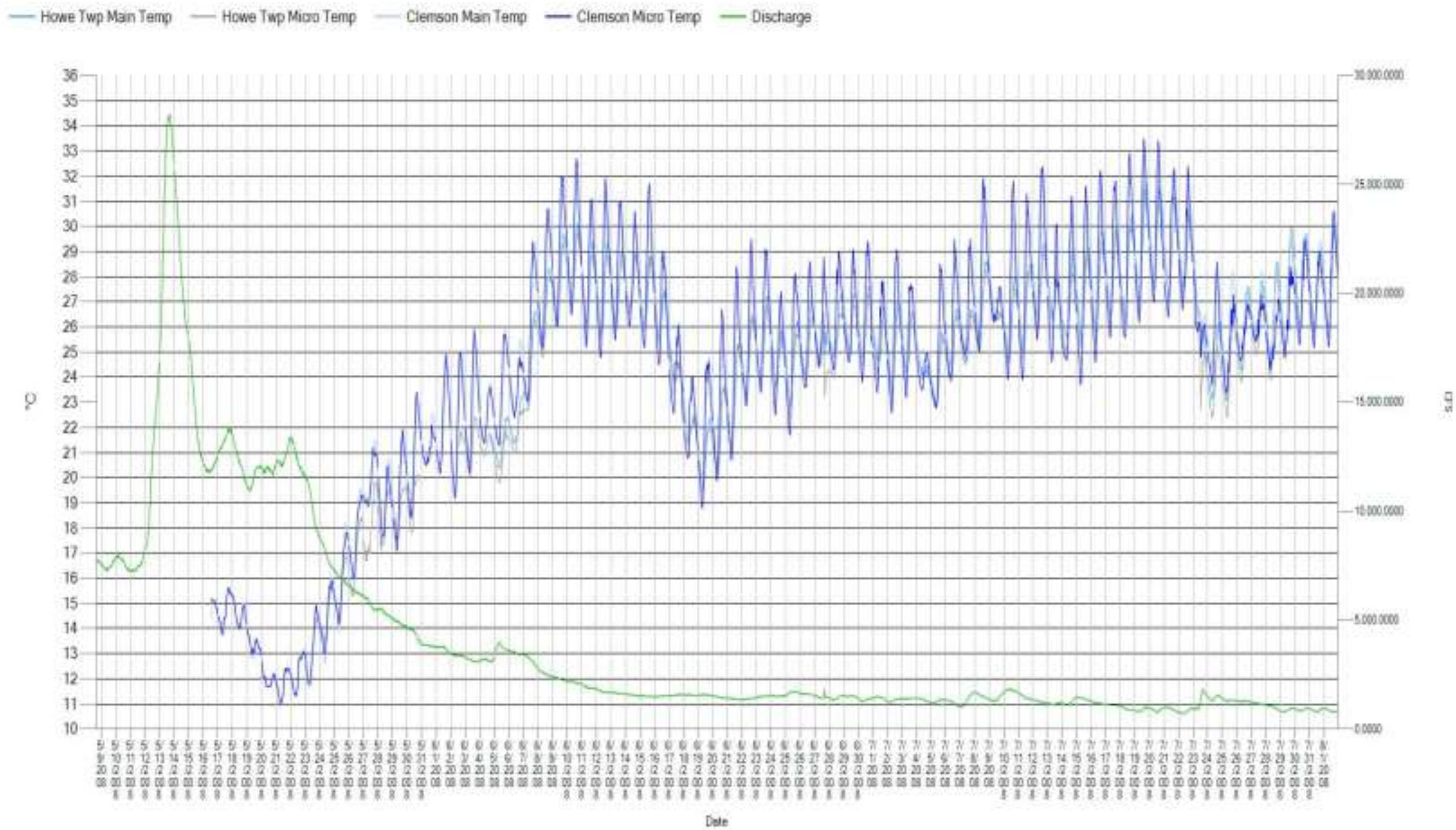


Figure 1. 2008 Water temperature at Howe Twp (main channel and micro habitat) and Clemson (main channel and micro habitat) vs. discharge.

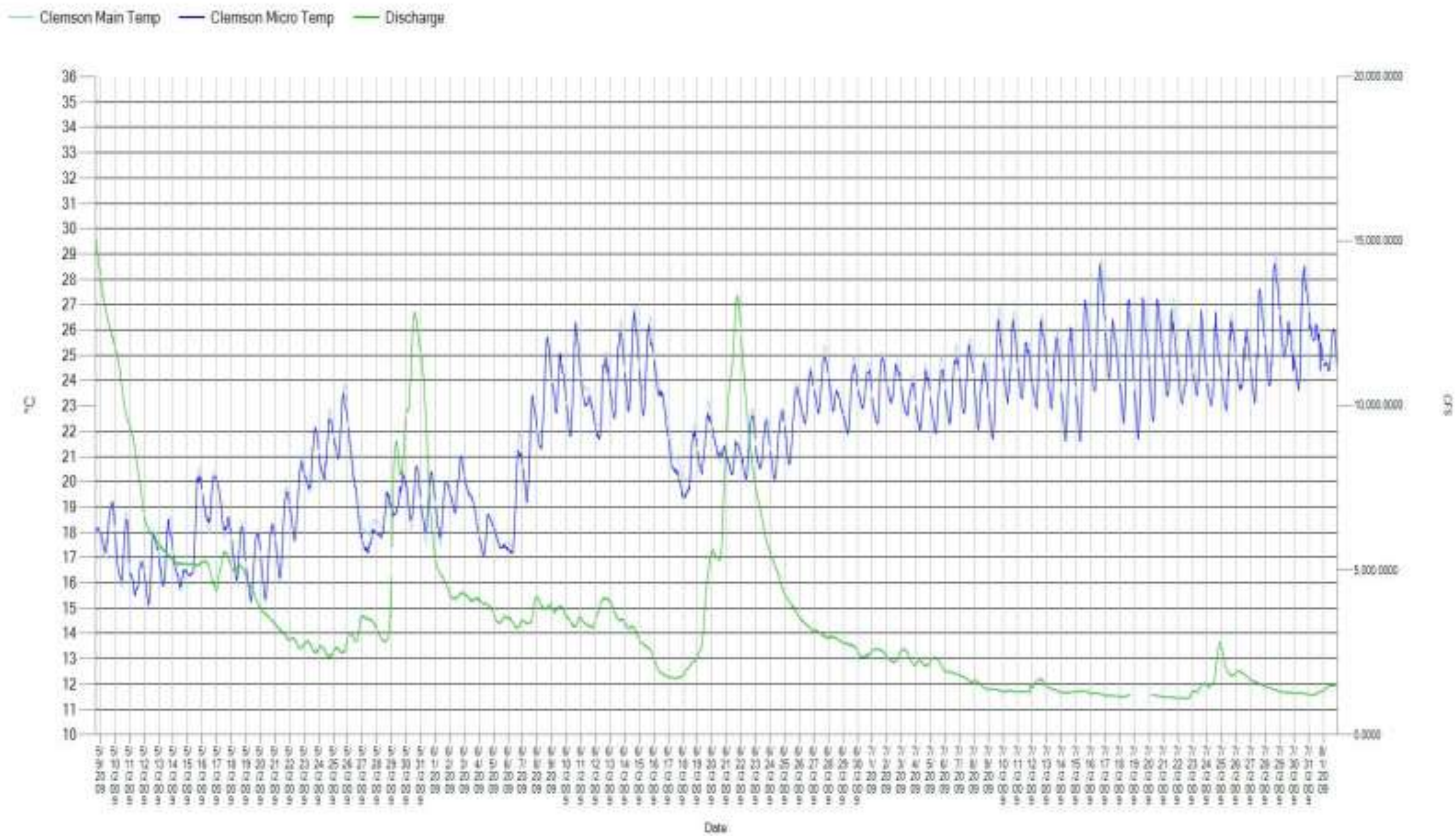


Figure 2. 2009 Water temperature at and Clemson (main channel and micro habitat) vs. discharge.

In 2008 water temperatures at all Howe Twp. and Clemson Island sites exceeded and maintained temperatures above 16˚C on/after May 26th and should have initiated SMB spawning. Discharge after May 26th consistently decreased with little evidence of major elevated discharge events that would have resulted in an unsuccessful spawn (Figure 1). Nests and eggs could have been exposed to sediment contamination or near-shore water column contamination from about May 26th for as long as about 10 days (June 6th). Discharge for at least the next 35 days was stable and YOY SMB may have dispersed from near-shore/micro habitats approximately July 1st – July 11th. A review of when PFBC YOY were collected and YOY length data could be consulted to verify this hypothetical timeline.

In 2009 water temperature at Clemson Island exceeded and maintained temperatures above 16˚C on/after May 19th and should have initiated SMB spawning. Discharge steadily decreased for approximately 9 days, which may have been enough time for eggs to hatch. After 10 days a major flow event occurred, which would have impacted those fish that had successfully spawned. As of June 2nd flows had decreased and a "2nd" spawn could have occurred. After a maximum of 10 days (June 12th), eggs could have hatched. Another major flow event occurred on June 19th, which could have again disrupted a successful spawn for many, but given the 7-day period there may have been just enough opportunity for swim-up to occur (Figure 2). Nests and eggs could have been exposed to sediment contamination or near-shore water column contamination for two periods (May 19th – May 28th and June 2nd – June 12th).

Conclusion:

Discharge and water temperature variability in any given year/season could dictate the magnitude of affect from a candidate cause and could help explain the variability of YOY disease and maybe SMB recruitment at sites from year to year.

Candidate Cause 2: Intraspecific Competition

Worksheet: 3

Title: Stressor-Response Relationships of Smallmouth Bass (WS #3)

Agency: Pennsylvania Fish and Boat Commission

Candidate Cause: Intraspecific competition resulting from density-dependent factors associated with populations at or above carrying capacity negatively impacted the condition of SMB and resulted in increased susceptibility to disease and mortality.

Introduction:

Condition indices offer fishery scientists a tool to evaluate the effects of various management strategies and, indirectly, ecological interactions in fish populations and communities (Murphy and Willis 1991). When combined with other information including density, condition data provide fishery scientists a more complex understanding of population dynamics (recruitment, growth, and mortality) and environmental influences (Pope and Kruse 2007). Wege and Anderson (1978) proposed the use of relative weight (W_r) to evaluate fish condition. W_r is a comparison of the weight of fish in a population to the standard weight (W_s) for that species. Standard weight is a length-derived estimate of weight of a fish based on length-weight regressions from a number of different populations. Kolander et al. (1993) developed the standard weight equation for SMB from 50 populations from 19 states, including populations from the Susquehanna and Juniata rivers. W_r of fish in a population as a whole or in different components of the population (total length groups in this instance) vary with resource availability. When W_r values are well below 100 for an individual or a component of the population, problems may exist in food or feeding conditions; when W_r values are well above 100, fish may not be making the best use of surplus prey (Anderson and Neumann 1996). A population or subpopulation with an average relative weight less than 80 is likely resource limited (Pope et al. 2010).

The intent of this analysis was to evaluate W_r in subject reaches between the pre-2005 and post-2005 periods and among comparison reaches over the same time periods; looking for broad shifts in condition that may indicate resource limitations resulting from, at least in part, intraspecific competition. Specifically, if intraspecific competition were influencing abundance, we would expect to see (1) pre-2005 W_r values at subject reaches near or below 80; (2) a marked increase in condition at subject reaches during the post-2005 period; and (3) W_r values at subject sites would be different than those at comparison sites during the same periods.

Data:

Data used for analysis included individual SMB length and weight data from riverine populations across the Commonwealth collected by Pennsylvania Fish and Boat Commission (PFBC) during boat electrofishing surveys. Data were aggregated by river-reach to increase sample sizes. Only post-1990 data were included as that was the time period when consistent length-weight data was available for case reaches (middle Susquehanna River and lower Juniata River).

Analysis and Results:

Relative weight values were generated using a revision of the standard weight equation for SMB (Kolander et al. 1993). W_r values were generated for all adult SMB with individual length and weight data from each river reach. Data were grouped into 25 mm total length (TL) bins by year to assess whether changes were specific to different sizes of fish within the population. Only fish greater than 150 mm TL were included in the analysis as suggested by Kolander et al. (1993). Mean W_r per TL group per year (error bars indicate ± standard deviation) was calculated and plotted graphically to assess whether temporal shifts during the study period occurred.

Results varied by TL bin and by year for each of the river reaches included in the analysis. Temporal comparisons of W_r at the middle Susquehanna River (Figure 1) and lower Juniata River (Figure 2) demonstrate that W_r during the study period was at or near the 100 “benchmark” for nearly all TL bins, indicating good fish condition, overall. W_r values at the middle Susquehanna River were similar to those at the Allegheny River (Figure 3) and the upper Susquehanna River (Figure 4) during the study period. W_r values at the Delaware River (Figure 5) were generally lower than those of the middle Susquehanna River during the study period. W_r values at the West Branch Susquehanna River were generally lower, especially for larger TL groups, than at the middle Susquehanna River for most of the study period (Figure 6). Lower Juniata River W_r values where similar to those at the upper Juniata River (Figure 7) during the study period.

Conclusion:

The relative weight (W_r) data from the middle Susquehanna River and lower Juniata River suggest that fish condition during the pre-2005 period was not especially low as a result of intraspecific competition. If the abundance of adult SMB during the pre-2005 period had exceeded the carry capacity, it would have resulted in reduced condition at the subject reaches in temporal comparisons (pre-2005 and post-2005). The mean W_r data for the subject reaches is similar between periods despite decreases in abundance of Smallmouth Bass in the post-2005 period. Additionally, mean W_r at subject reaches was similar to comparison reaches through the 1990-2013 time period. This suggests that conditions within subject reaches were not appreciably different than those encountered in comparison reaches during the same time frame.

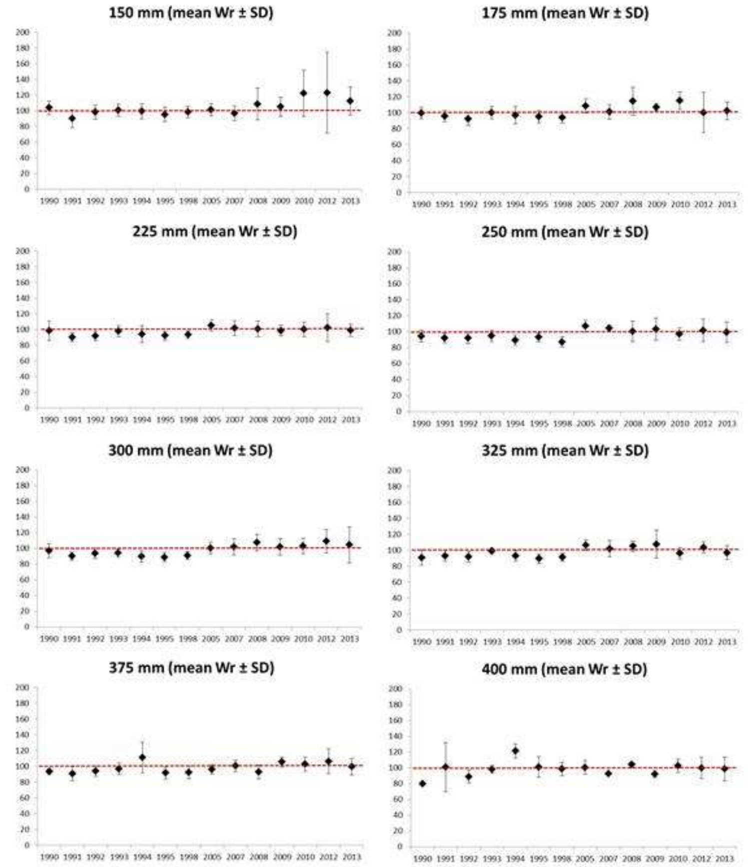


Figure 1: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the middle Susquehanna River (case) from 1990 to 2013 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.

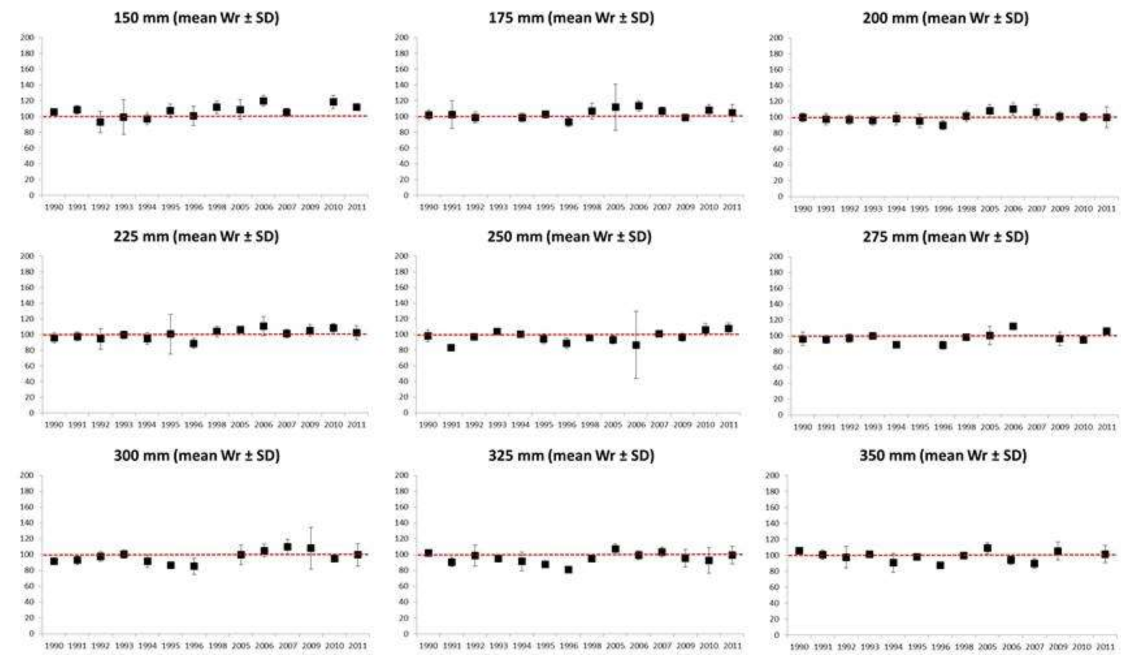
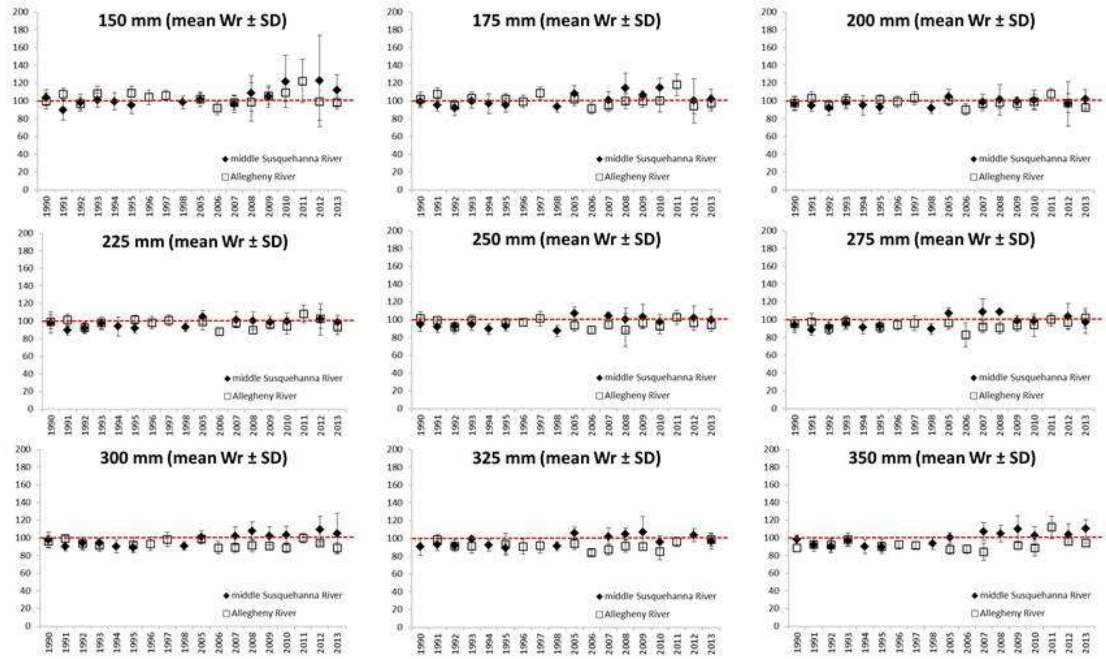


Figure 2: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the lower Juniata River (case) from 1990 to 2011 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.



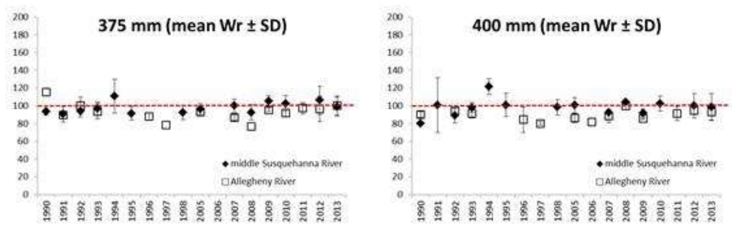
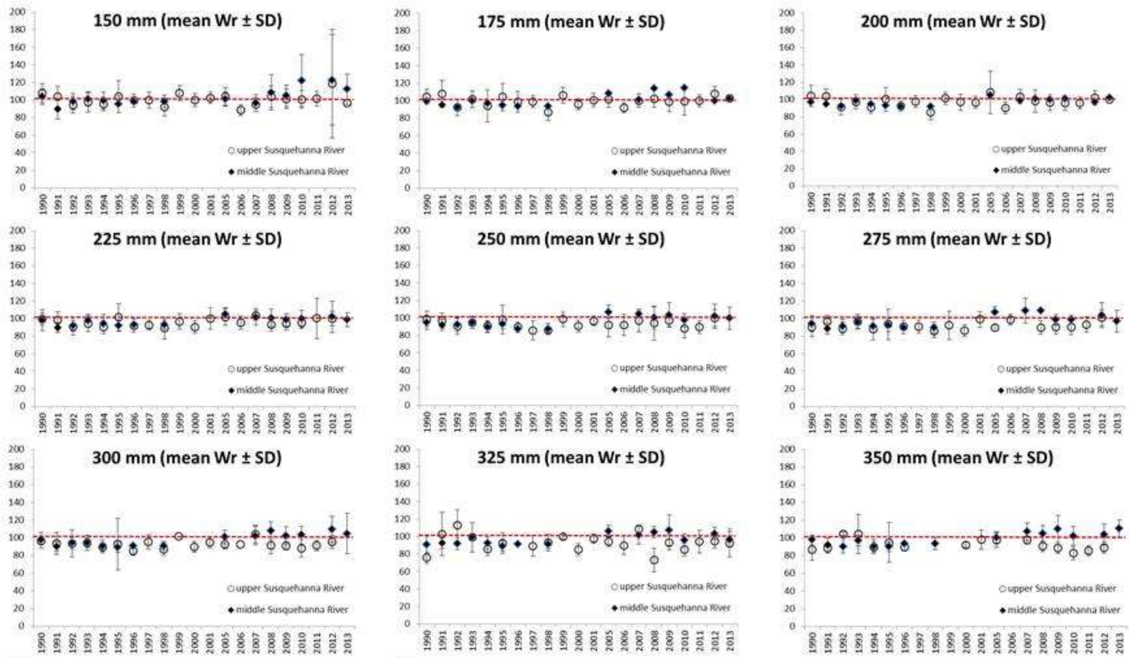


Figure 3: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the middle Susquehanna River (case; black diamonds) and Allegheny River (comparison; open squares) from 1990 to 2013 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.



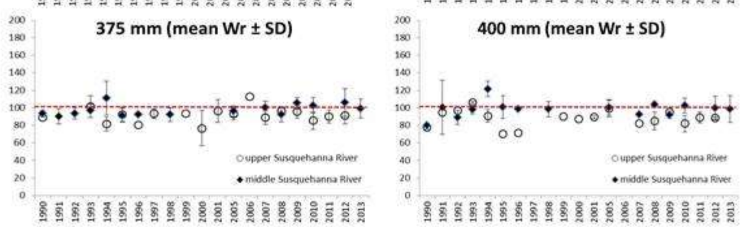


Figure 4: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the middle Susquehanna River (case; black diamonds) and upper Susquehanna River (comparison; open circles) from 1990 to 2013 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.

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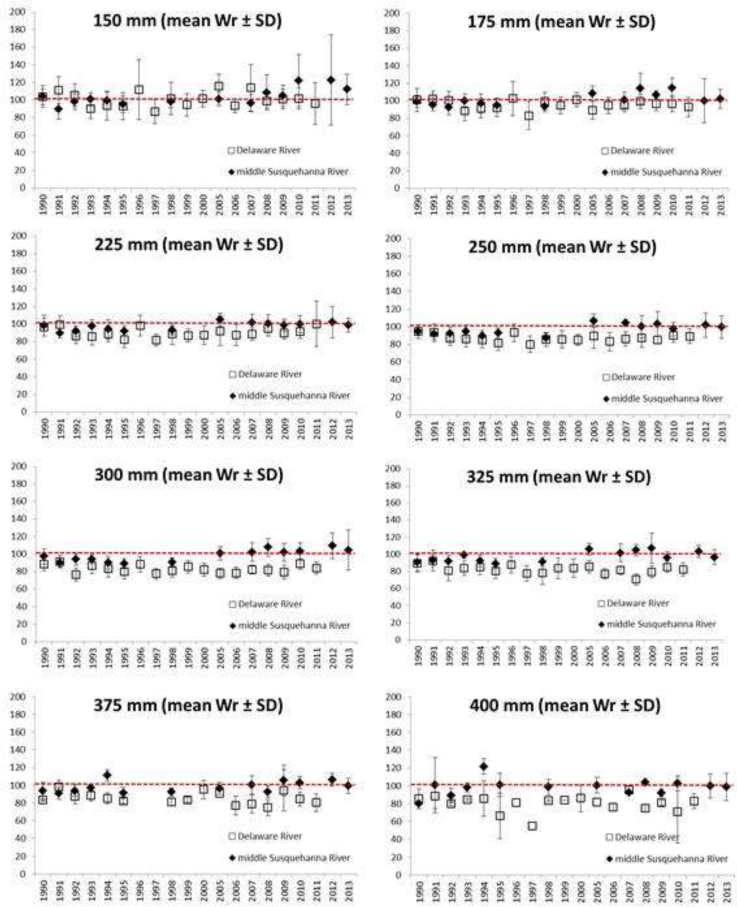
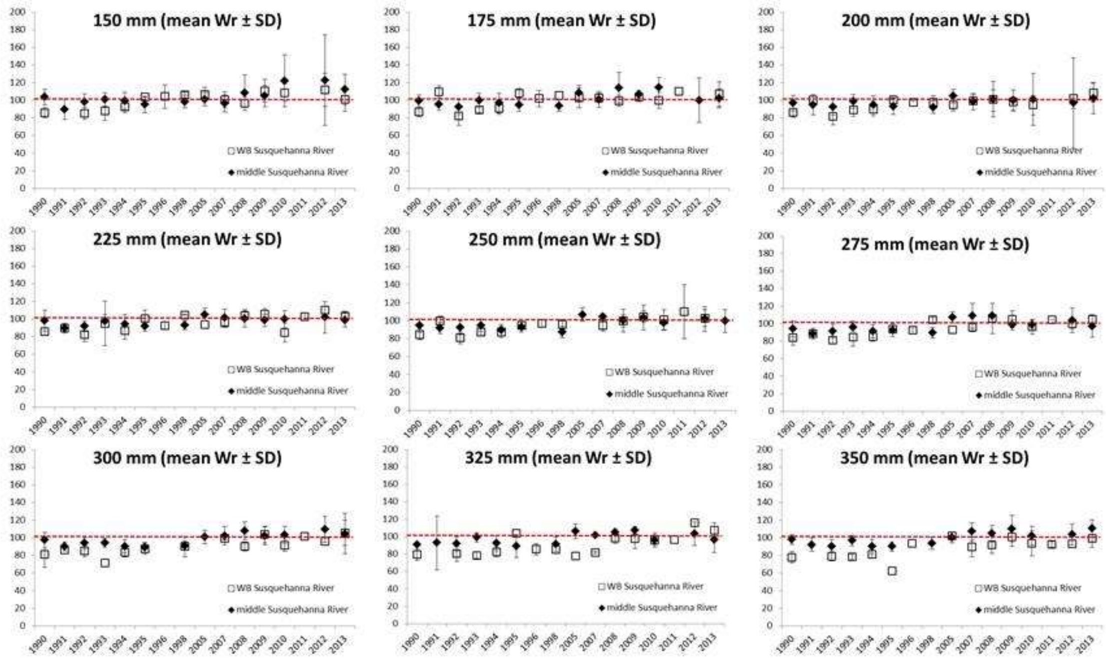


Figure 5: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the middle Susquehanna River (case; black diamonds) and Delaware River (comparison; open squares) from 1990 to 2013 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.



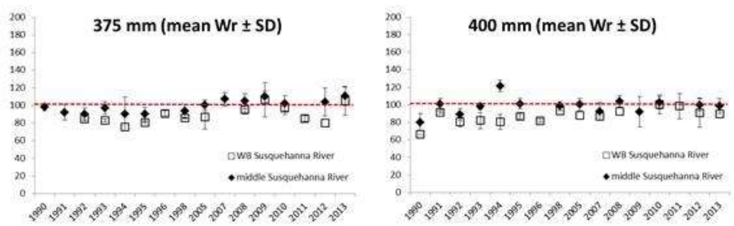


Figure 6: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the middle Susquehanna River (case; black diamonds) and West Branch Susquehanna River (comparison; open squares) from 1990 to 2013 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.

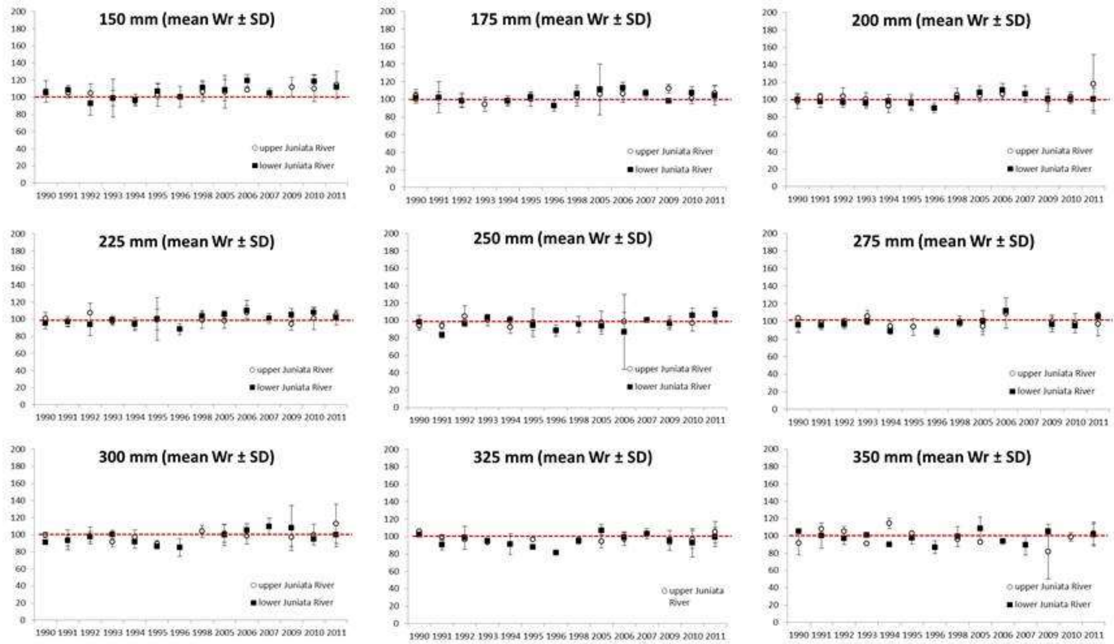


Figure 7: Mean relative weight (Wr) of Smallmouth Bass of different total length groups at the lower Juniata River (case; black squares) and upper Juniata River (comparison; open circles) from 1990 to 2011 (X-Axis). Red dotted line indicates 100% threshold value. No data available for period 1999 – 2004.

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Murphy, B.R., & D.W. Willis. 1991. Application of relative weight (W_r) to western warmwater fisheries. Pages 243-248 *in* J.L. Cooper and R.H. Hamre, editors. Warmwater fisheries symposium I. U.S. Forest Service General Technical Report RM-207, Fort Collins, Colorado.

Wege, G.J., & R.O. Anderson. 1978. Relative weight (W_r): a new index of condition for Largemouth Bass. Pages 79-91 *in* G. Novinger and J. Dillard, editors. New approaches to the management of small impoundments. American Fisheries Society, North Central Division, Special Publication 5, Bethesda, Maryland.

Candidate Cause 3: Interspecific Competition

Worksheet: 5

Title: Analysis of Fish Communities in the Susquehanna River Basin (WS #5)

Agency: Pennsylvania Department of Environmental Protection

Candidate cause: Increased non-native species decrease Smallmouth Bass recruitment, Age 1+

Introduction:

The structure and function of a fish community is regulated by multiple biotic and abiotic factors. The most influential of the biotic factors are typically thought to be predation and competition (Sih et al. 1985, Persson, 1988). Interspecific competition, hereafter referred to simply as competition, occurs when individuals from different species compete over the same resources (Larkin 1956, MacArthur and Levins 1967, Abrams 1983).

The introduction and spread of aquatic invasive species can have multiple effects on ecosystems and can lead to habitat degradation, loss of native flora and fauna (D'Antonio 2001, King 1984, Arim et al. 2006) and disease vectoring and proliferation (Andow et al.1990). Invasive species have been identified as the second leading cause, next to habitat degradation, of imperiled fishes in the United States (Wilcove et al.1998) and this conclusion is similar worldwide (Clavero and Garcia-Berthou 2005).

The rapid density increases brought about by the exponential growth rates of an invasive species (Arim et al. 2006) provide the pathway for increased competition with native fauna. This competition with invasive species has been identified as a major source, albeit not the only pathway, for native species declines and ecological disturbance (Gurevitch and Padilla 2004, Winfield 2012). Competition between SMB and the invasive Mimic Shiner, *Notropis volucellus* (MS) within the Susquehanna River drainage, could result in reduced recruitment of YOY SMB into the adult population. SMB are most likely to have increased competition with MS in their early ontogeny due to both habitat and diet overlap with MS. During this time, the diets of the two species are similar progressing from phytoplankton and zooplankton to invertebrates before SMB begin the ontogenetic shift to piscivory and a predator-prey relationship develops. This critical growth, survival and ontogenetic shift to piscivory period has been identified as a potential source of complex population dynamics for bass (Turchin 2003, Aday et al. 2009).

Data:

The Pennsylvania Department of Environmental Protection (DEP), collected fishes throughout the Susquehanna River basin, and neighboring basins, in 2013 and 2014. Surveys were conducted using a semi-quantitative sampling protocol for both wadeable (stream) and non-wadeable (river) methodologies, as is common in many community-level assessments. Sites were chosen based on proximity to the Pennsylvania Fish and Boat Commission's (PFBC) historic SMB sampling areas, and spread throughout the basin to provide coverage. These data were augmented, as appropriate, with data sets collected during the development of the semi-quantitative protocol in 2008 and 2009. Additional community data was provided by the Susquehanna River Basin Commission (SRBC). All community data were then compiled and analyzed to identify potential biotic relationships between species and trophic guilds.

Analysis and Results:

Fish community data were organized based on species occurrence, species contribution to a feeding guild, and native/non-native status. Regression analysis was performed on these data to identify potential relationships between and among categories. Results indicate a strong relationship between the invertivore/piscivore guild and the insectivore guild (figure 1), demonstrating a predator-prey relationship. The Mimic shiner generally comprises the major percentage within the insectivore guild, especially in the larger river systems. The relationship of SMB and Mimic shiner (figure 2), is another example of a predator-prey relationship yet the relationship of two other fishes within the invertivore/piscivore guild, Rock bass, *Ambloplites rupestris,* and the Redbreast sunfish, *Lepomis auritus*, to Mimic shiner is inverse (figures 3-4) to that of the SMB-Mimic shiner relationship. Similarly, the significant relationship of SMB to the insectivore guild, Mimic shiner excluded (figure 5), is inverse of the relationship of SMB to Mimic shiner.

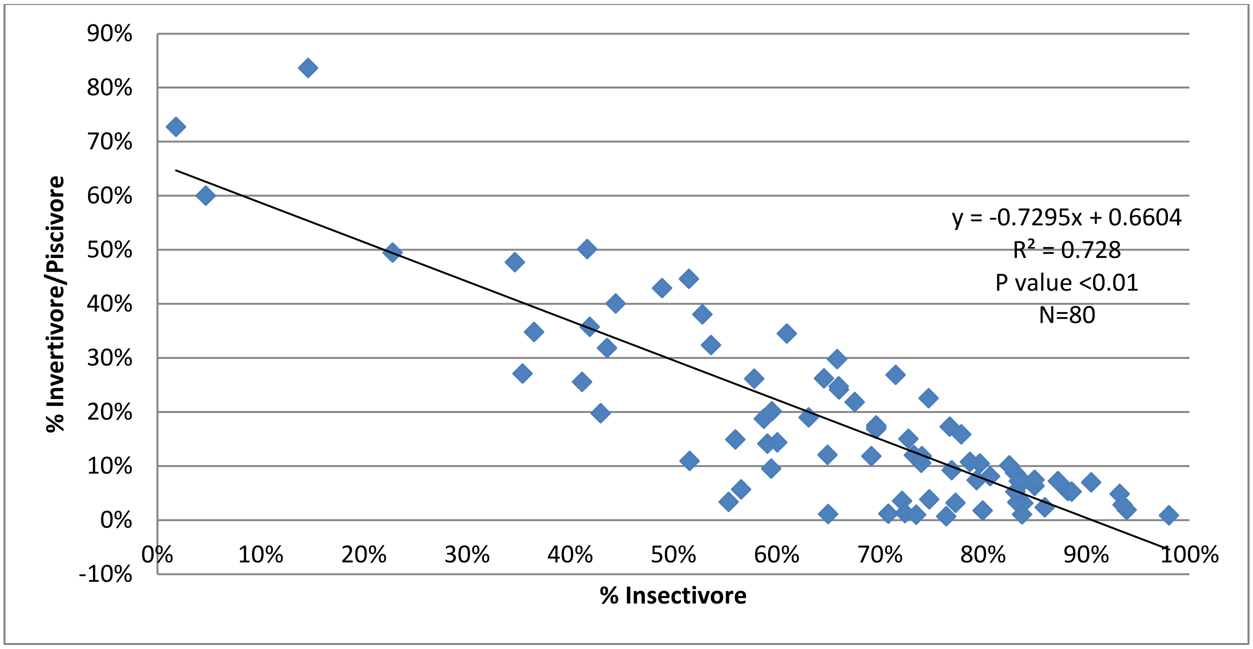


Figure 1. The relationship between the percent invertivore/piscivore to the percent insectivore.

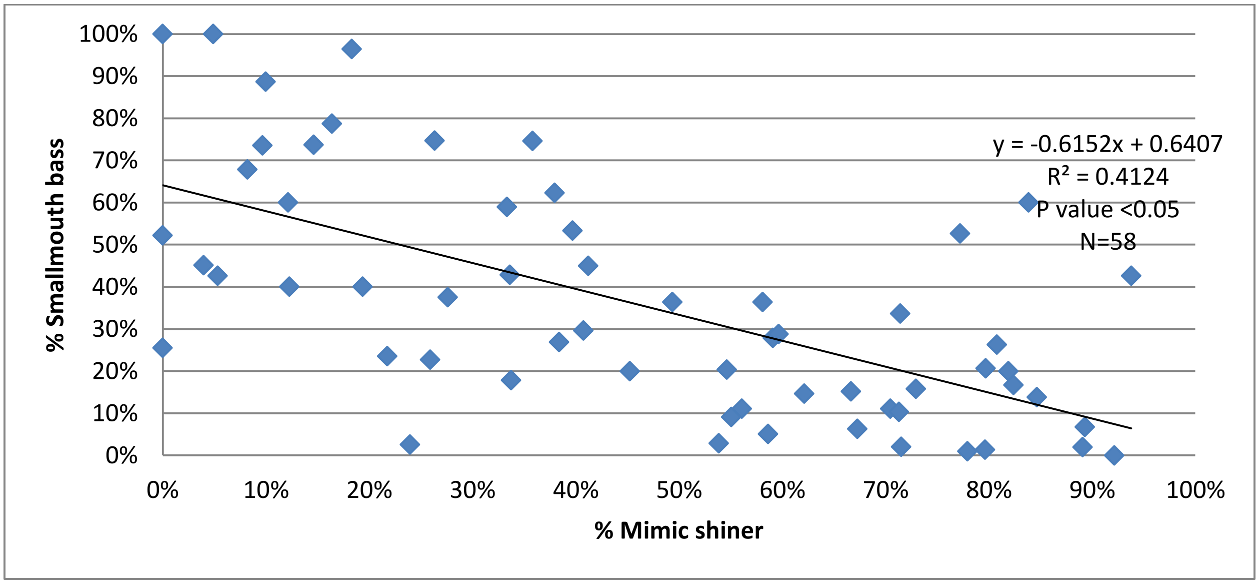


Figure 2. The relationship between percent SMB to the invertivore/piscivore guild and the percent mimic shiner to the insectivore guild. Sites located upstream of dams removed.

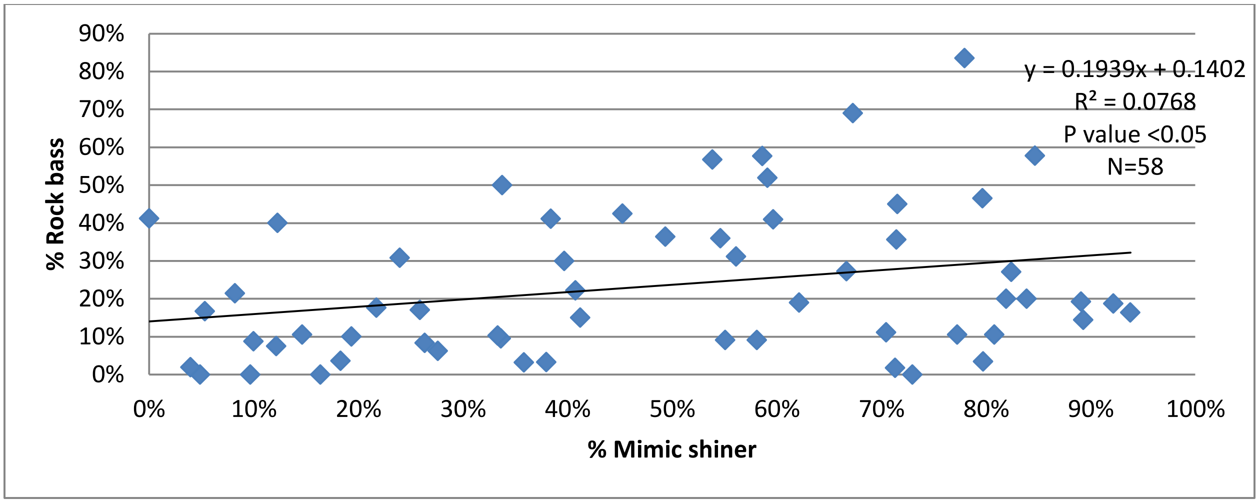


Figure 3. The relationship between percent Rock bass to the invertivore/piscivore guild and the percent mimic shiner to the insectivore guild. Sites located upstream of dams removed.

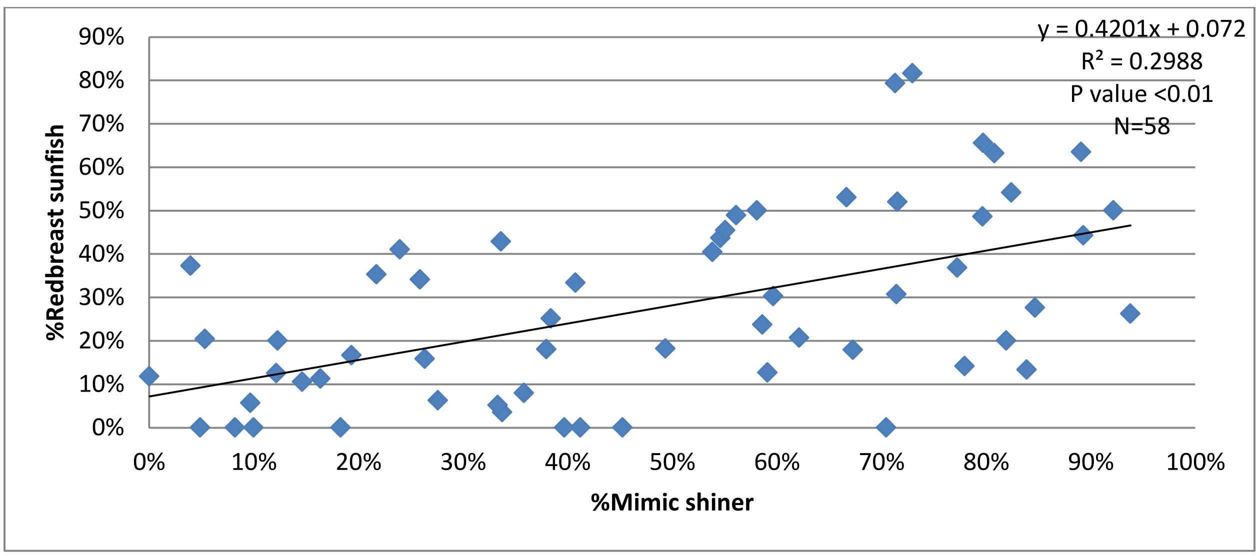


Figure 4. The relationship between percent Redbreast sunfish to the invertivore/piscivore guild and the percent mimic shiner to the insectivore guild. Sites located upstream of dams removed.

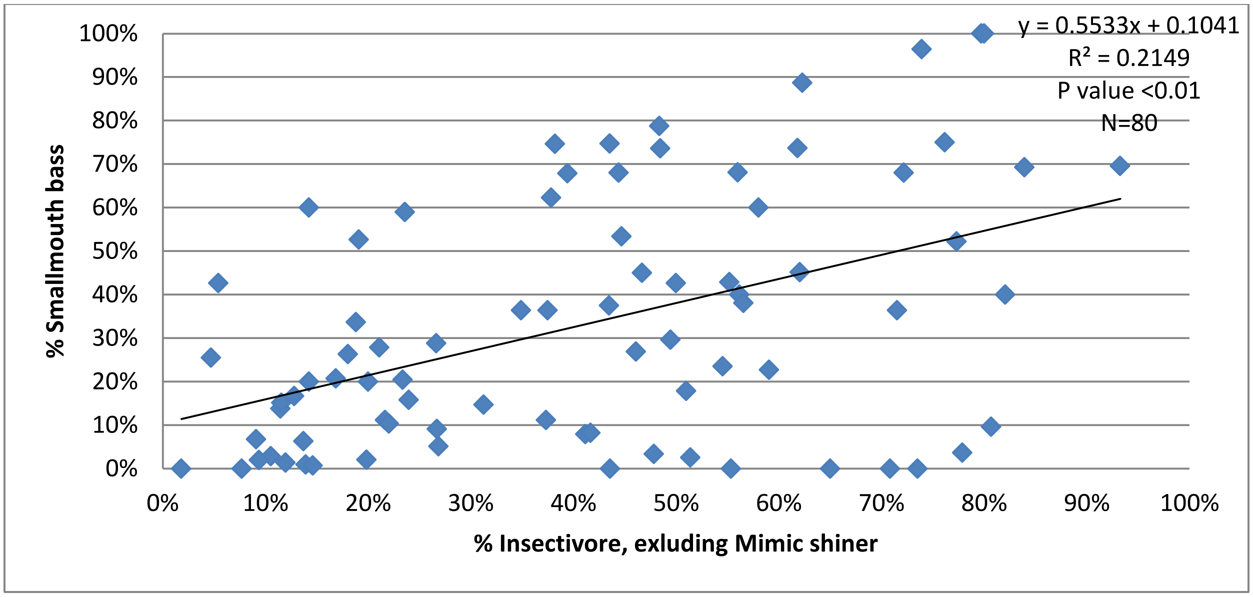


Figure 5. The relationship between percent SMB to the invertivore/piscivore guild and the percent insectivore guild, excluding Mimic shiner.

Candidate Cause 4: YOY Food Quality

Worksheets: 24 and 25

Title: Apparent decline in Susquehanna Smallmouth Bass and increase in the Non-native Rusty Crayfish (WS #24)

Agency: United States Geological Survey

Candidate cause: Decreased YOY food quality (e.g., food high in thiaminase) either kills YOY directly, or increases YOY susceptibility to disease.

Introduction:

An adequate supply of essential dietary nutrients is necessary for survival and well-being. An essential nutrient deficiency of vitamin B_1 (thiamine) has been documented in several aquatic top predator species in association with population declines. In these affected populations dietary thiaminase, an enzyme that destroys thiamine has been found to be involved. The diet of SMB is purported to be high in crawfish. The apparent decline in Susquehanna SMB population seemed to correspond with an increase in non-native rusty crawfish (*Orconectes rusticus*). Crayfish are reported to contain thiaminase which raised the following questions. Is thiaminase activity sufficiently elevated in rusty crayfish to cause thiamine deficiency? Is there a difference in thiaminase activity between rusty and native crayfish? Are there other sources of thiaminase in the diet of SMB that might lead to thiamine deficiency?

Data:

Pennsylvania Department of Environmental Protection, collected SMB food item samples in 2014 for prey items to be measured for thiaminase activity and periphyton for fatty acid analysis. Sites both within the Susquehanna basin and outside the basin (Allegheny, Delaware Rivers) were included. Prey item samples and periphyton were not necessarily collected at the same locations. Thiaminase analysis was conducted by USGS, Wellsboro, PA. Mimic shiner abundance figures from Tim Wertz, PA-DEP.

Analysis and Results:

The diet of SMB adults was determined. Empty stomachs were identified in 16.5% of the SMB. In those fish with identifiable contents, SMB stomachs contained crayfish (57.5%), fish (28.1%) and macro-invertebrates (37.1%). The diet of Susquehanna SMB is high in crayfish as reported.

Mean thiaminase activity in non-native rusty crayfish (0.39 µmol/g/min), native crayfish *O. obscurus* (0.41µmol/g/min) and in native crayfish *C. bartoni* (0.70 µmol/g/min) was low. There was variation in activity among sites but was biologically unimportant with respect to causing thiamine deficiency (Table 1). Thiaminase activity equal to or less than 1.5 µmol/g/min in prey items has not been associated with thiamine deficiency. Thiaminase activity between 2.0 and 2.9 µmol/g/min may lead to deficient state. Thiaminase activity greater than 2.9 µmol/g/min can result in thiamine deficiency. Thiaminase in non-native Mimic shiner (*Notropis volucellus*) was 53.2 µmol/g/min. This high activity would lead to thiamine deficiency assuming that the percentage of the diet reflected the percentage of SMB with fish in their stomachs. It is unknown if the percentage of SMB stomachs with fish (28%) is co-incidental or has any relationship with 28 % of the SMB eggs analyzed that had thiamine levels below 8 nmol/g which would make fry susceptible to secondary effects of thiamine deficiency. Thiaminase activity in Mimic shiner varied among sites. Thiaminase activity at Tuscarora Creek and Swatara was higher than in fish from Pine creek. Mean thiaminase activity in two groups of darters (Percina sp., 0.55 µmol/g/min; Etheostoma sp. 0.81µmol/g/min) was low and unlikely to lead to thiamine deficiency. However Blackside Darter (*P. maculate)* thiaminase activity (2.44 µmol/g/min) needs further confirmation as its activity could lead to thiamine deficiency (Table 2). It is unknown if YOY consume YOY Mimic shiner and this warrants further investigation. Mimic shiner and SMB are reported to hatch at approximately the same time and same general location. The overlap of these two species and their inherent size differential may provide opportunity for YOY SMB to consume YOY Mimic shiner.

Furthermore, expansion of non-native Mimic shiner populations appear to be exponential (Figure 1) in the tributaries. Population increase of Mimic shiner in large river areas was found to increase linearly (Figure 2). In both cases the increased availability of forage fish containing thiaminase suggests that SMB may have an increased source of dietary thiaminase that could affect their thiamine status.

Conclusion:

This candidate cause relating food quality especially food sources containing significant thiaminase activity merit continued investigation.

Table 1. Susquehanna basin crayfish thiaminase activity

Orconectes rusticus (Rusty)	N	µmol/g/min
Mifflintown Boat Access at Juniata	10	0.46
Shady Nook Boat Access at Susquehanna	8	0.38
West Fairview Boat Access at Susquehanna	8	0.32
Orconectes obscurus (Native)		
Kettle Creek	5	0.41
Cambarus bartonii (Native)		
Kettle Creek	2	0.63
Spring Mills at Penns Creek	7	0.72

Table 2. Susquehanna River forage fish thiaminase activity.

		N	µmol/g/min
Mimic shiner	*Notropis volucellus*		
	Tuscarora Creek	22	58.1
	Swatara Creek	17	65.0
	Pine Creek	12	45.5
	Pine Creek	10	31.3
Darters	*Percina* sp.		
	Shield Darter - *P. peltata*	11	0.21
	Common Logperch - *P. Caprodes*	4	1.02
	Blackside Darter - *P. maculata*	1	2.44
	Etheostoma sp.		
	Greenside Darter - *E. blenniodes*	10	0.99
	Johnny Darter - *E. nigrum*	3	0.61
	Variegate Darter - *E. variatum*	10	0.79
	Banded Darter - *E. zonale*	11	0.73

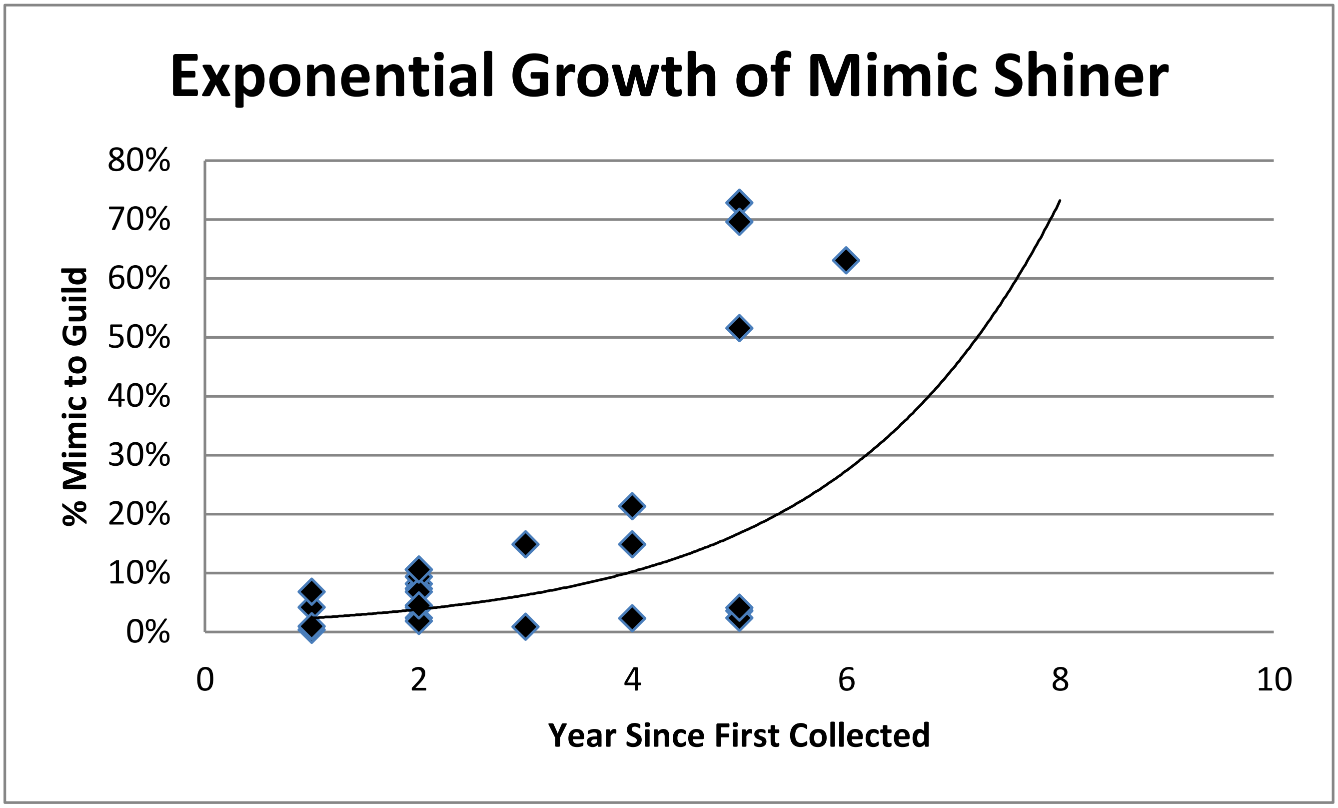


Figure 1. Mimic Shiner Exponential Growth in the Susquehanna Drainage
In order to delineate an approximate year of invasion, sample sites were chosen based on the following criteria. Sample locations should document an "absence" (year=0), followed by subsequent years of "presence" (year=1+). Sample sites that contained no measure of "absence", yet contained multiple years of record were included if they met the following criteria. Sites were included if the first survey within the data set contained Mimic shiner where their contribution to guild was < 5%. This first record was assumed to be year=1. Sites Chosen, with Years of Record included: Buffalo Creek, 2010-2014; Lycoming Creek, 2008-2013; Wyalusing Creek, 2008-2013; Loyalsock Creek, 2008-2014; Driftwood Branch Sinnemahoning Creek, 2012-2013; Tioughnioga River, 2008-2011; and Bennett Branch Sinnemahoning Creek 2012-2013. (Graph/data from Tim Wertz)

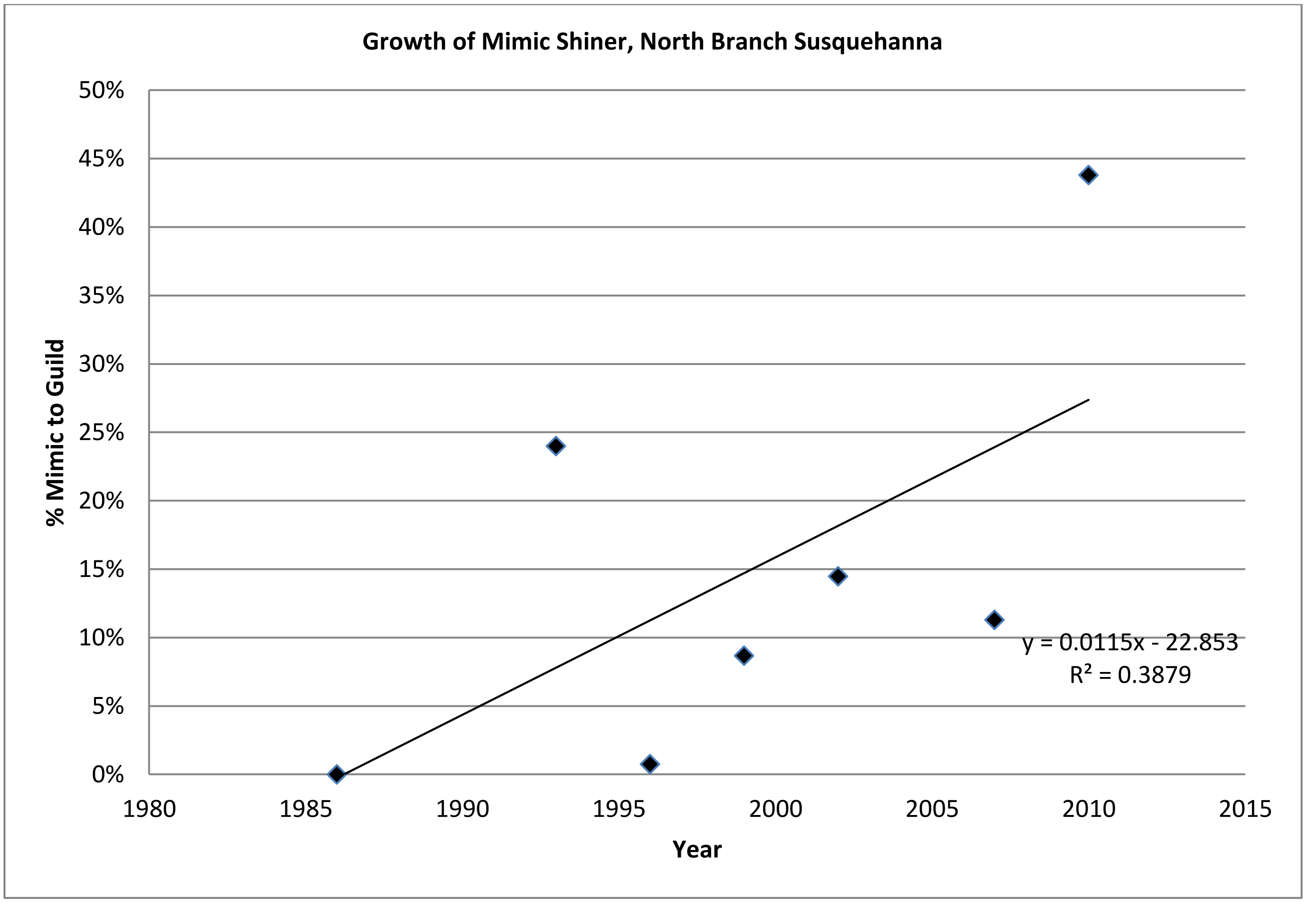


Figure 2. Growth of Mimic shiner at Non-wadeable Sites
Sites were chosen using different criteria, compared to tributaries, based on the period of record since initial collection on the Main stem of the Susquehanna and Sherman's Creek done in 1978. Site needed to have been collected using quantitative or semi-quantitative methodology of fish community. Site data spanned multiple years at the same collection location(s). Site was considered unusable if no "absence" period was recorded. Data were from Shroud Institute and Proctor & Gamble reports for the North Branch Susquehanna, 1986-2010. (Graph/data from Tim Wertz)

Title: Impacts of decreased food quality on YOY Smallmouth Bass and on Adults susceptibility to disease (WS #25)

Agency: The United States Geological Survey

Candidate cause: Decreased YOY food quality (e.g., poor fatty acid ratios) increases YOY and adults susceptibility to disease.

Introduction:

An adequate supply of essential dietary nutrients is necessary for survival and well-being. Essential fatty acids are reported to be important in immune function (Fenton et al. 2013). Arachidonic acid, in the omega-6 (ω6) family of fatty acids, is a precursor for pro-inflammatory eicosanoid. When a pathogen invades an organism, the pro-inflammatory response signals the immune system and a cascade of events kill and eliminate the invading pathogen. In a healthy situation a balance of omega-3 (ω3) modulates or down regulates the inflammatory response. A healthy ratio of ω3 to ω6 ranges between approximately 1:1 to 2:5.

Data:

Pennsylvania Department of Environmental Protection, collected periphyton for fatty acid analysis. Sites within Pennsylvania including the Susquehanna and Allegheny Rivers were included. Fatty acid analysis was conducted by USGS, Wellsboro, PA. Periphyton fatty acid composition in streams was associated with four land use categories. The ratio of ω3:ω6 fatty acids were calculated.

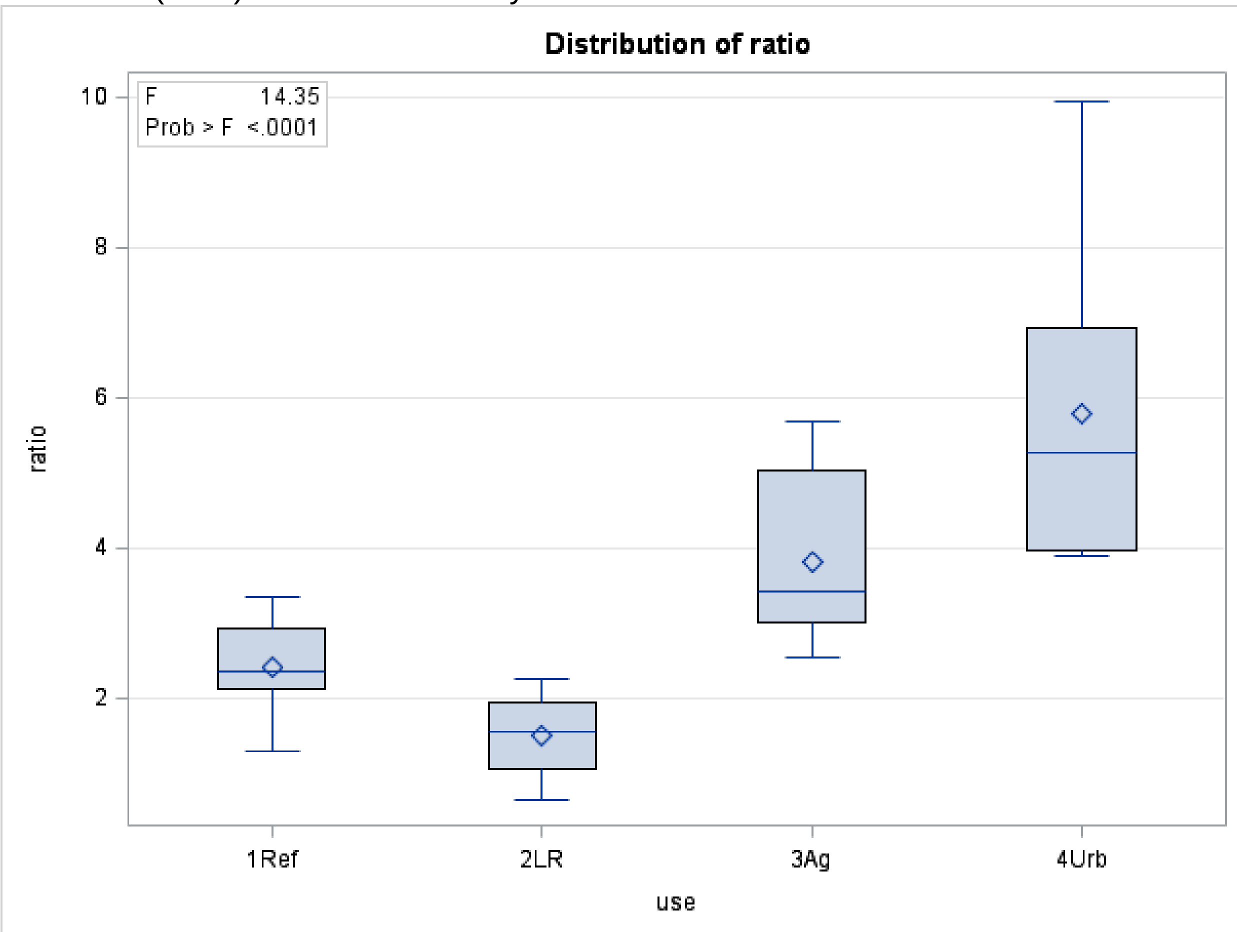
Analysis and Results:

Periphyton fatty acid analysis found that the ratio ω3:ω6 were within expected range for natural health and normal immune response in reference sites and large river sampled sites (Figure 1). However the ratio of ω3:ω6 was higher in sites with agriculture (3.8) and urban (5.8) land use. If these ratios persisted up the food chain, fish with these higher ratios potentially may not produce a strong pro-inflammatory response and would more likely succumb to the invading pathogen. When looking closer at the individual sites within the CADDIS designated area, all CADDIS sites were classified as large River (Susquehanna River (HBG East), Susquehanna River (HBG middle), Juniata River (Newport West), Juniata River (Newport East) and the ω3:ω6 ratios were within the normal range (Table 1). Thus there appears to be little evidence to support the hypothesis that fatty acid imbalance is a causal factor in the decline of lower Susquehanna River SMB within the CADDIS framework. However outside the CADDIS designated area these fatty acid imbalances may explain some of the diseased SMB observed, especially in urban associated streams.

Literature Cited:

Fenton, J. I., N. G. Hord, S. Ghosh, & E. A. Gurzell.   2013. Immunomodulation by dietary long chain omega-3 fatty acids and the potential for adverse health outcomes. Prostaglandins Leukotrines Essential Fatty Acids. 89(6):379-90. doi: 10.1016/j.plefa.2013.09.011.

Figure 1. The ratios ω3:ω6 in reference (1Ref), large rivers (2LR), agriculture (3Ag), and urban (4Urb) sites within Pennsylvania in 2014.



The ratios ω3:ω6 were 2.4, 1.5, 3.8, and 5.8 in reference, large rivers, agriculture and urban sites, respectively.

Table 1. Sum of stream periphyton omega-6, omega-3 fatty acids and the ratio of ω3:ω6 across land use classification in Pennsylvania.

Site	Land use	Sum of ω6	Sum of ω3	Ratio ω3/ω6
West Branch Susquehanna River	Large River	11.87	7.5	0.632
Susquehanna River (HBG East)	Large River	11.62	11.22	0.966
Susquehanna River (HBG middle)	Large River	10.4	11.76	1.131
Juniata River (Newport West)	Large River	9.73	15.02	1.544
Allegheny River (West)	Large River	8.59	13.49	1.570
Allegheny River (East)	Large River	7.72	13.96	1.808
Juniata River (Newport East)	Large River	7.39	15.24	2.062
Juniata River (Lewistown)	Large River	7.78	17.5	2.249
Browns Run	Reference site	12.71	16.18	1.273
Grays Run	Reference site	8.83	18.78	2.127
Pine Creek	Reference site	7.4	17.38	2.349
Kettle Creek	Reference site	6.27	18.4	2.935
Loyalsock Creek	Reference site	5.92	19.71	3.329
Wyalusing Creek	Agriculture	6.7	16.92	2.525
Chillisquaque Creek	Agriculture	5.62	15.8	2.811
Buffalo Creek	Agriculture	7.09	21.36	3.013
Raccoon Creek	Agriculture	6.38	20.17	3.161
Tohickon Creek	Agriculture	5.52	18.81	3.408
Tuscarora Creek	Agriculture	5.88	20.67	3.515
Cooks Creek	Agriculture	3.91	19.71	5.041
Kishacoquillas Creek	Agriculture	5.03	26.04	5.177
Jacks Creek	Agriculture	4.3	24.45	5.686
Wissahickon	Urban	5.39	21.01	3.898
Neshaminy Creek	Urban	4.24	16.86	3.976
Skippack Creek	Urban	4.82	20.42	4.237
Towamencin Creek	Urban	5.09	26.75	5.255
Skippack Creek	Urban	3.07	19.22	6.261
Indian Creek	Urban	3.02	20.9	6.921
Indian Creek	Urban	1.94	19.27	9.933

Candidate Cause 5: Egg Quality

Worksheet: 26

Title: Impacts of decreased food quality on Smallmouth Bass Eggs (WS #26)

Agency: United States Geological Survey

Candidate cause: Decreased SMB egg quality, especially eggs deficient in thiamine, either kills YOY directly, or increases YOY susceptibility to disease.

Introduction:

Apparent reduction in SMB population within the lower Susquehanna River is an issue of concern. Nutritional status and supply of essential dietary nutrients play an important role in survival and well-being of fish. Essential nutrient deficiency of the vitamin B_1 (thiamine) has been documented in several aquatic top predator species in association with population declines. Deficient (low) egg thiamine results in fry mortality after hatch in numerous salmonid species and alligators (McDonald et al. 1998; Brown et al. 2005; Honeyfield et al 2005, 2008). Furthermore immune dysfunction occurs in fish hatched from eggs containing low thiamine or fish with low thiamine status. Dysfunctional T-Cell activity can result in an increase in disease carrier individuals thus subjecting the population to a greater risk of disease outbreaks.

Data:

Pennsylvania Department of Environmental Protection, USGS, and others collected SMB eggs in 2013 and 2014 for thiamine analysis. Eggs were collected at three sites in Pa and two sites in MD in 2013. In 2014 eggs were collect at 14 sites in PA. Sites both within the Susquehanna basin and outside the basin (Allegheny, Delaware Rivers) were included. Thiamine analysis was conducted by USGS, Wellsboro, PA.

Analysis and Results:

Egg thiamine in 2013 from two sites in MD and three sites in PA were analyzed. SMB eggs from Chillisquaque Creek, PA contained less than half the concentration compared to eggs collected from the South Branch of the Potomac River in MD (Table 1). Maryland SMB mean total egg thiamine where no YOY mortality has been reported was higher than eggs from PA. In 2014 mean SMB egg total thiamine ranged from 7.81 to 16.36 nmol/g (Table 2). Egg thiamine values greater than 8 nmol/g are fully thiamine replete. Eggs with thiamine concentration between 1.5 and 8.0 nmol/g result in fry that are susceptible to secondary effects of low thiamine such as immune dysfunction. Overt fry mortality occurs when egg thiamine is less than 1.5 nmol/g. A closer look at individual egg thiamine values revealed that overall 28% of the egg lots contained concentrations less than 8 nmol/g and no egg thiamine concentration were below 1.5 nmol/g (Figure 1). Thus 28% of the females produced eggs that would be susceptible to the secondary effects of low thiamine. More importantly within the defined CADDIS area of the lower Susquehanna River, 37% females produced eggs containing less than 8 nmol/g (Figure 2). Outside the defined area only 14% of the females produced eggs with less than 8 nmol/g. Mean egg total thiamine in defined area was less (9.3 nmol/g) than outside the defined area (11.2 nmol/g). While overt fry mortality from low thiamine does not appear to be an issue, more than one quarter of the eggs may result in fry afflicted with secondary effects of low thiamine. It is interesting to note that as the Mimic shiner increase as a percentage of the prey fish base, SMB egg thiamine significantly decreases (Figure 3). There are no data on thiamine status of YOY. In conclusion it appears that the secondary effects of low thiamine could potentially affect SMB population by reducing disease resistance capability and therefore increasing morbidity and mortality (Ottinger et al. 2012, 2014).

Literature Cited:

Brown, S. B., J. D. Fitzsimons, D. C. Honeyfield & D. E. Tillitt. 2005. Implications of Thiamine Deficiency in Great Lakes Salmonines. Journal of Aquatic Animal Health 17:113-124.

Honeyfield, D. C., J. P. Hinterkopf, J. D. Fitzsimons, D. E. Tillitt, J. L. Zajicek & S. B. Brown. 2005. Development of Thiamine Deficiencies and Early Mortality Syndrome in Lake Trout by Feeding Experimental and Feral Fish Diets Containing Thiaminase. Journal of Aquatic Animal Health 17:4-12

Honeyfield, D. C., J. P. Ross, D. A. Carbonneau, S. P. Terrell, A. R. Woodward, T. R. Schoeb, H. F. Percival, & J. P. Hinterkopf. 2008. Pathology, physiological parameters, tissue contaminants, and tissue thiamine in morbid and healthy Central Florida adult American alligator (*Alligator mississippiensis*). Journal of Wildlife Diseases 44:280-294.

McDonald, G., J. Fitzsimons, & D.C. Honeyfield (Eds). 1998. Early life stage mortality syndrome in fishes of the Great Lakes and Baltic Sea. Symposium 21. American Fisheries Society, Bethesda, MD.

Ottinger, C. A., D. C. Honeyfield, C. L. Densmore, & L. R. Iwanowicz. 2012 Impact of Thiamine Deficiency on T-cell Dependent and T-cell Independent Antibody Production in Post-Yearling Lake Trout (*Salvelinus namaycush*). Journal of Aquatic Animal Health, 24: 258-273.

Ottinger, C. A., D. C. Honeyfield, C. L. Densmore & L. R. Iwanowicz. 2014. In vitro immune functions in thiamine-replete and -depleted Lake trout (*Salvelinus namaycush*). Fish & Shellfish Immunology 38: 211-220.

Table 1. Smallmouth bass egg total thiamine in 2013

Maryland	N	nmol/g	Mean
Potomac, South Branch	11	21.1	19.7
Monocacy River	13	16.0	
Pennsylvania			
Chillisquaque Creek	4	9.5	13.8
Mahantango Creek, West Br.	4	16.4	
Wyalusing Creek	5	15.2	

Table 2. Smallmouth bass egg total thiamine in 2014

	N	Mean	SD
Susquehanna at Marrietta	13	7.81	3.47
Bald Eagle Creek	5	8.24	1.32
Tuscarora Creek	8	8.83	2.43
Allegheny at Franklin	7	8.95	2.72
Juniata River at Newport	8	9.39	3.64
Chillisquaque Creek	5	9.68	2.37
Susquehanna at Harrisburg	9	10.12	1.57
Swatara (Juna. at Harper)	6	10.76	2.22
West Branch Susquehanna	5	10.99	1.67
Susquehanna at Shady Nook	9	10.99	3.40
Loyalsock Creek	5	11.09	4.11
Bald Eagle Creek-Control	12	13.11	3.35
Pine Creek	6	13.18	4.58
Kettle Creek at Rt 144 Bridge	5	14.54	2.31
Delaware River at Morrisville	5	16.36	7.46
Overall	131	10.54	3.61

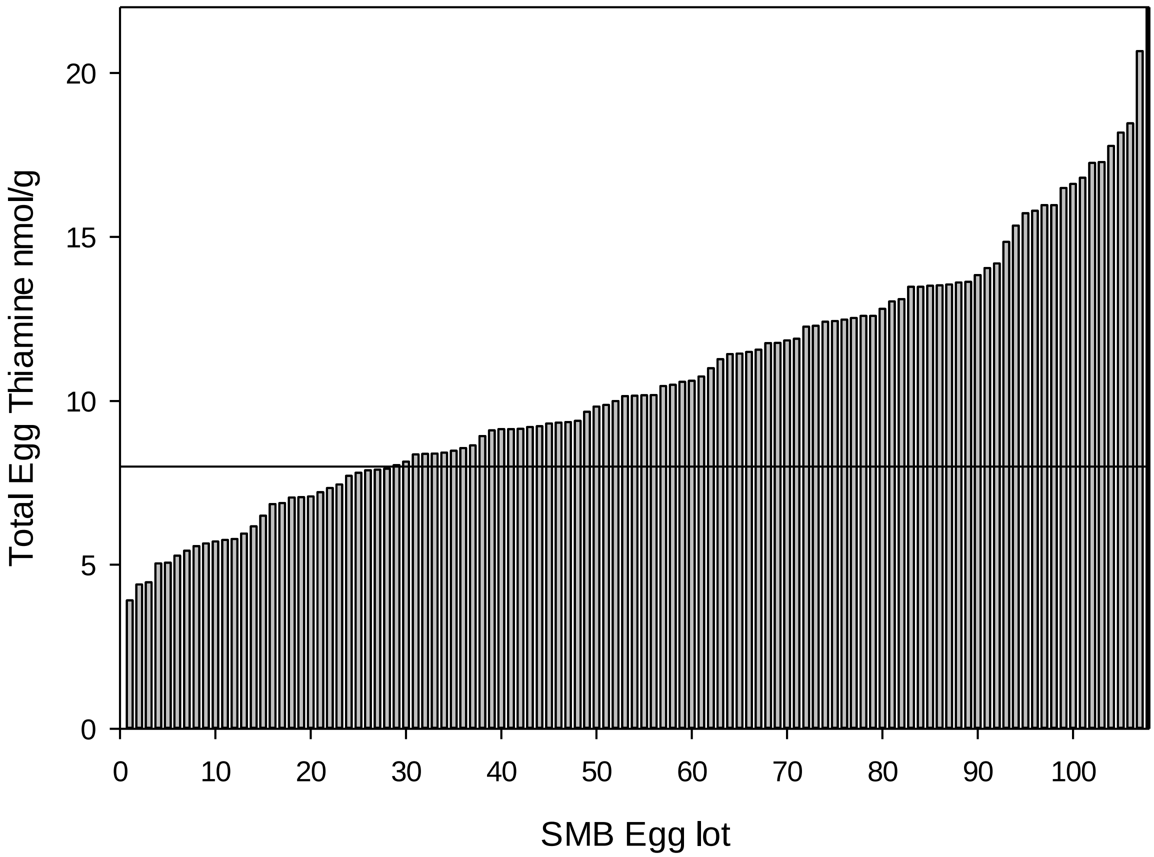


Figure 1. Smallmouth bass egg total thiamine analyized in 2014. Control line in graph is drawn at 8 nmol/g. Egg thiamine values above the line are fully thiamine replete. Values below the line are susceptable to effects of low thiamine. 28% are below 8nmol/g.

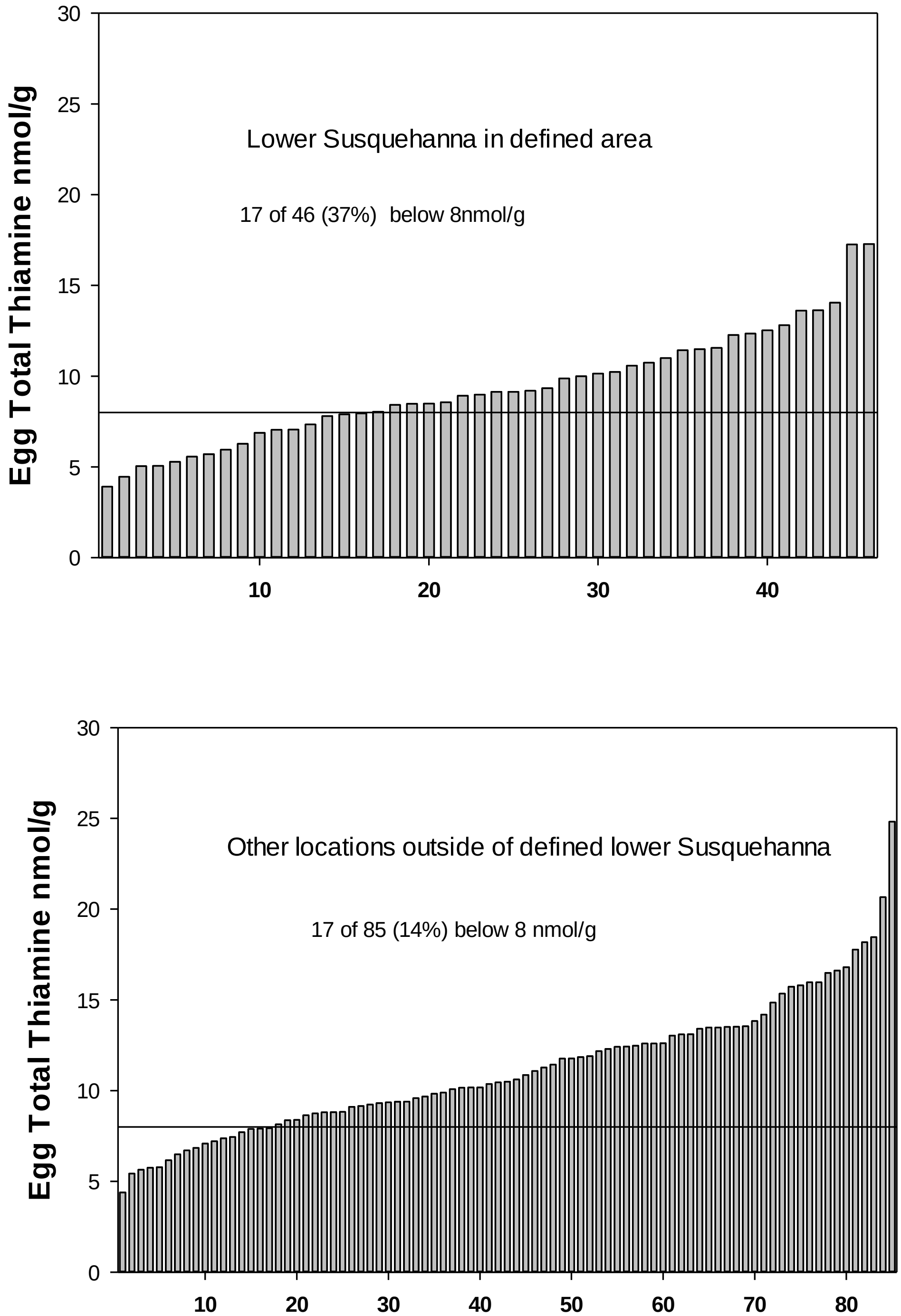


Figure 2. Egg total thaimine in SMB in defined CADDIS area (upper panel, Juniata River at Newport, Susquehanna at Shady Nook, Harrisburg, and Marietta) and outside the defined area (lower panel). Control line in graphs are drawn at 8 nmol/g. Egg thiamine values above the line are fully thiamine replete. Values below the line are susceptable to effects of low thiamine.

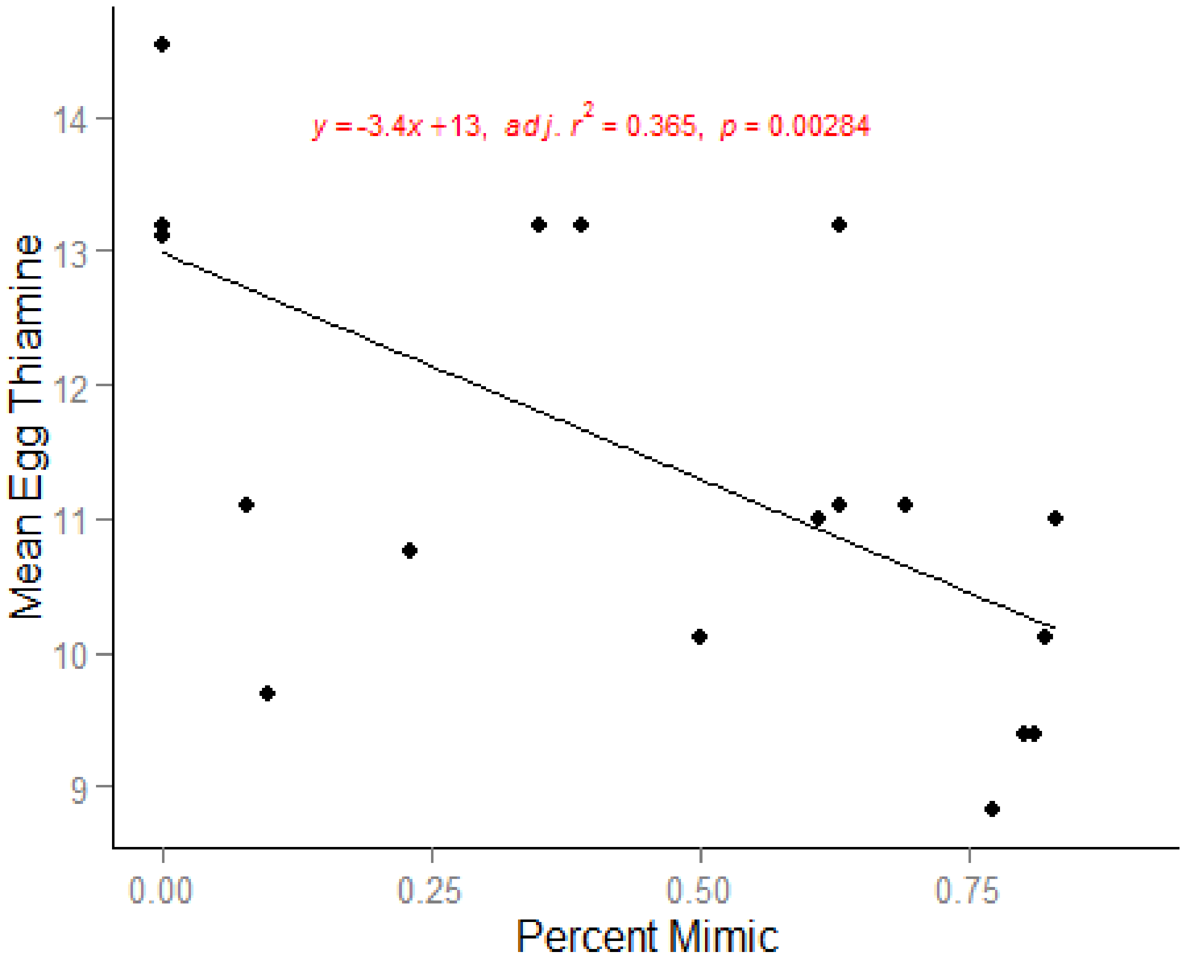


Figure 3. Relationship between Mimic shiner abundance and Smallmouth bass mean egg thiamine in the Susquehanna River and its tributaries.

Candidate Cause 6: YOY Habitat

Worksheets: 34, 35 and 36

Title: Impacts of algal blooms on YOY Smallmouth Bass (WS #34)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: Increased algal blooms after periods of low flow in the basin may reduce YOY late summer habitat and either increase YOY susceptibility to disease or directly cause YOY mortality.

Introduction:

If YOY smallmouth bass habitats in the CASSDIS area are prone to algal bloom formation in the summer months after periods of low flow, then YOY smallmouth bass may be subject to stressful conditions caused either by bloom or periods of high algae die-off. Increases in algal growth is commonly associated with increased water temperature and photosynthetic activity, which results in elevated diurnal swings in dissolved oxygen and pH. In addition, YOY may be more susceptible to cyanotoxins during increased algae growth or die-offs if the algae species composition consists of cyanobacteria strains that release cyanotoxins. Small mouth bass YOY have limited motility during their early stages and may not be able to avoid stressful conditions caused by increased algae growth.

Data:

During the month prior to July 9, 2012, discharge measured at the Harrisburg USGS gage was steadily declining and was below 10,000 cfs for the 10 days prior to July 9 (Figure 1). Maximum daily temperatures exceeded 30°C for 10 days prior to July 9, 2012 and the mean daily temperature exceeded 28°C during this interval. In response to angler complaints about nuisance algae during this period, the Pennsylvania Fish and Boat Commission (PFBC) conducted a flyover of the Susquehanna River from Sunbury to Wrightsville, PA. Photographs were taken along the route and depicted high algae growth at various locations throughout the basin on July 9, 2012 (Figure 2). The 2012 flyover photographs showed dense algae growth at Rockville, Harrisburg, and Calver Island among other locations. Higher algae growth tended to occur near shore habitats, back channel areas, and along the shorelines of small islands.

Additional photographs of a filamentous algae bloom on the Susquehanna River at Rockville were taken by Geoff Smith from the PFBC on August 12, 2012 coinciding with a fish pathology survey where YOY small mouth bass with abnormalities were identified (Figure 3).

In addition, PFBC conducted fish surveys at Swatara Creek at Harpers Tavern on July 16, 2012 coinciding with a fish kill report. Photos taken by Geoff Smith indicated high algae growth at this location and followed by a period of low flow as measured by the USGS gage at Harpers Tavern (Figure 4). The discharge at the Swatara Creek gage was at or below the median daily discharge statistics on record (year=96) from 6/18/12 to 7/16/12.

Aerial photography, collected by the PA DEP Southcentral regional office of the Susquehanna River and Juniata River within the CADDIS study area on October 4, 2007 after a summer and early fall of relatively low discharge, showed algal bloom formation at several sites (Figure 5). The surface blooms were concentrated along shoreline habitats and between islands on the river.

Conclusion:

The photographs are evidence that algae blooms occurred in potential YOY smallmouth bass habitat throughout the CADDIS study area of the Susquehanna River and the Juniata River in 2007 and 2012 after periods of relatively low flow conditions and higher temperatures in the summer and early fall months. There is circumstantial evidence that diseased YOY smallmouth bass co-occur with algal blooms in the basin. However, additional studies are needed to characterize the species composition and extent of algae blooms on the YOY small mouth bass habitat. A reasonable hypothesis is that the presence of a high accumulation of algae in YOY habitat post-hatching may cause stressful conditions during the first months of life for the smallmouth bass either during the bloom formation or during periods of high algal and/or cyanobacteria die-offs. Possible conditions induced by algae growth and die-offs include lower dissolved oxygen concentrations; increases in pH, turbidity, and temperature; and/or the potential production of cyanotoxins which can affect YOY small mouth bass through absorption from the water column or through feeding on a diet based heavily on zooplankton, which is typical for smallmouth bass early life stages. Since YOY smallmouth bass have limited motility and are somewhat restricted to shallow, near shore areas with slow moving water (Chaplin and Crawford 2012), the effects of the increased algae growth and decay in these locations on YOY smallmouth bass may be significant.

Figure 1: USGS 0157050 gage information for Discharge, pH, Temperature, and Dissolved Oxygen from June 2, 2012 to August 25, 2012.

A B

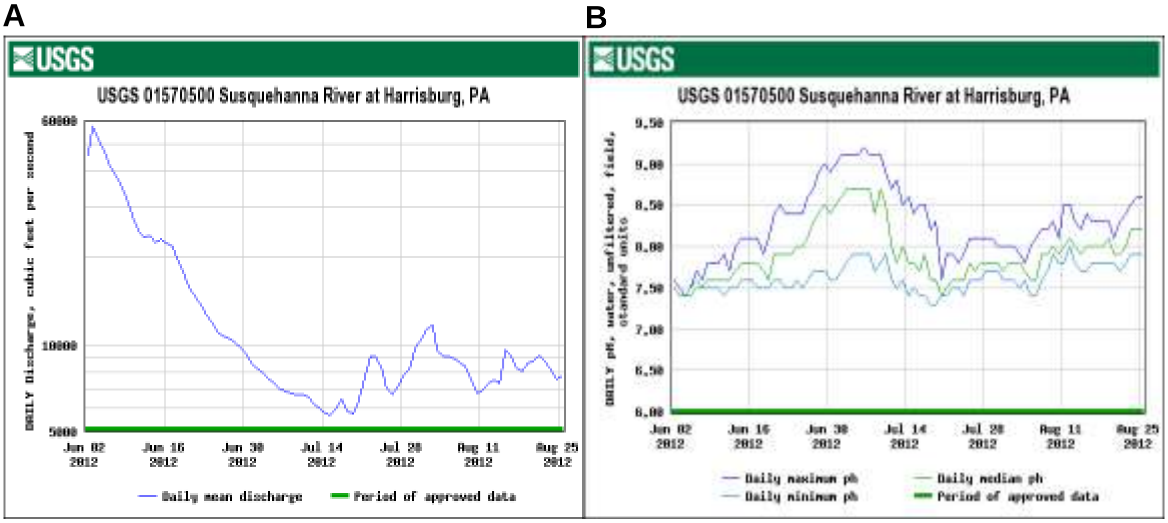


Figure 2: Aerial photographs taken by PFBC on 7/9/2012 by plane at the Susquehanna River locations A) upstream riffle at Rockville B) Susquehanna River at Duncannon C) Island off Front Street upstream of City Island D) between I-81 Bridge and City Island E) Upstream Edge of Calver Island.

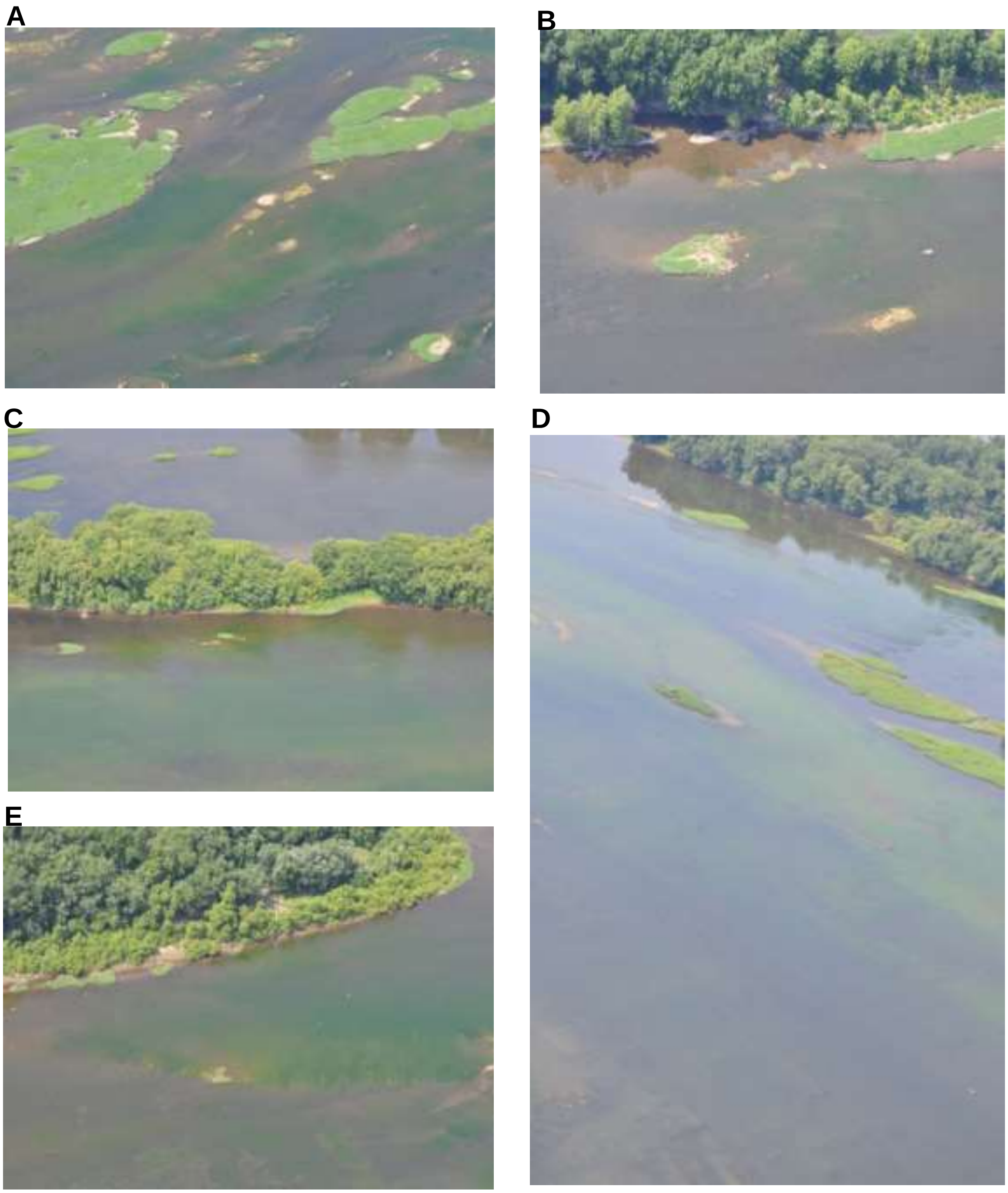


Figure 3: Photos collected on 8/13/2012 at Rockville on the Susquehanna River to coincide with Pennsylvania Fish and Boat Commission fish pathology investigation of A) algae growth in near shore YOY habitat and B) YOY smallmouth bass with abnormalities.

A

B

(photos taken by Geoff Smith, PFBC)

Figure 4: Discharge measured at USGS Gage 01573000 of Swatara Creek at Harper Tavern from June 2, 2012 to July 16, 2012 and photographs taken by Geoff Smith on July 6, 2012 at Harper Tavern after report of a fish kill.

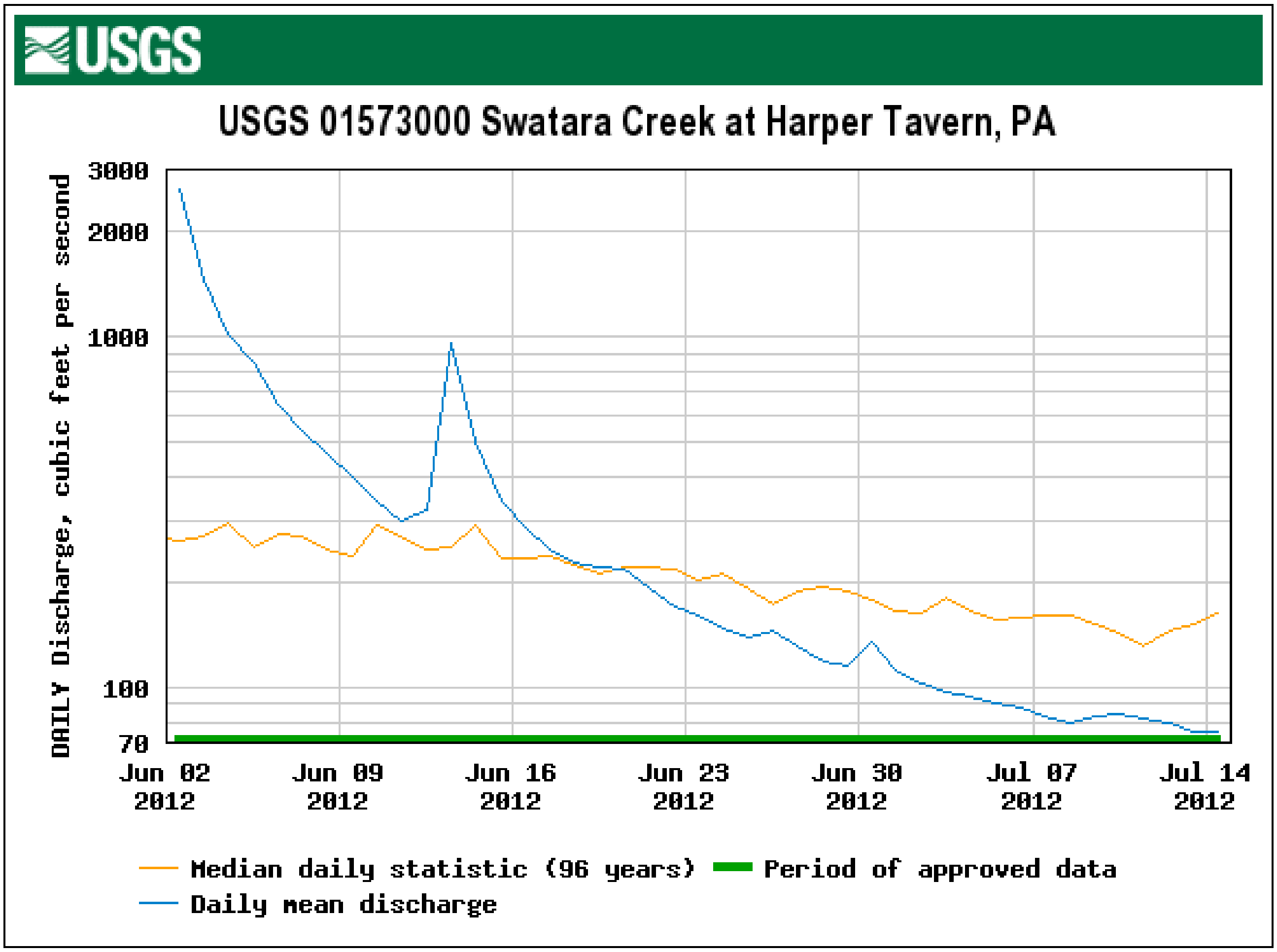
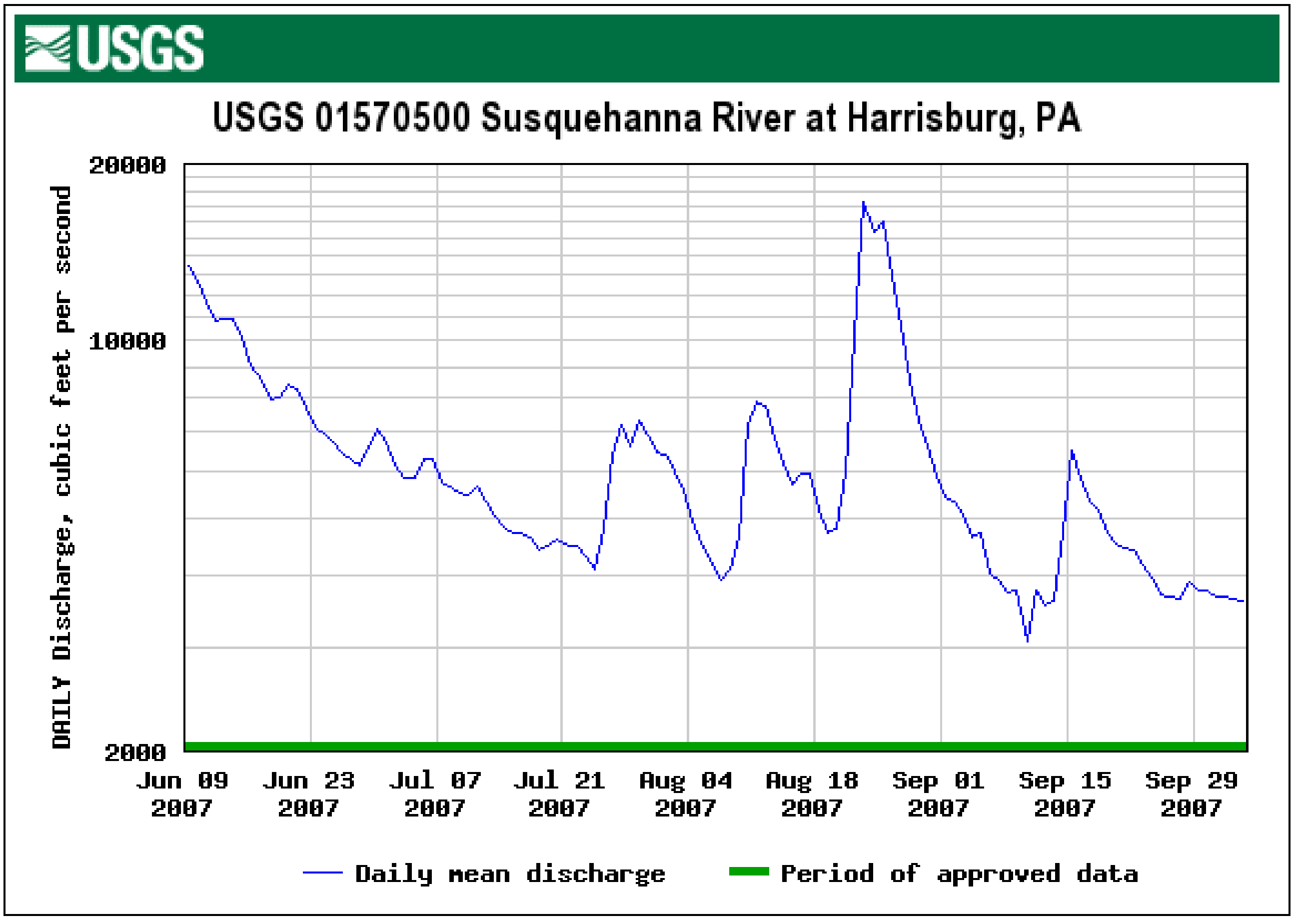


Figure 5: USGS discharge data from USGS 01570500 Susquehanna River gage at Harrisburg from June 1, 2007 to October 4, 2007 A) and aerial photographs of CADDIS area collected on 10/4/2014 by DEP Southcentral Regional Office by plane at B) Juanita River C) reach Sunbury to McKees Flats D) Reach Dock Street to York Haven, E) and F) Reach York Haven to Holtwood.

A



B

C

D

E

F

Title: Algal and Cyanobacterial Toxins (WS #35)

Agency: Pennsylvania Department of Environmental Protection

Candidate Causes: Algae, Cyanobacteria, & YOY Habitat

Proximate Stressor(s): Algal and cyanobacterial toxins either kill YOY directly, or increase YOY susceptibility to disease, and reduce recruitment of Susquehanna smallmouth bass into age class 1 (<u>Proximate Stressor #11</u>), and decrease in YOY habitat via increased algal growth either kill YOY directly or increase YOY susceptibility to disease (<u>Proximate Stressor #6</u>)

Introduction:

PA DEP periphyton community data collected from the Juniata and Susquehanna Rivers in 2013 documented the presence of cyanobacteria genera that may have the potential to produce cyanotoxins. In addition, preliminary adult SMB liver analysis results suggest that SMB in the Juniata River Basin show signs of cyano-toxicity. Thus, it's reasonable to assume that cyanobacterial toxins may have, or may be, contributing to the decline of the SMB fishery in the Susquehanna River Basin.

Although it appears cyanotoxins may possibly be having an influence on the SMB fishery in the Susquehanna River Basin, existing data is not suitable for drawing concrete conclusions about the impact cyanotoxins may have had, or may currently be having, on the Susquehanna River Basin SMB fishery. However, existing data may be useful for exploring relationships between SMB young-of-year (YOY) survey results and the environmental conditions typically associated with increased growth rates of toxic strains of cyanobacteria. In general, as water temperatures increase from 10 to 30 C in freshwater ecosystems, the algal group with the highest growth rates shifts from diatoms to green algae to cyanobacteria. In addition, as surface waters become enriched with nutrients, phosphorus in particular, there tends to be a shift in the algal community towards dominance by cyanobacteria.

In addition to affecting algal growth rates and community structure, elevated water temperature and phosphorus levels have been shown to influence the cellular toxin content of multiple genera of cyanobacteria. Davis and others (2009) examined the effects of elevated temperature and nutrient levels on the growth rates of toxic and non-toxic strains of the cyanobacteria *Microcystis.* They observed that experimentally elevated temperatures in the range of 25 – 30 C had a greater impact (i.e., increased growth rates) on toxic strains of *Microcystis* than it did on non-toxic strains of *Microcystis*. Furthermore, they observed that concurrent increases in temperature and phosphorus concentrations yielded the highest growth rates of toxic *Microcystis* cells, concluding that increasing temperature and nutrient levels may additively promote the growth of toxic, rather than non-toxic, populations of *Microcystis*, leading to blooms with higher cyanotoxin (microcystin) content.

We used existing Pennsylvania Fish and Boat Commission (PFBC) SMB YOY data in conjunction with chemical water quality data from the DEP water quality monitoring network(WQN) to determine if environmental conditions generally associated with increased abundance of toxic cyanobacteria (elevated temperature and phosphorus levels) coincide with the decline of the smallmouth bass fishery in the defined CADDIS area (Susquehanna River at Harrisburg and Juniata River at Newport), as expressed by YOY catch-per-unit-effort (CPUE) values.

In addition to studying the relationships between the environmental conditions generally associated with increased abundance of toxic cyanobacteria and SMB YOY CPUE values, we also used existing data to explore relationships between indicators of increased algal growth and CPUE values (Proximate Stressor #6). Increased algal growth is commonly associated with increased water temperature and photosynthetic activity, which results in elevated diurnal swings in dissolved oxygen and pH, and greater extreme values of these parameters (e.g., minimum DO and maximum pH values), both of which are potential stressors to SMB. We used continuously monitored water temperature, dissolved oxygen (DO), and pH data (CIM data) as a surrogate measure for algal growth to see if this data suggests a possible relationship between increased algal growth and the decline of the SMB fishery in the CADDIS area, based on YOY CPUE conditions.

Data:

We used PFBC annual directed YOY CPUE survey data from the Susquehanna River near Harrisburg (Rockville) and the Juniata River near Newport (Greenwood) to characterize or estimate reproductive success of SMB in the defined CADDIS area from 1988 to 2013. Similar data were utilized from the Delaware River near Morrisville (Yardley) as an out of basin control. SMB YOY station locations did not necessarily coincide exactly with WQN station locations, and for each WQN station, data from the closest SMB YOY station with a record of data adequate for assess changes in YOY conditions over time (approximately 1990 – 2013), were used to characterize YOY conditions at the WQN station.

We used WQN temperature, pH, and nutrient data, collected at each station either monthly or every other month. All WQN stations used in this analysis were located at USGS stream discharge monitoring stations. USGS daily mean values were used in stream discharge-related analyses. The water quality data used in this analysis reflect the water quality conditions at the WQN station in general, and were not specifically collected in YOY habitat. Thus, it’s unclear how accurately the WQN data reflect the chemical water quality conditions of the specific areas that were sampled in the YOY survey. However, for the purpose of this exercise, it is assumed that general patterns in environmental conditions observed at a particular WQN station during a given time period (e.g. significantly elevated water temperature in a specific month) were most likely also occurring in the YOY habitat associated with that particular WQN station.

Water temperature, pH, and total phosphorus data from the WQN stations on the Juniata River at Newport and the Delaware River at Morrisville are extensive throughout the timeframe of this analysis (approximately 1990 – 2013). However, this is not the case for other forms of phosphorus, nitrogen, or the WQN station on the Susquehanna River at Harrisburg. The Harrisburg WQN station was inactive from March 1995 through September 2012. Thus, our conclusions about the potential influence of toxic cyanobacteria and algal growth conditions (proximate stressors #11 and #6) on SMB YOY in the defined CADDIS area are heavily influenced by total phosphorus data from the Juniata River at or near Newport.

We used CIM data collected between 15 June and 18 July in 2012 and 2013 to characterize temperature, DO, and pH conditions during, and shortly after, SMB spawning at Harrisburg (west sonde), Newport (north sonde), and Morrisville (west sonde).

Analysis and Results:

We used the following data analysis approach: first, we identified the years 1991 through 1994 as a period of relatively high YOY CPUE values, and 2005 – 2011 as a period of relatively low YOY CPUE values recorded in the CADDIS area, and targeted our analysis in these periods of dramatically differing YOY CPUE results (Figure 1).

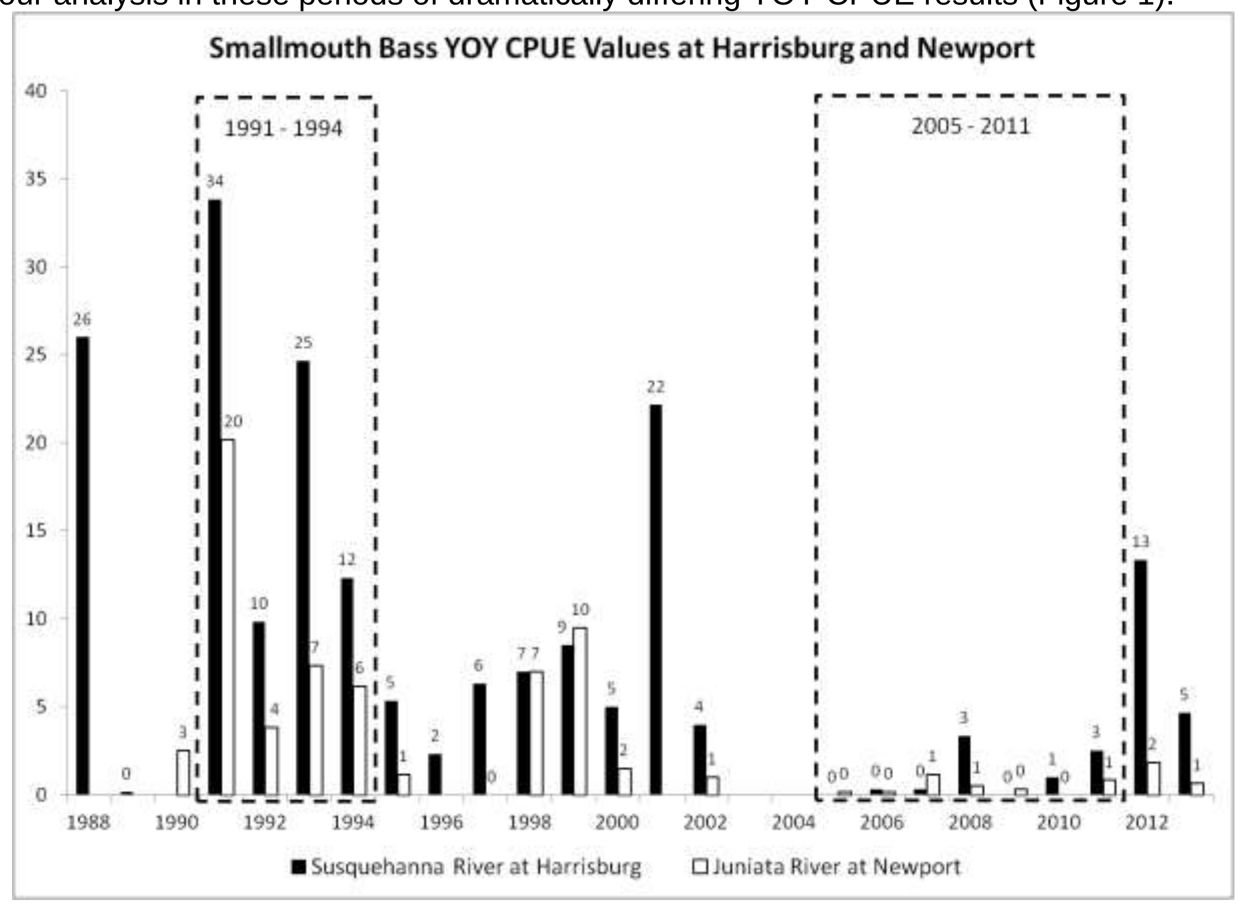


Figure 1. SMB YOY Catch per Unit Effort Data from the Susquehanna River at Harrisburg and the Juniata River at Newport.

Then, we reduced the potential confounding influence of June stream discharge conditions on YOY CPUE values. Smith and others (2005) observed significant, non-linear relationships between mean June stream discharge and recruitment success of SMB in the James, Rappahannock, and Shenandoah Rivers in Virginia, with the strongest year-classes produced in years with moderate June flows (usually within 40% of the long-term mean). They also reported that stream-specific optimal discharge conditions for spawning and fry SMB may fall within a relatively narrow range. For Harrisburg and Newport, we identified the years with relatively low June flows during each of the two time periods, and used data collected during those years in our analysis (Figure 2 (for Harrisburg) and Table 1). Since the temporal pattern of relatively high vs. relatively low CPUE values observed in the CADDIS area shown in Figure 1, was not observed at Morrisville, we used data from selected years with high CPUE values vs. selected years of low CPUE values, from a pool of all years with relatively low June flows (Figure 3 and Table 1). This process resulted in the formation of a group of high CPUE samples, and a group of low CPUE samples, from each WQN station, with each group being collected under similar stream discharge conditions. Similarly, we used CIM data collected during years with relatively low June flows in our analysis (Table 2; Figures 2 and 3, Harrisburg and Morrisville, respectively).

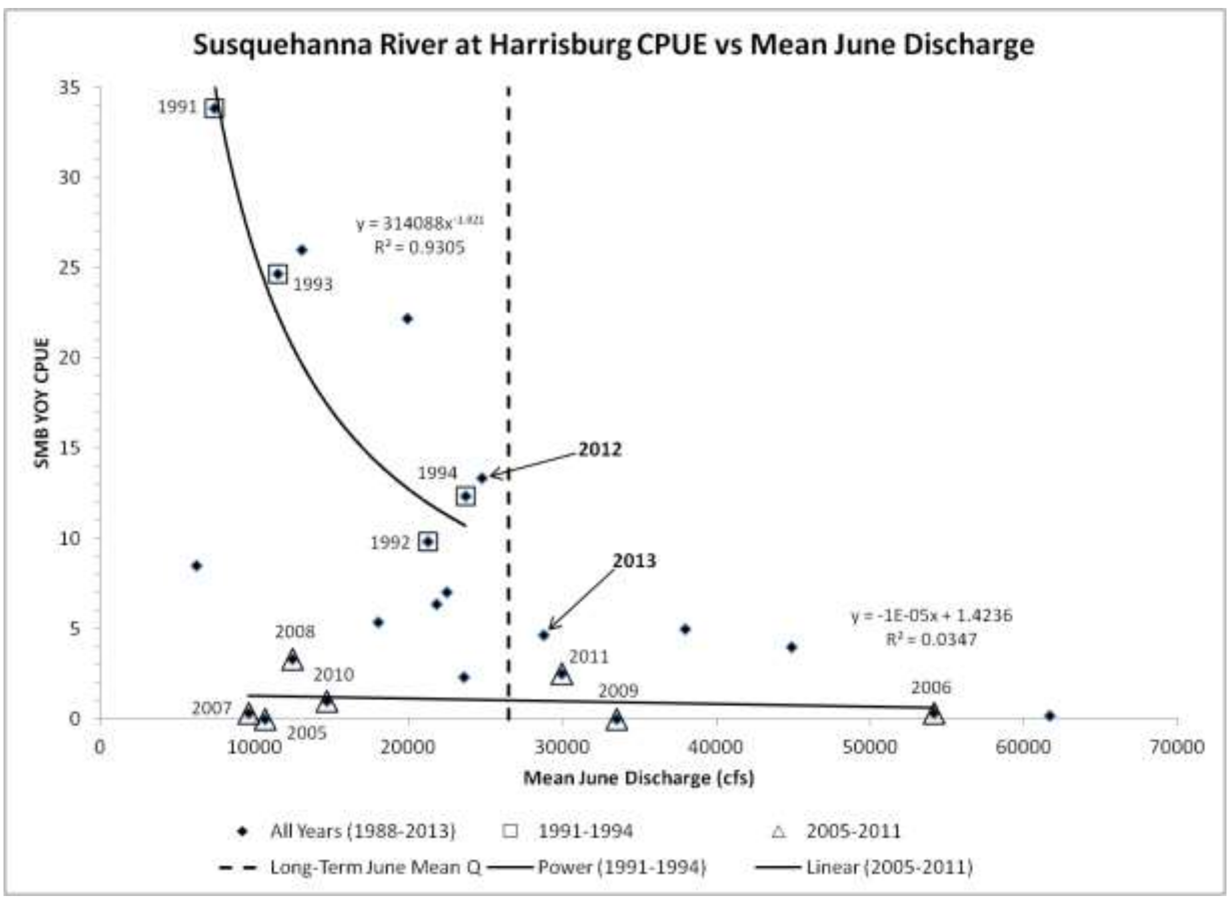


Figure 2. SMB YOY CPUE vs. Mean June Discharge Data from the Susquehanna River at Harrisburg.

Table 1. High CPUE and Low CPUE Sample Groups from WQN Stations. Each of the Sample Groups from a Given WQN Station, Consists of Data Collected During Years with Similar June Stream Discharge Conditions.

Analysis Group	Historical Max SMB YOY CPUE	Relative SMB YOY CPUE
Susquehanna River at Harrisburg (1991-1994)	33.83	High (9.83 – 33.83)
Susquehanna River at Harrisburg (2005, 2007, 2008, 2010)		Low (0.00 – 3.33)
Juniata River at Newport (1991-1994)	20.17	High (3.38 – 20.17)
Juniata River at Newport (2005, 2007, 2008, 2010)		Low (0.00 – 1.17)
Delaware River at Morrisville (1997, 2007, & 2008)	17.00	High (11.50 – 17.00)
Delaware River at Morrisville (1993, 1994, 2004, & 2010)		Low (0.00 – 2.17)

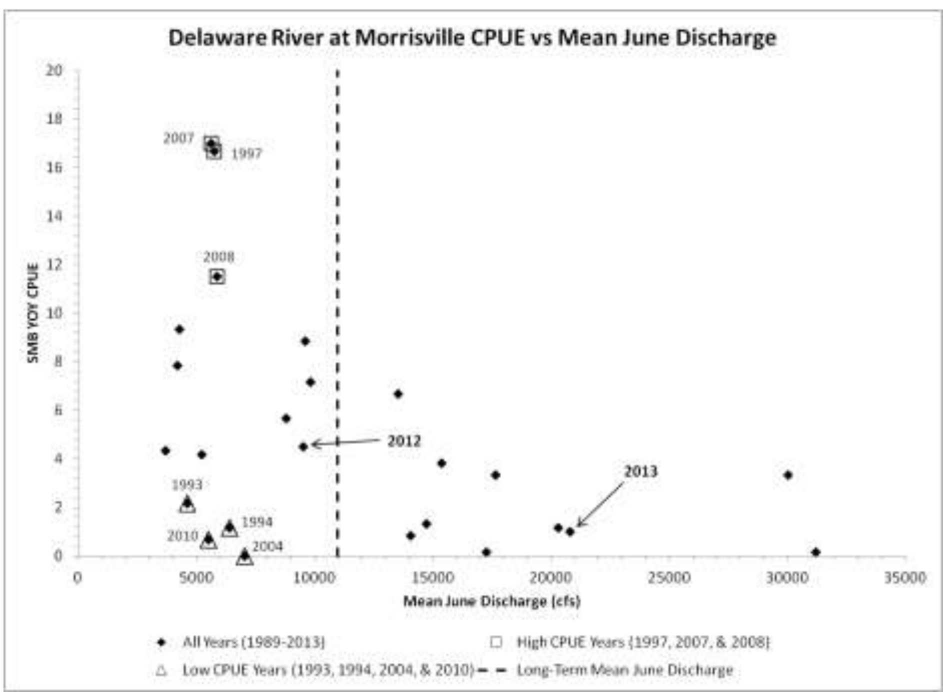


Figure 3. SMB YOY CPUE vs. Mean June Discharge Data from the Delaware River at Morrisville.

Table 2. Relative SMB YOY CPUE Conditions at WQN Stations with Mean June Stream Discharge Conditions below the Long-Term Mean (1973 – 2013) Used in Continuous Instream Monitoring (CIM) Data Analyses.

Analysis Group	**Mean June Discharge (cfs)**	**Long-Term Mean June Discharge (cfs) (1973 – 2013)**	**Relative SMB YOY CPUE**
Susquehanna River at Harrisburg (2012)	24,760	26,496	High (13.33)
Juniata River at Newport (2013)	2,546	3,388	Low (0.67)
Delaware River at Morrisville (2012)	9,511	10,978	Moderate (4.50)

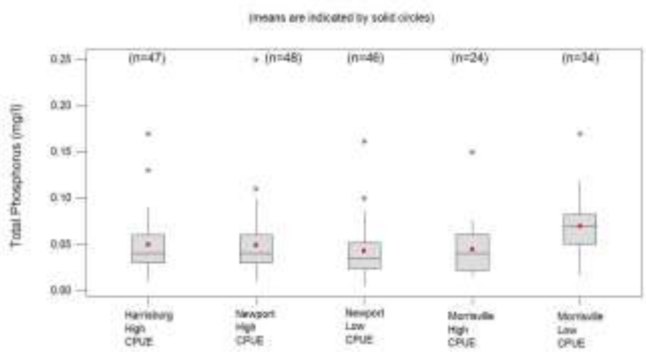
Next, we used parametric and non-parametric analyses to determine if there were significant differences in water temperature (Temp), total phosphorus (TP), and pH conditions of the analysis groups shown in Table 1. We conducted one set of analyses using Temp, TP, and pH values collected throughout the entire year (ANOVA), and a second set of analyses (Kruskal-Wallis test) using only data collected under warm-water conditions (Temp ≥ 20 C). We also reviewed the limited amount of total orthophosphate, total nitrate-N (total NO3-N), and total nitrogen (TN) data collected at the three WQN stations, during the same timeframe.
Based on the analyses we conducted, there is no difference in the Temp or pH conditions of any of the analysis groups. However, low CPUE values co-occur with elevated TP values at the Delaware River Morrisville WQN station, but not in the Susquehanna CADDIS area (Figure 4), but based on a very limited amount of total orthophosphate data, there is no difference in the total orthophosphate concentrations at Newport during low CPUE years and Morrisville during either high or low CPUE years (Figure 5).

Nitrogen data from Harrisburg and Newport was limited to total NO3-N during high CPUE years (1991-1994), and TN data collected at Newport during low CPUE years (2005-2010). Thus, no real comparison of nitrogen conditions during high CPUE years vs. low CPUE years was made. However, the available nitrogen data suggest that nitrogen conditions at Newport during high CPUE years (NO3-N mean=1.20 mg/l, n=48) were relatively similar to those during low CPUE years (TN mean=1.59 mg/l, n=47). It's unlikely the TN conditions observed at Newport during low CPUE years were related to the low CPUE values during that time period. For example, in an analysis of the relationships between nutrient concentrations and biological responses in medium to large rivers, the Minnesota Pollution Control Agency (MPCA, 2008) reported that at TN values less than 1.5 – 2.0 mg/l, biological metric values exhibited no apparent relationship with TN. They also reported that biological metrics showed reduced numbers of sensitive (intolerant) taxa and an increase in tolerant taxa, and a shift in the fish community from piscivores to omnivores at TN values greater than 6 mg/l.

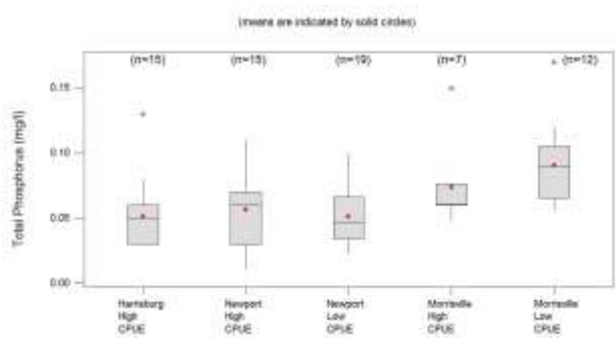
Lastly, the very limited CIM data we used as a surrogate measure for algal growth do not suggest a relationship between increased algal growth and the decline of the SMB fishery in the CADDIS area, based on YOY CPUE conditions. Our analysis, using data collected during years with mean June discharge values below the long-term mean flow, showed no meaningful co-occurrence of reduced relative CPUE values and the diurnal Temp, DO, or pH conditions typically associated with elevated algal productivity and photosynthetic activity (Figures 6, 7, and 8).

Conclusion:

The analyses we conducted yielded no definitive information that indicates algal or cyanobacterial toxins or decreased young of year habitat via increased algal growth, coincide with reduced young of year catch-per-unit-effort values and the decline of the smallmouth bass fishery in the defined CADDIS area (Susquehanna River at Harrisburg and Juniata River at Newport).



(A)



(B)

Figure 4. Total Phosphorus Values Collected Throughout the Entire Year (A) and Under Warm Water Conditions (Temp ≥ 20 C) Associated with Relatively High SMB YOY CPUE Values, and Relatively Low YOY CPUE Values in the Defined CADDIS Area (Harrisburg and Newport) and Morrisville (Entire Year Data ANOVA p = 0.003, Warm-Water Data Kruskal-Wallis Test p=0.001).

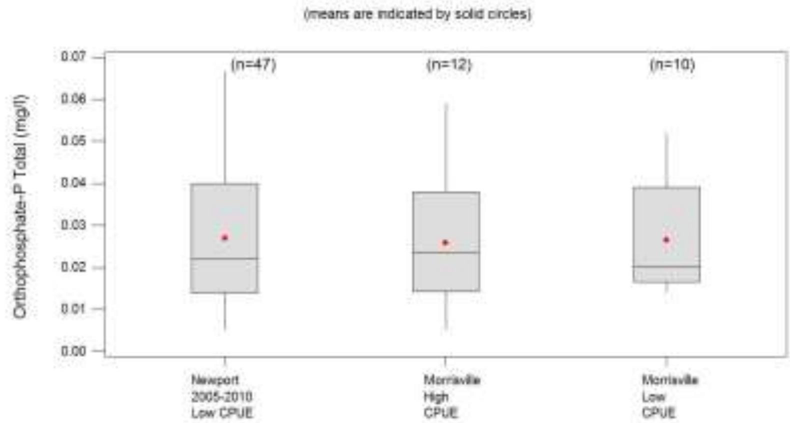


Figure 5**.** Total Orthophosphate Values Associated with Relatively Low SMB YOY CPUE Values in the Defined CADDIS Area at Newport, and Relatively High and Relatively Low YOY CPUE Values at Morrisville (Kruskal-Wallis Test p=0.957).

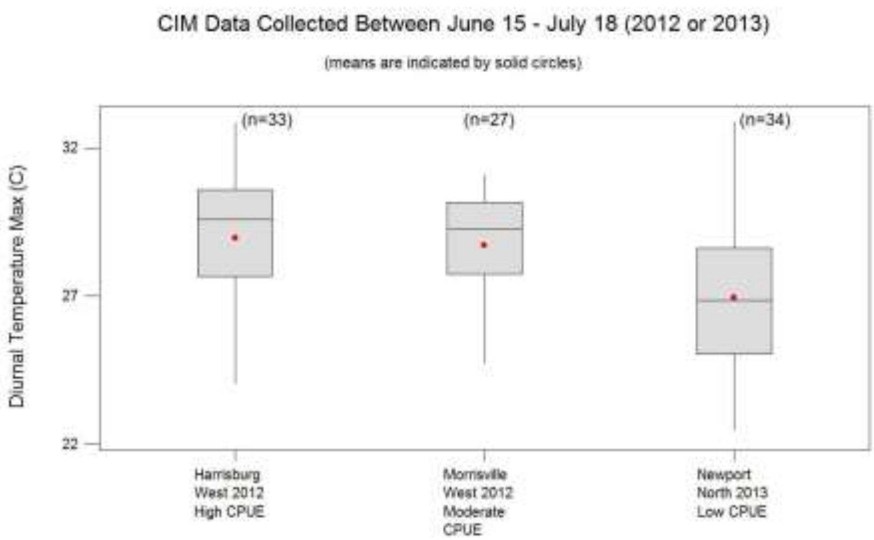
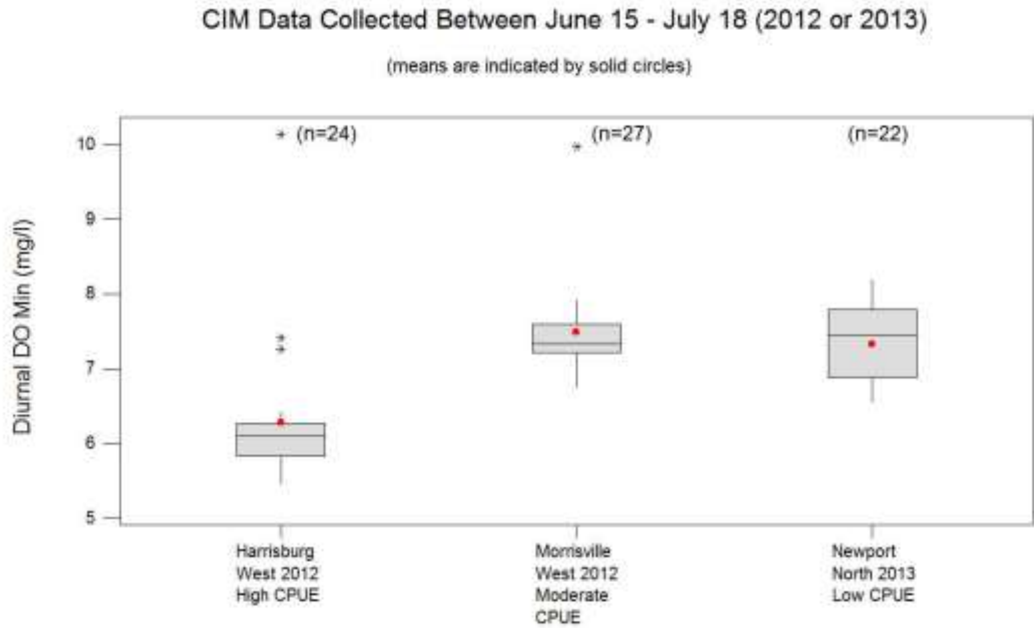
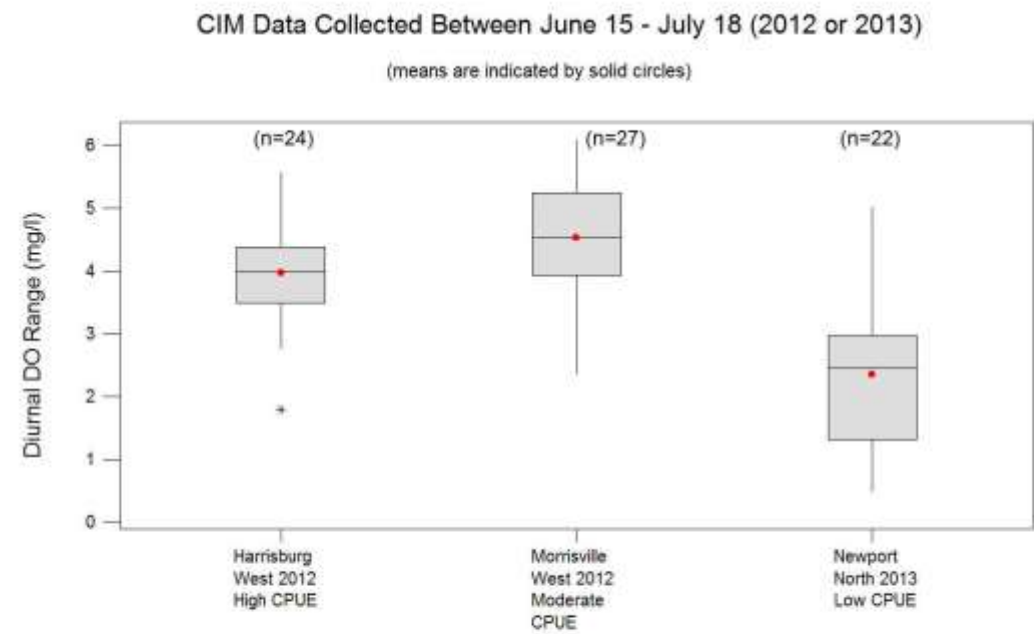


Figure 6. Diurnal Maximum Temperature Values Recorded in the Defined CADDIS Area (Harrisburg and Newport) and at Morrisville (Kruskal-Wallis Test p=0.001).

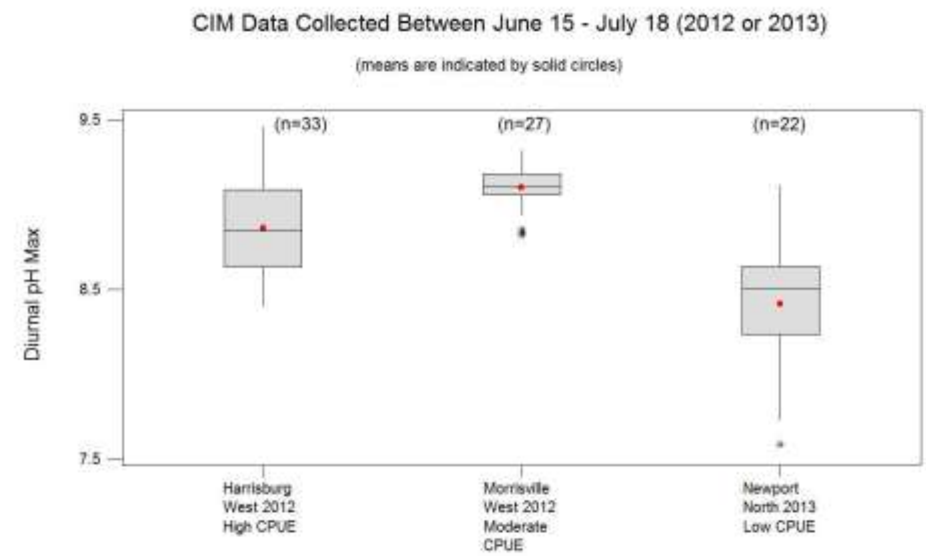


(A)

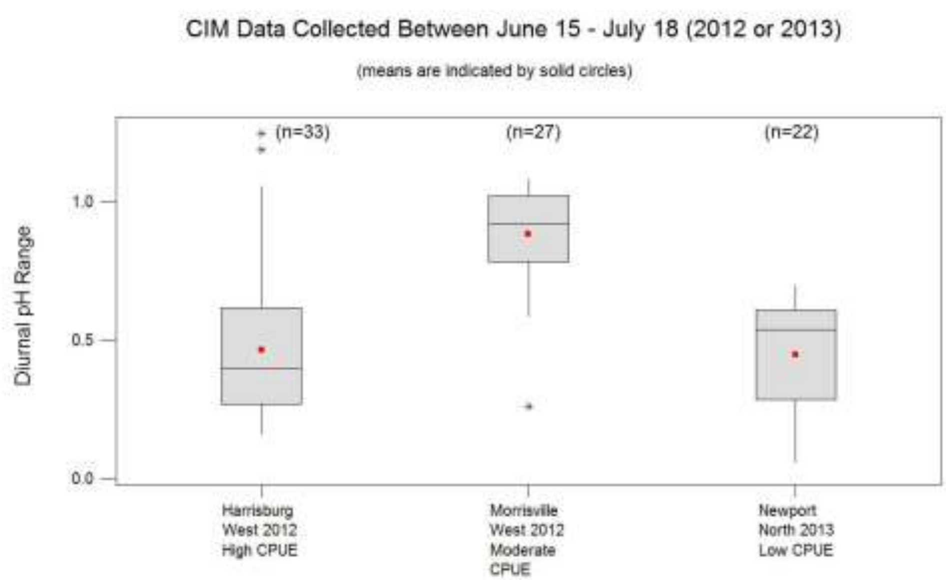


(B)

Figure 7. Diurnal Minimum Dissolved Oxygen Values (A) and Diurnal Dissolved Oxygen Range Values (B) Recorded in the Defined CADDIS Area (Harrisburg and Newport) and at Morrisville (Kruskal-Wallis Test DO Min p=0.000, and DO Range p=0.000).



(A)



(B)

Figure 8. Diurnal pH Maximum Values (A) and Diurnal pH Range Values (B) Recorded in the Defined CADDIS Area (Harrisburg and Newport) and at Morrisville (Kruskal-Wallis Test pH Max p=0.000, and pH Range p=0.000).

Literature Cited:

Davis, W.D., D.L. Berry, G.L. Boyer, & C.J. Gobler. 2009. The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae: 8:715-725.

MPCA. 2008. Relation of nutrient concentrations and biological responses in Minnesota streams:applications for river nutrient criteria development. Minnesota Pollution Control Agency. Saint Paul, MN.

Smith, S.M., J.S. Odenkirk, & S.J. Reeser. 2005. Smallmouth bass recruitment variability and itrelation to stream discharge in three Virginia rivers. North American Journal of Fisheries Management 25:1112-1121.

Title: Decreases in late summer YOY habitat as a result of reduced late summer flows and YOY Mortality (WS #36)

Agency: Pennsylvania Department of Environmental Protection

Stressor #6: Criteria, Co-occurrence, and Spatial Comparison Worksheet

Candidate Cause: Decreases in late summer YOY habitat as a result of reduced late summer flows may increase YOY mortality

Introduction:

Lower summer flows during certain years in the CADDIS area may result in higher temperatures and reduced dissolved oxygen concentrations in smallmouth bass YOY habitat after spring hatching. During the first few months of life, which is a critical period for survival and development for YOY SMB, YOY inhabit localities in river systems which are characterized by Chaplin and Crawford (2012) as microhabitats with relatively shallow, slow-moving water in near shore areas. The YOY microhabitats generally have lower dissolved oxygen concentrations then nearby main channel locations (Chaplin et al 2009). The U.S. Environmental Protection Agency established a dissolved oxygen criterion of 5.0 mg/L to be protective of early life stages of warm water fishes (U.S. EPA, 1986). Many studies show that Centrarchid (bass family) juveniles may be the most sensitive of all warm water fishes to low DO concentrations. At or below dissolved oxygen concentrations of 4.5 mg/L, SMB hatchling and larvae survival was observed to be significantly reduced (Siefert et al., 1974; Spoor, 1984). Lethal and sublethal effects of reduced D.O. (less than 5 mg/L) witnessed in laboratory experiments were, in general, directly related to exposure times which ranged from hours to days (Mount, 1964; Doudoroff & Shumway, 1970; Siefert et al., 1974; Spoor, 1984). Stressful conditions for YOY would be expected at microhabitat locations with higher temperatures and where dissolved oxygen concentrations recede below the criterion during the critical period in low flow years resulting in a weaker year class (reduced recruitment into age class 1) or greater susceptibility of YOY to disease.

Data:

The data used for this worksheet is a summary of the USGS study conducted from 2008 to 2010 to investigate the effect of stream flow on YOY microhabitat conditions at stations on the Delaware River at Trenton, N.J., Susquehanna River below Clemson Island, Susquehanna River at Harrisburg, Juniata River at Newport, Juniata River at Howe Township Park, and Allegheny River at Acmetonia (Chaplin and Crawford 2012). Dissolved oxygen, water temperature, pH, and specific conductance were compared at seven stations from May 1 to July 31 in 2008, 2009, and 2010. Sondes were deployed at each station in YOY SMB microhabitat and in pairs at 2 stations in 2008 to compare main-channel habitats and YOY habitats of the Susquehanna River below Clemson Island and Juniata River near Howe Township Park. In addition 2 sondes were deployed at stream gages to characterize main channel habitats of the Susquehanna River at Harrisburg and Juniata River at Newport.

Analysis and Results:

Main channel and microhabitat comparisons within CADDIS area

Median streamflow during the critical period (May 1 through July 31) in the Susquehanna River at Harrisburg (USGS 01570500) for the three years of the USGS study was 13,100 ft^3/s in 2008, 26,300 ft^3/s in 2009, and 14,300 ft^3/s in 2010.

In the microhabitat at station C4 (Clemson Island, Susquehanna River) of the USGS study, the minimum dissolved oxygen concentrations in 2008 was below 5.0 mg/L for 31 days during the critical period and was below 5.0 mg/L for periods lasting up to 8.5 hours (Chaplin and Crawford, 2012 Figure 1). In 2009, a year with higher stream flow during the critical period, dissolved oxygen concentrations was at or below 5.0 mg/L for only 2 days at the same station.

At station C6 (Juniata River microhabitat near Howe Township Park) of the USGS study, the concentration of dissolved oxygen was less than 5.0 mg/L for 20 days and periods up to 7 hours in 2008 and was less than 5.0 mg/L on 11 days for periods up to 8 hours in 2010.

In general, microhabitats in the Susquehanna River and Juniata River for small mouth bass YOY have lower dissolved oxygen concentrations during the critical period than nearby main channel locations (Chaplin and Crawford 2012).

Comparison of CADDIS area conditions to out of basin stations

Chaplin and Crawford (2012) concluded that for the critical period of each year particularly during June and July, dissolved oxygen was consistently lower and water temperatures were consistently warmer in the Susquehanna River at Harrisburg (C8) than in the Delaware River at Trenton (C1) and Allegheny River at Acmetonia (C10). In 2008, daily minimum DO during the critical period fell below 5.0 mg/L at both Juniata River stations and the station on the Susquehanna River at Harrisburg but not at the station on the Delaware River at Trenton (C1) or the Allegheny River station at Acmetonia (C10). In addition, Chaplin and Crawford found that dissolved oxygen concentrations receded below 5.0 mg/L for 5 days in 2008 and 14 days in 2010 but did not recede below the U.S. EPA criterion at C1 or C10 during the study duration.

In 2008, the basin stations tended to have warmer daily maximum temperatures during the critical period then out of basin stations (Figure 2). Chaplin and Crawford reported that median daily maximum temperatures at C8 were 2.0 °C warmer than those at C1 in 2008, 2.7 °C warmer in 2009 and 1.6 °C warmer in 2010. Median daily maximum temperatures were also warmer for all three years at C8 vs. C10 (2008 by 3.1 °C, 2009 by 1.2 °C and 2010 by 3.4 °C).

Conclusion:

The USGS study conducted by Chaplin and Crawford (2012) supports the hypothesis that diminished YOY habitat in the basin during years of low stream flow may be contributing to more stressful conditions for YOY smallmouth bass and the decline in recruitment of YOY small mouth bass into age class 1 in the basin. In 2008 and 2010, the low flow years, dissolved oxygen concentrations fell below the U.S. EPA criterion for dissolved oxygen concentrations that would be protective of early life stages of warm water fishes in YOY microhabitat areas on the Susquehanna River at Clemson Island and on the Juniata River at Howe Park and tended to be lower than nearby main channel locations. In addition, main channel locations at 3 out of 4 basin sites (Susquehanna River at Harrisburg, Juniata River at Howe Park, and Juniata River at Newport) experienced DO concentrations during the critical period lower than the U.S. EPA criterion in 2008 and 2010. Basin sites typically were warmer than the two out-of-basin controls during the critical period and and dissolved oxygen concentrations were higher at the two out-of-basin sites. Dissolved oxygen concentrations at the two out-of-basin control sites did not recede below 5.0 mg/L during the study.

Literature Cited:

Chaplin, J.J., & Crawford, J.K., 2012. Streamflow and water-quality monitoring in response to young-of-year smallmouth bass *Micropterus dolomieu*) mortality in the Susquehanna River and major tributaries, with comparisons to the Delaware and Allegheny Rivers, Pennsylvania, 2008–10: U.S. Geological Survey Open-File Report 2012–1019, 39 p.

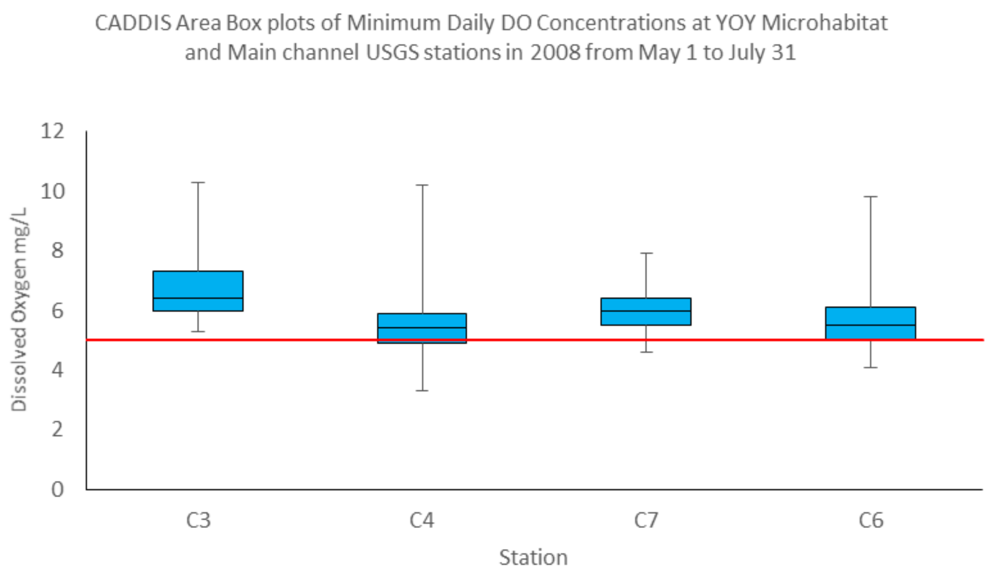
Chaplin, J.J., Crawford, J.K., & Brightbill, R.A., 2009. Water quality monitoring in response to young-of-the-year smallmouth bass (*Micopterus dolomieu*) mortality in the Susquehanna River and major tributaries, Pennsylvania: 2008: U.S. Geological Survey Open-File Report 2009-1216, 59 p.

Doudoroff, P. & D.L. Shumway. 1970. Dissolved Oxygen Requirements of Freshwater Fishes. Food and Agriculture Organization of the United Nations. Fisheries Technical Paper 86.

Siefert, R.E., A.R. Carlson & L.J. Herman. 1974. Effects of Reduced Oxygen Concentrations on the Early Life Stages of Mountain Whitefish, Smallmouth Bass, and White Bass. The Progressive Fish Culturist. 36(4):186-190.

Spoor, W.A. 1984. Oxygen requirements of larvae of smallmouth bass, *Micropterus dolomieui* Lacépède. Journal of Fish Biology. 25:587-592.

U.S. Environmental Protection Agency. 1986. Ambient water quality criteria for dissolved oxygen: Washington, D.C., U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Criteria and Standards Division, EPA 440/5-86-003, April 1986, 46 p.



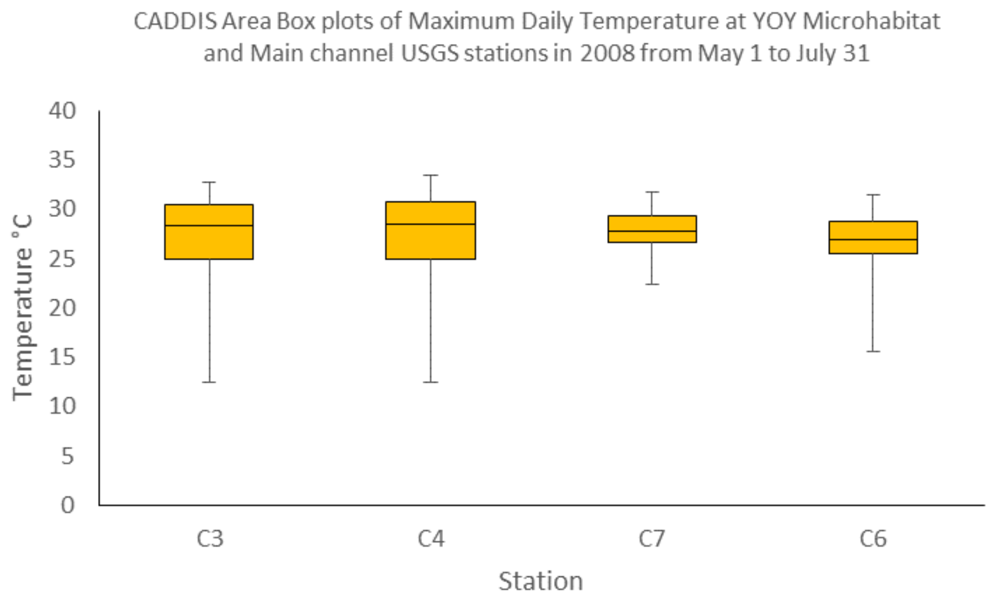
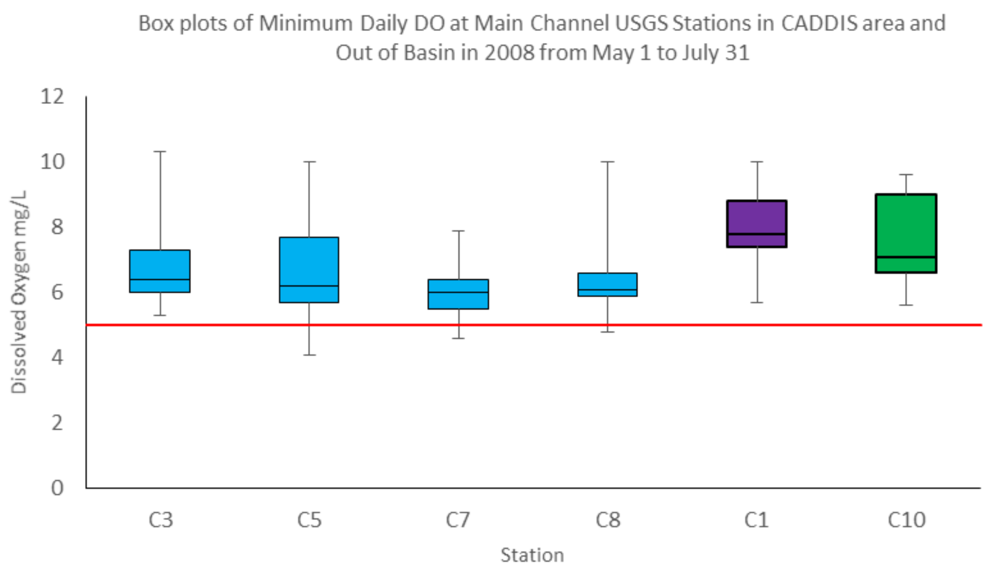


Figure 1. Reproduced from summary statistics in Chaplin and Crawford (2012), Appendix 1, p.28. Main channel stations are C3 (Susquehanna River below Clemson Island) and C7 (Juniata River near Howe Township Park). YOY microhabitat stations are C4 (Susquehanna River at Clemson Island) and C6 (Juniata River near Howe Township Park). Minimum daily dissolved oxygen concentrations (mg/L) and maximum daily temperature (degrees Celsius) in 2008 from May 1 to July 30 are compared between the four locations.



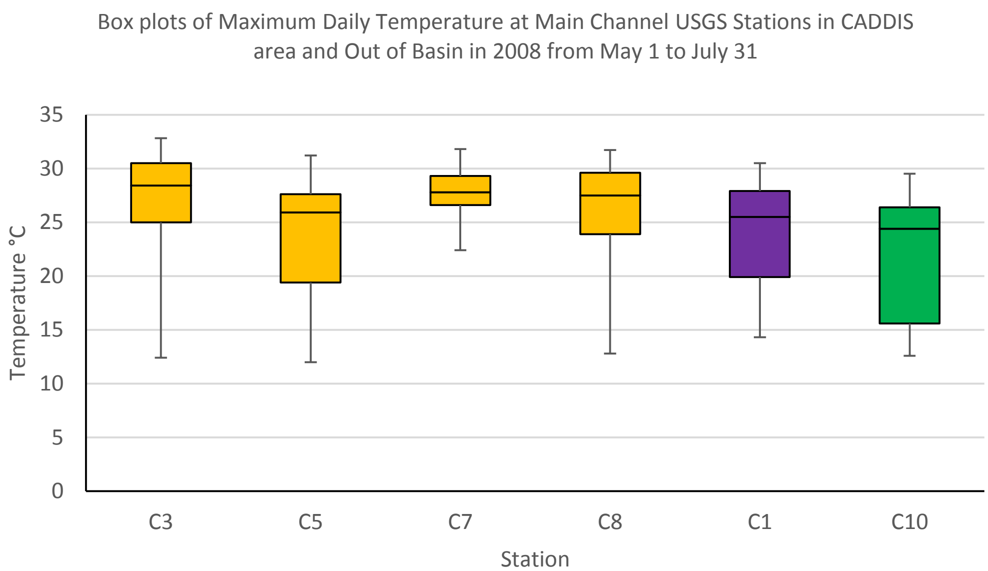


Figure 2. Reproduced from summary statistics in Chaplin and Crawford 2012, Appendix 2, p.34. Main channel stations in the CADDIS area are C3 (Susquehanna River below Clemson Island), C5 (Juniata River at Newport), C7 (Juniata River near Howe Township Park), C8 (Susquehanna River at Harrisburg). Main channel stations at the out of basin areas are C1 (Delaware River at Trenton) and C10 (Allegheny River at Lock and Dam 3, Acmetonia) Minimum daily dissolved oxygen concentrations (mg/L) and maximum daily temperature (degrees Celsius) in 2008 from May 1 to July 30 are compared between the locations.

Candidate Cause 7: Temperature

Worksheets: 7, 8, 9, 10, 11, and 12

Title: Continuous data from SRBC Early Warning System Dataset for Rockville site (WS #7)

Agency: Susquehanna River Basin Commission

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

A comparison of continuous instream monitoring data from Rockville showing some combination of max, min and average pH and temperature was analyzed. Data is used to compare trends in pH and temp swings/maxes pre and post 2005 when the SMB issue was first reported. Complete data sets are uploaded onto Environmental Science Connector. Only max pH and average temp is shown here.

Data:

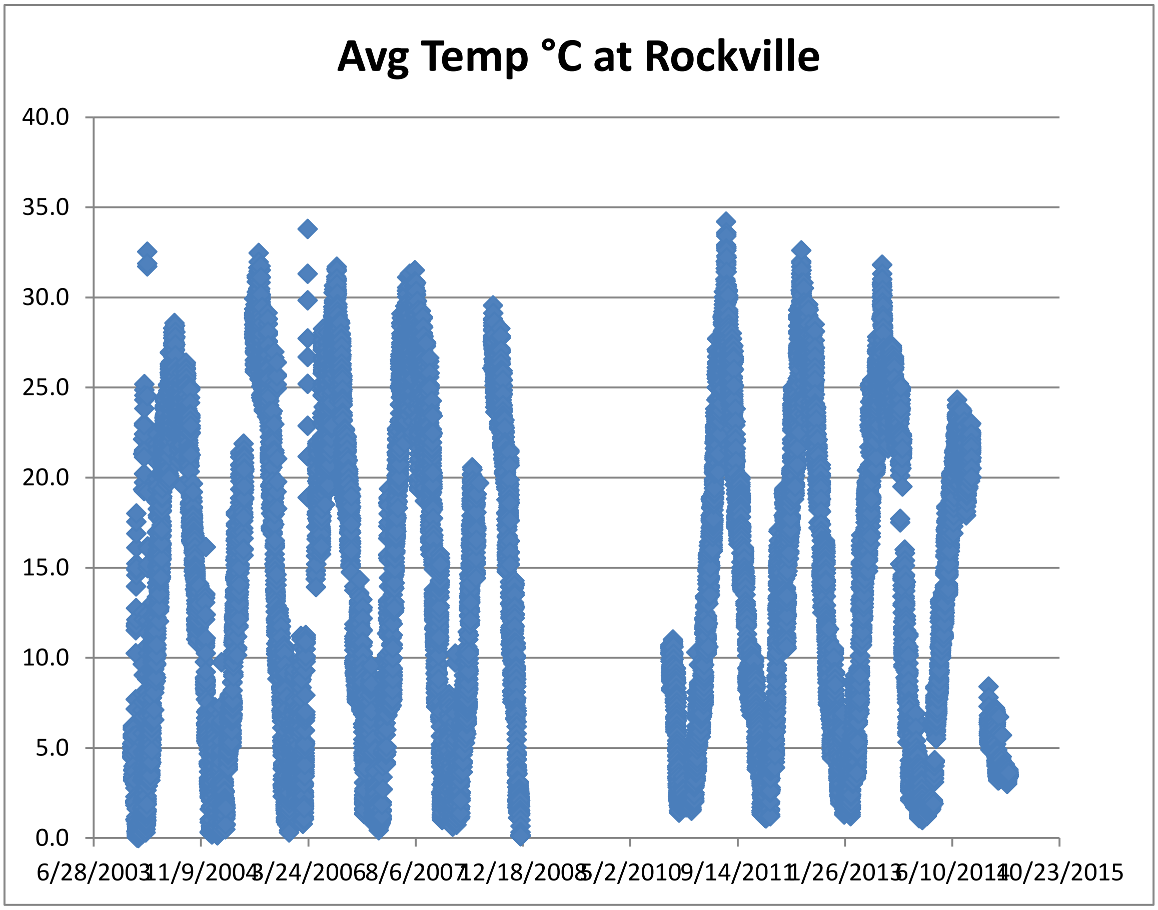
SRBC has facilitated data collection upstream of numerous major water supply intake points through its Early Warning System (EWS) program. At Rockville on the Susquehanna River, a site is located downstream of Rockville RR bridge on the left descending bank (approx. 200 ft from shore). (40.33629 -76.906294). Flow through sensors are maintained by United Water of Pennsylvania and serviced by HACH. They are serviced and calibrated frequently.

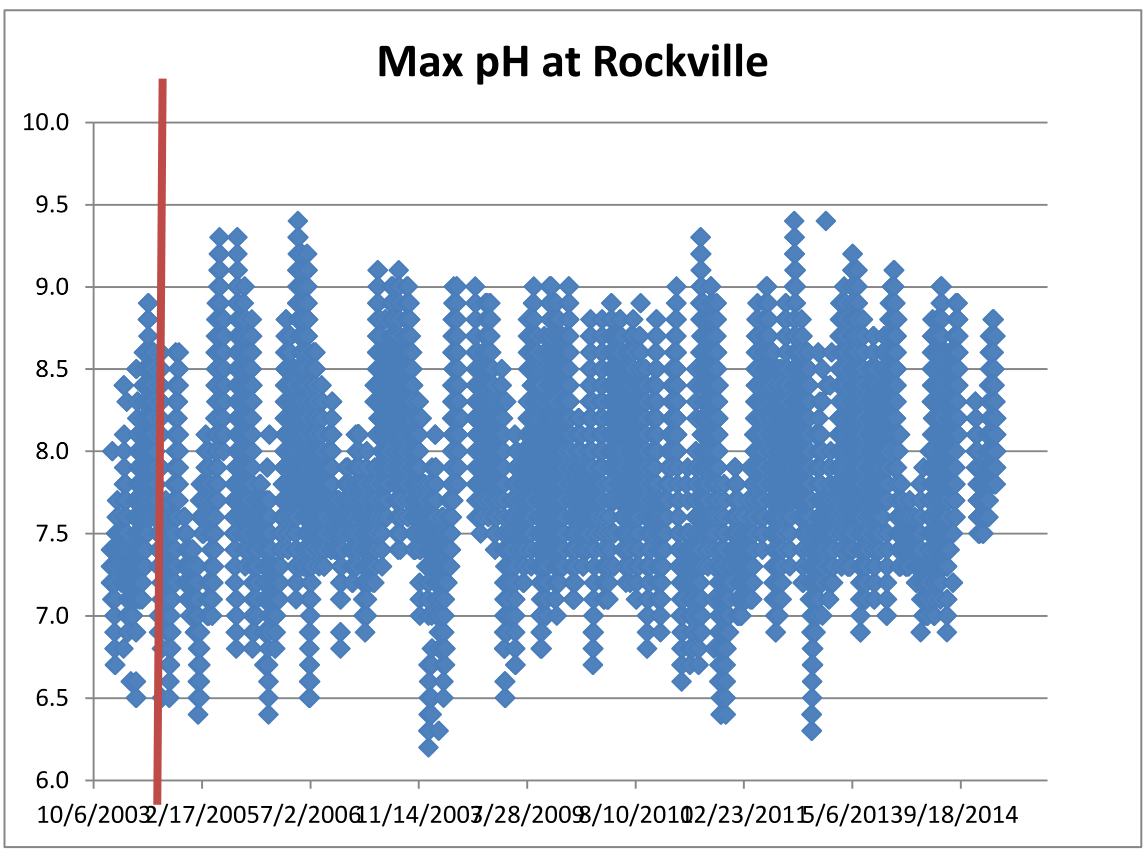
Data has been archived by SRBC and was compiled for CADDIS. QA/QC was done to eliminate values of no data and invalid data (i.e. pH = 20)

Analysis and Results:

Average temperature data for period of record is shown in Figure 1. The mean value of readings (taken every 15 min) over 4 hour period is shown. Data were reported every four hours. There does not appear to be much difference in annual temperature swings before 2005 or after

Figure 2 shows the maximum pH value for the period of record. This shows the max value recorded in a four hour increment prior to the reported value. Note: no pH values over 9.0 were seen prior to 2005. The red line shows where 2005 data starts.


Avg Temp °C at Rockville
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
6/28/2003
11/9/2004
3/24/2006
8/6/2007
12/18/2008
5/2/2010
9/14/2011
1/26/2013
6/10/2014
10/23/2015


Max pH at Rockville
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
10/6/2003
2/17/2005
7/2/2006
11/14/2007
3/28/2009
8/10/2010
12/23/2011
5/6/2013
9/18/2014

Title: Continuous data from SRBC Early Warning System Dataset for Danville site (WS #8)

Agency: Susquehanna River Basin Commission

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

A comparison of continuous instream monitoring data from Danville showing some combination of max, min and average pH and temperature was analyzed. The location is outside of the study area for poor recruitment of SMB. Complete data sets are uploaded onto the Environmental Science Connector, with only max pH and average temp shown here.

Data:

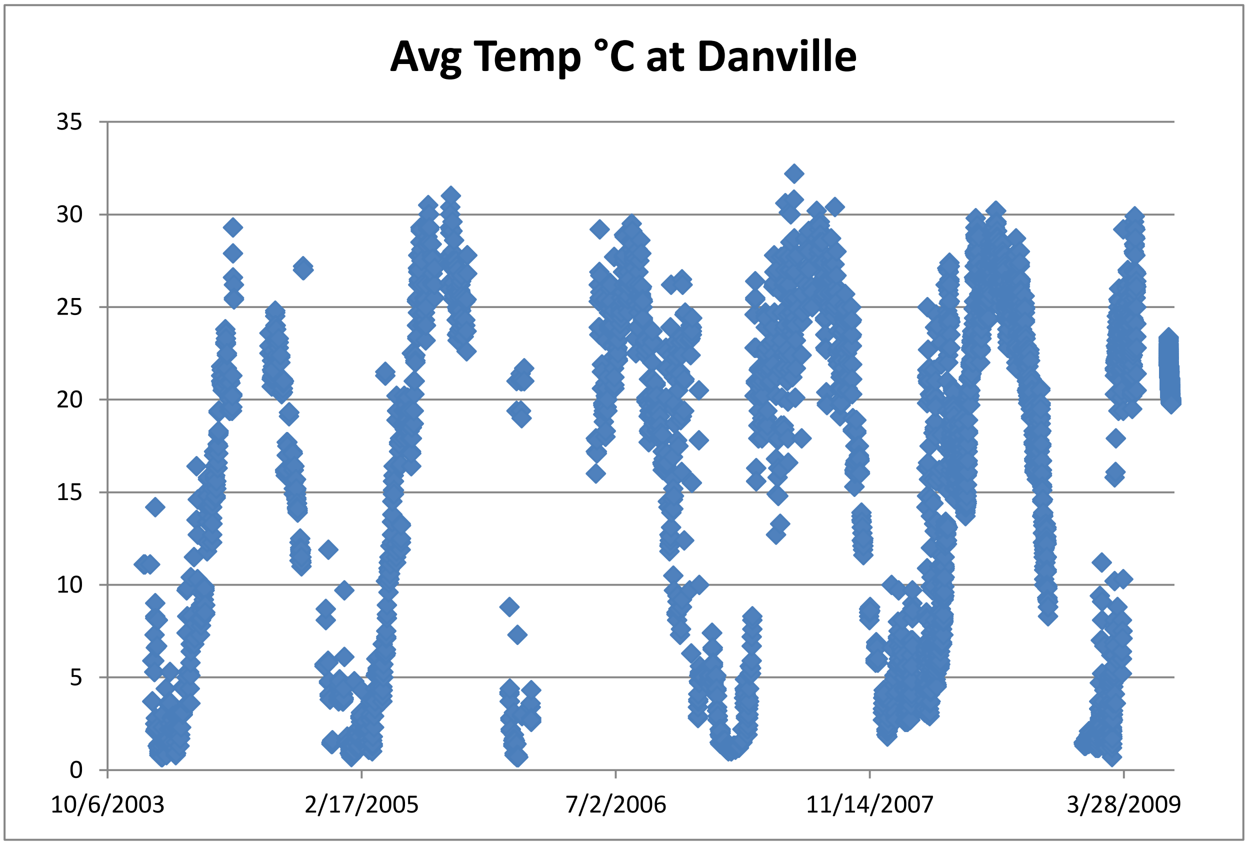
SRBC has facilitated data collection upstream of numerous major water supply intake points through its Early Warning System (EWS) program. This site is located near Danville soccer park on the right descending bank (40.945972 -76.606287) and data is collected using flow through sensors.

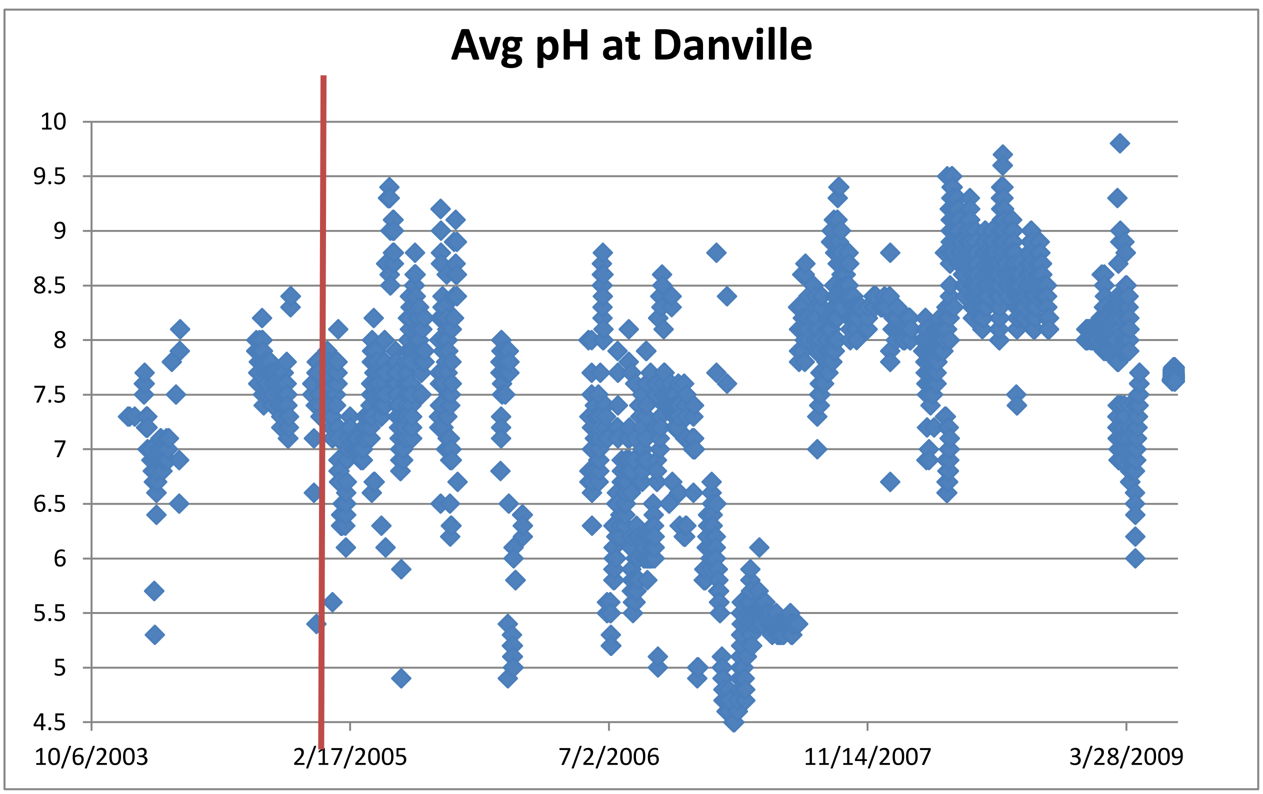
Data has been archived by SRBC and was compiled for CADDIS. QA/QC was done to eliminate values of no data and invalid data (i.e. pH = 20)

Analysis and Results:

Average temperature data for period of record is shown in Figure 1. This shows the mean value of readings (taken every 15 min) over a 4 hour period. Data was reported every four hours (through June 2009, when 15 minute readings started). There does not appear to be much difference in annual temperature swings before 2005 or after.

Figure 2 shows average pH values for the period of record. The average value recorded for four hour increments (readings taken every 15 min) prior to reported value is shown. Note: no pH values over were 9.0 prior to 2005 at Danville. The red line shows where 2005 data starts.


Avg Temp °C at Danville
35
30
25
20
15
10
5
0
10/6/2003
2/17/2005
7/2/2006
11/14/2007
3/28/2009


Avg pH at Danville
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
10/6/2003
2/17/2005
7/2/2006
11/14/2007
3/28/2009

Title: Continuous data from SRBC Early Warning System Dataset for Columbia site (WS #9)

Agency: Susquehanna River Basin Commission

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

A comparison of continuous instream monitoring data from Columbia showing some combination of max, min and average pH and temperature was analyzed. The goal was to compare trends in pH and temp swings/maxes pre and post 2005, when SMB issue were first reported. Complete data sets are uploaded onto the Environmental Science Connector with only max pH and average temp shown here.

Data:

SRBC has facilitated data collection upstream of numerous major water supply intake points through its Early Warning System (EWS) program. The site at Columbia is located downstream of the 462 Bridge "Veterans Memorial Bridge" along the left descending bank (40.028556 -76.508715) and is managed by Columbia Water Company.

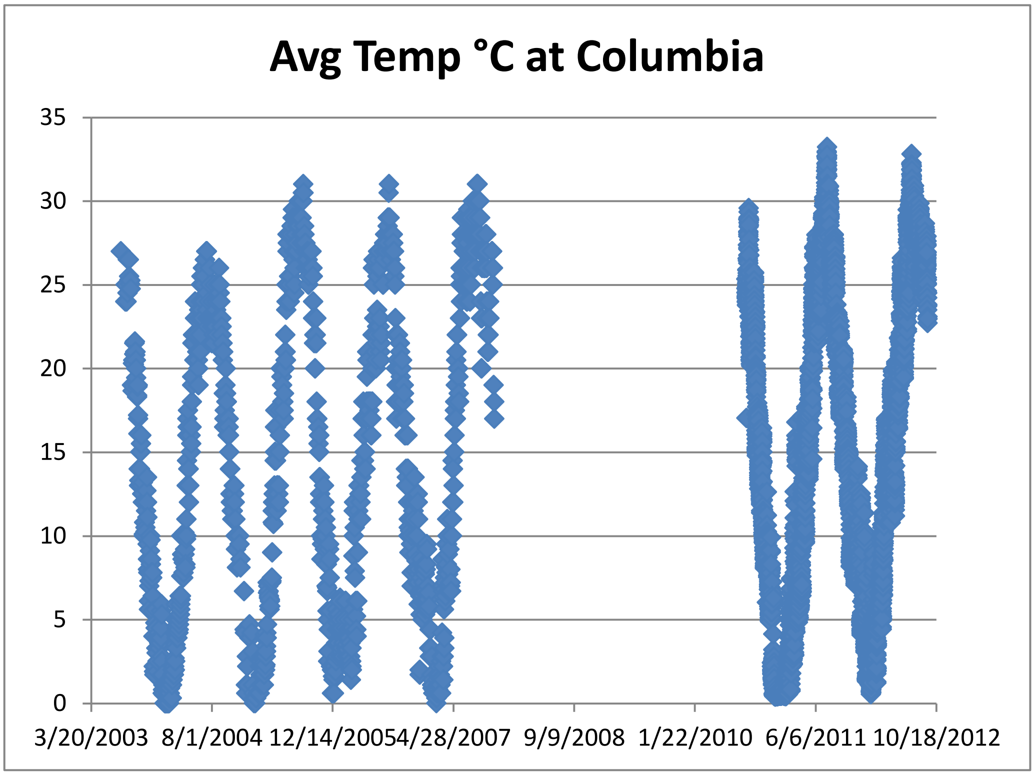
Data has been archived by SRBC and compiled for CADDIS. QA/QC was done to eliminate values of no data and invalid data (i.e. pH = 20)

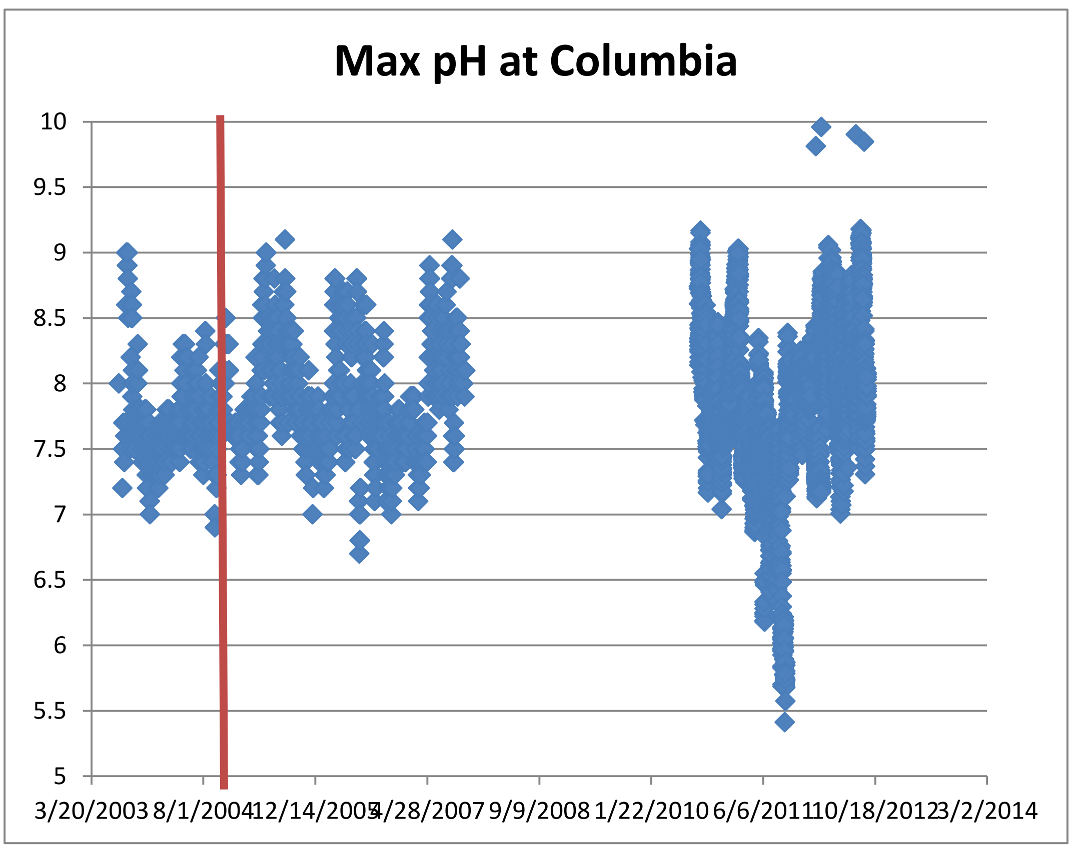
There were a few large data gaps where data were unavailable.

Analysis and Results:

Average temperature data for period of record is shown in Figure 1. The first part of the data set is the mean value of readings (taken every 15 min) over a 4 hour period. Data were reported every four hours. Starting in 2010, readings were recorded every 15 minutes . There does not appear to be much difference in annual temperature swings before 2005 or after.

Figure 2shows the maximum pH values for the period of record. The max value recorded in four hour increments prior to the reported value is shown. Starting in 2010, data were recorded every 15 minutes. There were few readings greater than 9.0 over the period of record.


Avg Temp °C at Columbia
35
30
25
20
15
10
5
0
3/20/2003
8/1/2004
12/14/2005
4/28/2007
9/9/2008
1/22/2010
6/6/2011
10/18/2012


Max pH at Columbia
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
3/20/2003
8/1/2004
12/14/2005
4/28/2007
9/9/2008
1/22/2010
6/6/2011
10/18/2012
3/2/2014

Title: Intra and inter site comparison of water temperature using Data from the Case (WS #10)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction: High water temperatures may contribute directly or indirectly to recruitment of YOY into age class 1+ by lower solubility of dissolved oxygen, direct stress resulting from an inability to acclimate to warming water temperatures, potential for greater pathogen populations, and magnified physiological responses to other stressors. If higher water temperatures exist in YOY microhabitats compared to the main channel, then it's possible that these microhabitat conditions are more stressful for SMB.

Data:

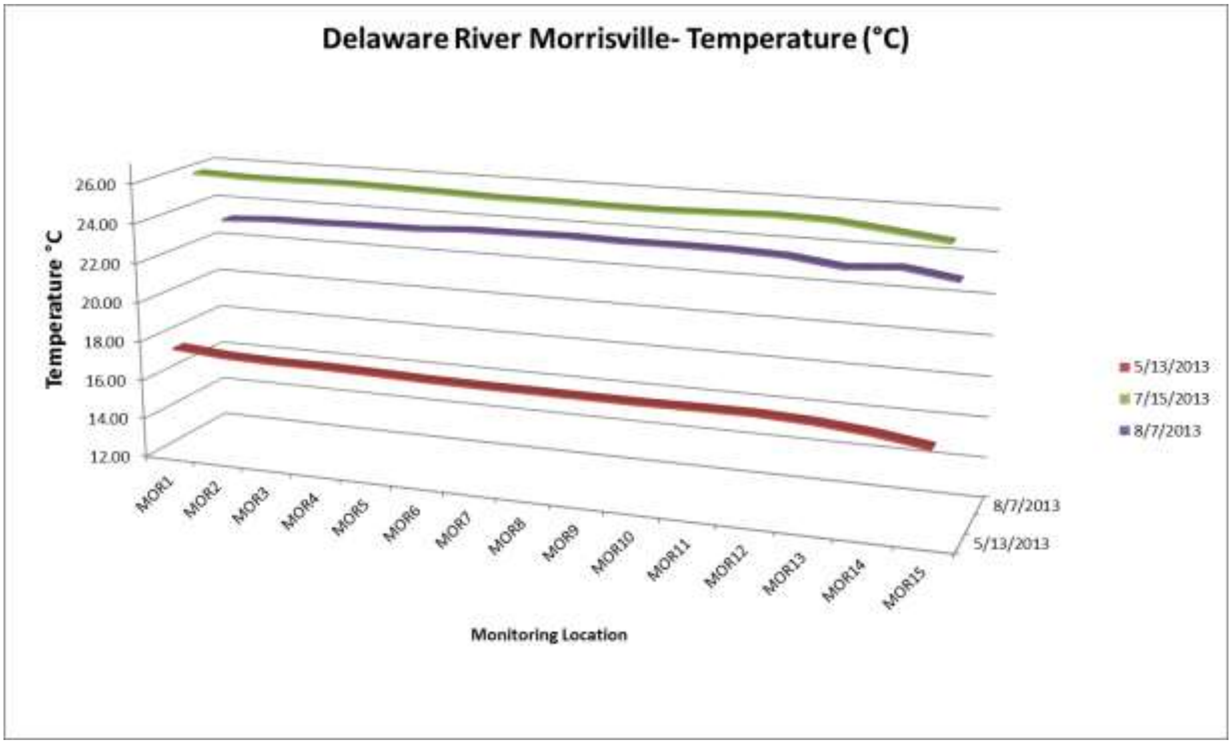
The Pennsylvania Department of Environmental Protection collected discrete water quality data along transects at various sites within and outside of the defined CADDIS area during 2013. Sites outside the define CADDIS area included the Delaware River at Morrisville, Susquehanna River at Danville, Susquehanna River at Marietta, and the Juniata River at Lewistown. Sites within the defined CADDIS area included the Susquehanna River at Browns Island, Susquehanna River at Rockville, Susquehanna River at City Island, and the Juniata River at Newport.

Analysis and Results:

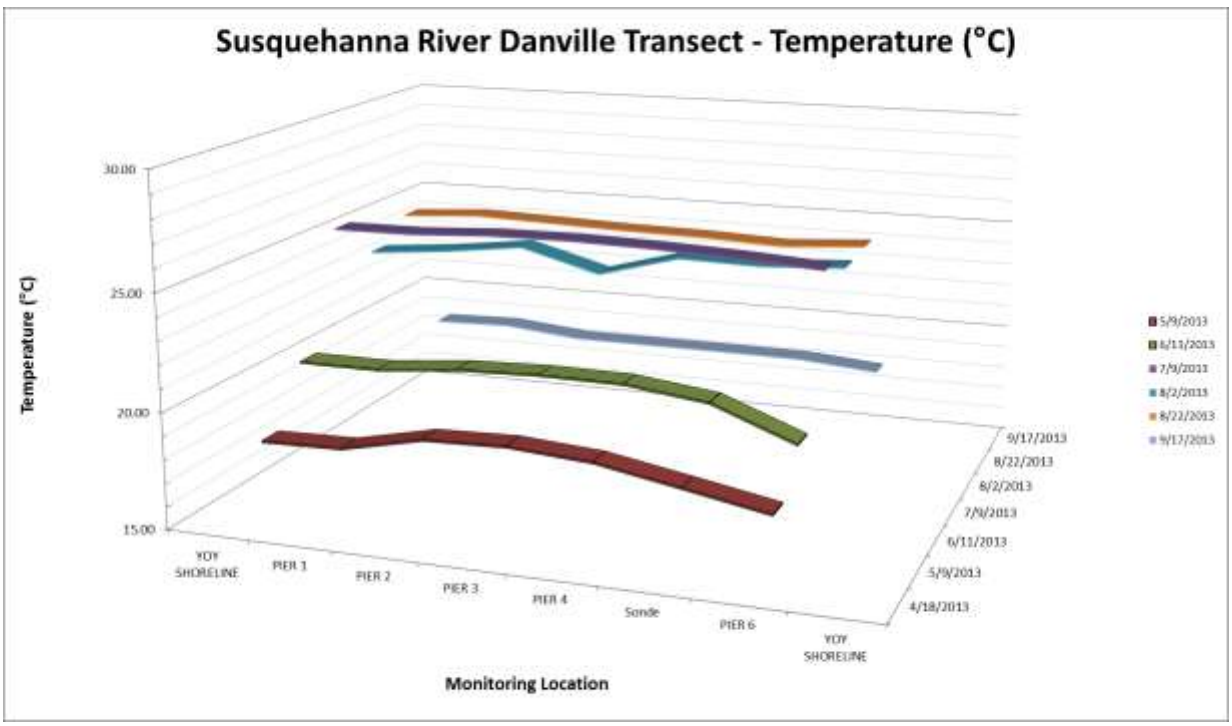
Transect data were plotted for each site to visually compare bank (or YOY) habitats to main channel habitats (see figures below). Results suggest that water temperatures were not drastically different between the habitat types at each site. Microhabitats did not always have higher water temperatures than main channel habitats, but were variable with slight consistently toward cooler temperatures. These findings are consistent with the habitat comparisons made by USGS in the Juniata River and Susquehanna River in 2008 (Chaplin et al. 2009). There were also no visual differences between habitats within versus outside the defined CADDIS area.

Conclusion:

The 2013 sampling year was characterized as relatively average-to-wet. Without more data, especially during lower flow years, it is difficult to determine how temperatures between the microhabitat and main channel habitat may differ. Additionally, like Chaplin et al. (2009) suggest, ability of smallmouth bass to acclimate to increasing temperature makes it difficult to determine a specific water-temperature threshold where stress may occur. Therefore, any small differences between microhabitats and main channel water temperatures are not likely to be directly affecting SMB.

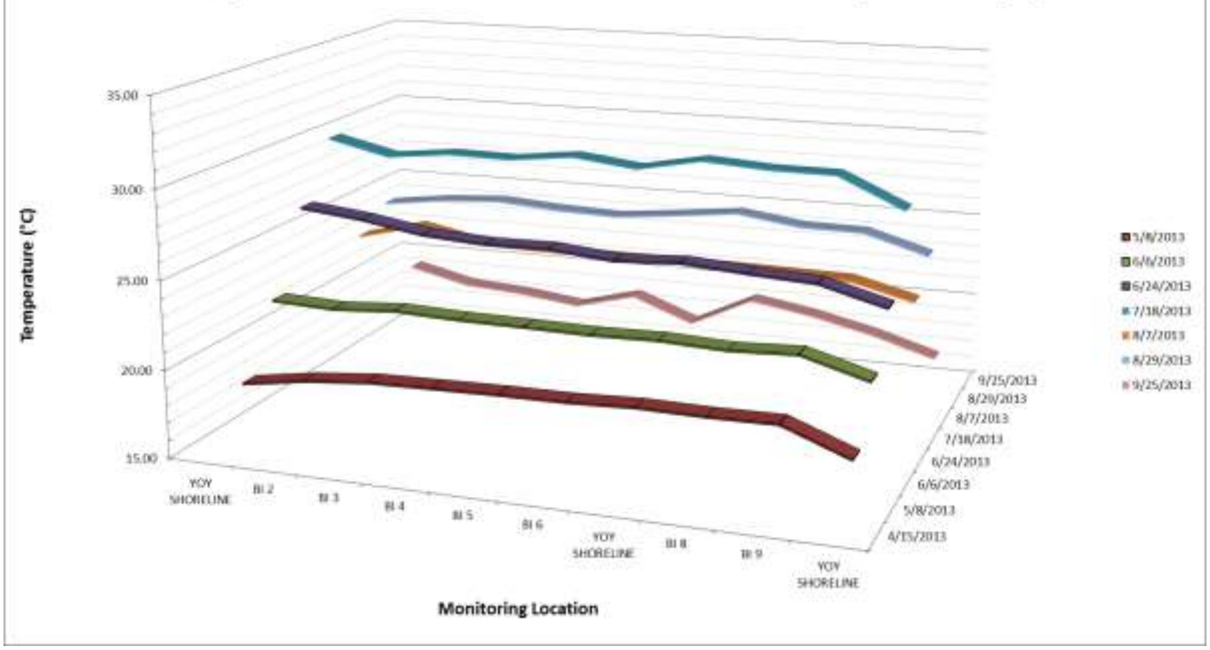


Delaware River Morrisville- Temperature (°C)
Temperature °C
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
MOR1
MOR2
MOR3
MOR4
MOR5
MOR6
MOR7
MOR8
MOR9
MOR10
MOR11
MOR12
MOR13
MOR14
MOR15
8/7/2013
5/13/2013
Monitoring Location
5/13/2013
7/15/2013
8/7/2013

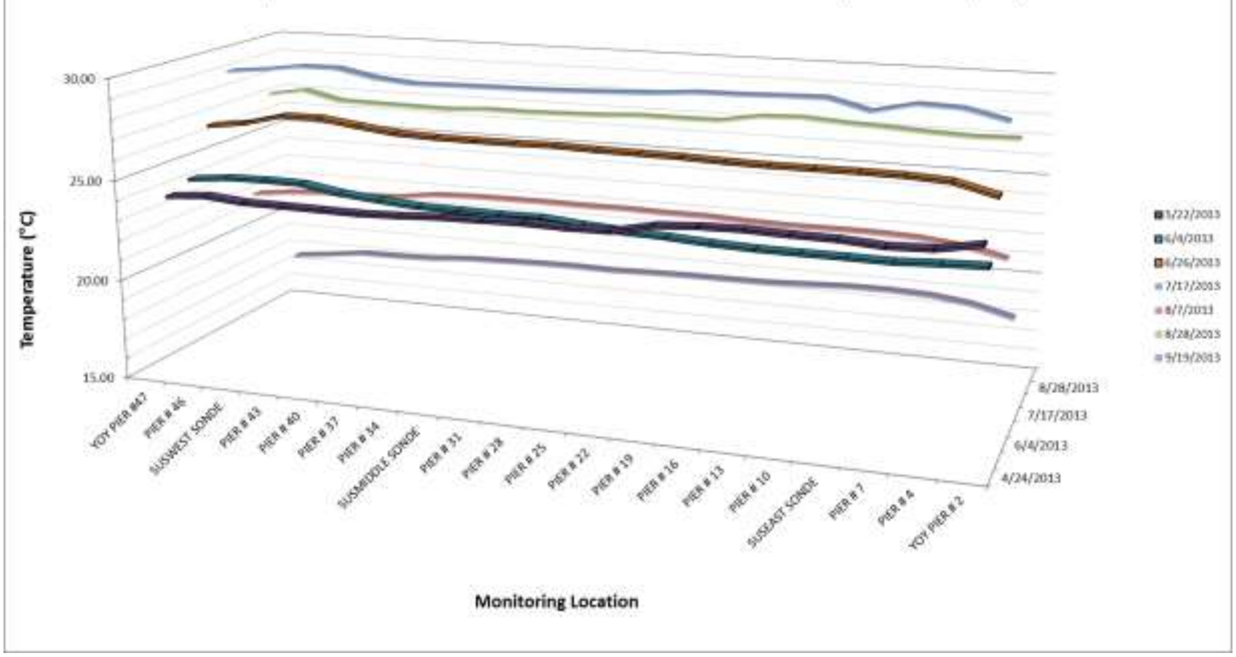


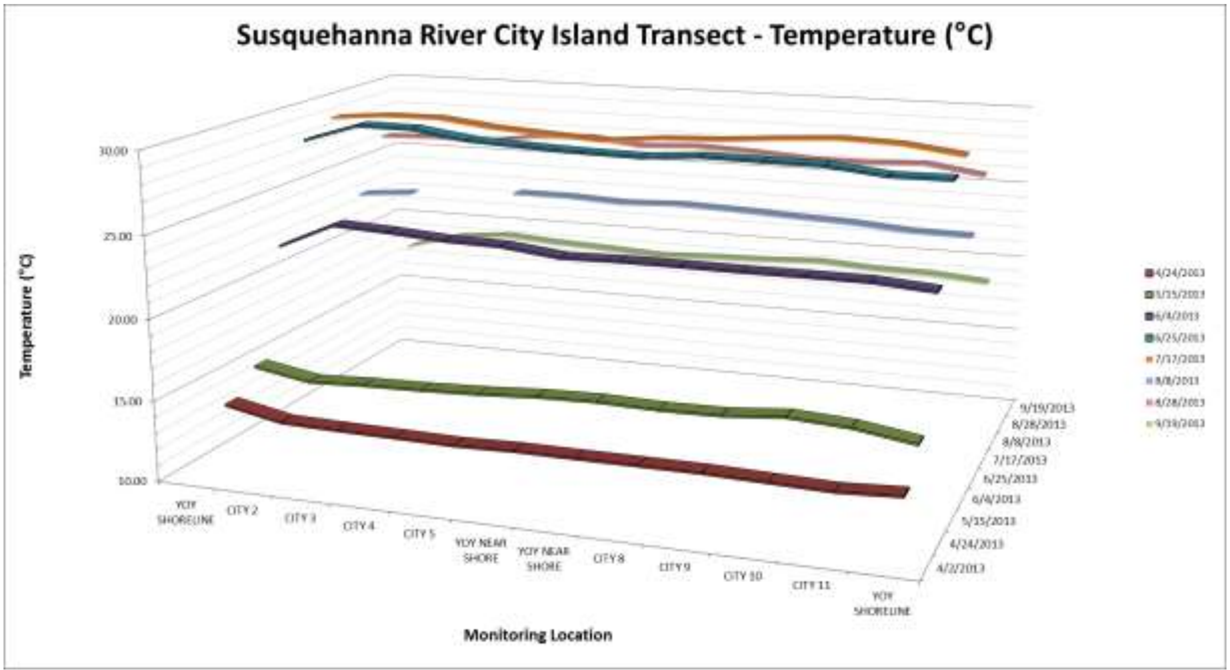
Susquehanna River Danville Transect - Temperature (°C)
Temperature (°C)
30.00
25.00
20.00
15.00
YOY SHORELINE
PIER 1
PIER 2
PIER 3
PIER 4
Sonde
PIER 6
YOY SHORELINE
9/17/2013
8/22/2013
8/2/2013
7/9/2013
6/11/2013
5/9/2013
4/18/2013
Monitoring Location
5/9/2013
6/11/2013
7/9/2013
8/2/2013
8/22/2013
9/17/2013

Susquehanna River Brown's Island Transect - Temperature (°C)



Susquehanna River Rockville Transect - Temperature (°C)




Susquehanna River City Island Transect - Temperature (°C)
Temperature (°C)
30.00
25.00
20.00
15.00
10.00
YOY SHORELINE
CITY 2
CITY 3
CITY 4
CITY 5
YOY NEAR SHORE
YOY NEAR SHORE
CITY 8
CITY 9
CITY 10
CITY 11
YOY SHORELINE
Monitoring Location
9/19/2013
8/28/2013
8/8/2013
7/17/2013
6/25/2013
6/4/2013
5/15/2013
4/24/2013
4/2/2013
4/24/2013
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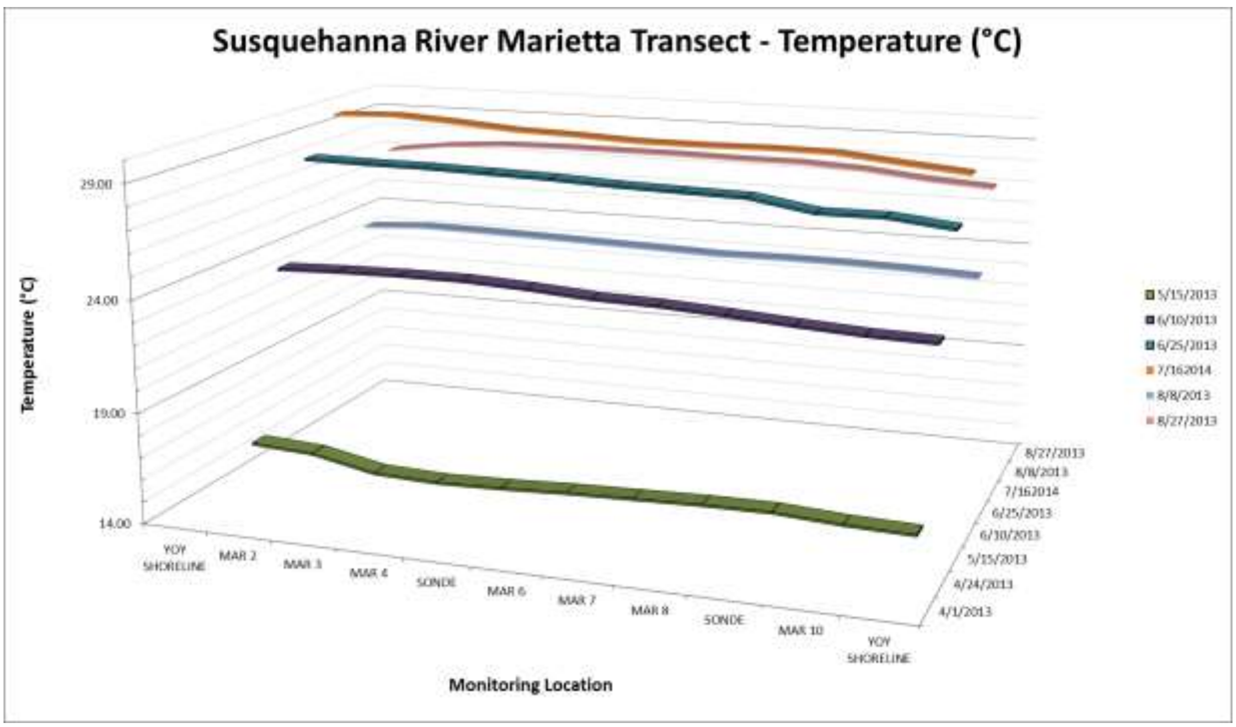
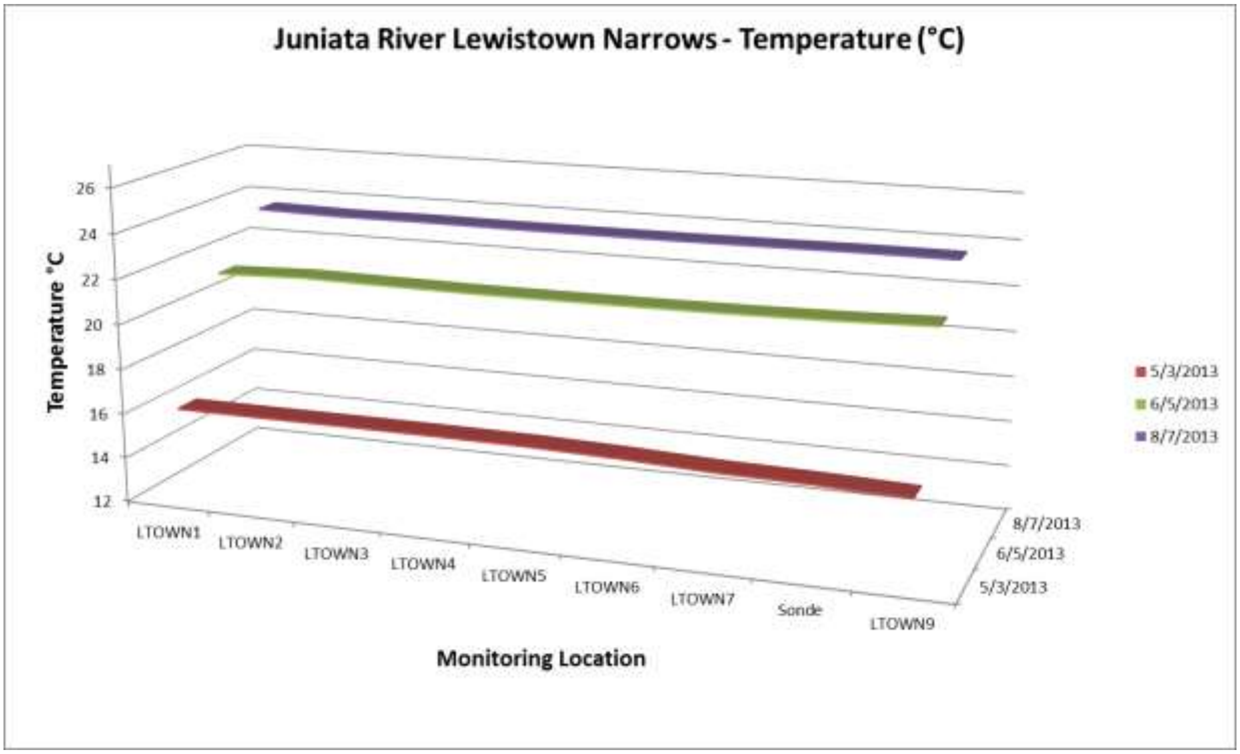
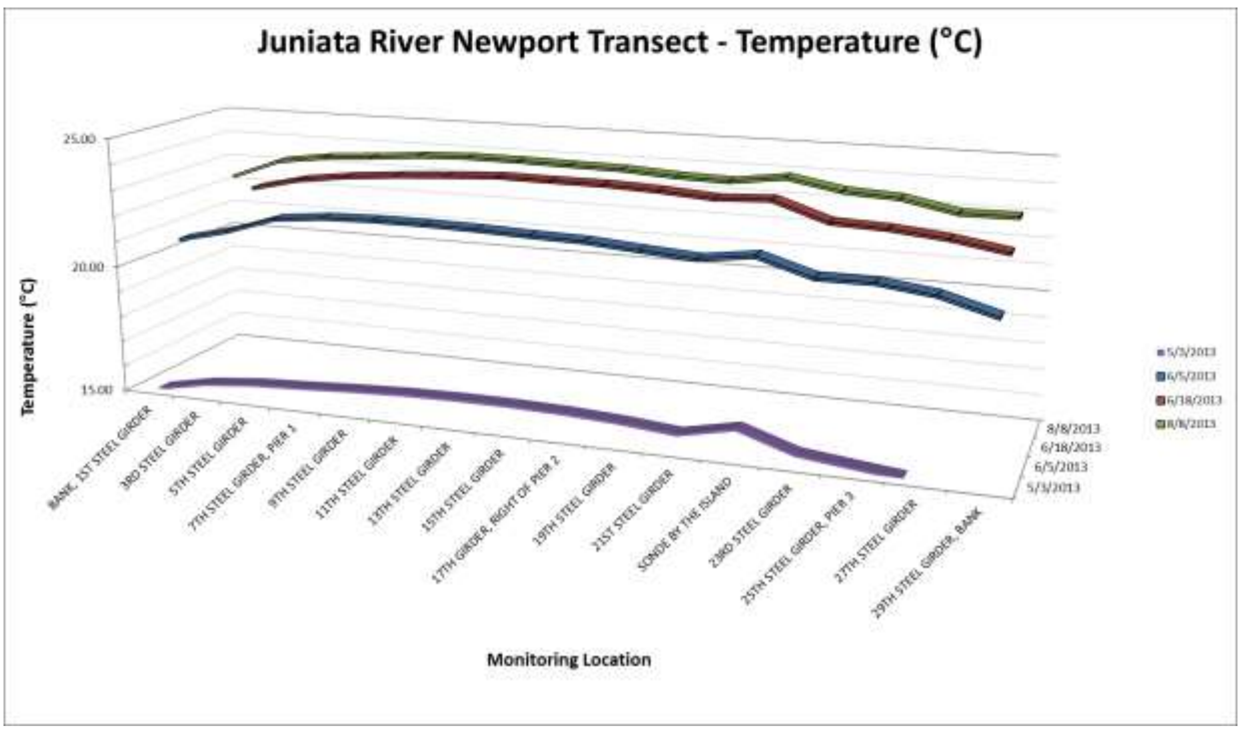
6/4/2013
6/25/2013
7/17/2013
8/8/2013
8/28/2013
9/19/2013


Susquehanna River Marietta Transect - Temperature (°C)
Temperature (°C)
29.00
24.00
19.00
14.00
YOY SHORELINE
MAR 2
MAR 3
MAR 4
SONDE
MAR 6
MAR 7
MAR 8
SONDE
MAR 10
YOY SHORELINE
Monitoring Location
8/27/2013
8/8/2013
7/162014
6/25/2013
6/10/2013
5/15/2013
4/24/2013
4/1/2013
5/15/2013
6/10/2013
6/25/2013
7/162014
8/8/2013
8/27/2013





Literature Cited

Chaplin, J.C., J.K. Crawford, and R.A. Brightbill. 2009. Water-quality monitoring in response to young-of-the-year Smallmouth Bass (*Micropterus dolomieu*) mortality in the Susquehanna River and major tributaries, Pennsylvania: 2008, U.S. Geological Survey Open-File Report 2009-1216.

Title: Temperature Criteria of Small Mouth Bass (WS #11)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

High, low or rapidly fluctuating water temperatures may directly affect recruitment of YOY into age class 1+.

Wrenn (1980) suggests that the upper lethal limit of YOY SMB is approximately 37°C. Yet, higher water temperatures (around 29°C) during this life stage may be beneficial for rapid growth and development (Zweifel et al. 1999). Additionally, water temperatures during the first summer are positively correlated with year class strength. This relationship most likely exists because energy reserves used to survive the first winter are produced in the summer when food is abundant and the water temperature is most suitable for growth (Horning and Pearson 1973, Clady 1975, Shuter et al. 1980).

Landsman et al (2011) suggest that egg hatching and YOY larval success is unaffected when water temperatures rapidly (within one hour) fluctuate between -7 to +8°C beyond normal conditions. However, significant mortality did occur when eggs were rapidly exposed to water temperatures 13°C above expected conditions. Therefore, Landsman et al (2011) demonstrate that eggs and larvae of SMB are resilient to acute water temperature changes. It was also noted that SMB young were slightly more resilient to decreasing water changes than increasing water changes.

Although SMB eggs and larva may be able to withstand fluctuating water temperatures, sudden drops in temperature below 14°C can be lethal due to the male abandoning the nest (MacLean et al. 1981, Armour 1983).

Data:

The Pennsylvania Department of Environmental Protection and the U.S. Geological Survey collected continuous water temperature data during the spring and summer of 2012 and 2013. Stations within the defined CADDIS area included the Susquehanna River at Harrisburg (2012 and 2013) and Juniata River at Newport (2012 and 2013). Comparison sites within the basin included the Susquehanna River at Marietta (2013) and the Susquehanna River at Danville (2013). Data from Danville during 2012 do exist, but the site was not characterizing main channel conditions and is therefore not comparable to other stations. The Delaware River at Morrisville (2012 and 2013) was chosen as the out-of-basin comparison. The Allegheny River was also considered as an out-of-basin comparison, but this site is directly within the influence of French Creek and the Allegheny River is highly flow regulated. As a result, direct site comparison would be problematic.

Analysis and results:

Continuous water temperature data were analyzed at each site to determine the maximum observation, maximum daily ranges, and potential drops below 14°C after the spawn. Daily ranges were a surrogate measure for rapid fluctuations. If daily ranges exceeded 7°C, then further investigation into the magnitude and direction of that fluctuation would be necessary. Results suggest that water temperatures did not exceed 37°C and maximum daily ranges did not exceed 7°C at any sites during these two years (Table 1). There were insufficient data to confidently determine if water temperatures dropped below 14°C during or just after spawning occurred. However, no sites had recorded temperatures below 14°C by late May or early June.

Table 1. Maximum observed and maximum daily water temperatures at each site.

Site/Year	Maximum	Daily Maximum Range
Susquehanna River, Harrisburg West 2012	32.9	5.0
Susquehanna River, Harrisburg West 2013	31.5	5.5
Susquehanna River, Harrisburg Middle 2012	32.9	4.9
Susquehanna River, Harrisburg Middle 2013	33.2	5.0
Susquehanna River, Harrisburg East 2012	33.2	4.1
Susquehanna River, Harrisburg East 2013	32.6	4.0
Juniata River, Newport 2012	32.4	4.9
Juniata River, Newport 2013	33.7	5.6
Susquehanna River, Danville2013	30.6	3.7
Susquehanna River, Marietta West 2013	32.8	4.2
Susquehanna River, Marietta East 2013	32.6	3.1
Delaware River, Morrisville West 2012	31.1	4.0
Delaware River, Morrisville West 2013	31.6	4.1
Delaware River, Morrisville East 2012	31.3	5.4
Delaware River, Morrisville East 2013	30.8	3.4

Conclusion:

The 2013 sampling year was characterized as relatively average to wet. Without more data, especially during lower flow years, it is difficult to determine how extreme water temperature may become. However, based on the data provided, it seems unlikely that water temperature is directly affecting the recruitment of SMB into age class 1+.

Literature Cited:

Armour, C.L. 1993. Evaluating temperature regimes for protection of smallmouth bass. United States Department of the Interior Fish and Wildlife Service Resource Publication 191, Washington, D.C.

Causal Analysis of the Smallmouth Bass Decline in the Susquehanna and Juniata Rivers. 2015. Worksheet # 54 - STROUD Temperature cycling and maximum temperature effects on SMB.

Clady, M.D. 1977. Eariy survival and recruitment of smallrnouth bass in northern Michigan. Journal of Wildlife Management 39: 194-200.

Edwards, E. A., G. Gebhart, & O. E. Maughan. 1983. Habitat suitability information: smallmouth bass. U.S. Fish andWildlife Service FWS/OBS-82/10.36.

Horning, W.B. II, & Pearson, R.E. 1973. Growth temperature requirements and lower lethal temperatures for juvenile smallmouth bass (*Micropterus dolomieui*). Journal of the Fisheries Research Board of Canada 30: 1226-1 230.

Landsman, S. J., A. J. Gingerich, D. P. Philipp, & C. D. Suski. 2011. The effects of temperature change on the hatching success and larval survival of largemouth bass *Micropterus salmoides* and smallmouth bass *Micropterus dolomieu*. Journal of Fish Biology. 78:1200-1212.

Maclean, J.A., Shuter, B.J., Regier, H.A. & MacLeod, J.C. 1981 . Temperature and yearclass strength of smallmouth bass. Proceedings of the symposium on early life history of fish. International Council for the Exploration of the Sea, Denmark. 178: 30-40.

Shuter, B. J., J. A. MacLean, F E. J. Fry, & H. A. Regier. 1980. Stochastic simulation of temperature effects of first year survival of smallmouth bass. Transactions of the American Fisheries Society 109:1-34.

Wrenn, W. B. 1980. Effects of elevated temperature on growth and survival of Smallmouth Bass. Transactions of the American Fisheries Society. 109:617-625.

Zweifel, R.D., R. S. Hayward, & C. F. Rabeni. 1999. Bioenergetics insight into black bass distribution shifts in Ozark boarder region streams. North American Journal of Fisheries Management. 19:192-197.

Title: Impacts of High Temperatures on YOY (WS #12)

Agency: Pennsylvania Department of Environmental Protection.

Candidate Cause: High water temperatures either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

High water temperature causes dissolved oxygen to be less soluble and can be a stressor to YOY leaving them more susceptible to disease and predation. This analysis looks at whether temperatures have risen over time in portions of the Susquehanna River basin in the case, in-basin but out of case, and out-of-basin sites. If the baseline temperature in the study area of the case is now higher than it was in the past, this could make the conditions in the River more prone to harmful conditions during the summer months and when flows are low. If temperatures have not risen over time at sites in the case or have risen at all sites, then this weakens this candidate cause.

Data:

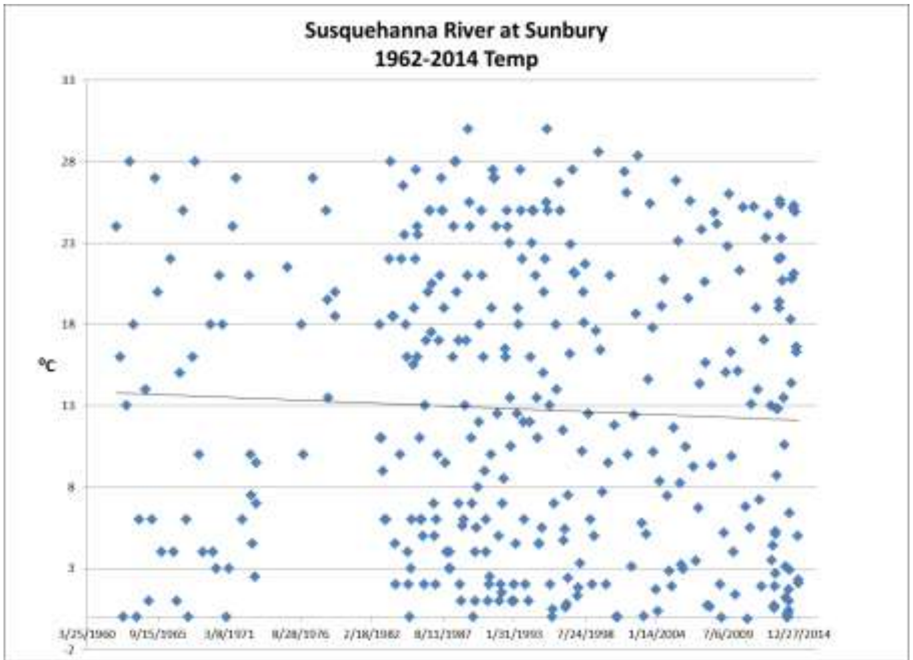
Temperature data was collected at the Pennsylvania Department of Environmental Protection's (PA DEP) Water Quality Network (WQN) stations during field visits, using handheld meters. Typically, multiple readings are taken across the stream/river depending on the width. The values are then averaged to obtain the result. The stations are sampled from 6 to 20 times per year depending on the station and time period. This data was downloaded from PA DEP's Sample Information System (SIS) and EPA's Storage and Retrieval Data Warehouse (STORET).

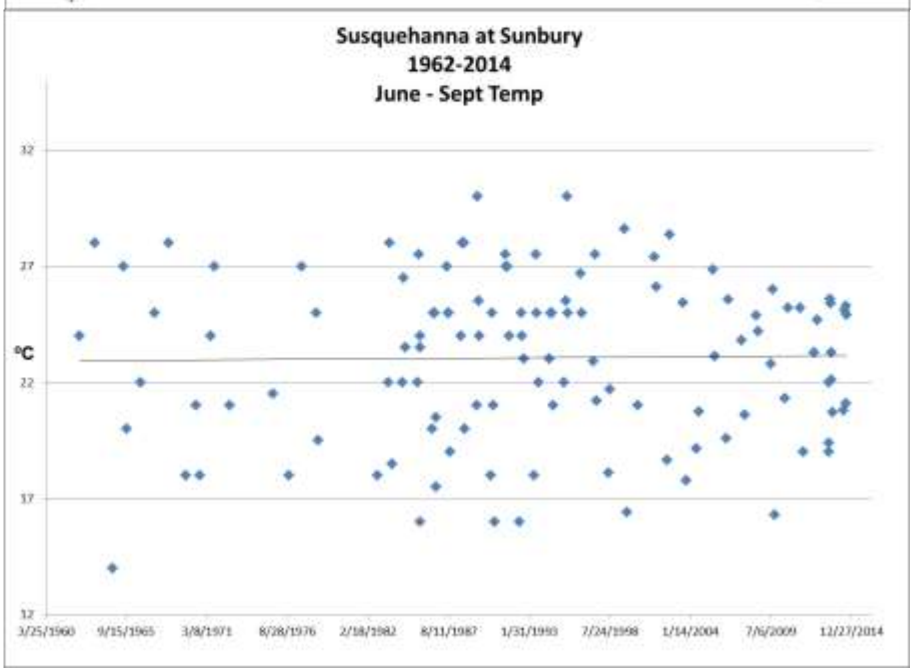
Analysis:

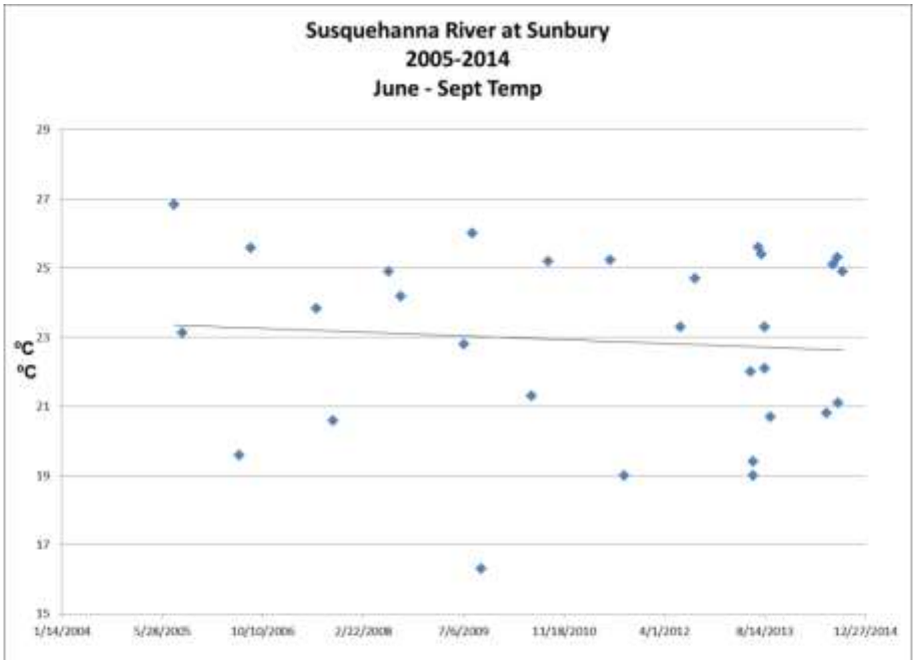
Data was analyzed at three in case sites: Juniata at Newport, Susquehanna River at Sunbury, and Susquehanna River at Harrisburg; two in-basin comparison sites: Susquehanna River at Marietta and Susquehanna River at Wilkes-Barre; and two out-of-basin sites: Allegheny River at Kennerdell and Delaware River at Morrisville. The entire history of temperature data was obtained for all stations and scatter plots were generated to view the data. Trendlines were added to show if temperature is increasing or decreasing over time. Graphs were also created to look at only the months of June-September and June alone. A shorter time period (2005-2014) was also looked at for the Susquehanna River at Sunbury and the Juniata River at Newport to see if the trends differed.

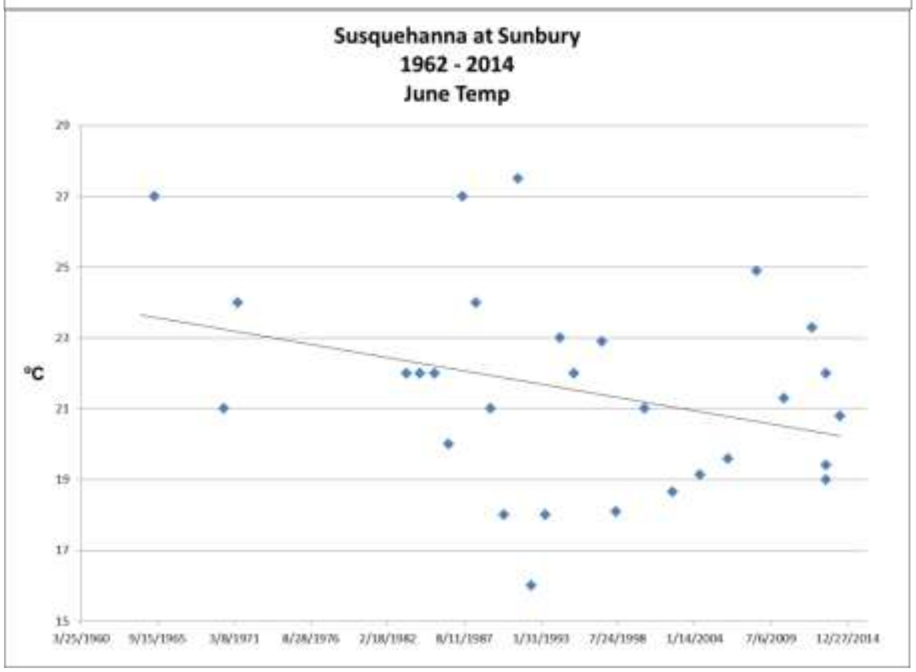
Results:

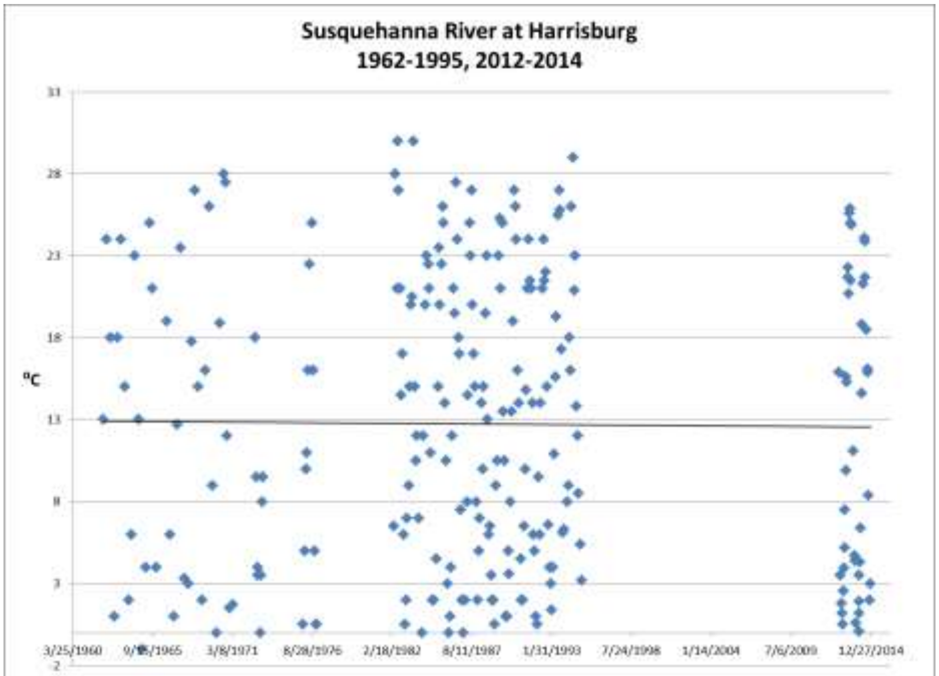
This section contains 23 scatter plots with trendlines for the 7 stations.


Susquehanna River at Sunbury
1962-2014 Temp
°C
33
28
23
18
13
8
3
-2
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

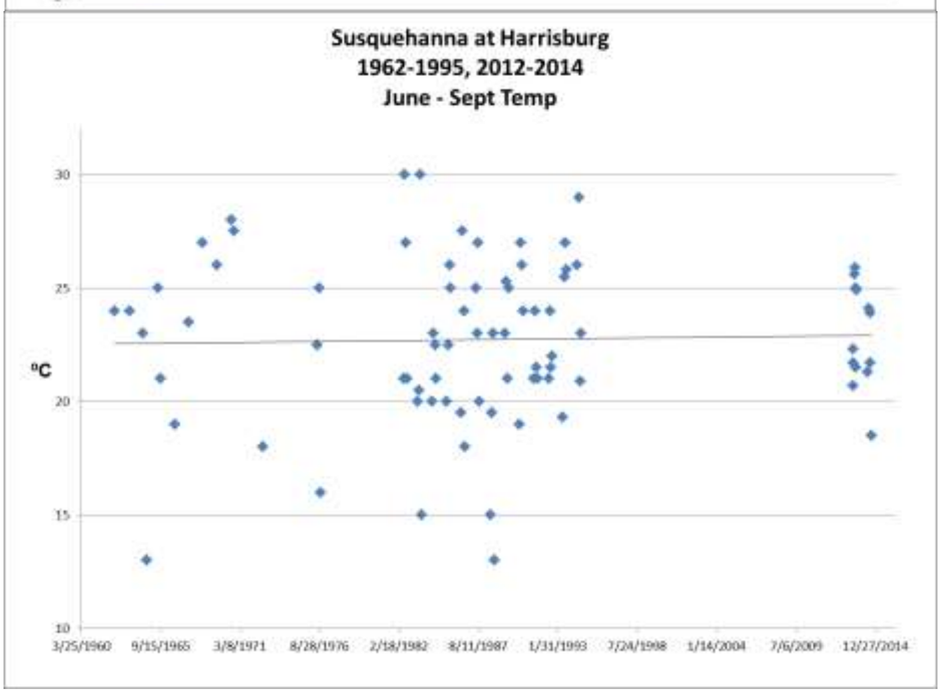

Susquehanna at Sunbury
1962-2014
June - Sept Temp
°C
32
27
22
17
12
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014


Susquehanna River at Sunbury
2005-2014
June - Sept Temp
°C
°C
29
27
25
23
21
19
17
15
1/14/2004
5/28/2005
10/10/2006
2/22/2008
7/6/2009
11/18/2010
4/1/2012
8/14/2013
12/27/2014

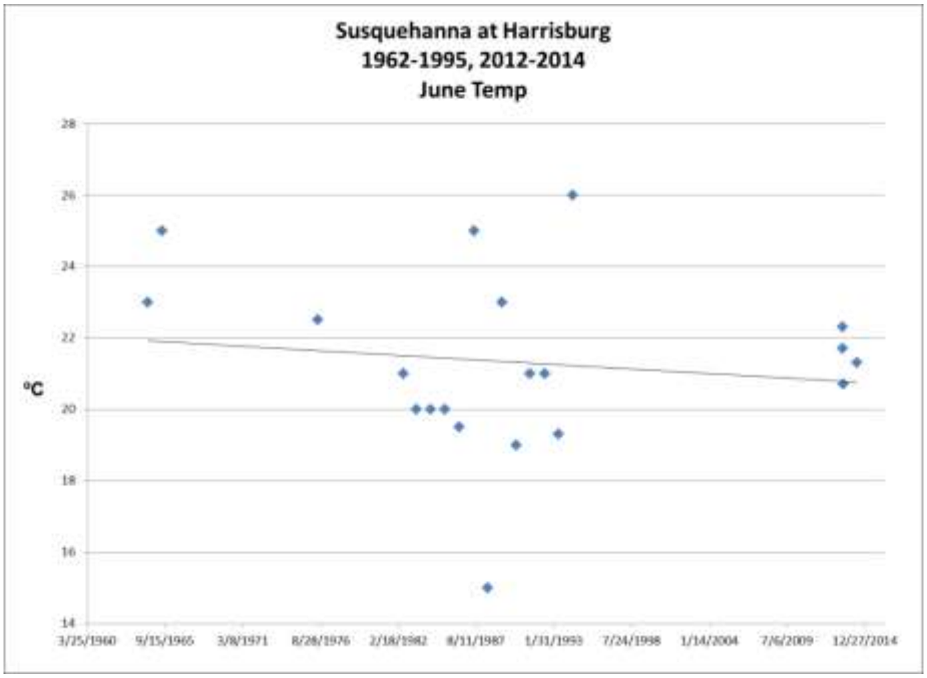

Susquehanna at Sunbury
1962 - 2014
June Temp
°C
29
27
25
23
21
19
17
15
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

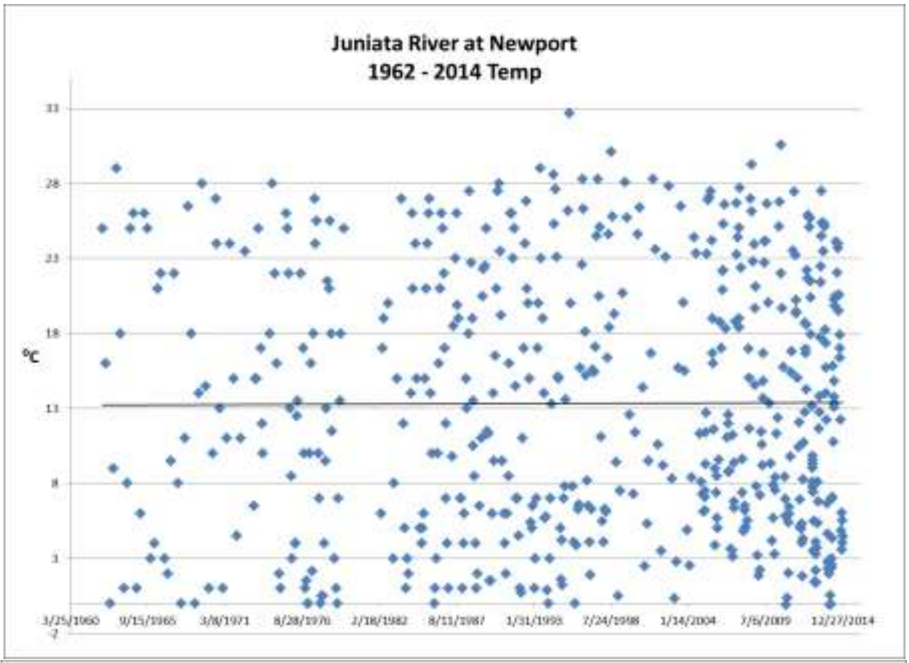


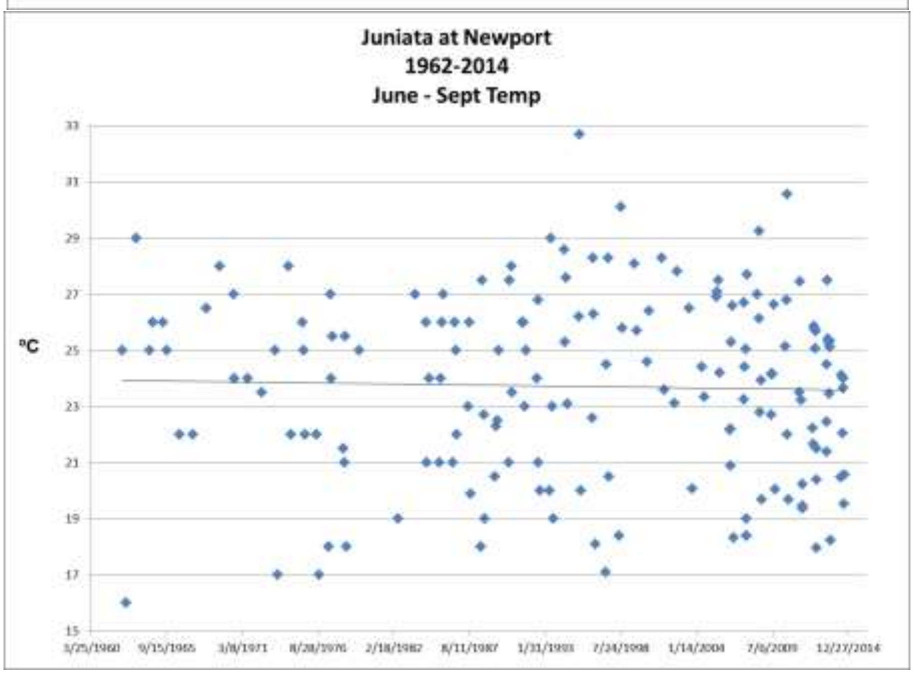
Susquehanna River at Harrisburg
1962-1995, 2012-2014
ºC
31
28
23
18
13
8
3
-2
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

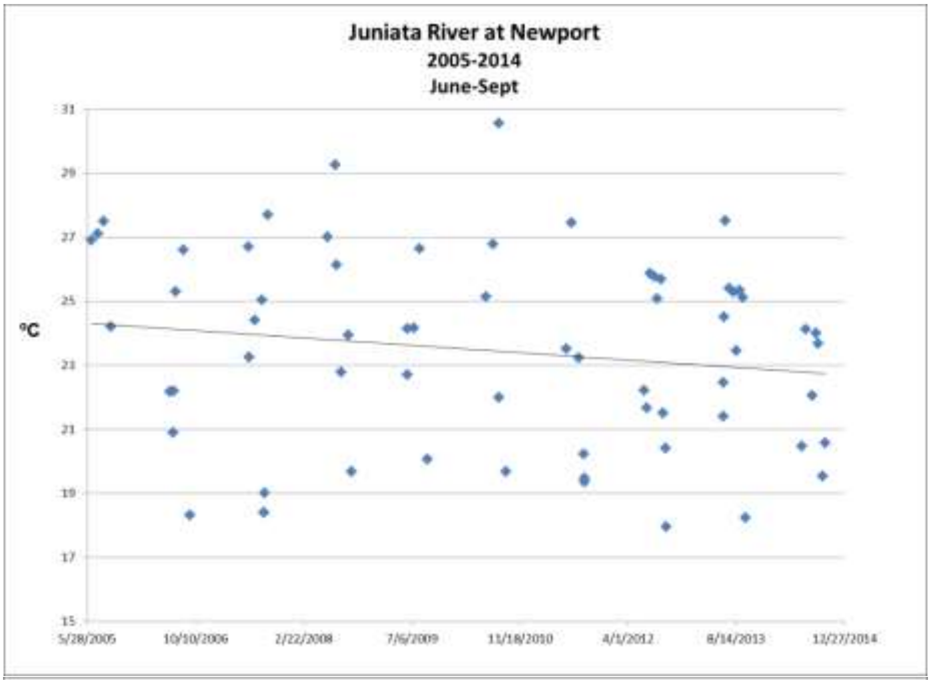


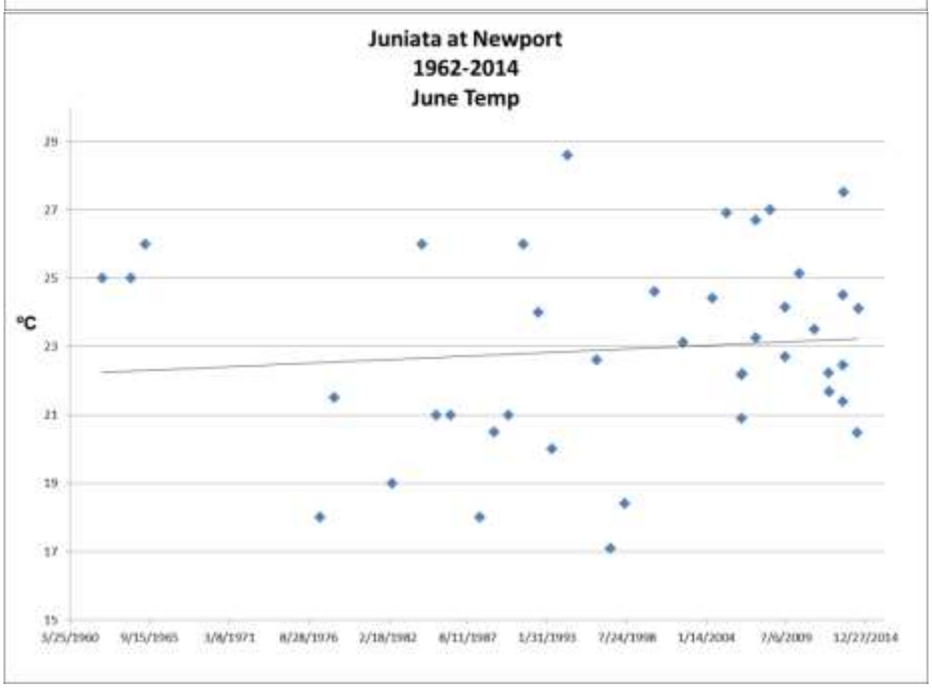
Susquehanna at Harrisburg
1962-1995, 2012-2014
June - Sept Temp
ºC
30
25
20
15
10
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

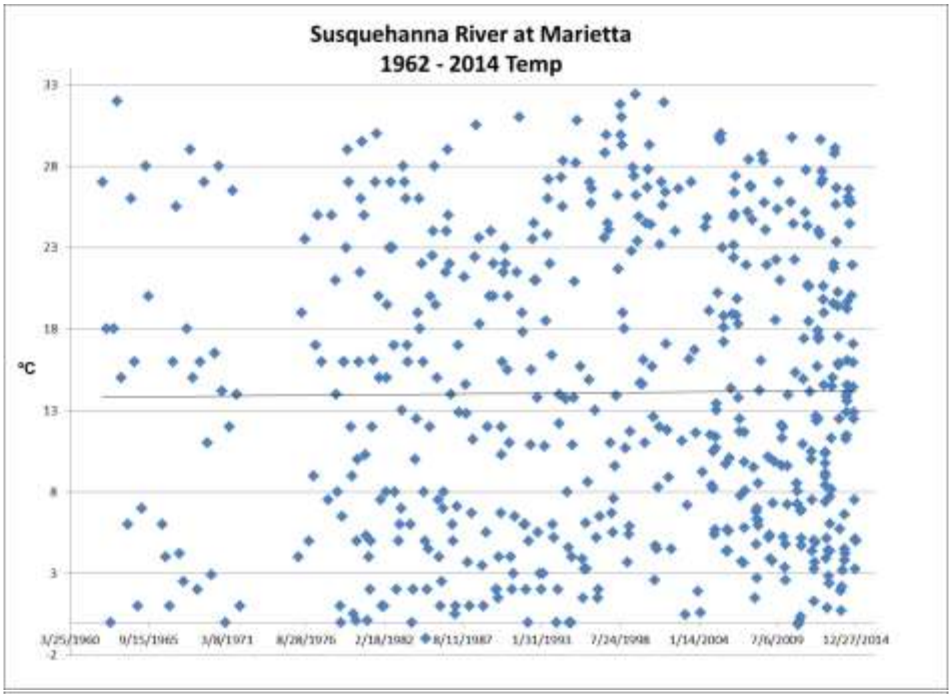

Susquehanna at Harrisburg
1962-1995, 2012-2014
June Temp
°C
28
26
24
22
20
18
16
14
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

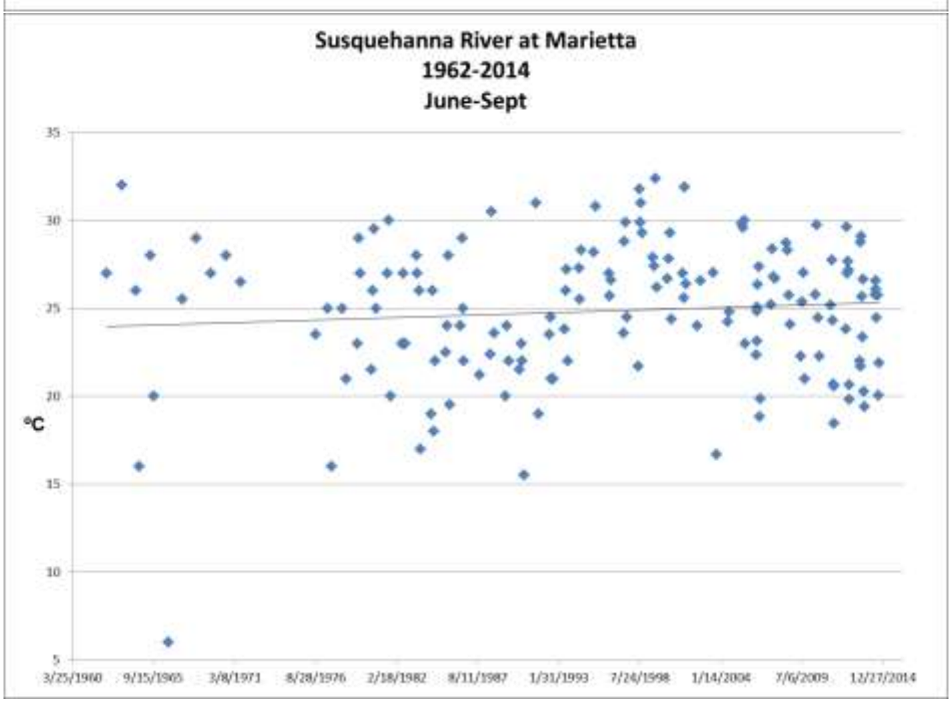

Juniata River at Newport
1962 - 2014 Temp
°C
33
28
23
18
13
8
3
-2
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014


Juniata at Newport
1962-2014
June - Sept Temp
°C
33
31
29
27
25
23
21
19
17
15
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014


Juniata River at Newport
2005-2014
June-Sept
°C


Juniata at Newport
1962-2014
June Temp
°C


Susquehanna River at Marietta
1962 - 2014 Temp
°C
33
28
23
18
13
8
3
-2
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014


Susquehanna River at Marietta
1962-2014
June-Sept
°C
35
30
25
20
15
10
5
3/25/1960
9/15/1965
3/8/1971
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8/11/1987
1/31/1993
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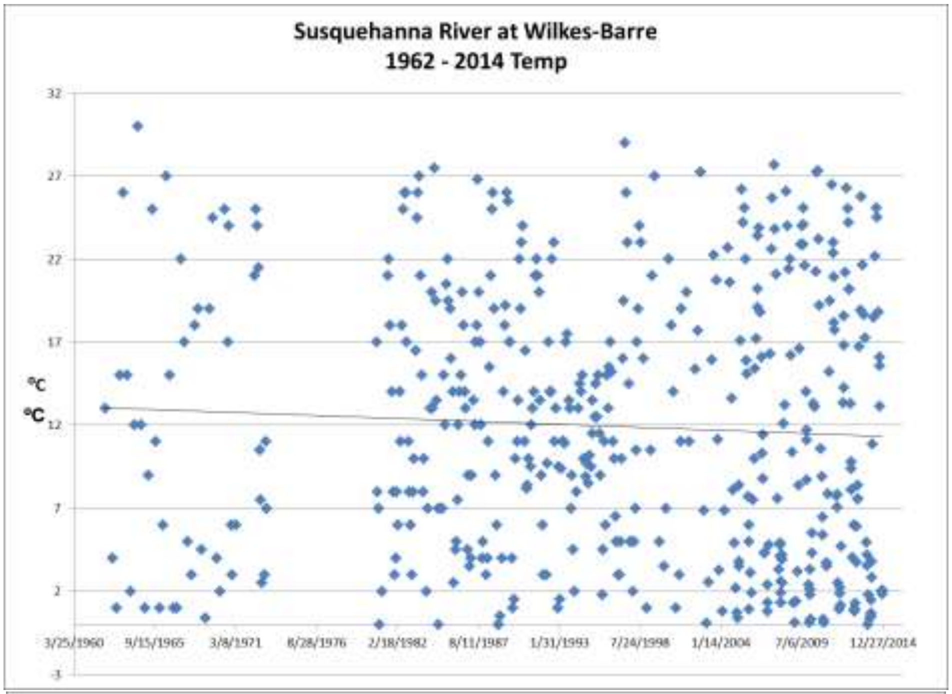
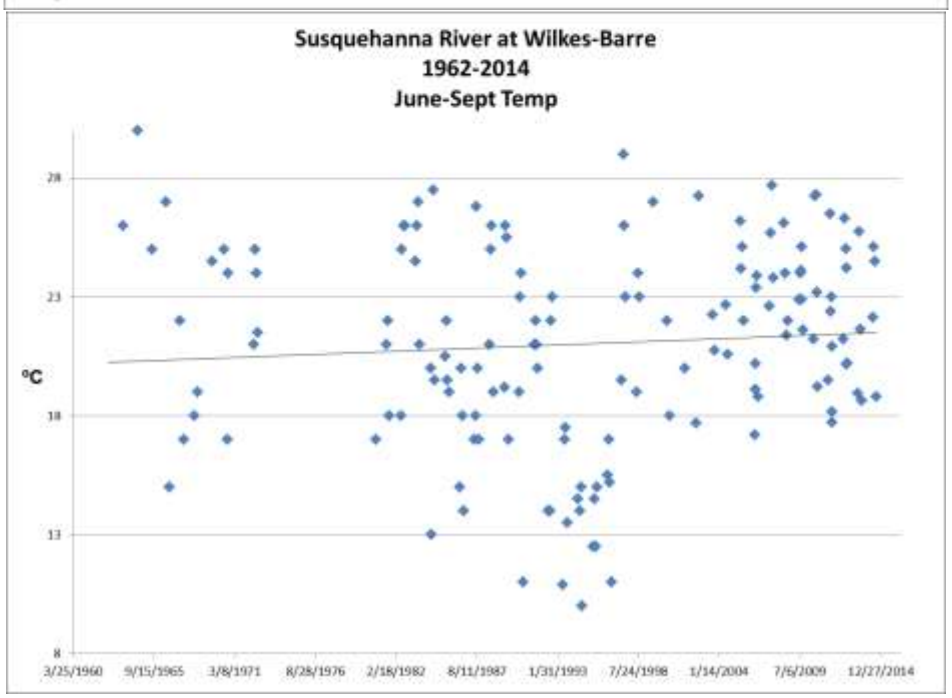
7/6/2009
12/27/2014


Susquehanna River at Wilkes-Barre
1962 - 2014 Temp
°C
°C
32
27
22
17
12
7
2
-3
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014


Susquehanna River at Wilkes-Barre
1962-2014
June-Sept Temp
°C
28
23
18
13
8
3/25/1960
9/15/1965
3/8/1971
8/28/1976
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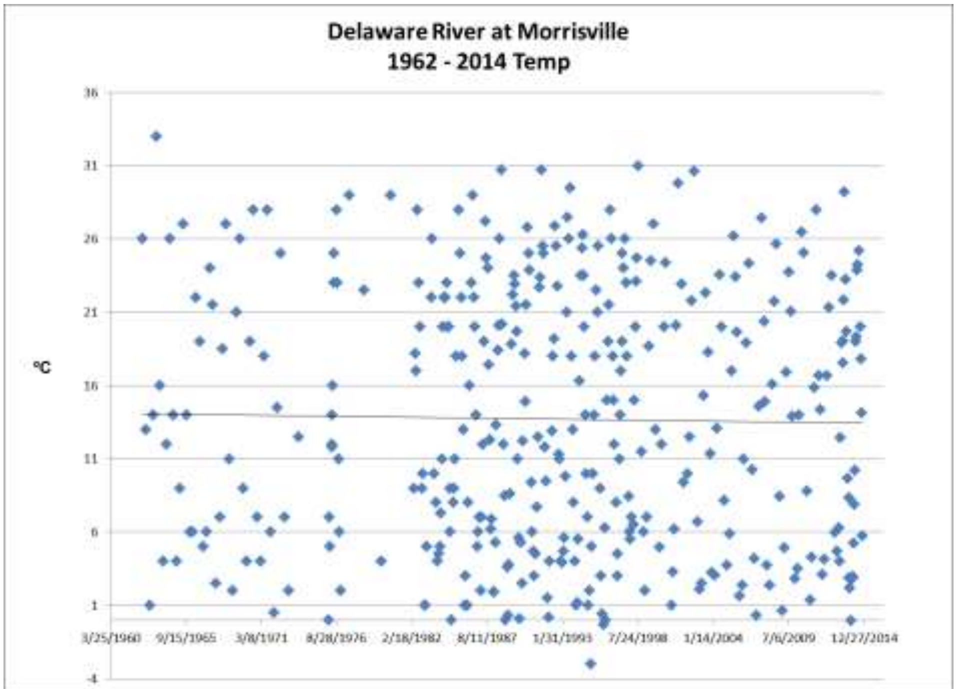
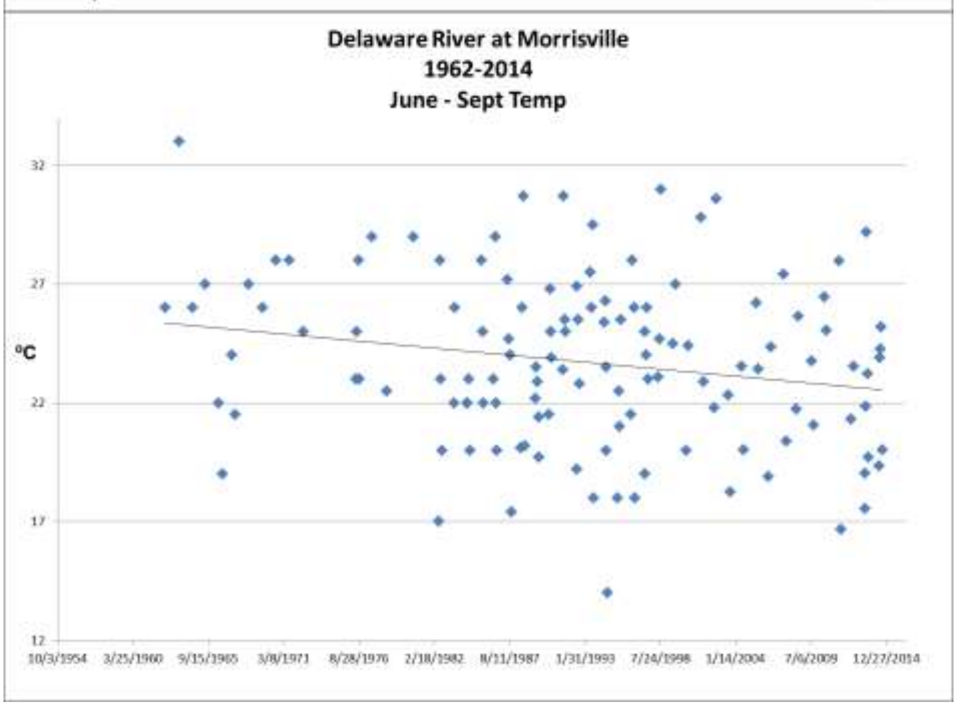
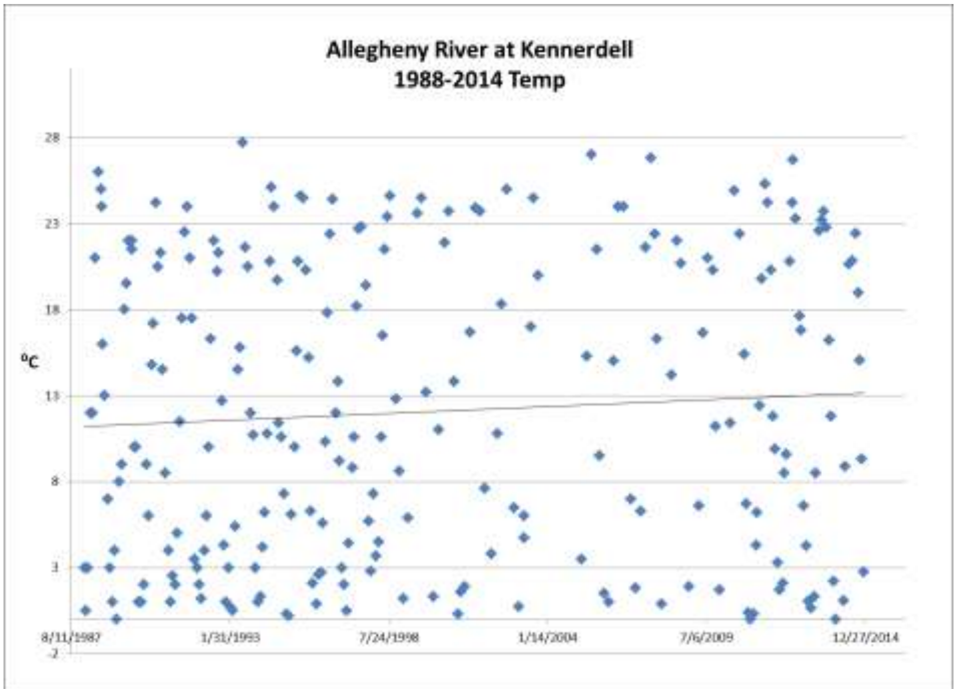
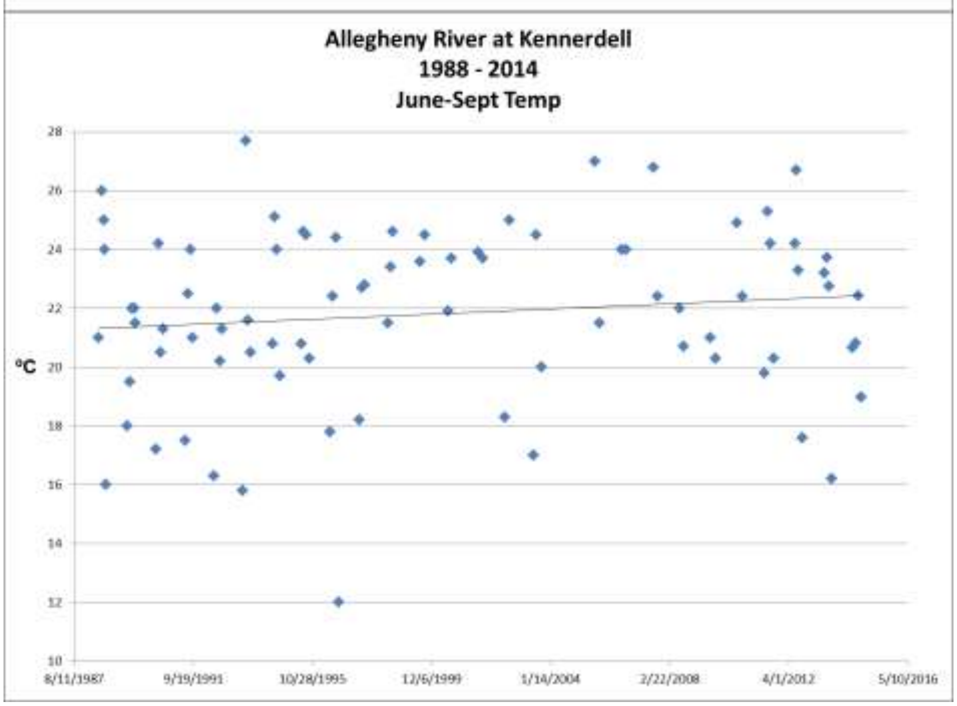
7/6/2009
12/27/2014


Delaware River at Morrisville
1962 - 2014 Temp
°C
36
31
26
21
16
11
6
1
-4
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

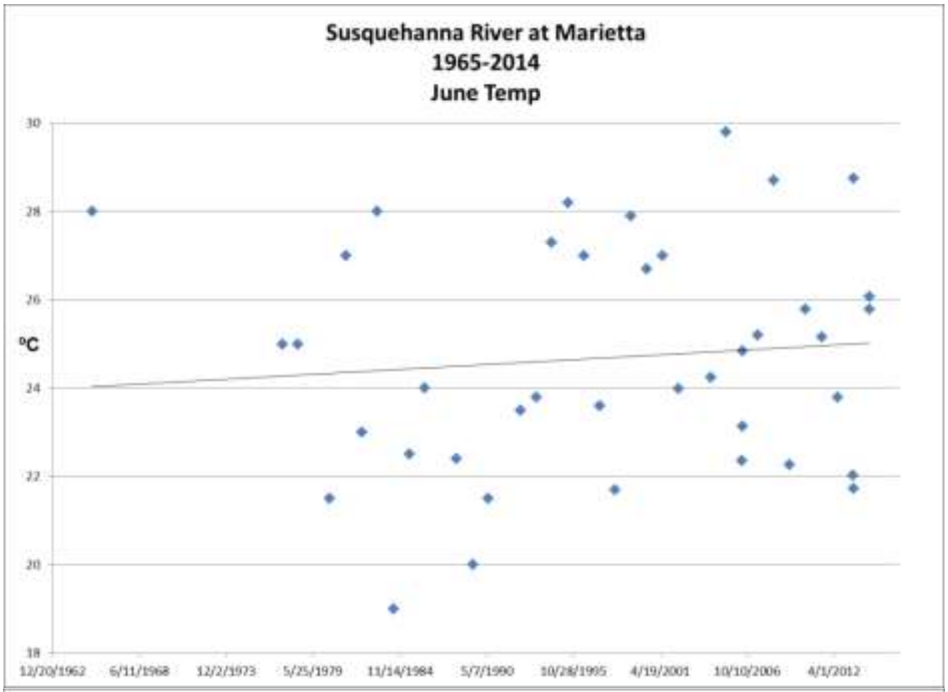

Delaware River at Morrisville
1962-2014
June - Sept Temp
°C
32
27
22
17
12
10/3/1954
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014



Allegheny River at Kennerdell
1988-2014 Temp
°C
28
23
18
13
8
3
-2
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014



Allegheny River at Kennerdell
1988 - 2014
June-Sept Temp
°C
28
26
24
22
20
18
16
14
12
10
8/11/1987
9/19/1991
10/28/1995
12/6/1999
1/14/2004
2/22/2008
4/1/2012
5/10/2016


Susquehanna River at Marietta
1965-2014
June Temp
°C
30
28
26
24
22
20
18
12/20/1962
6/11/1968
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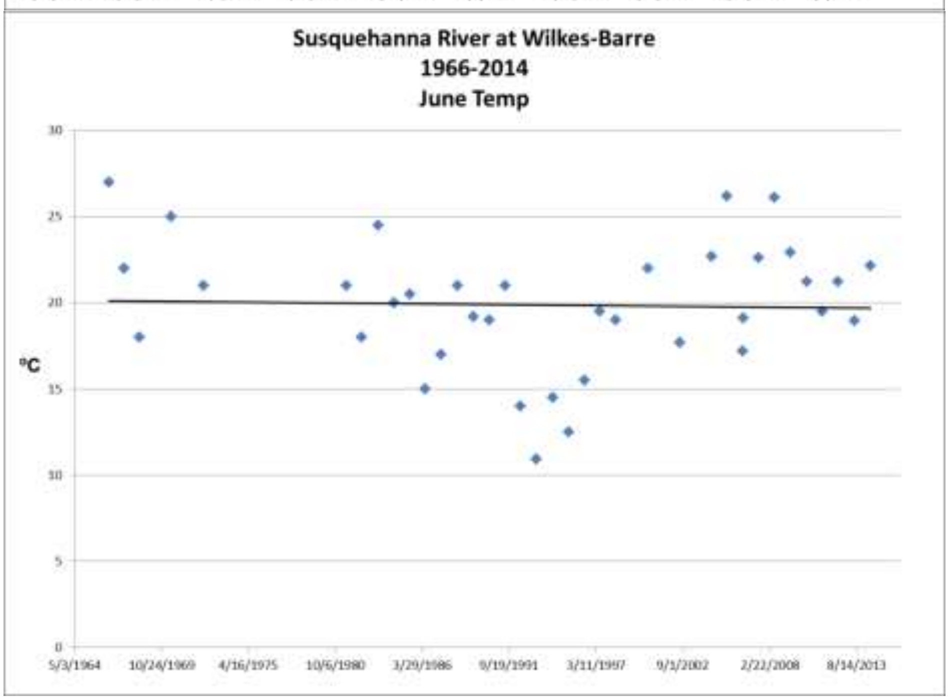
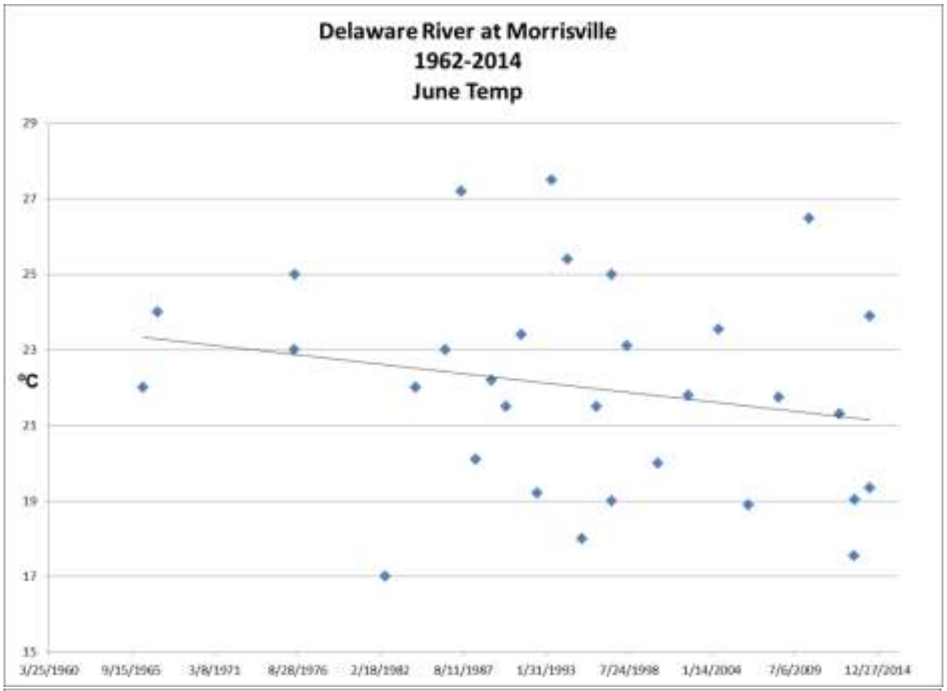
5/25/1979
11/14/1984
5/7/1990
10/28/1995
4/19/2001
10/10/2006
4/1/2012


Susquehanna River at Wilkes-Barre
1966-2014
June Temp
°C
30
25
20
15
10
5
0
5/3/1964
10/24/1969
4/16/1975
10/6/1980
3/29/1986
9/19/1991
3/11/1997
9/1/2002
2/22/2008
8/14/2013


Delaware River at Morrisville
1962-2014
June Temp
ºC
29
27
25
23
21
19
17
15
3/25/1960
9/15/1965
3/8/1971
8/28/1976
2/18/1982
8/11/1987
1/31/1993
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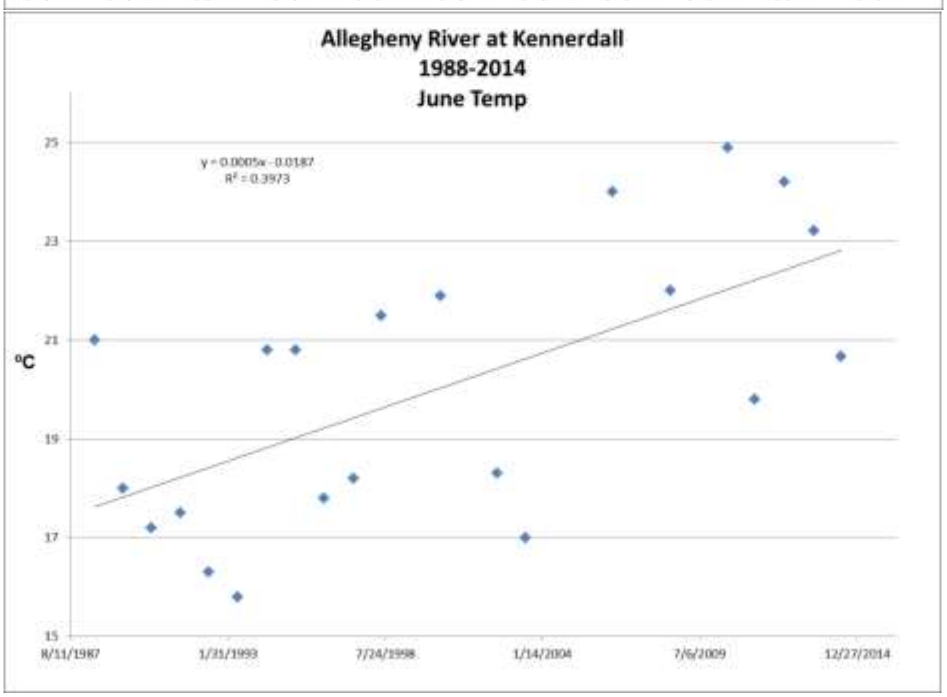
1/14/2004
7/6/2009
12/27/2014


Allegheny River at Kennerdall
1988-2014
June Temp
y = 0.0005x - 0.0187
R² = 0.3973
ºC
25
23
21
19
17
15
8/11/1987
1/31/1993
7/24/1998
1/14/2004
7/6/2009
12/27/2014

Conclusion:

When looking at discrete temperature data, keep in mind that each data point represents a snapshot of the temperature at a specific time of day. It does not account for the diel cycle of temperature and is not the average temperature for the day.

At the 3 stations in the case area (Juniata at Newport, Susquhanna at Sunbury, & Susquehanna at Harrisburg), most of the graphs showed no relationship between temperature and time or a very slight decreasing trend. The June data for the entire period of record at Juniata at Newport was the only slightly increasing trend. None of the trends from the stations in the case area were strong enough to make any conclusions.

For the out of case stations, the Susquehanna at Wilkes-Barre, Susquehanna at Marietta, and the Allegheny River at Kennerdell all had slightly increasing trends or showed no trend. The Delaware River at Morrisville showed slightly decreasing trends. The steepest trendline out of all the stations, including the in case stations, was for the June data at the Allegheny River site with an $R^2 = 0.3973$.

Based on this analysis, there is no trend when comparing temperature over time at any of the in case stations and the largest increasing trend was observed at an out-of-basin site. These results weaken the case that temperature has increased over time in the study area of this case.

Candidate Cause 8: pH

Worksheets: 13 and 14

Title: Impacts of High pH on Small Mouth Bass Survival (WS #13)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: High or variable pH either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

High pH in surface waters (above 9.0) can directly cause adverse physiological effects, possibly decreasing SMB survival and recruitment to age class 1+. Based off reviews published by the European Inland Fisheries Advisory Commission in 1969 and other sources, a range of 6.5 to 9.0 pH are expected to be harmless to aquatic life; although, the toxicity of other constituents may be affected within this range. Temporary exceedances of 9.0 are expected and do naturally occur in shallow and biologically productive waters. Therefore, it is important to look not only at the magnitude of the exceedance, but also the frequency and duration of those events.

Very little data exists on how SMB are specifically affected by high pH and at which age tolerance may or may not change. However, if pH is directly affecting SMB recruitment, then conditions within the defined CADDIS area should experience a greater magnitude duration and frequency of high pH than either in basin or out-of-basin comparison sites.

Data:

The Pennsylvania Department of Environmental Protection and the U.S. Geological Survey collected continuous pH data during the spring and summer of 2012 and 2013. Stations within the defined CADDIS area included the Susquehanna River at Harrisburg (2012 and 2013) and Juniata River at Newport (2012 and 2013). Comparison sites within the basin included the Susquehanna River at Marietta (2013) and the Susquehanna River at Danville (2013). Data from Danville during 2012 do exist, but the site was not characterizing main channel conditions and is therefore not comparable to other stations. The Delaware River at Morrisville (2012 and 2013) was chosen as the out-of-basin comparison. The Allegheny River was also considered as an out-of-basin comparison, but this site is directly within the influence of French Creek and the Allegheny River is highly flow regulated. Consequently, direct site comparison would be problematic.

Analysis and Results:

Continuous pH data at each site were analyzed using concentration duration frequency (CDF) curves. Thresholds of 8.0, 8.5, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5 and 9.6 were selected for mean duration site comparison. In 2012, the Delaware River experienced some of the most extreme pH conditions with respect to magnitude and duration (Table 1). Similar conditions were observed again in 2013 (Table 2).

Table 1. Mean duration comparisons for 2012. Sites in blue are comparison sites outside the defined CADDIS area.

Site	Mean Duration (hours) per Threshold								
	8.0	8.5	9.0	9.1	9.2	9.3	9.4	9.5	9.6
JuniataNewport	197.6	20.4	7.1	5.2	4.7	3.0			
SusquehannaHarrisburgWest	45.0	15.3	10.6	7.6	6.5	6.0	3.0	0.5	0.5
SusquehannaHarrisburgMiddle	11.2	6.0							
SusquehannaHarrisburgEast	30.6	12.8							
DelawareMorrisvilleWest	212.7	17.7	7.0	4.8	2.5	2.3			
DelawareMorrisvilleEast	21.3	11.8	9.5	7.7	6.0	4.4	3.7	2.6	1.5

Table 2. Mean duration comparisons for 2013. Sites in blue are comparison sites outside the defined CADDIS area.

Site	Mean Duration per Threshold								
	8.0	8.5	9.0	9.1	9.2	9.3	9.4	9.5	9.6
JuniataNewport	43.5	14.5	8.1	7.1	4.7				
SusquehannaHarrisburgWest	33.9	11.7							
SusquehannaHarrisburgMiddle	19.0	6.6	3.6	3.7	1.7	0.5			
SusquehannaHarrisburgEast	76.6	19.6	8.2	7.5	0.5				
DelawareMorrisvilleWest	12.6	6.9							
DelawareMorrisvilleEast	22.8	11.1							
SusquehannaMariettaWest	25.8	7.5							
SusquehannaMariettaEast	17.0	13.2							
SusquehannaDanville	24.9	22.2	6.5	4.4					

Conclusion:

These years (2012 and 2013) were characterized as relatively average-to-wet years. Without more data, especially during lower flow years, it is difficult to determine how extreme pH levels can rise and how long they potentially occur. Additionally, site conditions are characterizing more or less main channel conditions, and may not represent SMB YOY habitat. However, since the Delaware River does experience typically longer and higher pH threshold exceedances and does not exhibit poor YOY SMB recruitment, it is likely that high pH is not directly affecting SMB recruitment within the defined CADDIS area.

Title: Increases in pH on the Susquehanna River, Major Tributaries and Out-of-Basin (WS #14)

Agency: Pennsylvania Department of Environmental Protection

Temporal/Spatial Co-occurrence using Data from the Case

Candidate Cause: High or variable pH either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

An increasing trend in pH can cause significant changes in a riverine system by allowing select constituents to become more or less soluble and consequently more or less bio-available. For example, elevated pH can cause an increase in the ratio of dissolved phosphorus to total phosphorus, and also cause an increase in the toxic form of ammonia.

Data:

Discrete pH data were collected from the Department's Water Quality Network and EPA's STORET Database.

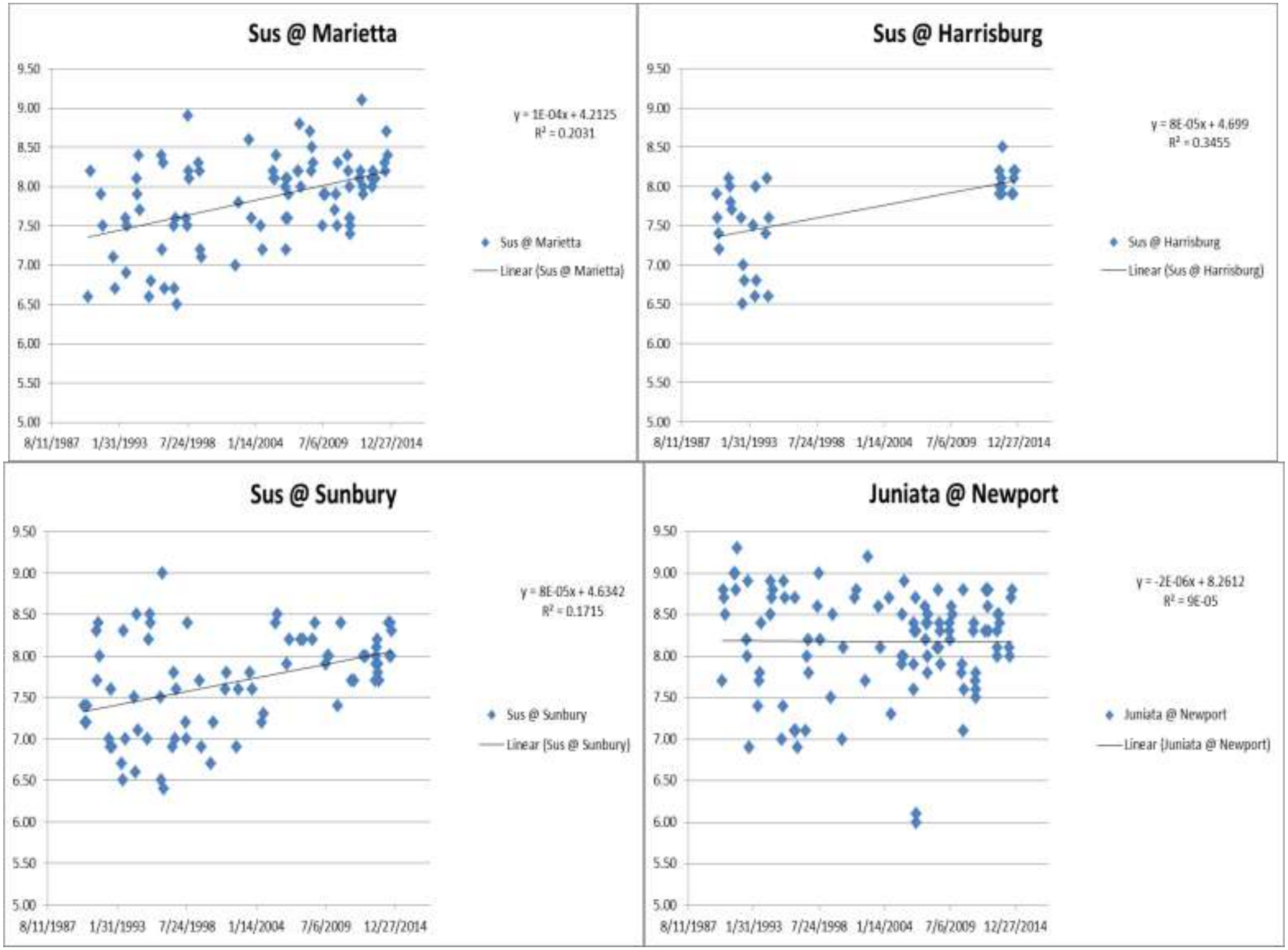
Analysis:

Data for months June through September were summarized for select stations and graphed using Microsoft Excel for periods 1990 – 2014 and again for periods 1995 – 2014, except for Susquehanna River at Harrisburg due to lack of data for the period 1995-2012. Linear trend lines were added to each figure as well as an R^2 and equation.

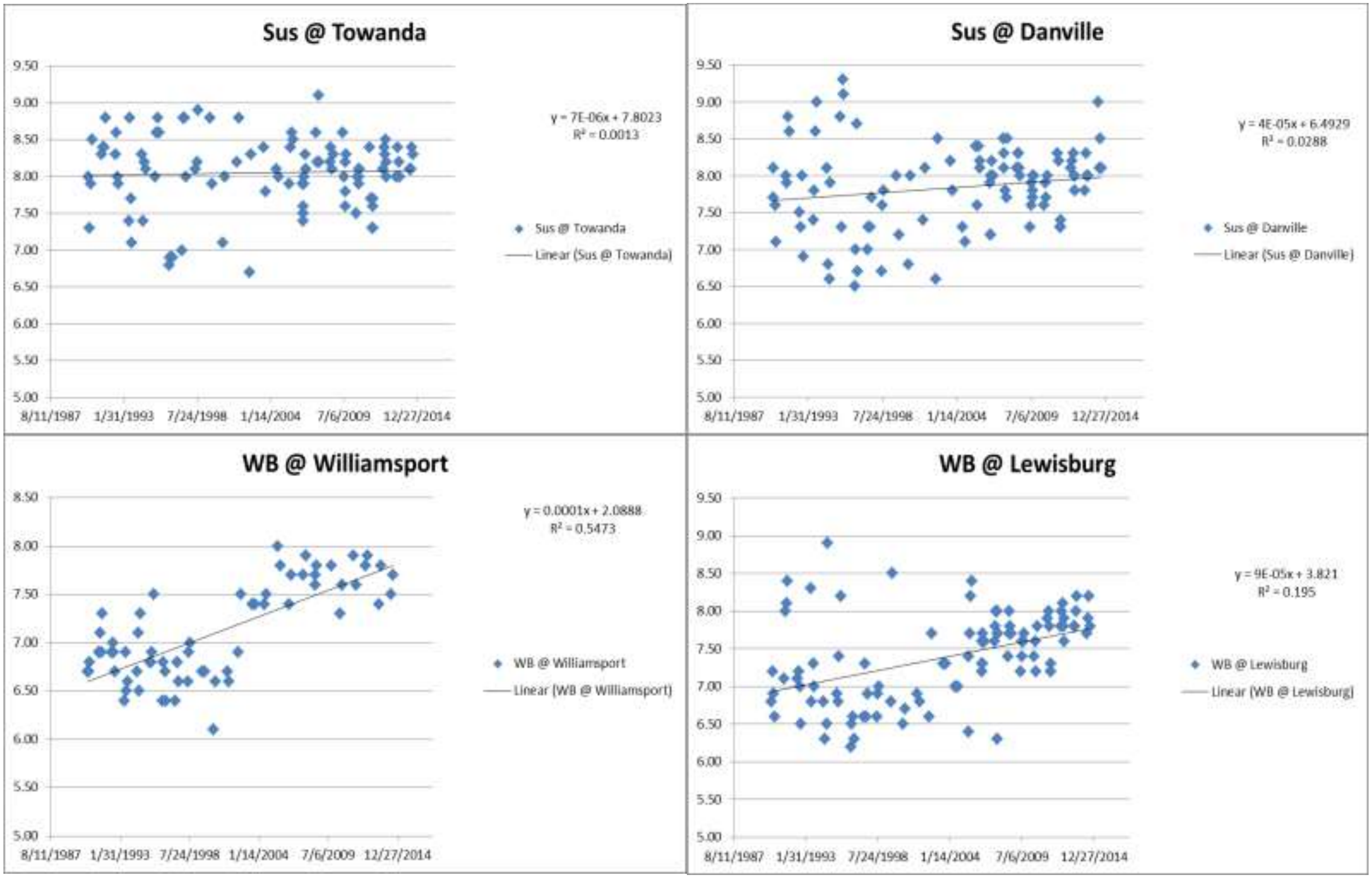
Results:

The discrete nature of the data and the natural diel variability of pH confounds the ability to characterize trends. While elevated pH values are not necessarily the focus of this worksheet, they should be considered when developing conclusions.

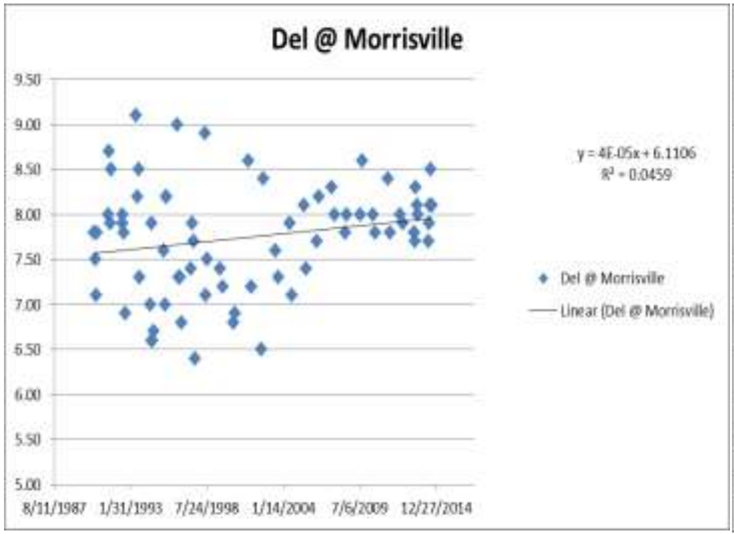
Discrete pH 1990-2014 Lower Mainstem and Juniata River

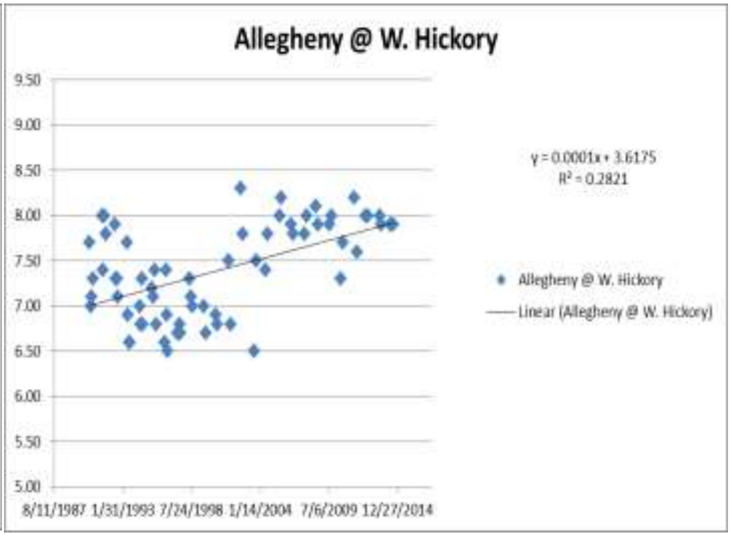


Discrete pH 1990-2014 North Branch and West Branch

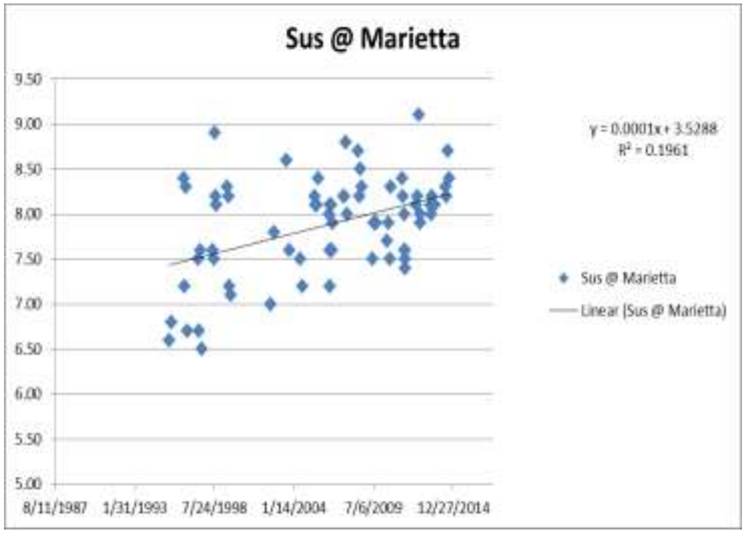


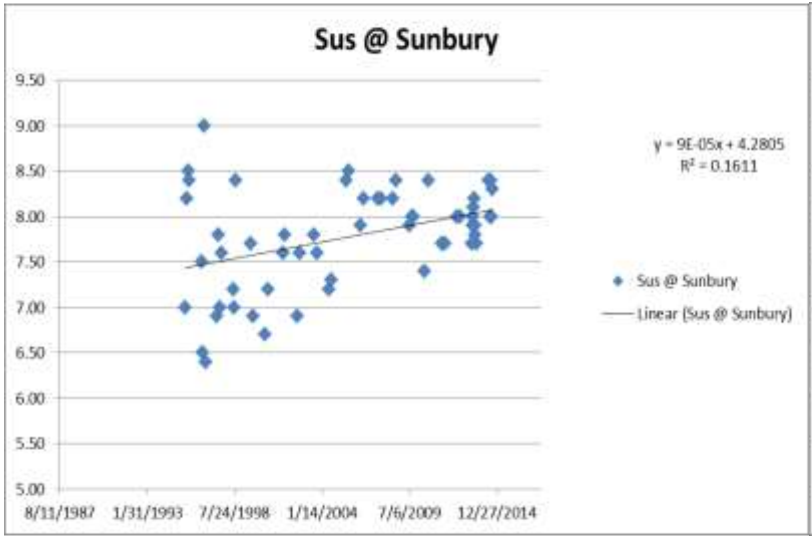
Discrete pH 1990-2014 Delaware and Allegheny

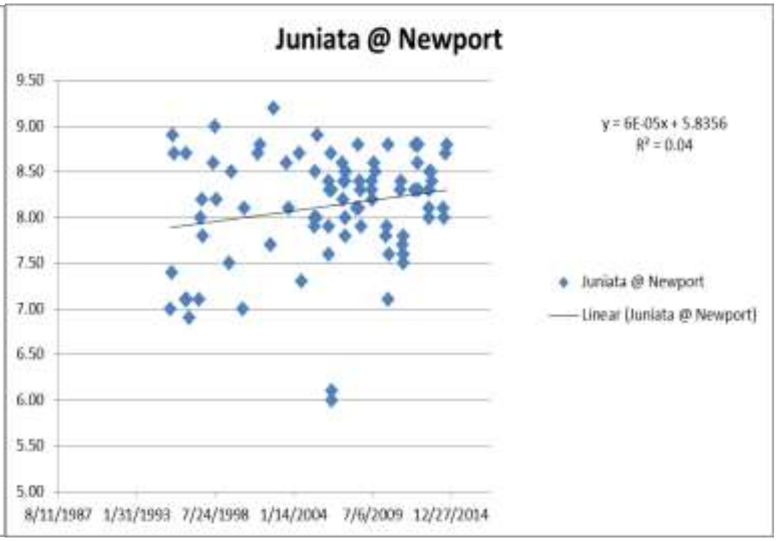




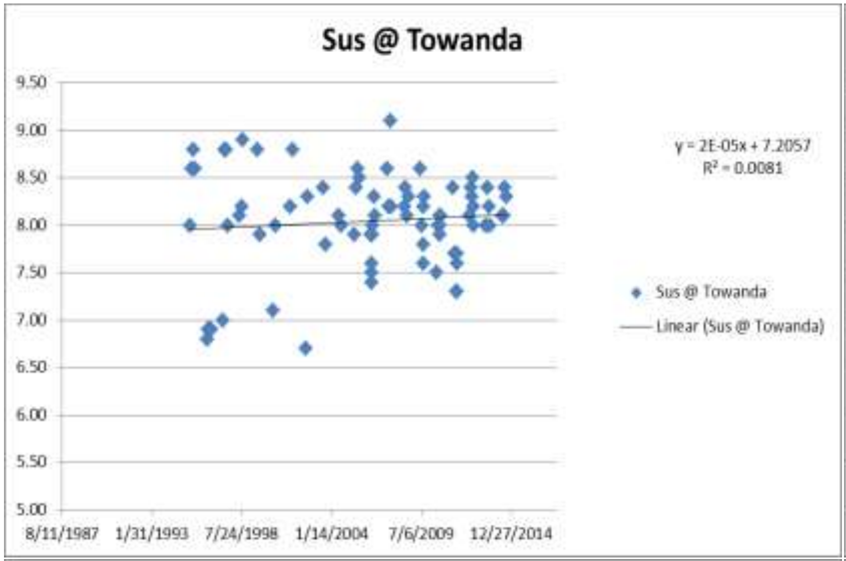
Discrete pH 1995-2014 Lower Mainstem and Juniata River

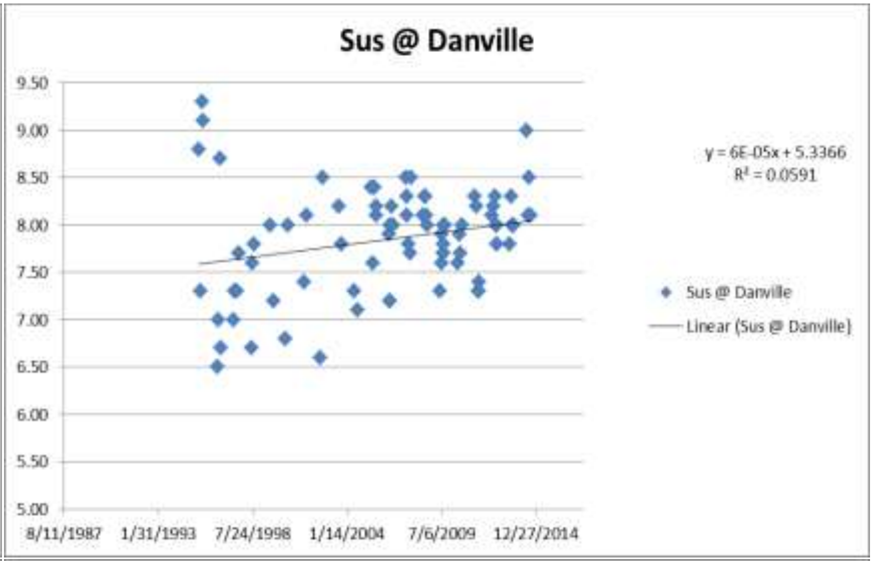


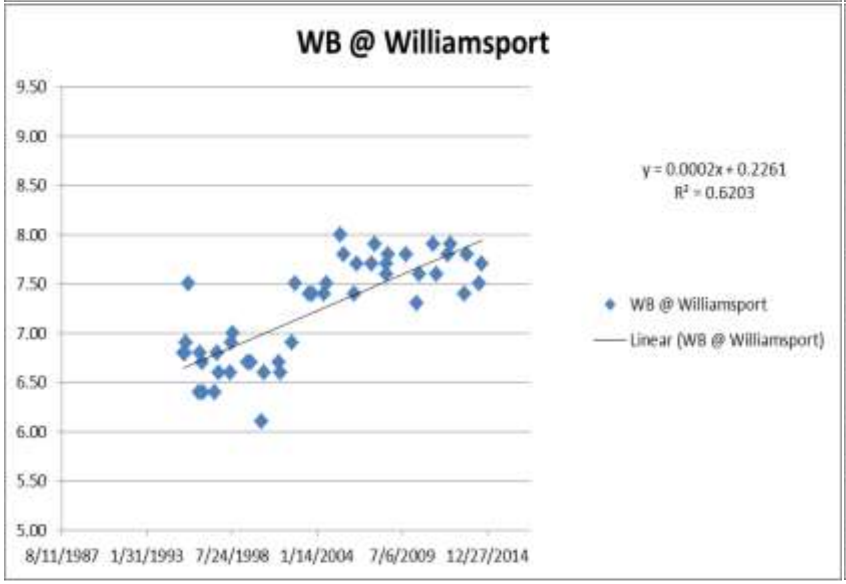


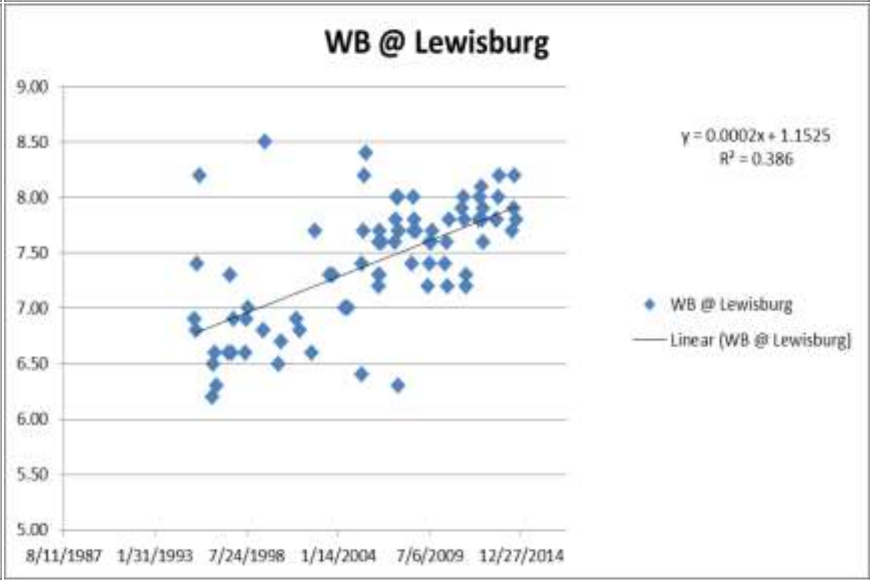


Discrete pH 1995-2014 North Branch and West Branch

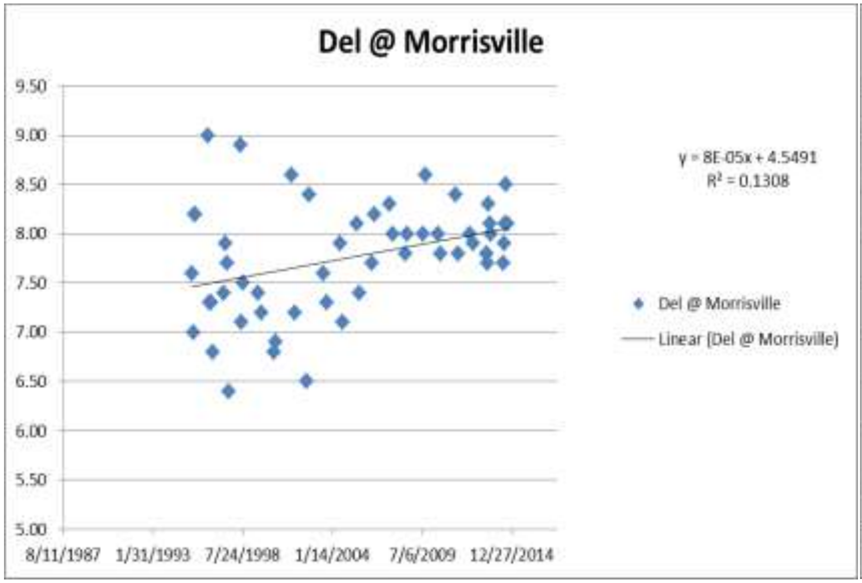


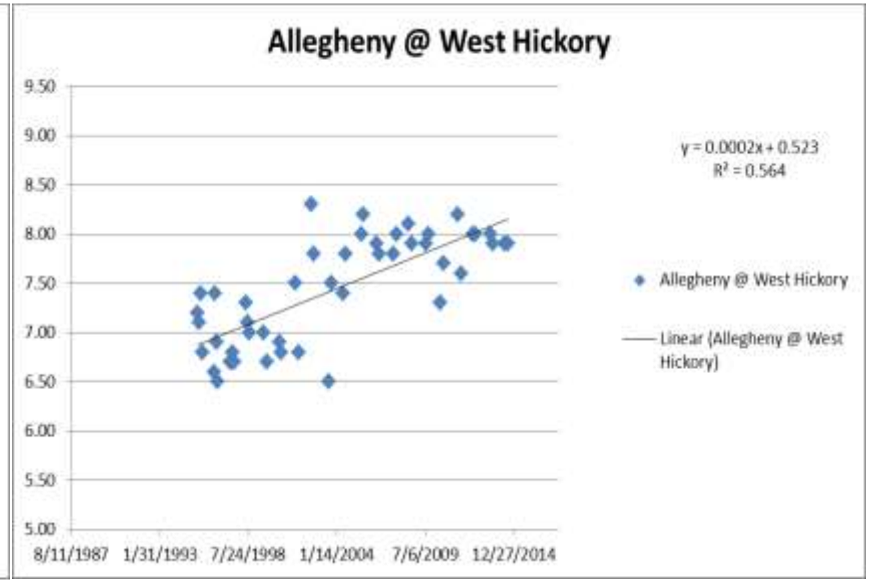






Discrete pH 1995-2014 Delaware and Allegheny





Conclusion:

Overall, it seems plausible to conclude that there is an increasing trend in pH on the West Branch Susquehanna River as depicted by the pH data at Williamsport and Lewisburg. Williamsport is located upriver of Lewisburg and increasing trends in pH (most likely due to acid mine drainage abatement) is more apparent. As the West Branch flows through Lewisburg and towards the confluence with the North Branch any AMD remediation affect becomes more dilute. An increasing trend in pH for the West Branch is stronger for the period 1995-2014 than it is for 1990-2014. Although an increasing trend exists, pH values for the West Branch were not above 8.5.

The figures do not support increasing trends on the Juniata River or the North Branch Susquehanna River, but pH values did exceed 9.0 even in the 1990's.

The Lower Mainstem site data from upriver down (Sunbury, Harrisburg & Marietta) visually depict an increasing trend, but R^2 values and slopes are less than those of West Branch Sites; pH values did exceed 9.0. It is important to note that the Susquehanna River from Sunbury to Marietta does not completely mix and pH values at Lower Mainstem sites are representative of the River across an incompletely mixed transect. Most if not all PFBC YOY sample sites Sunbury to York Haven are located on the West Shore of the River and are not necessarily exposed to conditions represented by the pH data.

The Delaware River at Morrisville data does not show strong support for an increasing trend in pH, but pH values did exceed 9.0 even in the 1990's.

The Allegheny River at West Hickory data does show strong support for an increasing trend in pH, especially 1995-2014; however, pH values did not exceed 9.0.

Candidate Cause 9: Dissolved Oxygen (DO)

Worksheets: 18, 19, 23, 51

Title: Impacts of Low Dissolved Oxygen on Small Mouth Bass Survival and Recruitment (WS #18)

Agency: Pennsylvania Department of Environmental Protection

Spatial comparisons using Data from the Case

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Low dissolved oxygen particularly for juvenile stages can cause lethal and sublethal affects, possibly decreasing SMB survival and recruitment to age class 1+.

Data:

The Pennsylvania Department of Environmental Protection and the U.S. Geological Survey collected continuous DO data during the spring and summer of 2012 and 2013. Stations within the defined CADDIS area included the Susquehanna River at Harrisburg (2012 and 2013) and Juniata River at Newport (2012 and 2013). Comparison sites within the basin included the Susquehanna River at Marietta (2013) and the Susquehanna River at Danville (2013). Data from Danville during 2012 do exist, but the site was not characterizing main channel conditions and is therefore not comparable to other stations. The Delaware River at Morrisville (2012 and 2013) was chosen as the out-of-basin comparison. The Allegheny River was also considered as an out-of-basin comparison, but this site is directly within the influence of French Creek and the Allegheny River is highly flow regulated. Consequently, direct site comparison would be problematic.

Analysis and Results:

Data were temporally truncated to reflect a period from June 1 to July 31. Daily minimums were calculated and graphed for each year for spatial comparisons (Figures 1 and 2).

Generally speaking, the Delaware River tended to have the highest daily minimum for both years. In 2012, the Juniata River had the lowest daily minimums, but the Susquehanna River at Marietta East had the lowest during 2013.

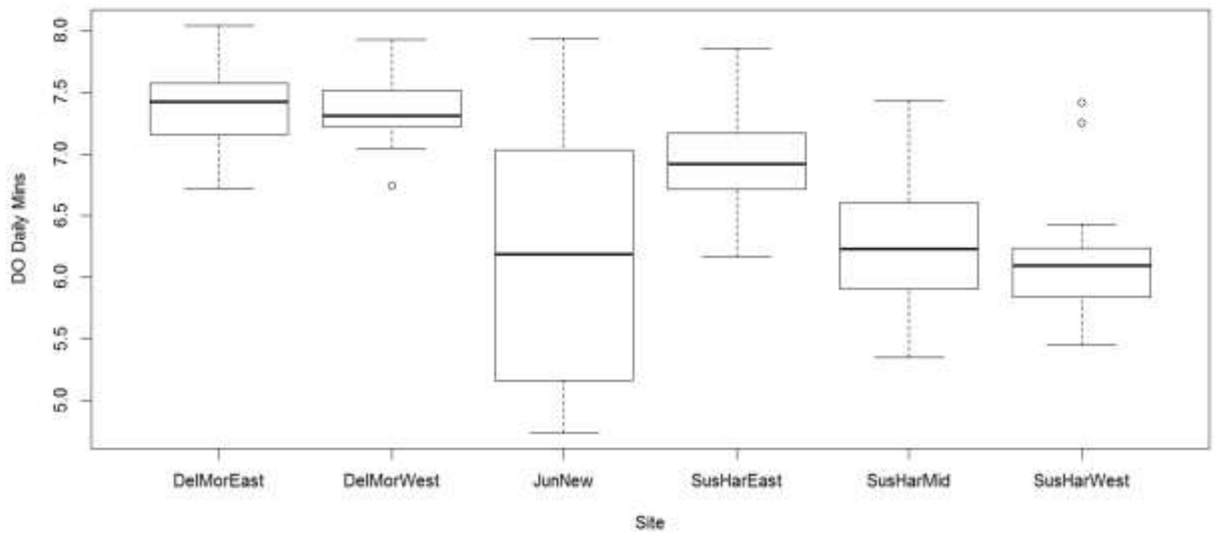


Figure 1. Spatial comparison of daily minimum DO concentrations (mg/L) from 6/1/2012 to 7/31/2012.

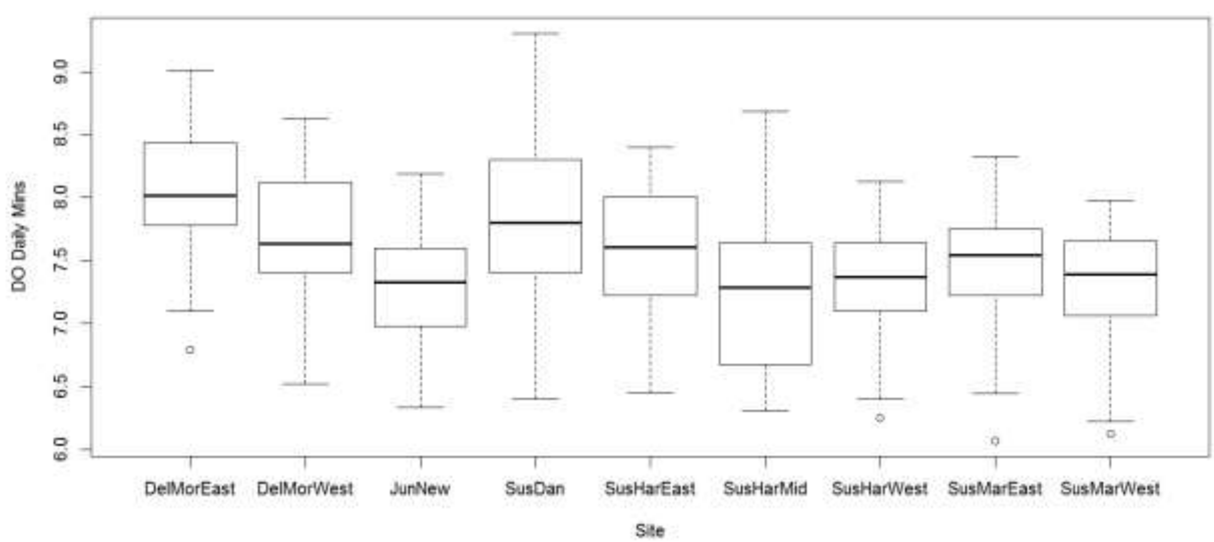


Figure 2. Spatial comparison of daily minimum DO concentrations (mg/L) from 6/1/2013 to 7/31/2013.

Conclusion:

Spatial comparisons suggest that the Delaware River tended to have higher minimum DO concentrations between all sites. However, this is far less noticeable in 2013. These data suggest that DO may become an issue during high temperatures and low flow conditions at certain locations.

Continuous data were collected within the main channel at each site. Consequently, they do not capture possible extremes that may exist along the banks.

Title: Impacts of Low Dissolved Oxygen on Small Mouth Bass Survival (WS #19)

Agency: Pennsylvania Department of Environmental Protection

Temporal Co-occurrence using Data from the Case

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Low dissolved oxygen, particularly during juvenile stages, can cause lethal and sublethal affects, possibly decreasing SMB survival and recruitment to age class 1+.

Many studies show that Centrarchid (bass family) juveniles may be the most sensitive of all warm water fishes to low DO concentrations. At or below DO concentrations of 4.5 mg/L, smallmouth bass hatchling and larvae survival was observed to be significantly reduced (Siefert et al., 1974; Spoor, 1984). Lethal and sublethal effects of reduced DO (less than 5 mg/L) witnessed in laboratory experiments were, in general, directly related to exposure times which ranged from hours to days (Mount, 1964; Doudoroff & Shumway, 1970; Siefert et al., 1974; Spoor, 1984).

Data:

The Pennsylvania Department of Environmental Protection and the U.S. Geological Survey collected continuous DO data during the spring and summer of 2012 and 2013. Stations within the defined CADDIS area included the Susquehanna River at Harrisburg (2012 and 2013) and Juniata River at Newport (2012 and 2013). Comparison sites within the basin included the Susquehanna River at Marietta (2013) and the Susquehanna River at Danville (2013). Data from Danville during 2012 do exist, but the site was not characterizing main channel conditions and is therefore not comparable to other stations. The Delaware River at Morrisville (2012 and 2013) was chosen as the out-of-basin comparison. The Allegheny River was also considered as an out-of-basin comparison, but this site is directly within the influence of French Creek and the Allegheny River is highly flow regulated. Consequently, direct site comparison would be problematic.

Analysis and Results:

Continuous DO data were graphed for every station and year separately (Figures 1-15) with a line drawn at 5 mg/L to indicate the threshold of concern. The Juniata River at Newport during 2012 was the only threshold exceedance observed. Consequently, a concentration duration and frequency (CDF) curve was used to quantify the observations. The CDF curve analysis indicated that there were six separate time periods where DO fell below 5.0 mg/L (Figure 16). Duration for these six events ranged between approximately 0.5 to 3.5 hours, with three events occurring for approximately 1.5 hours. All of these events occurred during the month of July 2012.

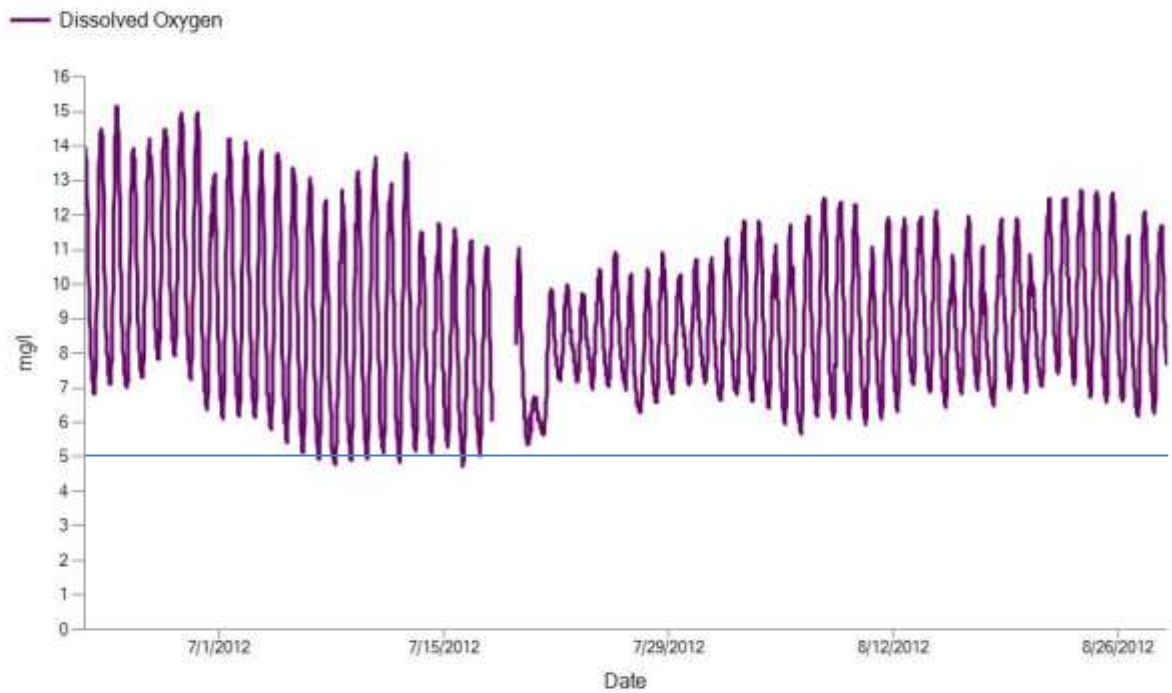


Figure 1. Continuous dissolved oxygen for Juniata River at Newport 2012.

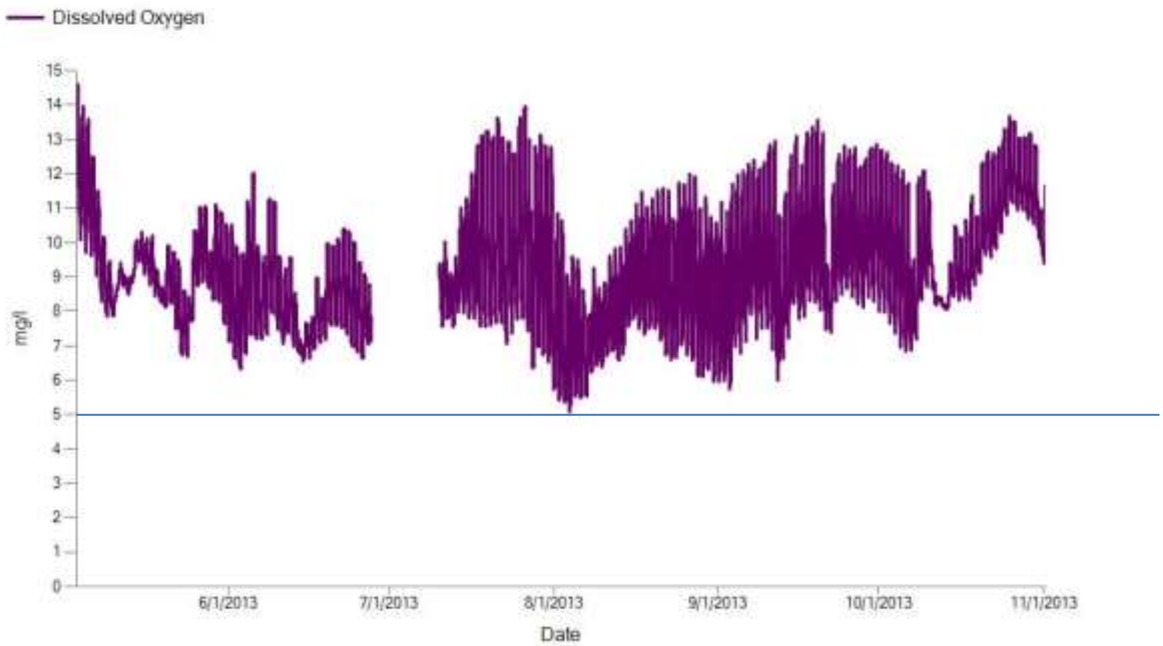


Figure 2. Continuous dissolved oxygen for Juniata River at Newport 2013.

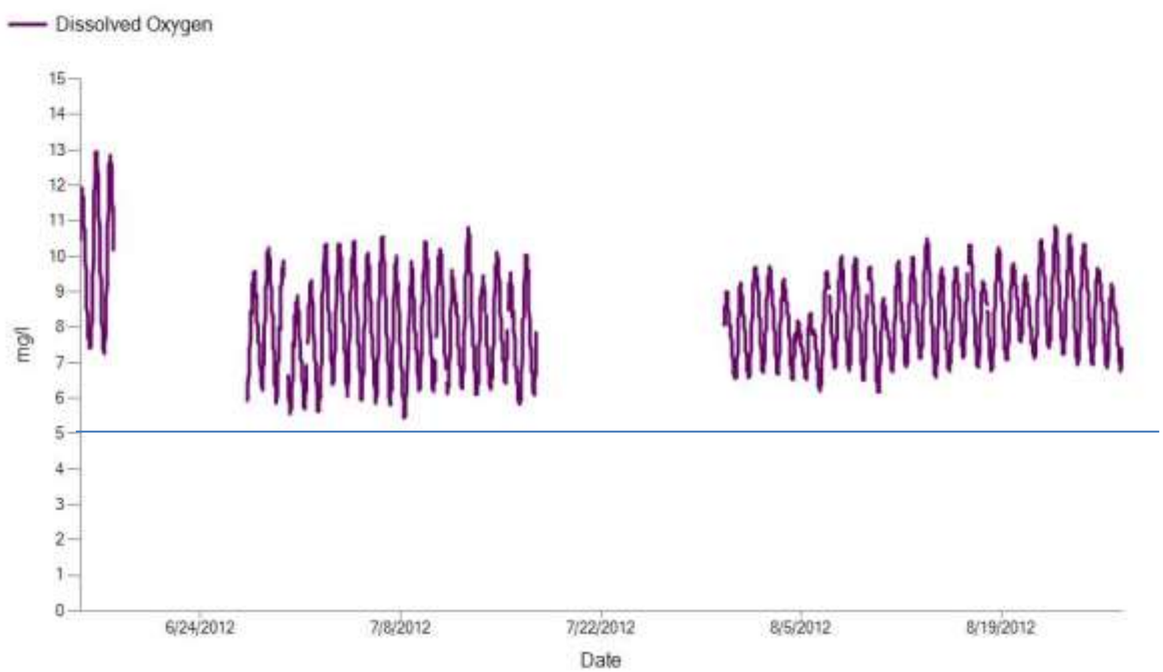


Figure 3. Continuous dissolved oxygen for Susquehanna River at Harrisburg West 2012.

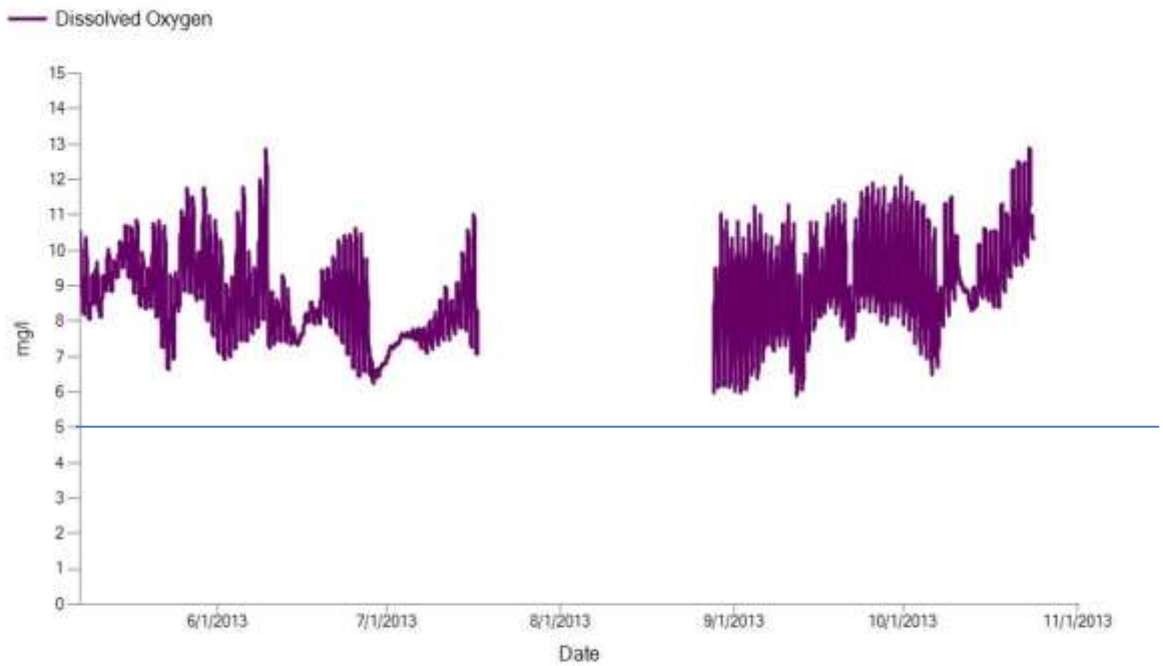


Figure 4. Continuous dissolved oxygen for Susquehanna River at Harrisburg West 2013.

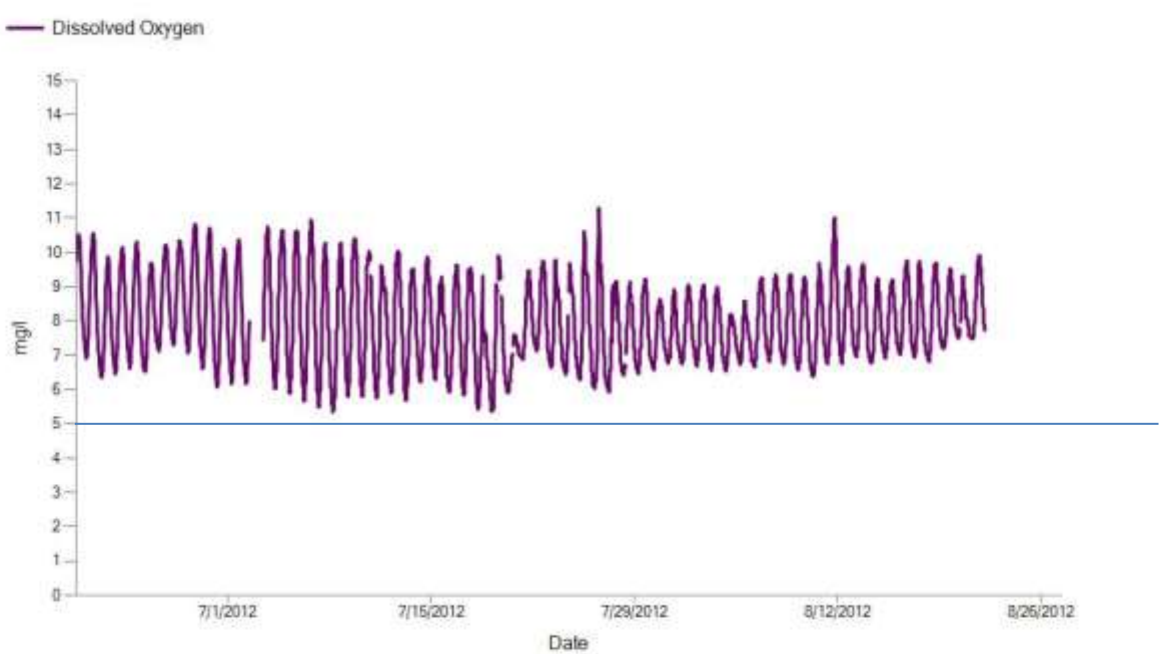


Figure 5. Continuous dissolved oxygen for Susquehanna River at Harrisburg Middle 2012.

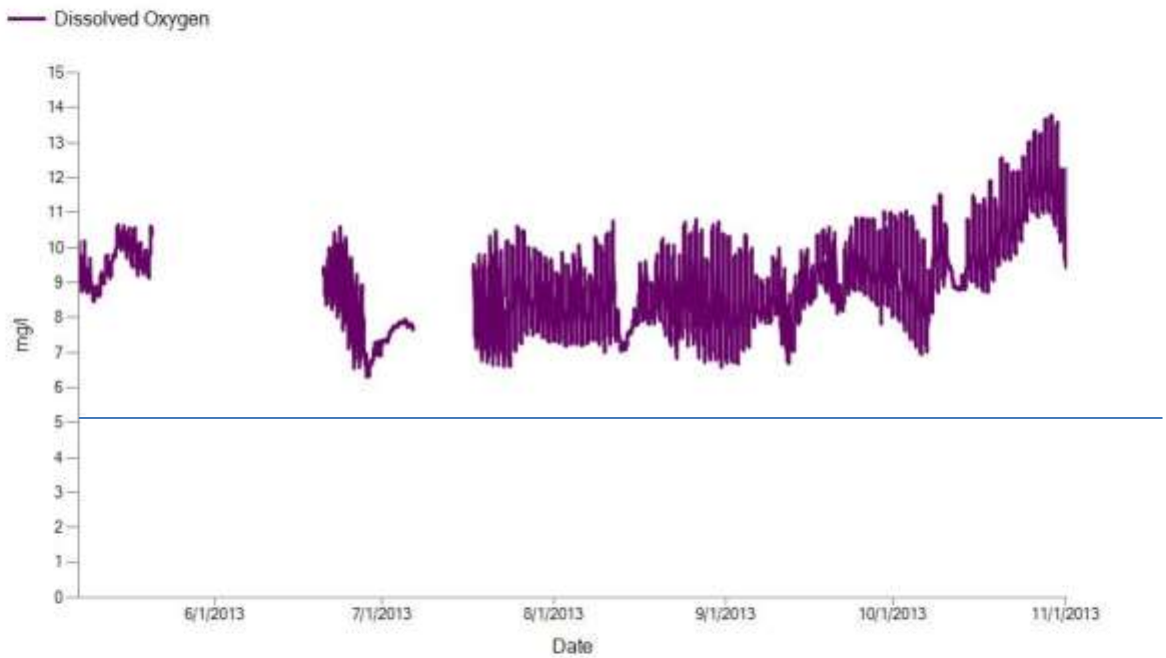


Figure 6. Continuous dissolved oxygen for Susquehanna River at Harrisburg Middle 2013.

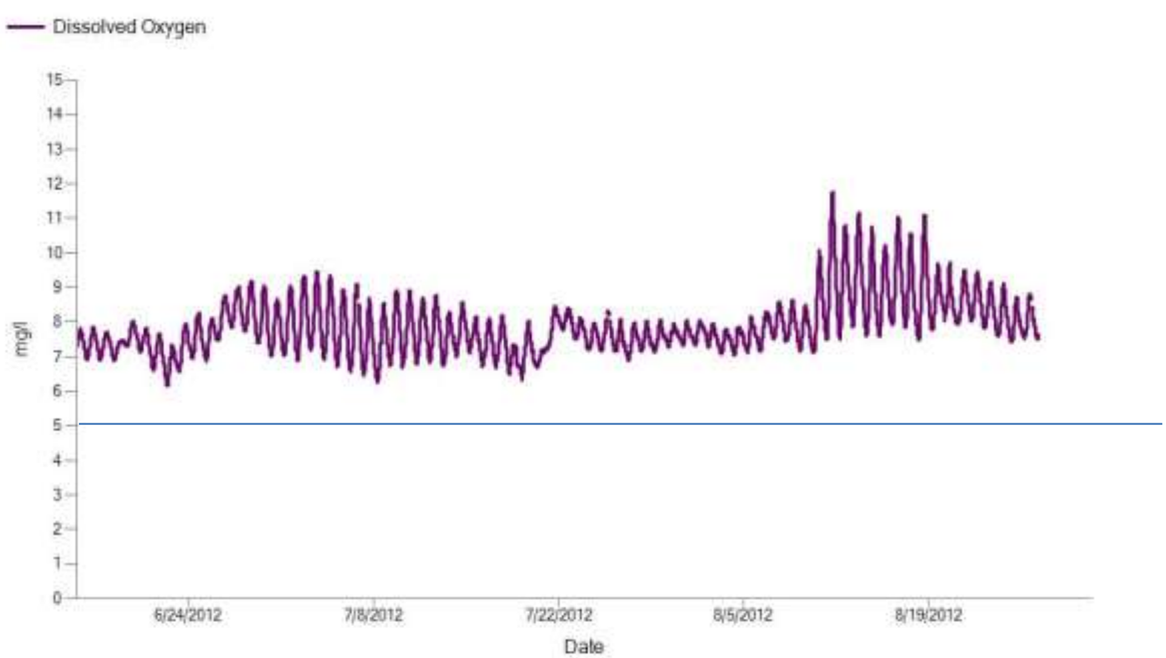


Figure 7. Continuous dissolved oxygen for Susquehanna River at Harrisburg East 2012.

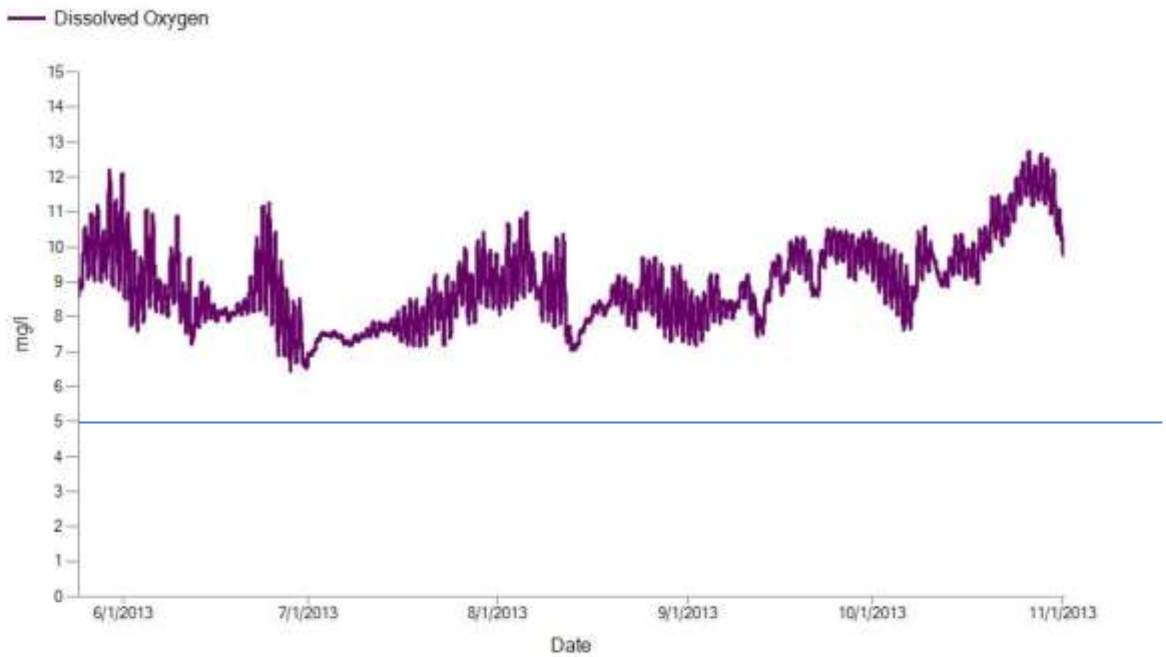


Figure 8. Continuous dissolved oxygen for Susquehanna River at Harrisburg East 2013.

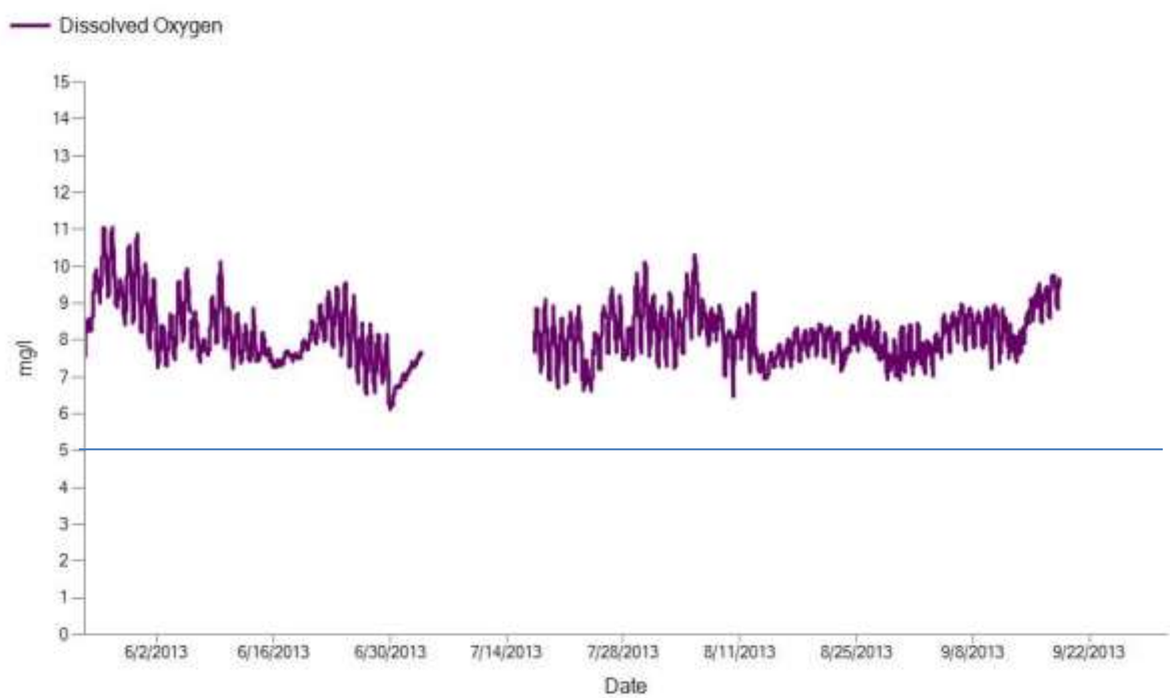


Figure 9. Continuous dissolved oxygen for Susquehanna River at Marietta West 2013.

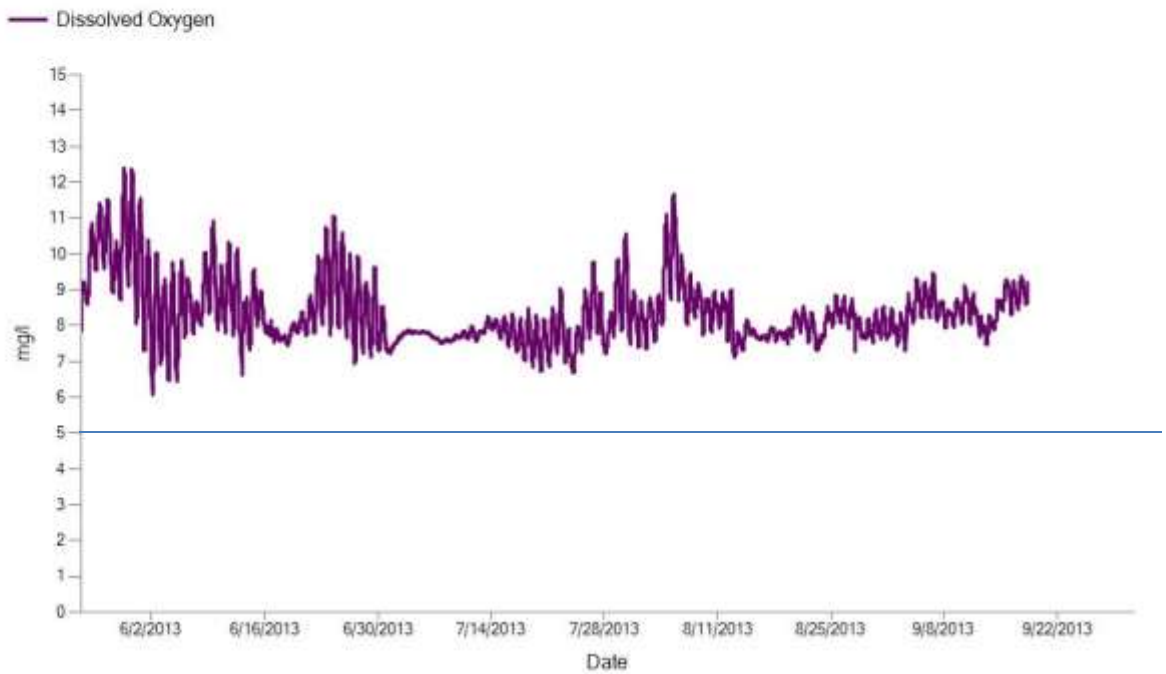


Figure 10. Continuous dissolved oxygen for Susquehanna River at Marietta East 2013.

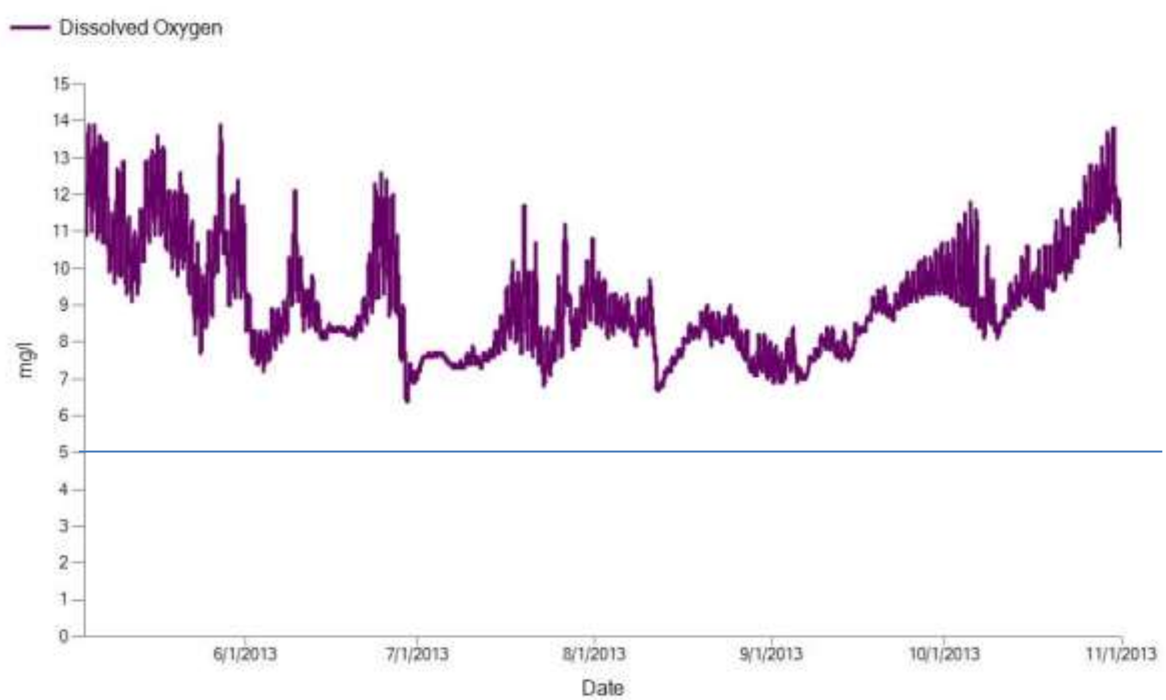


Figure 11. Continuous dissolved oxygen for Susquehanna River at Danville 2013.

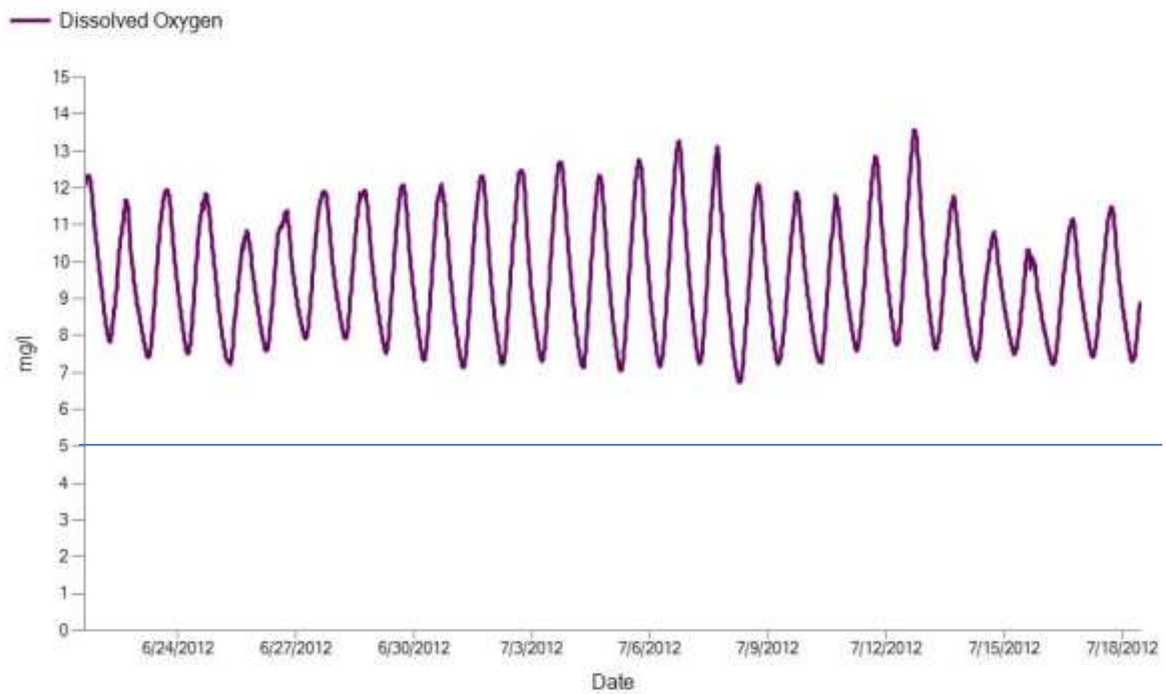


Figure 12. Continuous dissolved oxygen for Delaware River at Morrisville West 2012.

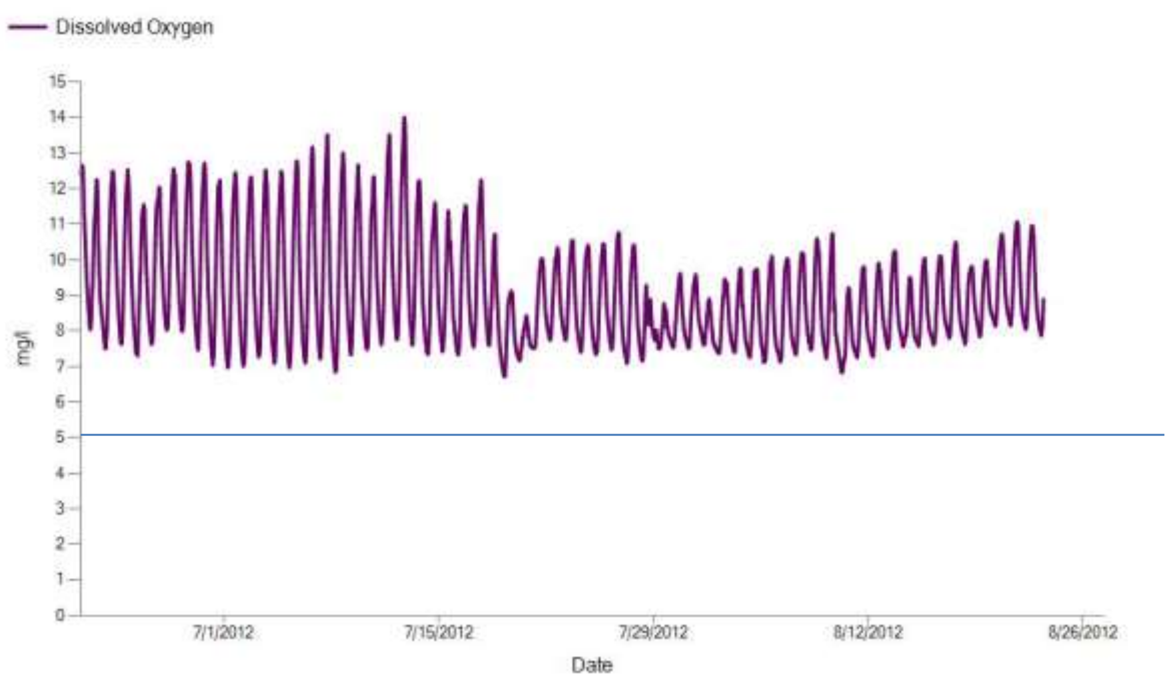


Figure 13. Continuous dissolved oxygen for Delaware River at Morrisville East 2012.

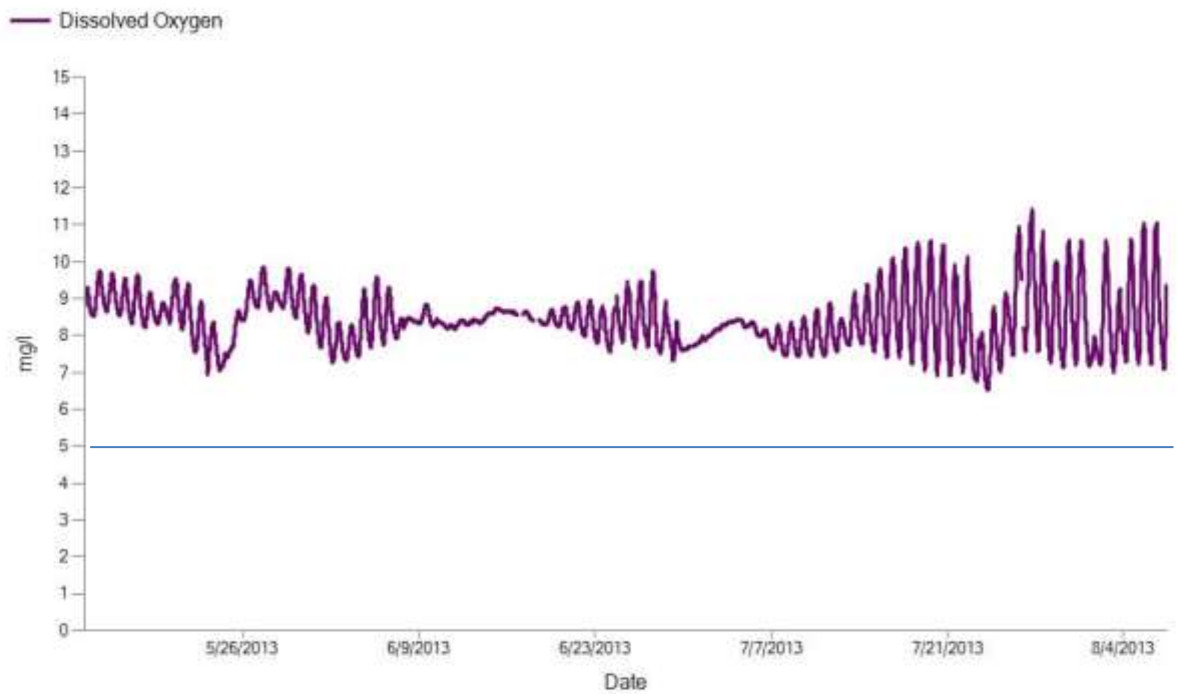


Figure 14. Continuous dissolved oxygen for Delaware River at Morrisville West 2013.

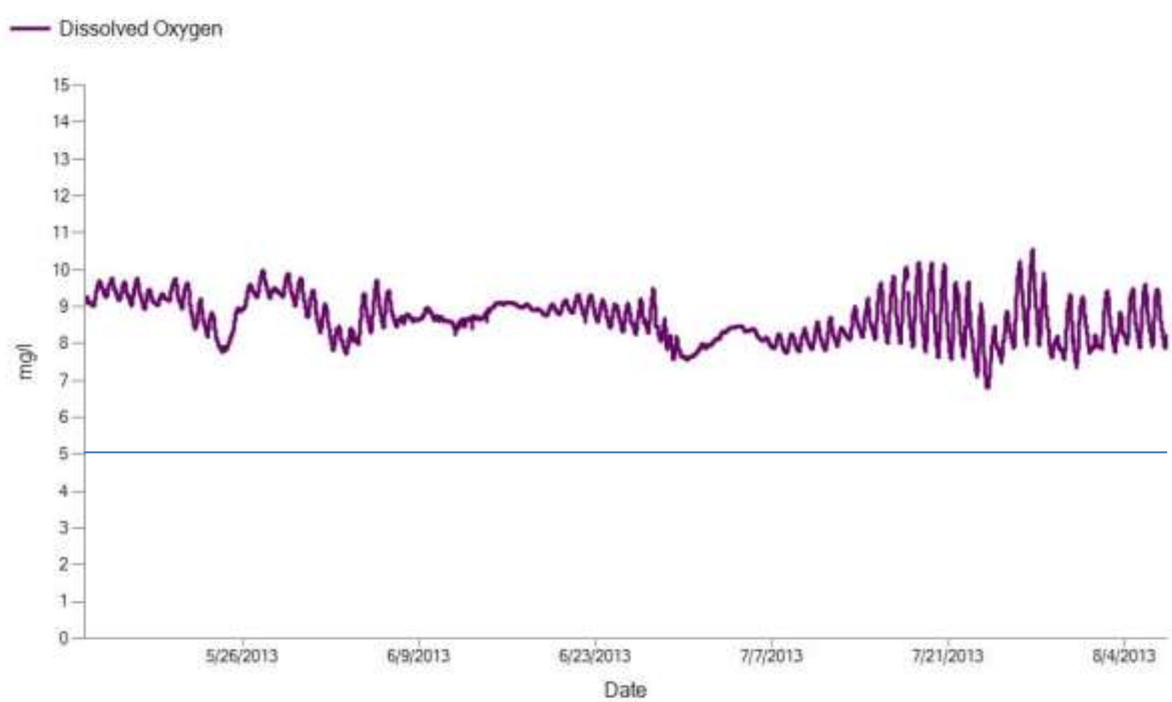


Figure 15. Continuous dissolved oxygen for Delaware River at Morrisville East 2013.

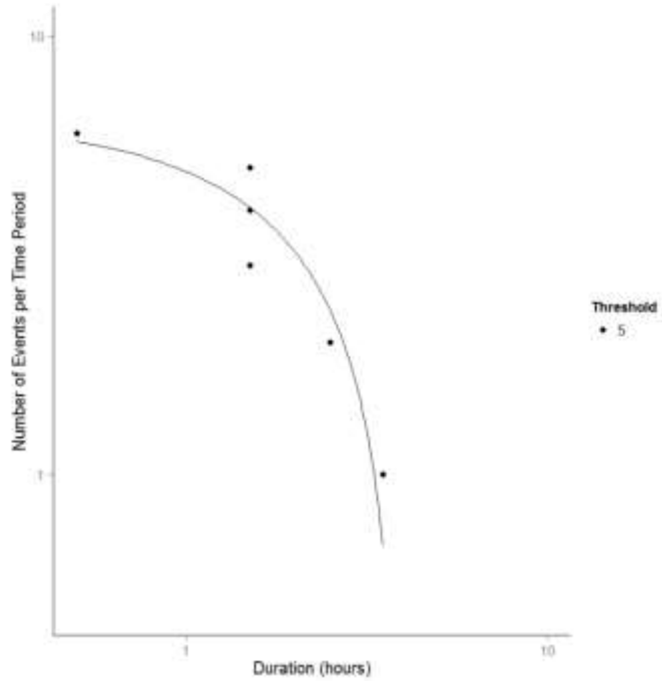


Figure 16. Concentration duration and frequency curve for the Juniata River at Newport 2012. The X and Y axis are in log scale. A threshold of 5 mg/L dissolved oxygen is shown.

Conclusion:

The Juniata River at Newport during 2012 was the only site to experience minimum DO concentrations that could have affected SMB.

Continuous data were collected within the main channel at each site. Consequently, they do not capture possible extremes that may exist along the banks.

Literature Cited:

Doudoroff, P. & D.L. Shumway. 1970. Dissolved Oxygen Requirements of Freshwater Fishes. Food and Agriculture Organization of the United Nations Fisheries Technical Paper 86.

Mount, D.I. 1964. Development of a System for Controlling Dissolved-Oxygen Content of Water.

Siefert, R.E., A.R. Carlson & L.J. Herman. 1974. Effects of Reduced Oxygen Concentrations on the Early Life Stages of Mountain Whitefish, Smallmouth Bass, and White Bass. 1974. The Progressive Fish Culturist. 36(4):186-190.

Spoor, W.A. 1984. Oxygen requirements of larvae of smallmouth bass, *Micropterus dolomieui* Lacépède. Journal of Fish Biology. 25:587-592.

Title: Extreme Discharge (Q) Events and Increases in Ortho-P Trends (WS #23)

Agency: Pennsylvania Department of Environmental Protection

Change in and/or unique patterns of extreme (high and low) flow events and PO4 trends

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Extreme flow events in a riverine system can cause dramatic, cyclical, and/or defining changes. High flow or flood events encourage sediment and nutrient transport longitudinally from upriver sources to downriver and laterally from riparian sources to in river (Ward, 1989). Low flow or extreme low flow, drought, can create a concentrating effect of water quality constituents and can elicit limiting conditions to riverine communities (Nilsson et al 2008). A typical riverine system experiences both extreme high and low discharge events and these events occur at some frequency, usually dependent on a number of factors including climate, geology, topography, soils, and vegetation (Poff et al 1997).

Data:

The Pennsylvania Department of Environmental Protection obtained hourly discharge data from the Harrisburg USGS gage station for water-years 1990 to 2014.

Analysis:

Discharge data (1990-2014) were plotted on a hydrograph in order to visually characterize discharge patterns. Discharge for 2001-2014 was initially plotted and discharge for 1991-1999 was overlaid with 2001-2009 to gain a comparable perspective of these two 10-year periods. Initial observations identified "wet years" vs. "dry years" and, in general, major discharge events (Figure 1). In order to identify low flow events, the scale was adjusted and events less than 4K cfs were identified in '91, 93, 95, 98, 99, 01, 02, 05, 07, & 10 (Figure 2). To identify the full magnitude of elevated discharge events, the scale was adjusted to 0-575K cfs and events greater than 275K were identified in '93, 94, 96, 98, 05, 06 08, 10, 11, & 12 (Figure 3). Flood discharge at Harrisburg is approximately 308-310K cfs. The presence of low (<4K cfs) and high (>275K cfs) discharge events in any given year were identified on PFBC's 'Catch rate (fish/h) of adult smb … at the middle Susquehanna River: 1990-2012' (Figure 4). The presence of low (<4K cfs) and high (>275K cfs) discharge events was also compared to ortho-phosphorus load trends (Figure 6).

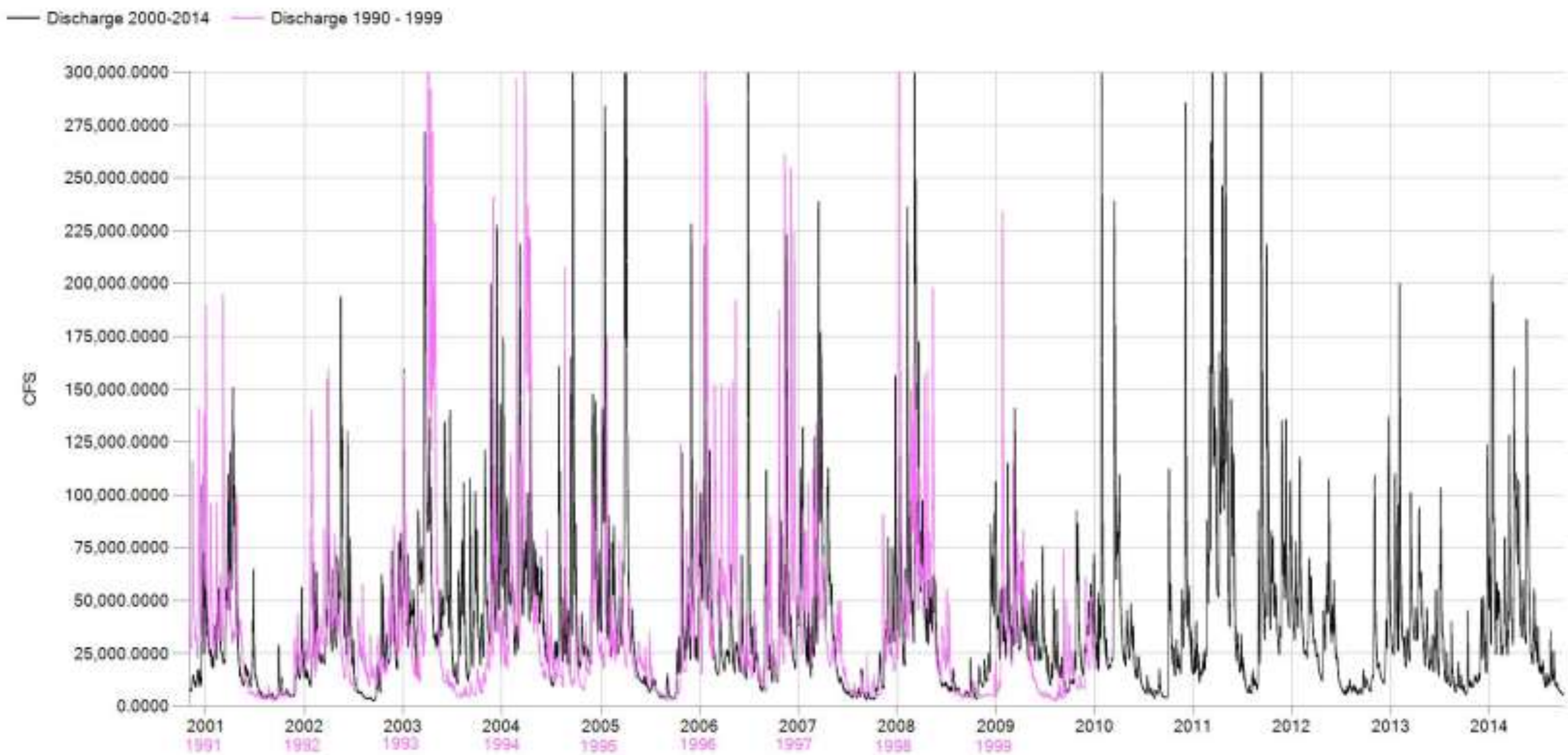


Figure 1. Discharge at Harrisburg 1991-2014, 0-300K cfs.

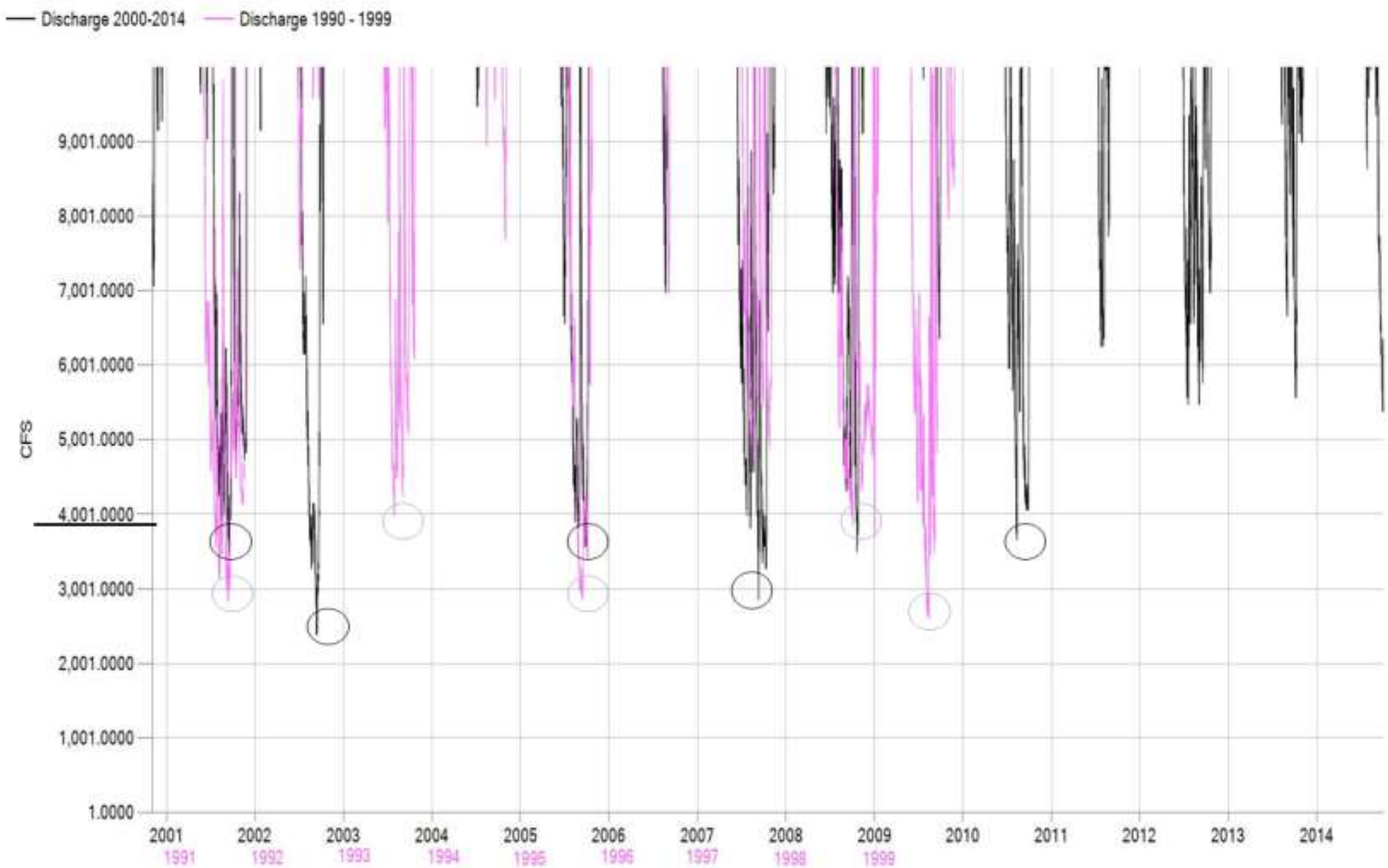


Figure 2. Discharge at Harrisburg 1991-2014, 0-10K cfs, with events < 4K identified.

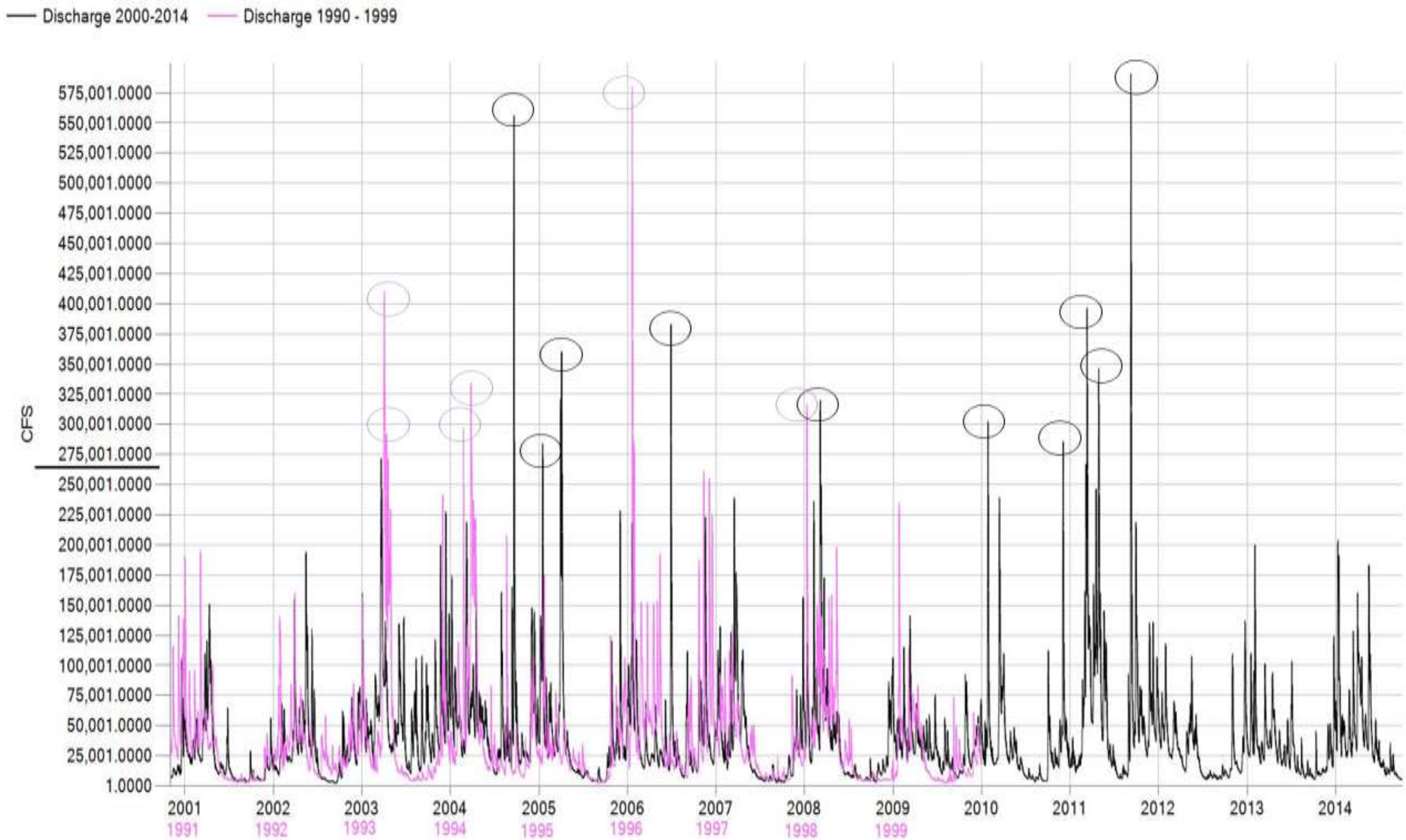


Figure 3. Discharge at Harrisburg 1991-2014, 0-575K cfs, with events >275K identified.

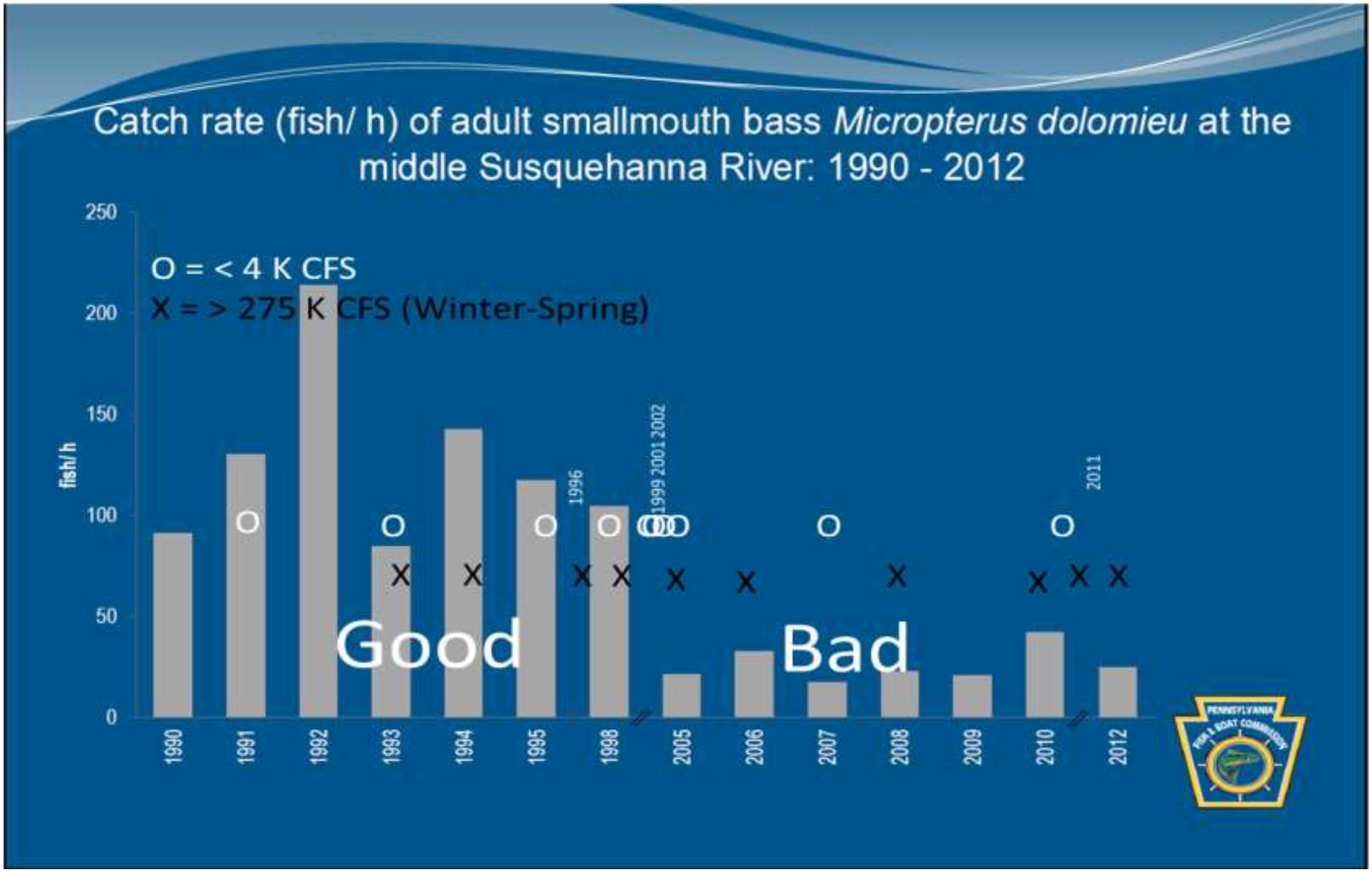


Figure 4. PFBC fall recruitment results for the middle Susquehanna River: 1990-2012 with the occurrence of low (<4K cfs) and high (>275K cfs) discharge events identified for given years.

Results:

From 1990 through 2010 flow events <4K cfs occurred at fairly regular intervals (every year to no more than two years apart). From 1993 through 1998 flow events >275K occurred 4 of 6 years, followed by a period of approximately 6 years without this elevated flow event and without a major flood event. This period post-1998 is consistent with the time smallmouth bass recruitment had been projected to have started to decrease. Low flow events in 2005 and 2007 correlate with some of the worst recruitment and the timing of such is just as high flow events begin to occur at more regular intervals (Figures 4 & 5). In recent years, recruitment has still been low, but trends may be showing increases, especially for 2011 – 2014 when major elevated discharge events have been occurring more frequently. Low discharge events (<4K) have not occurred since 2010.

Additionally, dissolved ortho-phosphorus trends in the Susquehanna River characterize increasing trends beginning in the mid-90s and peaking in 2004. And while there were no > 275K discharge events in 2003 and 2004, mean yearly mean discharge for 2003 and 2004 were above mean (Figure 6). This suggests flow patterns may be significantly driving other data patterns.

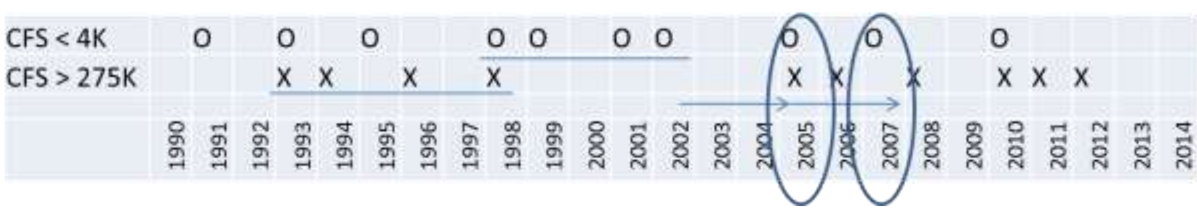


Figure 5. Occurrence of low (<4K cfs) and high (>275K cfs) discharge events identified for given years.

Conclusion:

Discharge and discharge patterns, especially flood and drought conditions, are major drivers in any riverine system. The timing of or lack of major elevated discharge events coupled with the presence of low discharge events in 1999 through 2004 correlates temporally with the decline in recruitment of smallmouth bass in the Susquehanna River.

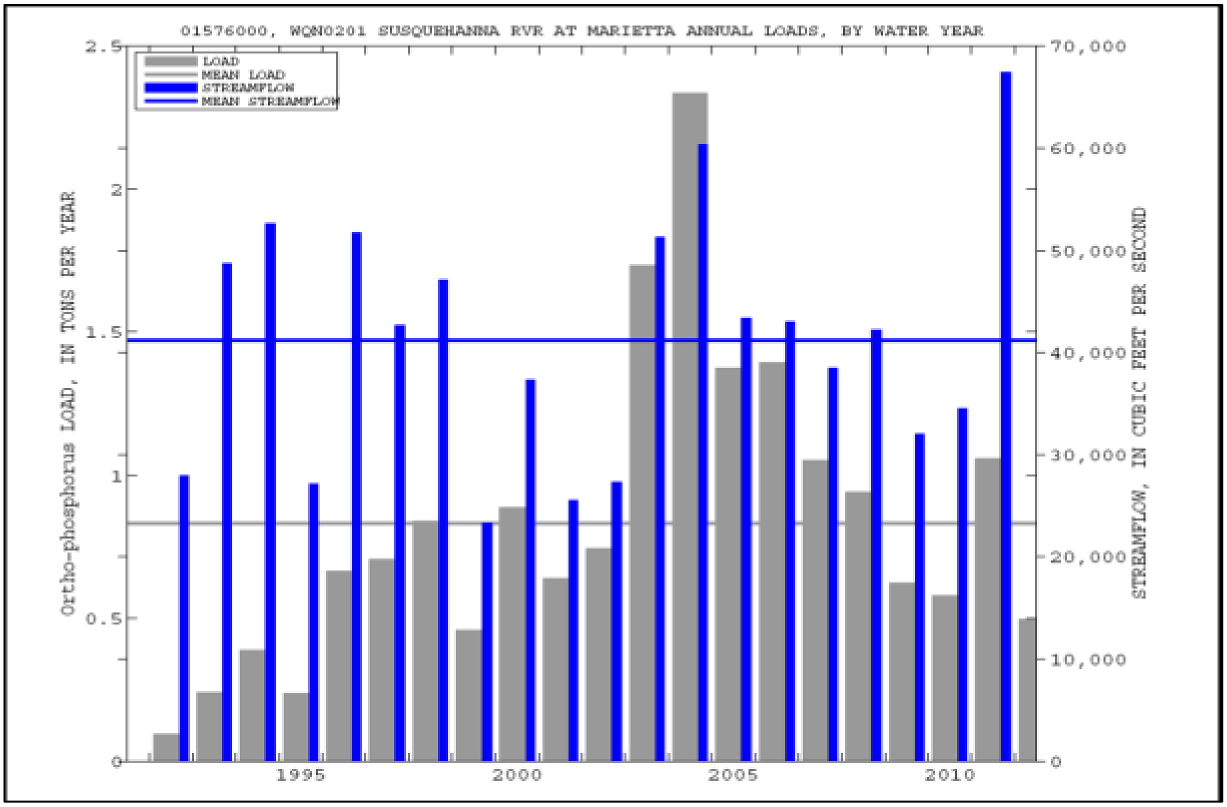


Figure 6. Ortho-phosphorus load and mean discharge trends for the Susquehanna River at Marietta 1990-2012

Title: Low Dissolved Oxygen and Decreasing Smallmouth Bass Survival and Recruitment (WS #51)

Agency: Susquehanna River Basin Commission

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Low dissolved oxygen, particularly for juvenile stages, can cause lethal and sublethal effects, possibly decreasing SMB survival and recruitment to Age Class 1+.

Data:

SRBC has collected field DO measurements as part of various projects on four locations on the Susquehanna River within the case study area (Fort Hunter, Halifax, McKees Half Falls, and Sunbury) and on the Juniata River just upstream of the confluence with the Susquehanna. DO measurements were also collected at two sites on the Susquehanna River that bracket the case study area—downstream at Wrightsville and upstream at Danville. DO measurements on the West Branch Susquehanna at Lewisburg are also provided. These measurements were taken with a handheld probe at several areas across the channel, with the median measurement presented. Most data from 1985-2008 were taken in June through September in a time ranging from the mid-morning through early afternoon. Data from 2011 and 2012 were collected in October and November.

Analysis and Results:

Figure 1 below shows the range of dissolved oxygen measurements for these sites from 1985 through 2012, although the record is spotty at any one particular site. DO values in 2005 are the lowest values seen in all of the data for each site. Fort Hunter and McKees Half Falls have the lowest values (5.55 mg/l and 5.19 mg/l, respectively) in 2005 of all the sites. Within the study area, values ranged from 5.19 mg/l to 6.98 mg/l (Halifax). Values before and after 2005 are difficult to compare because of the data gaps, but overall the values appear to fall within a reasonable range of each other. 2011 and 2012 data are taken in the Fall when temperatures are cooler (see Figure 2), so DO levels are not surprisingly higher.

Figure 2 illustrates water temperature within the case study area. None of these sites exhibited stressful (>30 C) water temperatures during these site visits. Temperatures in 2005 within the case study area were pretty consistent, ranging from 23.5 C (Sunbury) to 25.3 C (Halifax & Juniata River). Temperatures before and after 2005 fall within a reasonable range of each other.

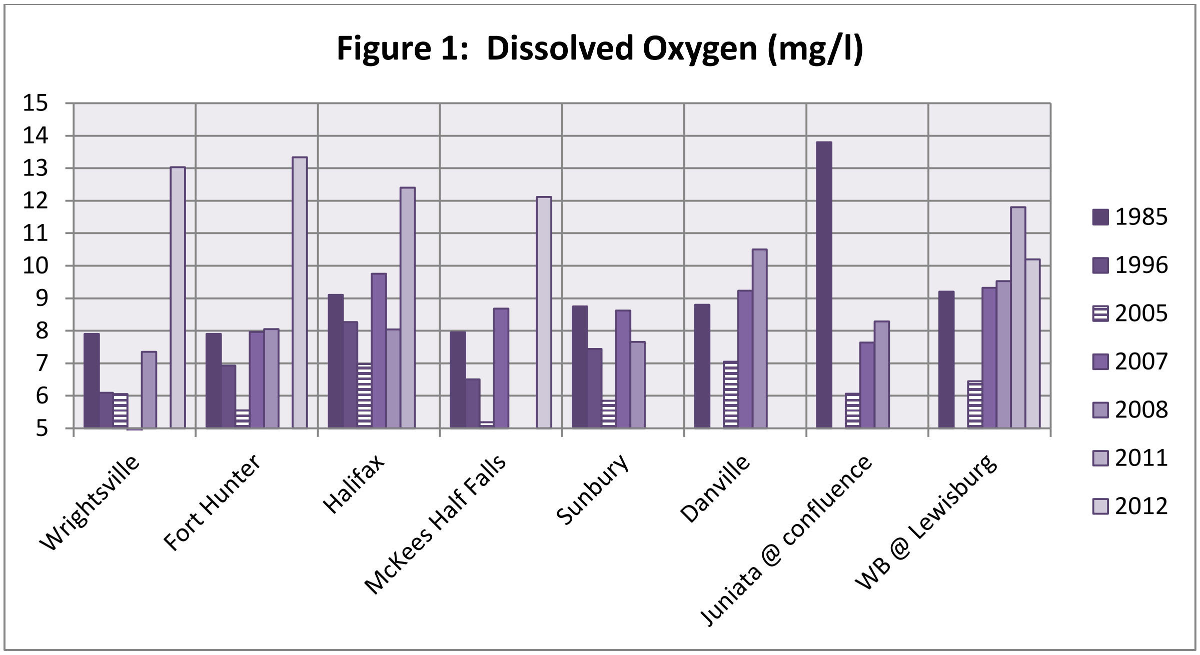


Figure 1: Dissolved Oxygen (mg/l)
15
14
13
12
11
10
9
8
7
6
5
Wrightsville
Fort Hunter
Halifax
McKees Half Falls
Sunbury
Danville
Juniata @ confluence
WB @ Lewisburg
1985
1996
2005
2007
2008
2011
2012

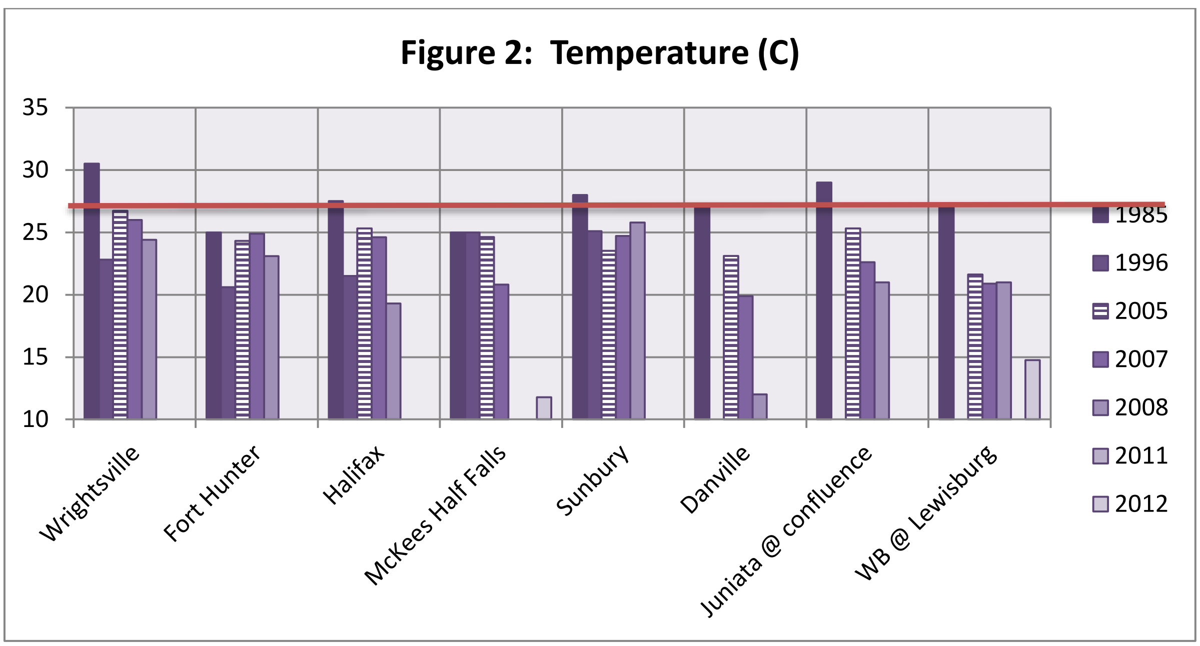


Figure 2: Temperature (C)
35
30
25
20
15
10
Wrightsville
Fort Hunter
Halifax
McKees Half Falls
Sunbury
Danville
Juniata @ confluence
WB @ Lewisburg
1985
1996
2005
2007
2008
2011
2012

Candidate Cause 10: Ammonia (NH_3)

Worksheets: 20, 21 22, and 52

Title: Impacts of Ammonia toxicity on the survival and recruitment of Smallmouth Bass (WS #20)

Agency: Pennsylvania Department of Environmental Protection

Spatial comparison using Data from the Case

Candidate Cause: Ammonia toxicity (increases by high pH and high temperature) either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Ammonia toxicity increases with pH and water temperature, possibly decreasing SMB survival and recruitment to age class 1+. Stressful summer conditions – specifically water temperatures exceeding 30°C and pH exceeding 9.0 – were observed at several locations on the Susquehanna River and Juniata River since 2008.

Laboratory methods for surface water samples measure both the un-ionized form (ammonia, NH_3) and the ionized form (ammonium, NH_4^+). The un-ionized NH_3 is much more toxic to aquatic organisms. As temperature and pH increase in the aquatic environment, the proportion of NH_3 to NH_4^+ also increases. With this understanding, formulas and laboratory measurements can be used to evaluate ammonia toxicity.

If ammonia toxicity is affecting SMB survival and recruitment to age class 1+, then stations within the defined CADDIS area should either have higher concentrations of ammonia, or be in closer proximity to a particular toxic threshold when temperature and pH are considered.

Data:

The Pennsylvania Department of Environmental Protection collected continuous water temperature and pH data during the spring and summer of 2012 and 2013. Stations within the defined CADDIS area included the Susquehanna River at Harrisburg (2012 and 2013), and Juniata River at Newport (2012 and 2013). The Susquehanna River at Marietta (2013) was used as an in basin comparison site. The Delaware River at Morrisville (2012 and 2013) was used as an out of basin comparison site.

Several ammonia grab samples were collected by PA DEP and USGS at stations where continuous data co-occurred. Ammonia grab samples were also collected by USGS where continuous data did not co-occur. These stations included the Susquehanna River at Falls, and the Susquehanna River at Great Bend.

Analysis and Results:

Ammonia samples from PA DEP and USGS for 2012 and 2013 were grouped together at each site and descriptive statistics were calculated (Table 1). All concentrations were relatively similar, with the Susquehanna at Marietta having the highest mean concentration. The Susquehanna at Harrisburg and Susquehanna at Great Bend had the lowest mean concentrations.

Continuous water temperature and pH data were applied to formulas in Table 3 of §93.7(a) in order to generate acute and chronic ammonia time series datasets. Generated criteria time series data are not directly applicable to water quality standards, but they can expose time periods when ammonia may become toxic. Tables 2 and 3 provide an approximation of the previously mentioned formula. As demonstrated by Table 3, there were no ammonia samples that approached acute thresholds. Therefore, the acute criterion time series was not included in the graphics.

Chronic ammonia criterion time series were compared to measured concentrations of ammonia (Figures 1-12) to determine if or when stream conditions became potentially stressful. There were no samples that exceeded the chronic threshold. However, at times, concentrations did approach the chronic threshold.

Table 1. Distribution of ammonia samples taken by PA DEP and USGS for 2012 and 2013. Values are total Ammonia as N (mg/L).

Location	Mean	sd	25%	50%	75%	100%	N
Juniata, Newport	0.033	0.017	0.020	0.030	0.040	0.090	56
Susquehanna, Harrisburg	0.023	0.013	0.016	0.020	0.030	0.057	29
Susquehanna, Marietta	0.037	0.024	0.020	0.030	0.050	0.100	51
Susquehanna, Great Bend	0.022	0.007	0.020	0.020	0.020	0.040	9
Susquehanna, Falls	0.025	0.012	0.020	0.020	0.023	0.060	12
Delaware, Morrisville	0.026	0.011	0.020	0.020	0.030	0.060	20

Table 2. Calculated chronic ammonia criterion based on the formula in Table 3 of §93.7(a). Highlighted concentrations (mg/L) indicate levels that could be expected at all sites based off the data in table 1.

Temperature	pH										
	8.5	**8.6**	**8.7**	**8.8**	**8.9**	**9**	**9.1**	**9.2**	**9.3**	**9.4**	**9.5**
25	0.164	0.135	0.113	0.095	0.080	0.069	0.060	0.053	0.047	0.042	0.039
26	0.155	0.128	0.107	0.090	0.077	0.066	0.058	0.051	0.046	0.041	0.038
27	0.146	0.121	0.101	0.086	0.073	0.063	0.055	0.049	0.044	0.040	0.037
28	0.138	0.114	0.096	0.081	0.070	0.061	0.053	0.047	0.043	0.039	0.036
29	0.130	0.109	0.091	0.078	0.067	0.058	0.051	0.046	0.042	0.038	0.036
30	0.123	0.103	0.087	0.074	0.064	0.056	0.050	0.045	0.041	0.037	0.035
31	0.117	0.098	0.083	0.071	0.062	0.054	0.048	0.043	0.040	0.037	0.034
32	0.111	0.093	0.079	0.068	0.059	0.052	0.047	0.042	0.039	0.036	0.034

33	0.105	0.089	0.076	0.065	0.057	0.050	0.045	0.041	0.038	0.035	0.033
34	0.100	0.085	0.072	0.063	0.055	0.049	0.044	0.040	0.037	0.034	0.032
35	0.095	0.081	0.069	0.060	0.053	0.047	0.043	0.039	0.036	0.034	0.032
36	0.091	0.077	0.066	0.058	0.051	0.046	0.041	0.038	0.035	0.033	0.032
37	0.086	0.074	0.064	0.056	0.049	0.044	0.040	0.037	0.035	0.033	0.031
38	0.083	0.071	0.061	0.054	0.048	0.043	0.039	0.036	0.034	0.032	0.031

Table 3. Calculated acute ammonia criteria based on the formula in Table 3 of §93.7(a). Concentrations (mg/L) reported by USGS and PA DEP were below these values for 2012 and 2013.

Temperature						**pH**					
	8.5	**8.6**	**8.7**	**8.8**	**8.9**	**9**	**9.1**	**9.2**	**9.3**	**9.4**	**9.5**
25	0.742	0.620	0.521	0.441	0.377	0.325	0.283	0.250	0.224	0.203	0.186
26	0.699	0.586	0.494	0.419	0.359	0.311	0.272	0.241	0.217	0.197	0.181
27	0.659	0.554	0.468	0.399	0.343	0.298	0.262	0.233	0.210	0.192	0.177
28	0.623	0.524	0.444	0.380	0.328	0.286	0.252	0.225	0.204	0.187	0.173
29	0.589	0.497	0.422	0.362	0.313	0.274	0.243	0.218	0.198	0.182	0.169
30	0.557	0.472	0.402	0.346	0.300	0.264	0.235	0.212	0.193	0.178	0.166
31	0.528	0.448	0.383	0.331	0.288	0.254	0.227	0.205	0.188	0.174	0.163
32	0.501	0.426	0.366	0.317	0.277	0.245	0.220	0.200	0.184	0.171	0.160
33	0.476	0.406	0.349	0.304	0.267	0.237	0.213	0.194	0.179	0.167	0.158
34	0.452	0.387	0.334	0.292	0.257	0.229	0.207	0.190	0.175	0.164	0.155
35	0.430	0.370	0.320	0.280	0.248	0.222	0.202	0.185	0.172	0.161	0.153
36	0.410	0.353	0.307	0.270	0.240	0.216	0.196	0.181	0.168	0.159	0.151
37	0.391	0.338	0.295	0.260	0.232	0.209	0.191	0.177	0.165	0.156	0.149
38	0.374	0.324	0.284	0.251	0.225	0.204	0.187	0.173	0.162	0.154	0.147

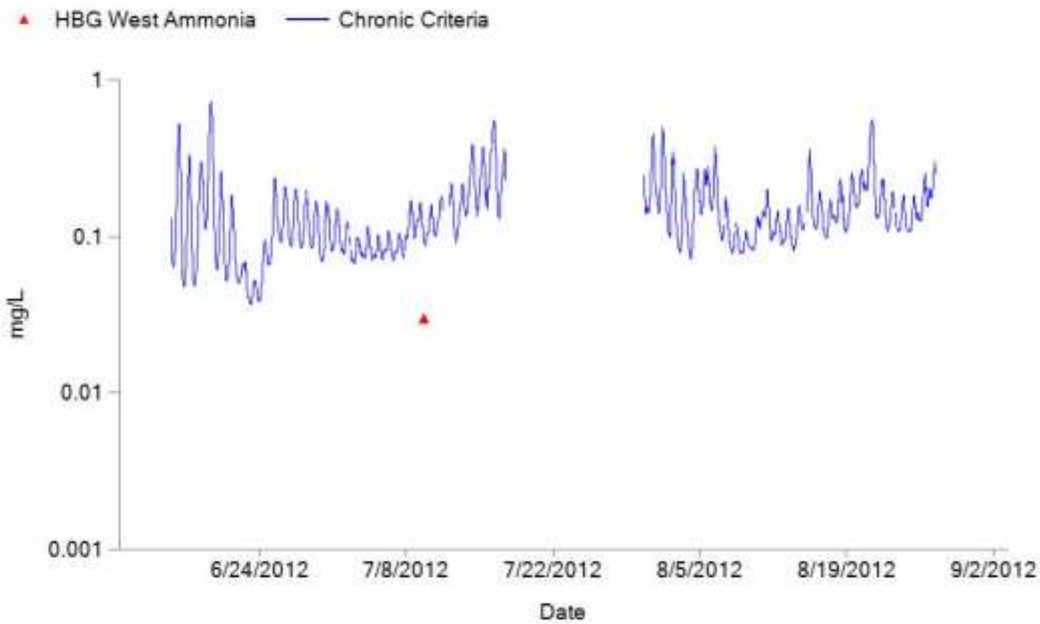


Figure 1. West site on the Susquehanna River at Harrisburg during the 2012 sampling year. The Y axis is in log scale.

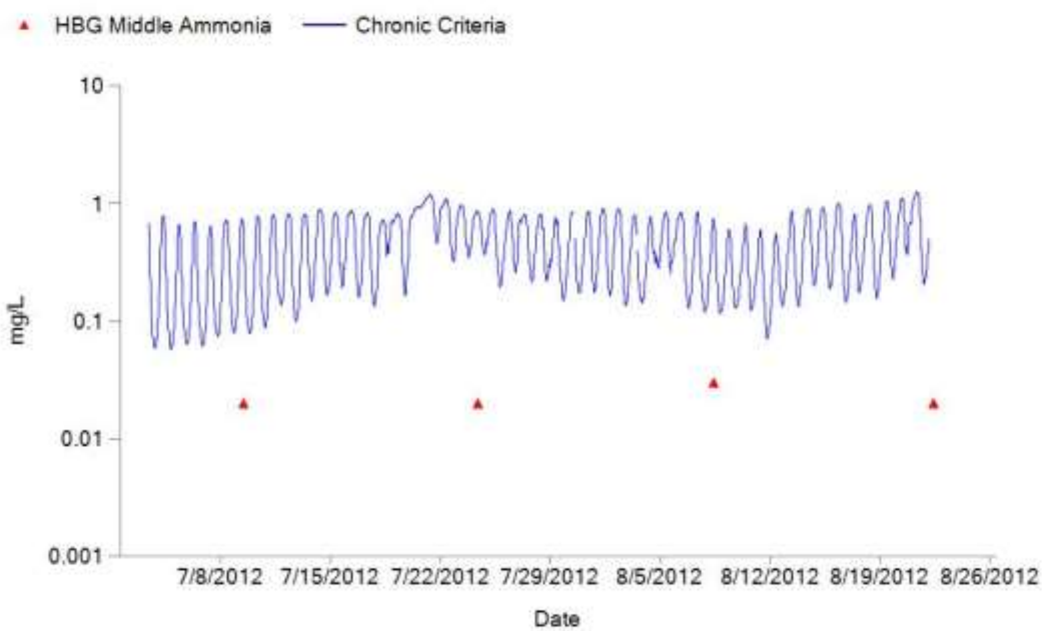


Figure 2. Middle site on the Susquehanna River at Harrisburg during the 2012 sampling year. The Y axis is in log scale.

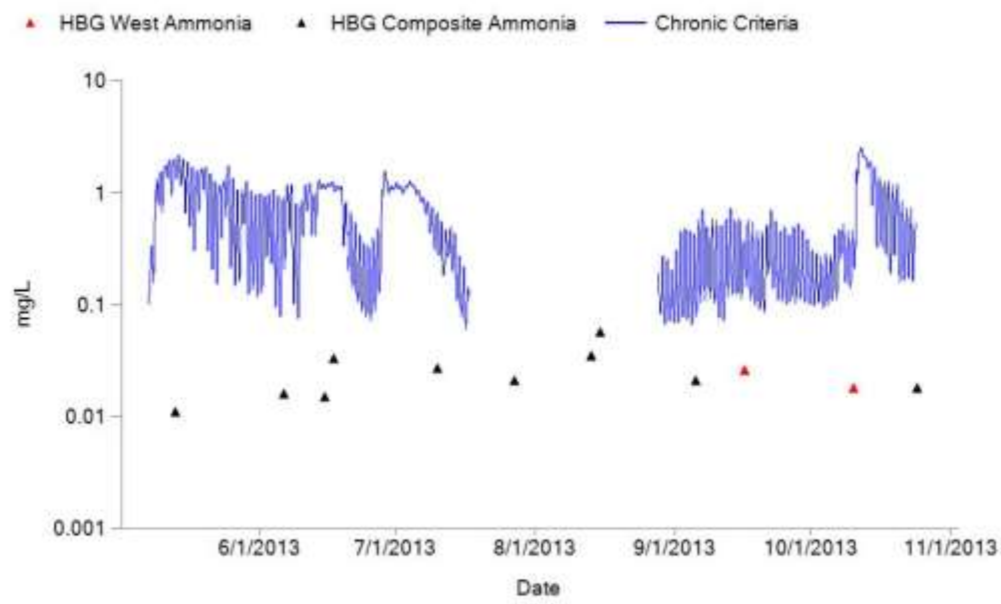


Figure 3. West site on the Susquehanna River at Harrisburg during the 2013 sampling year. The Y axis is in log scale.

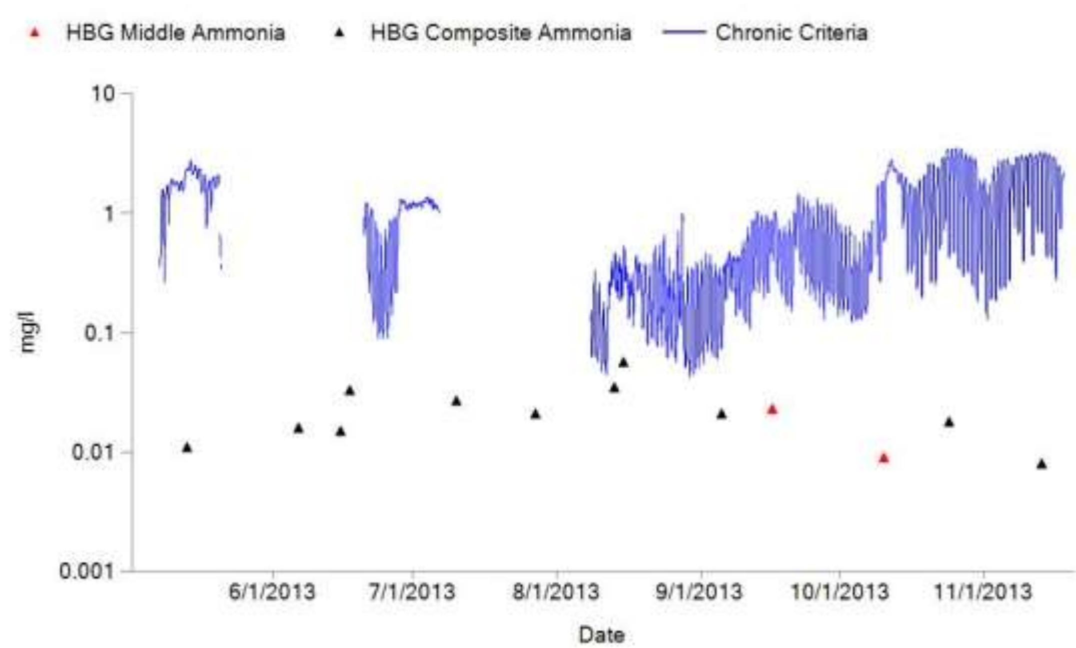


Figure 4. Middle site on the Susquehanna River at Harrisburg during the 2013 sampling year. The Y axis is in log scale.

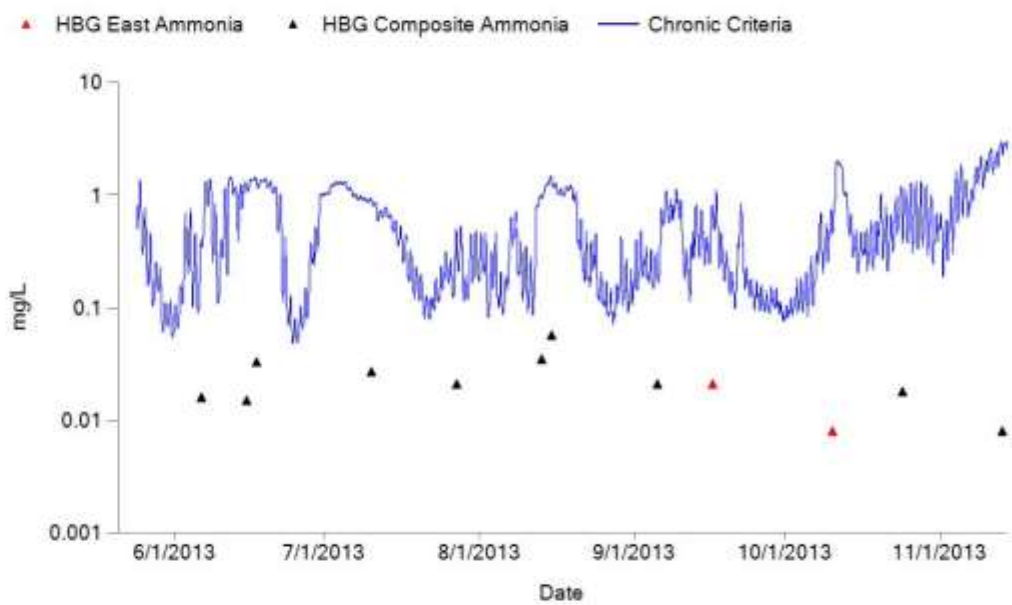


Figure 5. East site on the Susquehanna River at Harrisburg during the 2013 sampling year. The Y axis is in log scale.

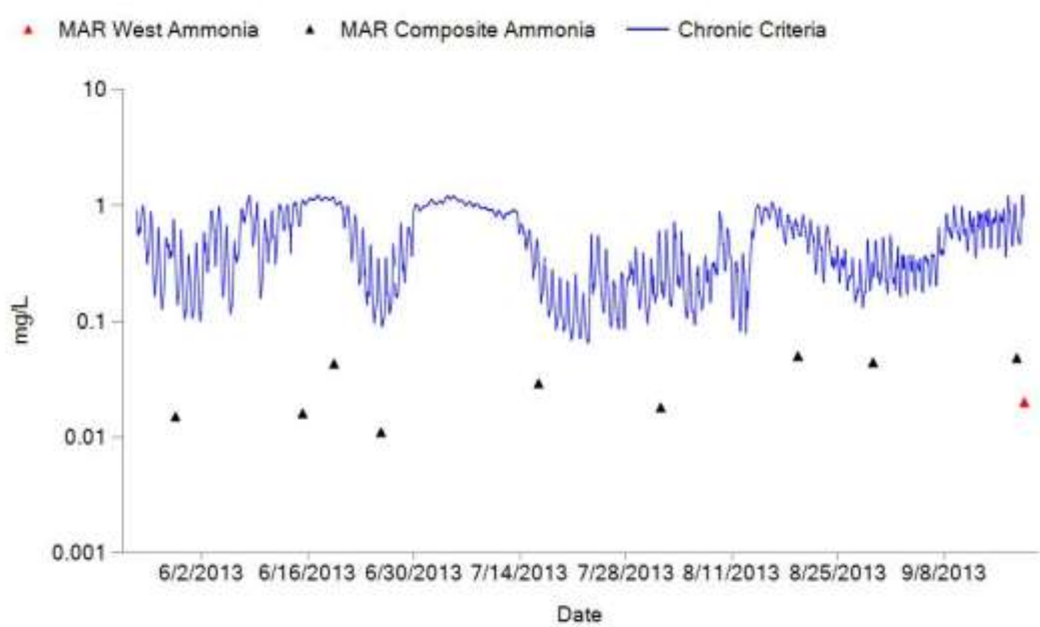


Figure 6. West site on the Susquehanna River at Marietta during the 2013 sampling year. The Y axis is in log scale.

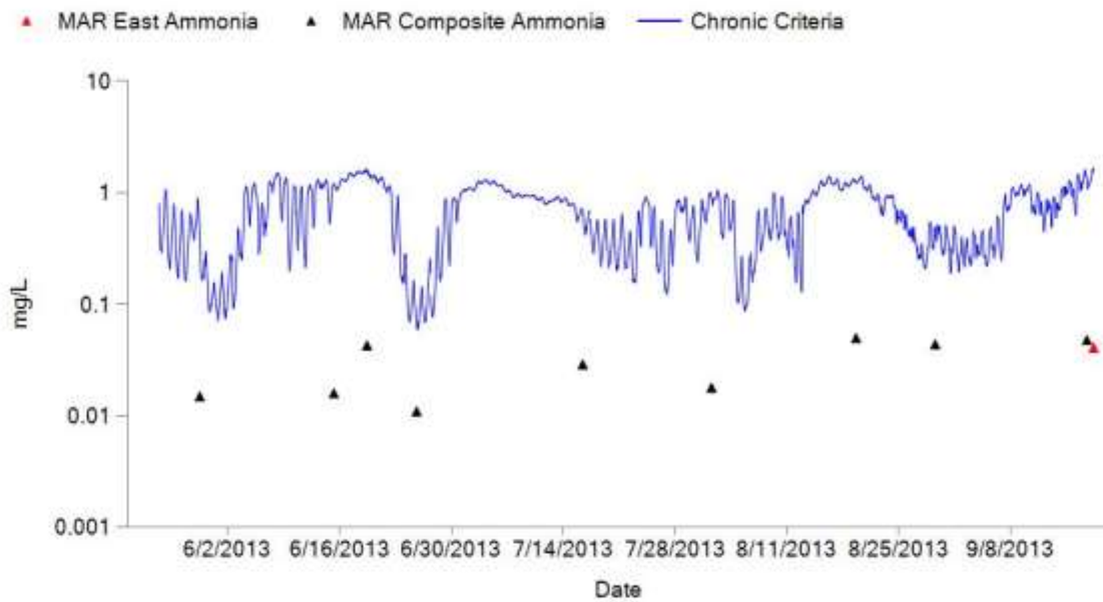


Figure 7. East site on the Susquehanna River at Marietta during the 2013 sampling year. The Y axis is in log scale.

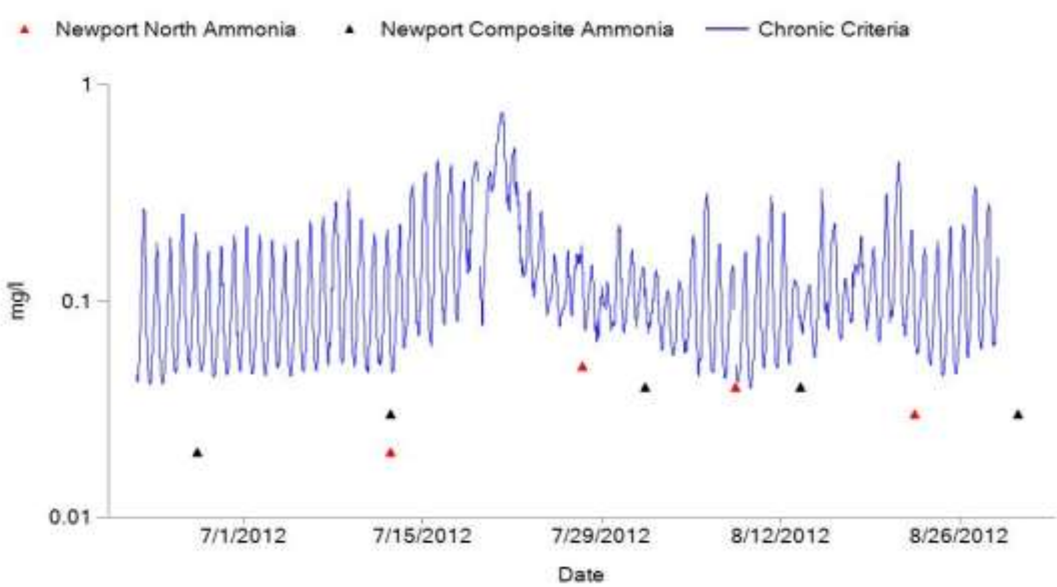


Figure 8. North site on the Juniata River at Newport during the 2012 sampling year. The Y axis is in log scale.

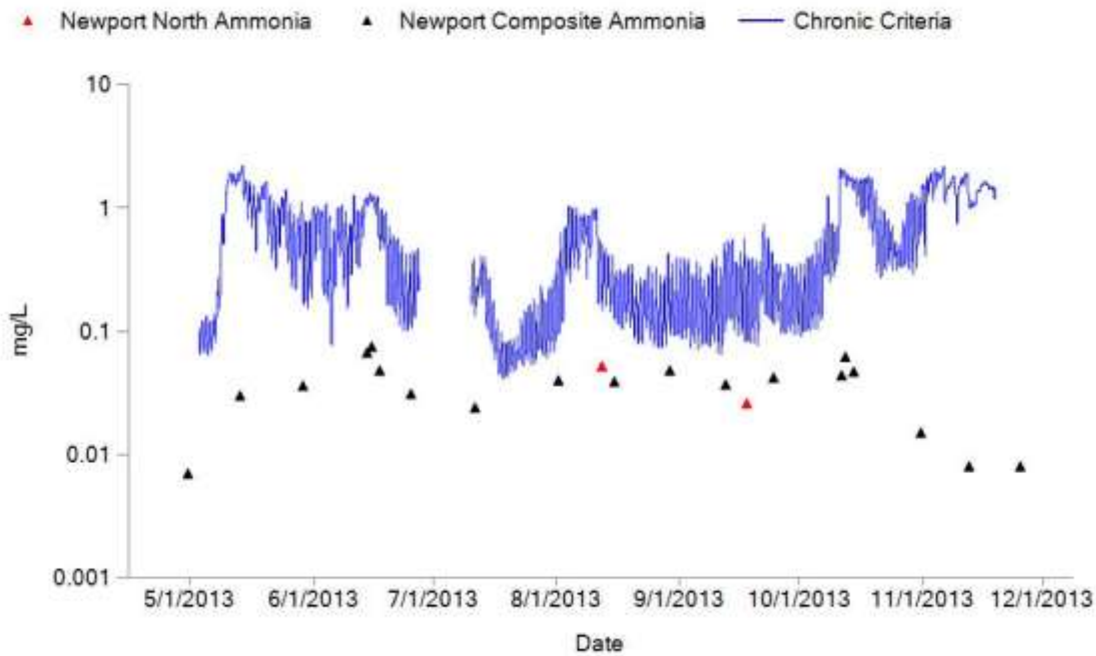


Figure 9. North site on the Juniata River at Newport during the 2013 sampling year. The Y axis is in log scale.

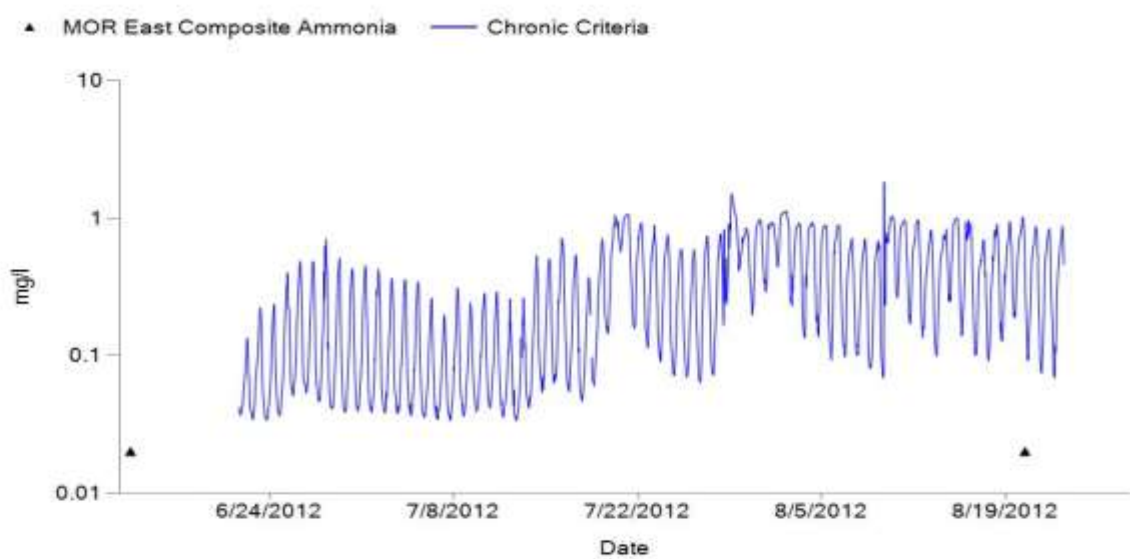


Figure 10. East site on the Delaware River at Morrisville during the 2012 sampling year. The Y axis is in log scale.

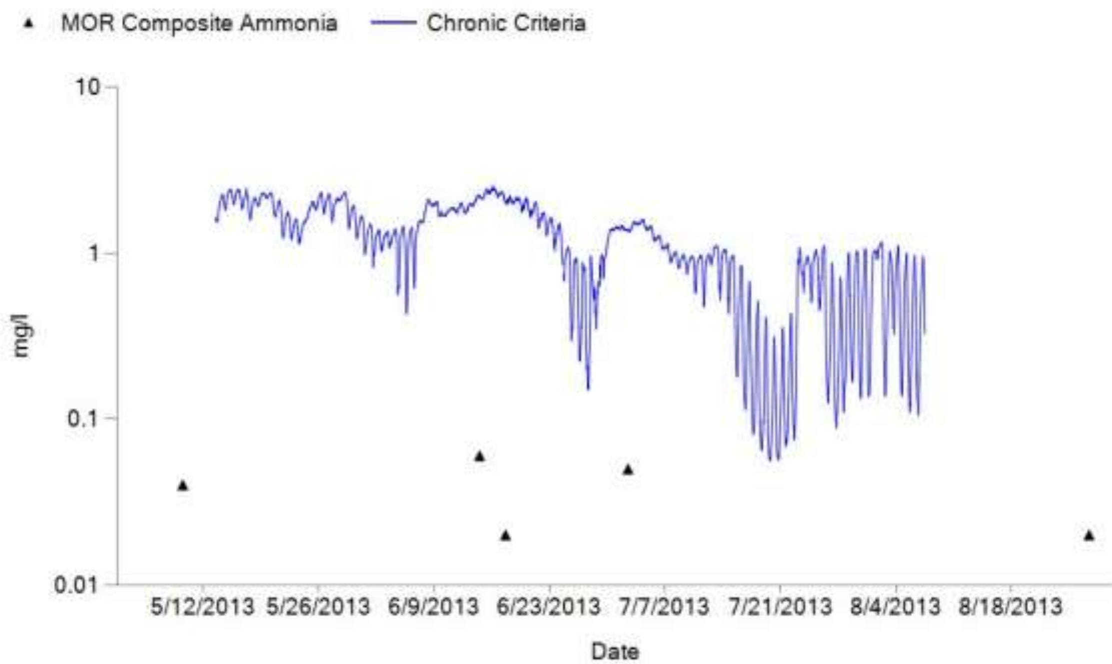


Figure 11. West site on the Delaware River at Morrisville during the 2013 sampling year. The Y axis is in log scale.

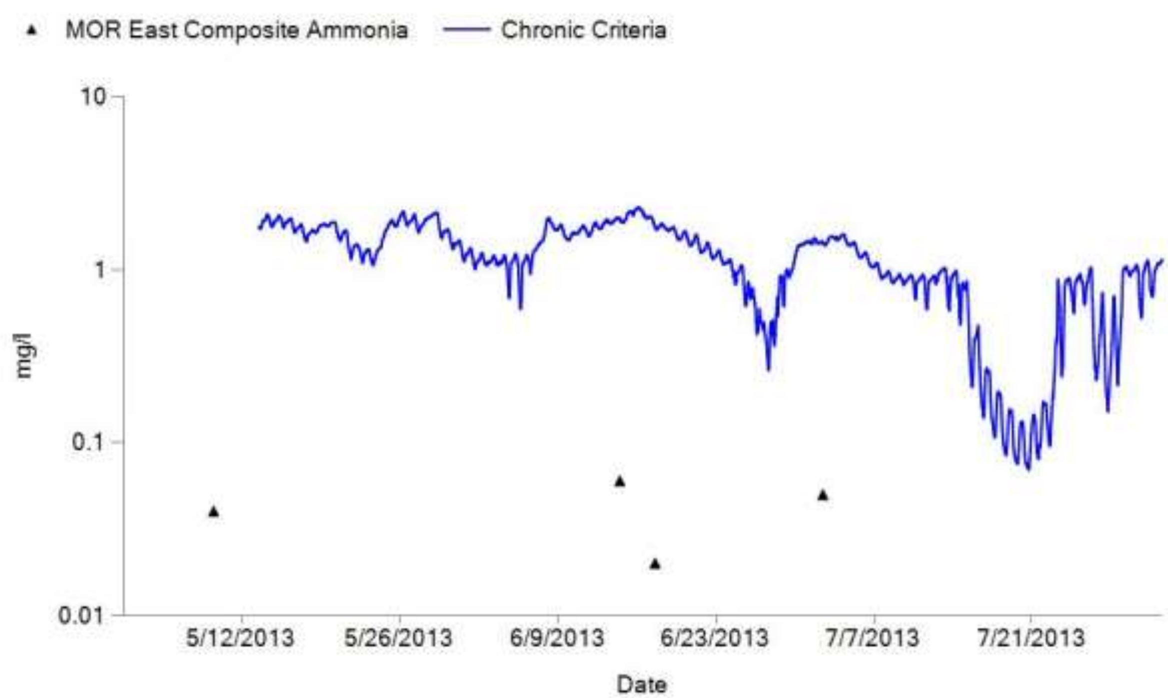


Figure 12. East site on the Delaware River at Morrisville during the 2013 sampling year. The Y axis is in log scale.

Conclusion:

Spatial comparison suggests there are no major differences between ammonia concentrations within the defined CADDIS sites and comparison sites. Additionally, comparisons between the chronic time series and measured concentrations suggest that there are no sites that are reaching ammonia concentrations of concern during the period analyzed. This evidence weakens the hypothesis that ammonia toxicity is affecting SMB survival and recruitment to age class 1+. However, it's important to note that these water quality measurements are not directly describing conditions that may occur within SMB YOY habitat.

Title: Toxicity of Ammonia to Early Life Stages of Smallmouth Bass (WS #21)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: Ammonia toxicity (increases by high pH and high temperature) either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

The toxicity of ammonia increases with pH and water temperature, possibly decreasing SMB survival and recruitment to age class 1+. However, the criteria used by PA DEP may not be the appropriate threshold to determine whether ammonia is becoming toxic to SMB specifically. Broderius and others (1985) evaluated the toxicity of ammonia to early life stages of SMB at varying levels of pH. These data should provide insight as to whether SMB YOY are generally more or less susceptible to the toxic effects of ammonia.

Data:

Broderius and others (1985) developed total ammonia chronic no-observed-effect levels of ammonia toxicity for pH levels, 6.60, 7.25, 7.83 and 8.68. All tests measured the variability of pH, but held temperature constant throughout the study (at 22°C). For comparison purposes, the chronic ammonia toxicity criterion was derived from formulas in Table 3 of §93.7(a).

Analysis and Results:

Chronic no-observed-effect levels from the publication were compared to the PA chronic ammonia criterion. Total ammonia concentrations were selected from the publication in order to compare PA criteria. The PA chronic ammonia criterion calculations were held to a consistent temperature of 22°C to be consistent with the 1985 study. Early life stages of SMB were substantially more tolerant to total ammonia concentrations at all reported pH levels than the PA criterion (Table 1).

Table 1. Comparison of chronic no-observed-effect levels in SMB to the PA chronic ammonia criterion at four pH levels.

pH	SMB Chronic No Observed Effect (mg/L)	PA Chronic Ammonia Criterion (mg/L)
6.60	17.4	2.10
7.25	14.4	1.43
7.83	14.6	0.83
8.68	2.40	0.14

Conclusion:

These data suggest that the early life stages of SMB are much more tolerant to the toxic effects of ammonia than the organisms that were used to develop the PA ammonia criteria.

Literature Cited:

Broderius, S., Drummond, R., Fiandt, J. & Russom, C. (1985), Toxicity of ammonia to early life stages of the smallmouth bass at four pH values. Environmental Toxicology and Chemistry, 4: 87

Title: Hypothesizing an increase in NH_3 within the Juniata/Lower Susquehanna Subbasins (WS #22)

Agency: Susquehanna River Basin Commission

Temporal and Spatial comparison using data from all of SRB

Candidate Cause: Ammonia toxicity (increases by high pH and high temperature) either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

This study looked at a hypothesis of an increase in NH_3 within the Juniata and/or Lower Susq Subbasins influencing SMB recruitment decline. Pre-2005 and post-2005 data were compared.

Data:

SRBC has collected data throughout the Susquehanna River basin since the mid-1980's at a wide range of sites. All NH_3 data collected anywhere within the Susquehanna River basin was pooled and separated by major subbasin (Chemung, Upper, West Branch, Middle, Lower and Juniata) and into two time periods, pre-2005 and post-2005.

All lab analyses reported as present below detection limit were included in the analysis by substituting the detection limit (which often varied across years/projects). This likely skews all data a little bit high but there were too many PBQs to ignore.

Data was further pared down to only include May-September.

Box plots and interval plots were used to display the data graphically. On boxplots, scales were modified to eliminate obvious outliers from view and make the box and whiskers more interpretable.

WQN data collected by SRBC is not included in these data.

Analysis and Results:

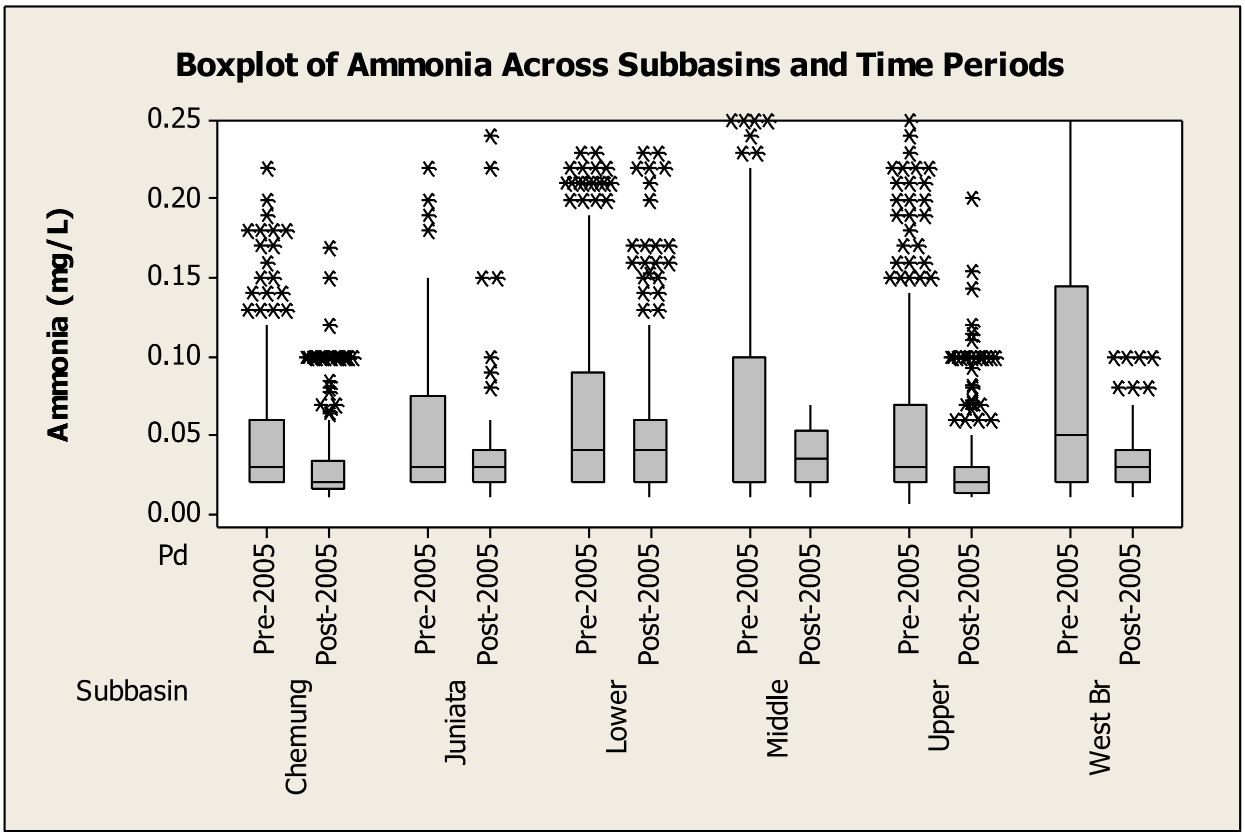
Data are not normally distributed, so nonparametric analyses were used.

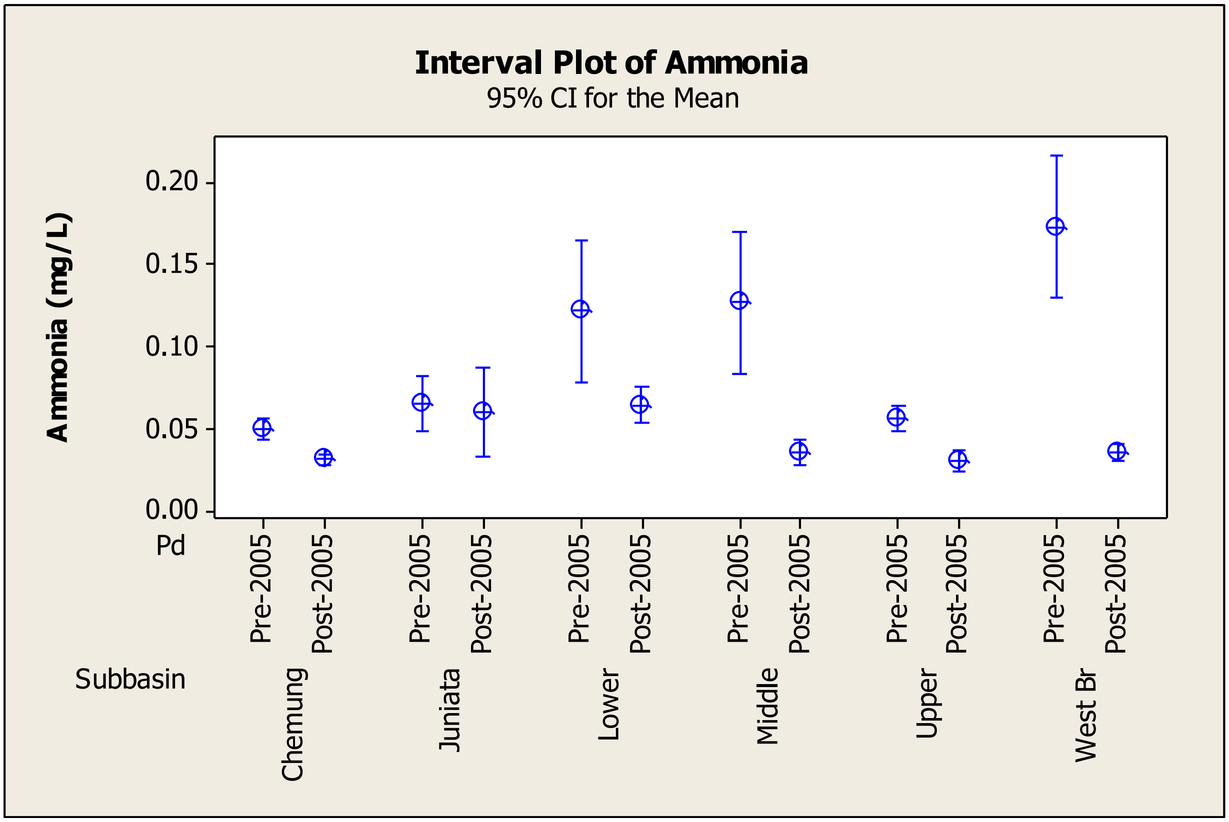
Table 1. Mann–Whitney Output for NH3 Differences Between pre- and post-2005 per subbasin (α=0.05)

	N pre	**N post**	**p value**	**Note**
Chemung	300	244	**0.0000**	pre higher
Juniata	105	59	0.2725	Not significant

Lower	566	380	**0.0006**	same median
Middle	358	26	0.8550	Not significant
Upper	503	328	**0.0000**	pre higher
West Branch	289	76	**0.0000**	pre higher

Pre-2005 ammonia values are consistently higher.




Interval Plot of Ammonia
95% CI for the Mean
Ammonia (mg/L)
0.20
0.15
0.10
0.05
0.00
Pd
Pre-2005
Post-2005
Subbasin
Chemung
Juniata
Lower
Middle
Upper
West Br

Title: Ammonia Toxicity and Decreasing Small Mouth Bass and Recruitment (WS #52)

Agency: Susquehanna River Basin Commission

Candidate Cause: Ammonia toxicity (increases by high pH and high temperature) either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

Ammonia toxicity increases with pH and water temperature, possibly decreasing SMB survival and recruitment to age class 1+.

Data:

SRBC collected ammonia data at one site on the Juniata River at Newport from 1984-present and total nitrogen at the four sites on the Susquehanna River within the case study area (Fort Hunter, Halifax, McKees Half Falls, and Sunbury) and on the Juniata River just upstream of the confluence with the Susquehanna. Total nitrogen measurements are also provided for two sites on the Susquehanna River that bracket the case study area—downstream at Wrightsville and upstream at Danville. Total nitrogen measurements on the West Branch Susquehanna at Lewisburg are also provided. These measurements were derived from a composite sample collected across the channel at each site and analyzed through either the DEP lab or ALS Analytical.

pH measurements and temperature were taken with a handheld probe at several areas across the channel, with the median measurement presented. Most data from 1985-2008 were taken in June through September in a time ranging from the mid-morning through early afternoon.

Analysis and Results:

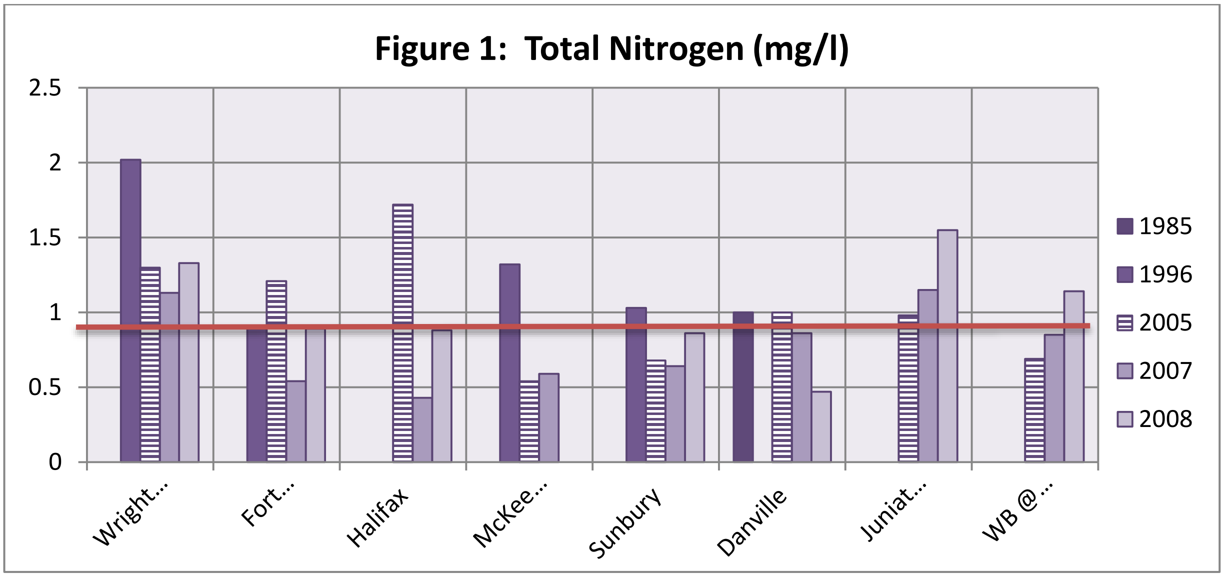
<u>Total Nitrogen</u>

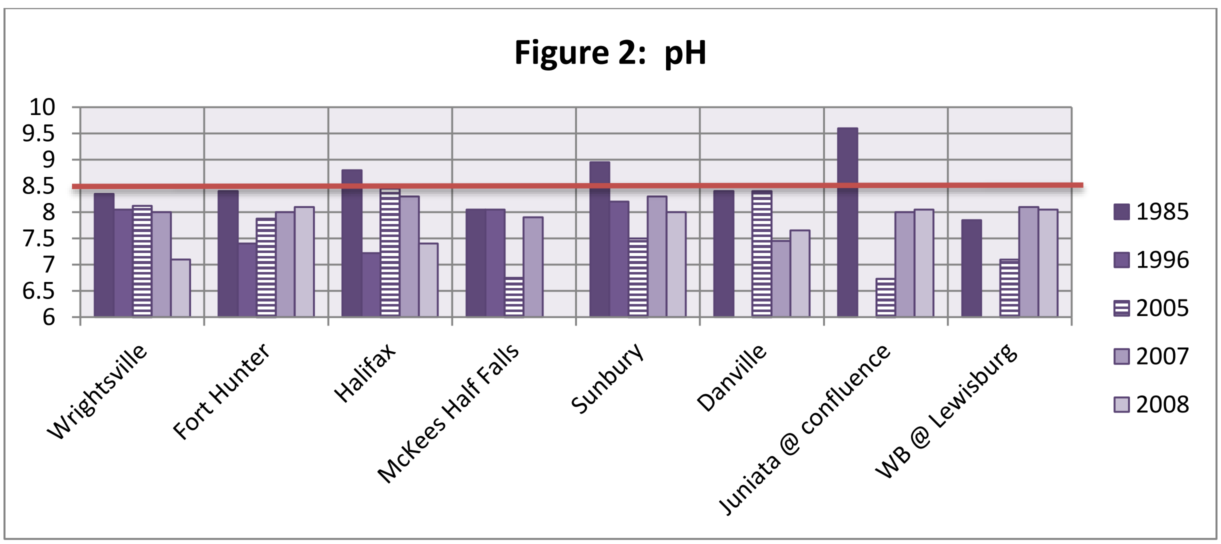
Figure 1 shows the range of total nitrogen measurements for these sites from 1985-2008. Many data gaps are present, but all sites were sampled for total nitrogen in 2005. Total nitrogen values for the case study area in 2005 range from 0.54 mg/l (McKees Half Falls) to a maximum value of 1.72 mg/l (Halifax), which is above the 1.0 mg/l expected background level. Fort Hunter also had an elevated total nitrogen value of 1.21 mg/l in 2005. Values greater than 1.0 mg/l were observed before and after 2005 within the case study area, with values on the increase at the Juniata River site through 2008.

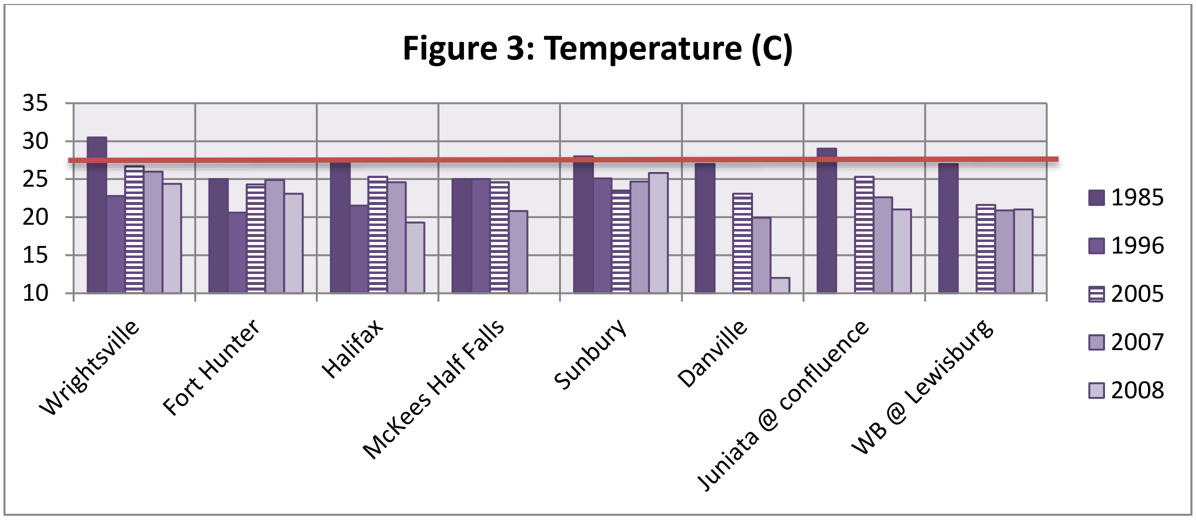
Figure 2 shows the range of pH in 2005 of 6.75 to 8.45 within the case study area. In 2005, Halifax had the highest pH value in the case study area of 8.45 as well as the highest value of total nitrogen (1.72 mg/l). pH values throughout the time period stay within a reasonable range of each other. pH values were highest in the study area in

1985, with Sunbury and Halifax having values above 8.5 and Juniata River having a value of 9.6.

Figure 3 shows temperature measurements for the same period of time.



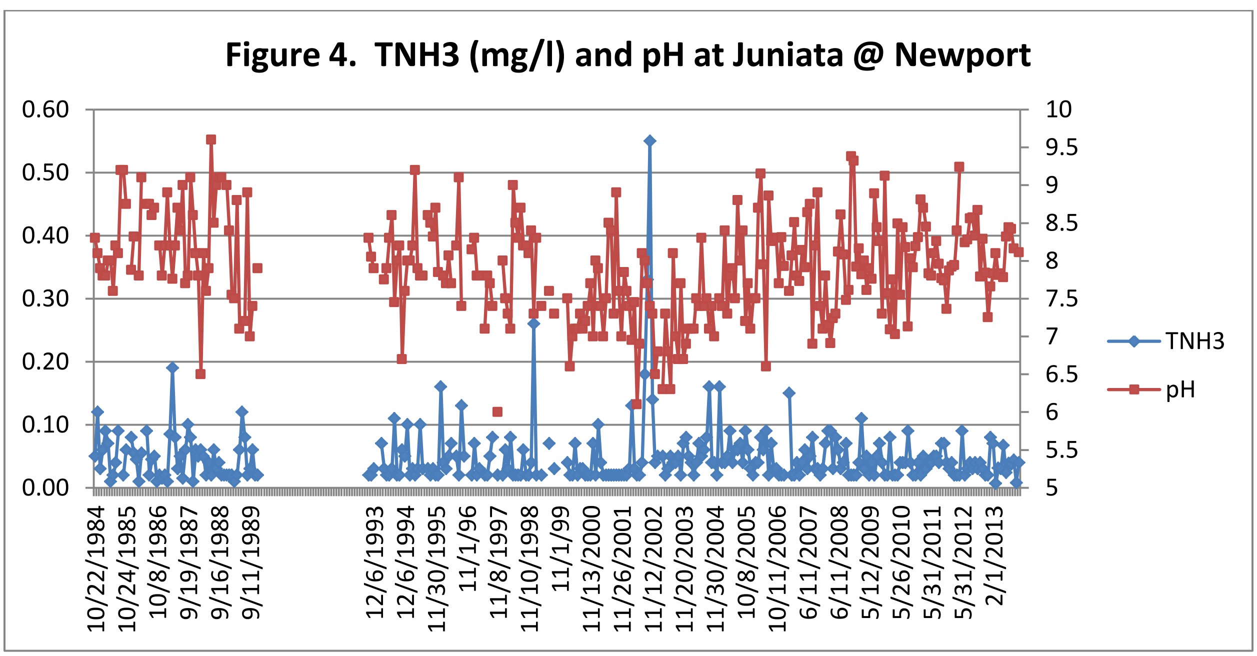




Ammonia

Data in Figure 4 were collected from the Juniata River at Newport from 1984-2013. While several months throughout this period had numerous samples taken throughout the month, only the highest ammonia value for that month was used for Figure 4. Ammonia ranged from 0.01 to 0.55 mg/l, and pH ranged from 6 to 9.6. Referring to Table 1 of the Ammonia_Criteria_PA DEP_Shull worksheet, no ammonia values that exceeded 0.14 mg/l (10 of them) also had an associated pH value greater than 8 (range of 7.1 to 8). Temperature for this period of time only exceeded 30 C on just two occasions in summer 1988.

Figure 5 shows TNH3 at Juniata River at Newport from 2005-2013.



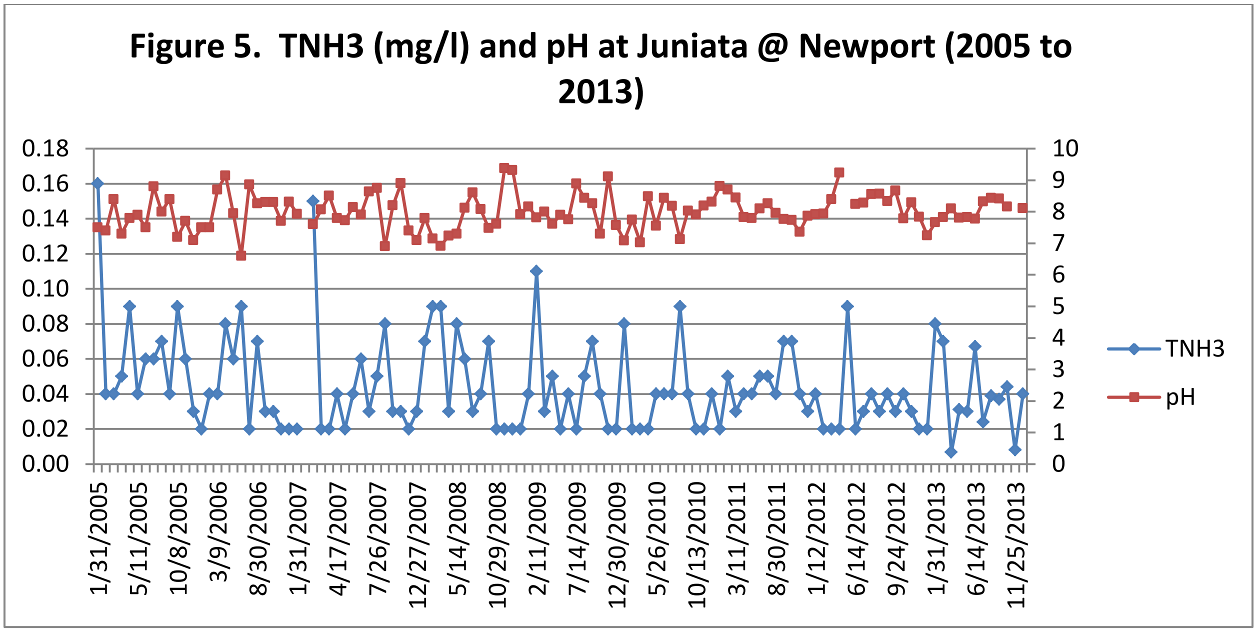


Figure 5. TNH3 (mg/l) and pH at Juniata @ Newport (2005 to 2013)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
10
9
8
7
6
5
4
3
2
1
0
1/31/2005
5/11/2005
10/8/2005
3/9/2006
8/30/2006
1/31/2007
4/17/2007
7/26/2007
12/27/2007
5/14/2008
10/29/2008
2/11/2009
7/14/2009
12/30/2009
5/26/2010
10/13/2010
3/11/2011
8/30/2011
1/12/2012
6/14/2012
9/24/2012
1/31/2013
6/14/2013
11/25/2013
TNH3
pH

Candidate Cause 11: Algal and Bacterial Toxins

Worksheets: 15, 16, 17, 32, 33, and 50

Title: Increases in Dissolved Organic Phosphorus in the Susquehanna River: 2003-2004 (WS #15)

Agency: Pennsylvania Department of Environmental Protection

Temporal Co-occurrence using Data from the Case

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

In an aquatic system, phosphorus is an essential nutrient for both plants and animals. Dissolved inorganic phosphorus (DIP) is the form required by plants for cellular growth and reproduction. It is readily available for uptake and under natural conditions usually occurs at low levels. Increased levels of DIP can cause algae blooms, low dissolved oxygen, and mortality of aquatic organisms including fish. High DIP could degrade the water quality and effect the recruitment of YOY SMB into age class 1+.

Data:

The Pennsylvania Department of Environmental Protection collaborates with the Susquehanna River Basin Commission and the United States Geological Survey (USGS) to collect water chemistry data as part of its Water Quality Network (WQN) monitoring. Dissolved inorganic phosphorus is one of the many parameters analyzed for at a subset of these WQN stations that are part of the Chesapeake Bay Non-Tidal Network. This data is collected monthly at 36 stations within the Susquehanna basin.

Analysis and Results:

Dissolved inorganic phosphorus data (and many other parameters) was downloaded from PA DEP's Sample Information System (SIS) and submitted to the United States Geological Survey (USGS) for trend and load analyses. The ESTIMATOR regression model was run to calculate loads and trends during the period of 1992-2012 and 2003-2012. Dissolved inorganic phosphorus was significantly increasing for the 1992-2012 time period. For some stations, the percent change was over 500%. During the 2003-2012 period, these same constituents were trending significantly down or were not significant (Tables 1 & 2).

Such a high percent change in DIP for the 1992-2012 timeframe, lead to further analyses by USGS. Using a new regression model called Weighted Regression on Time, Discharge, and Season (WRTDS), it was discovered that ESTIMATOR can overestimate loads because it squares the terms. This overestimation was magnified in the DIP trend results possibly due to how low in concentration DIP occurs and the environmental factors that confound this parameter (Figure 3). However, between 2003 and 2012 orthophosphate trends have significantly decreased. WRTDS is still showing the increasing trend in DIP starting around 2000 (Figure 4).

There was a large increase in DIP loads in 2003 and 2004 corresponding with the high flow years. This large increase in loads would have increased plant growth and lowered dissolved oxygen levels which could have affected the YOY SMB recruitment into age class 1+ in 2005.

Table 1. Dissolved inorganic phosphorus trend from 1992-2012

		DIP 1992-2012				
Station Name	**LCI**	**FA Trend**	**UCI**	**Error (%)**	**TAU-FA (p-value)**	**Significance**
SUSQUEHANNA RVR AT MARIETTA	138.1	208	298.2	14	1.1248 (<0.0001)	SIG-UP
W BR SUSQUEHANNA RVR AT LEWISBURG	94.6	186	320.1	21.7	1.0507 (<0.0001)	SIG-UP
SUSQUEHANNA RVR NR TOWANDA	399	528.7	692.1	12.5	1.8385 (<0.0001)	SIG-UP
SUSQUEHANNA RVR NR SUNBURY	-95.9	54	5617.7	532.2	0.4320 (0.8148)	NS
JUNIATA RV AT NEWPORT	130	206.6	308.6	15.8	1.1202 (<0.0001)	SIG-UP
PENNS CRK At PENNS CRK	-45.7	7693.2	1118290	1160.3	4.3558 (0.0856)	NS
ALLEGHENY RVR AT NATRONA	-95	87.6	6945.5	535.9	0.6292 (0.7337)	NS
TOWANDA CRK NR MONROETON	401.3	11006.9	245976.2	385.8	4.7102 (0.0029)	SIG-UP

Table 2. Dissolved inorganic phosphorus trend from 2003-2012

		DIP 2003-2012				
Station Name	**LCI**	**FA Trend**	**UCI**	**Error (%)**	**TAU-FA (p-value)**	**Significance**
SUSQUEHANNA RVR AT MARIETTA	63.5	-54.4	43.1	12	-0.7859 (<0.0001)	SIG-DOWN
W BR SUSQUEHANNA RVR AT LEWISBURG	-87	-78.3	64.1	29.5	-1.5301 (<0.0001)	SIG-DOWN
SUSQUEHANNA RVR NR TOWANDA	51.2	-39.2	24.2	11.9	-0.4970 (<0.0001)	SIG-DOWN
SUSQUEHANNA RVR NR SUNBURY	75.6	-60.6	36.4	27.6	-0.9310 (0.0001)	SIG-DOWN
JUNIATA RV AT NEWPORT	71.2	-65.1	57.8	10.3	-1.0540	SIG-DOWN

					(<0.0001)	
WQN0229 PENNS CRK At PENNS CRK	86.1	-74.7	53.7	36	-1.3737 (<0.0001)	SIG-DOWN
ALLEGHENY RVR AT NATRONA	58.6	-35.7	-0.1	25.2	-0.4415 (0.0497)	SIG-DOWN
TOWANDA CRK NR MONROETON	73.8	-60.9	41.7	22.6	-0.9398 (<0.0001)	SIG-DOWN

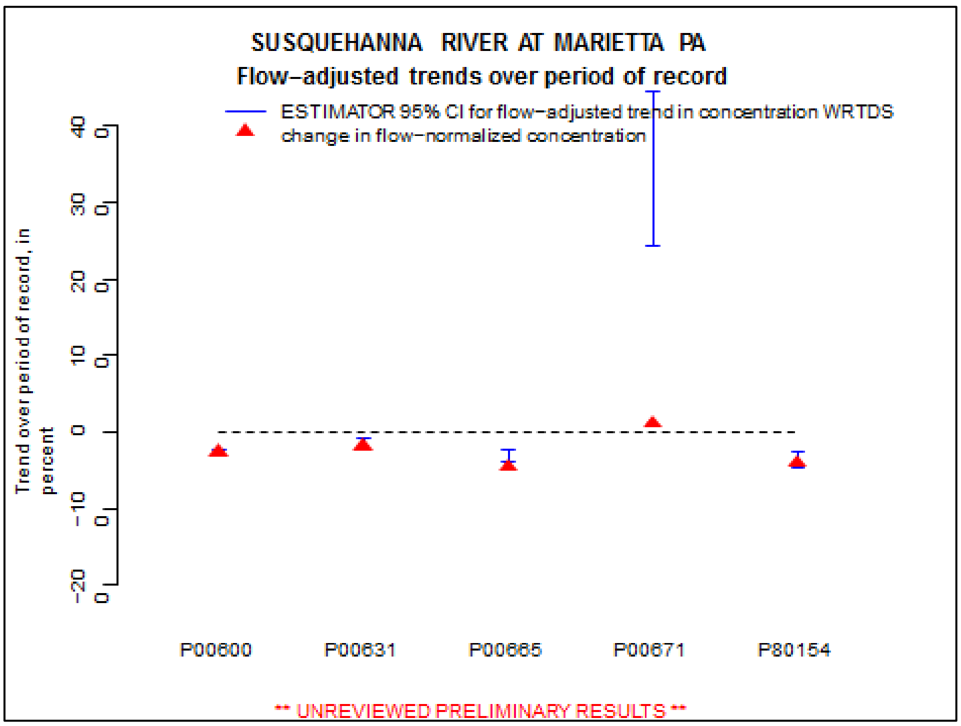


Figure was obtained from USGS New Cumberland PA office

Figure 3. Comparion of ESTIMATOR and WRTDS Trends (P00671 = DIP)

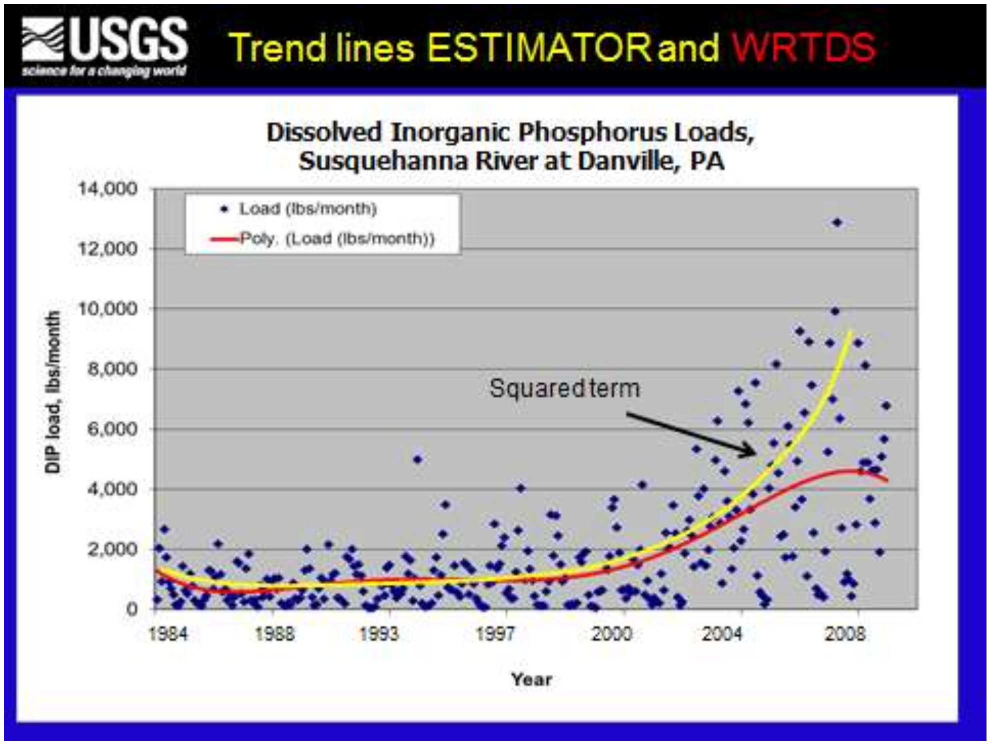


Figure was obtained from USGS New Cumberland PA office

Figure 4. Comparison of ESTIMATOR and WRTDS DIP trend

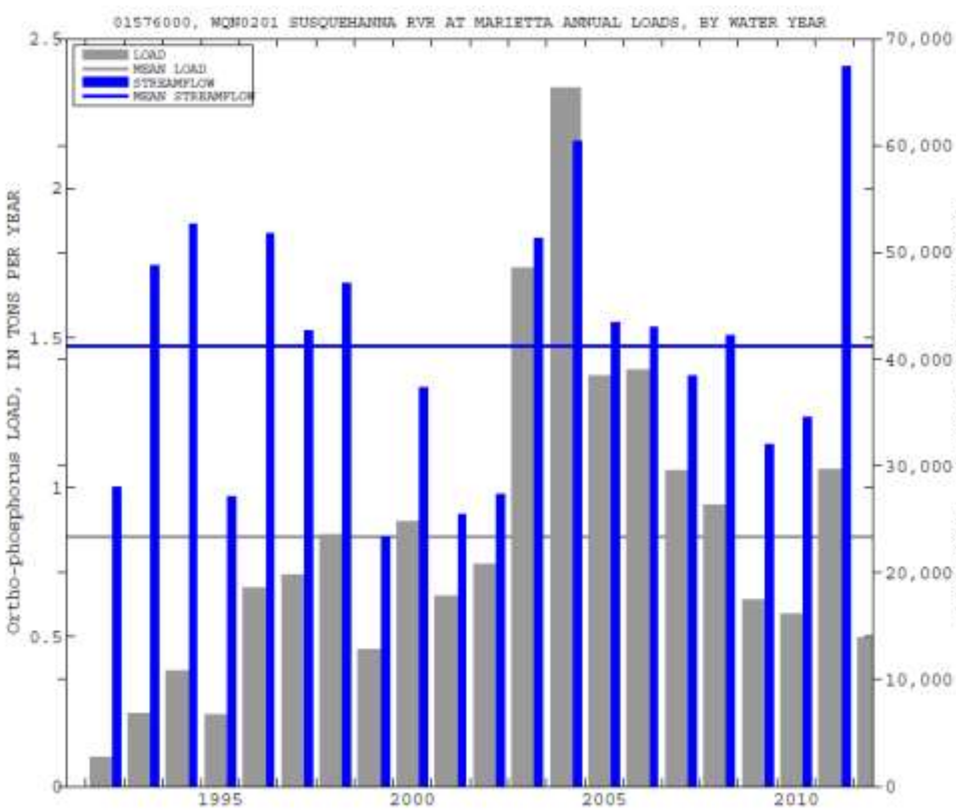


Figure 5. Dissolved inorganic phosphorus loads from 1992-2012

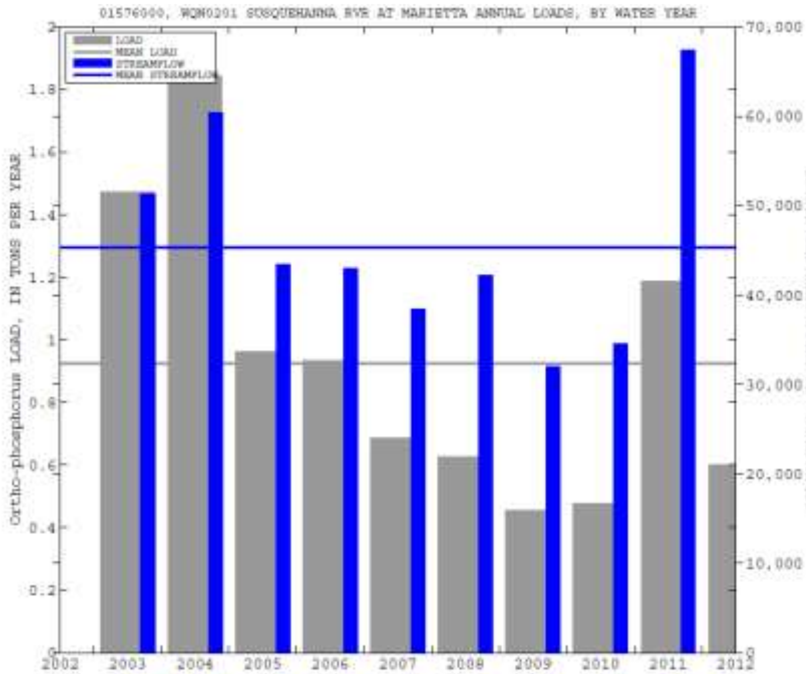


Figure 6. Dissolved inorganic phosphorus loads from 2003-2012

Conclusion:

Regression models show that dissolved inorganic phosphorus (DIP) trends have significantly increased in the last 20 years in the Susquehanna River basin. However, in the last 10 years the DIP trends have been significantly decreasing. There was a large spike in DIP loads at many locations in the Susquehanna River basin between 2003 and 2004. This large increase could have led to unfavorable water quality conditions for YOY smallmouth bass contributing to their poor 2005 recruitment into age class 1+.

Title: Hypothesizing an Increase in OrthoP within the Juniata/Lower Susquehanna Subbasins (WS #16)

Agency: Susquehanna River Basin Commission

Increasing TP in Case Area

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

This studies the hypothesis that an increase in TP within Juniata and/or Lower Susquehanna River subbasins influence SMB recruitment decline. Pre-2005 data and post-2005 data were compared.

Data:

SRBC has collected data throughout the Susquehanna River Basin since the mid-1980's at a wide range of sites. All TP data collected anywhere within the SRB was pooled and separated by major subbasin (Chemung, Upper, West Branch, Middle, Lower and Juniata) and into two time periods, pre-2005 and post-2005.

All values reported by the lab as "present below detection limit" were included in the analysis by substituting the detection limit (which often varied across years/projects). This likely skews all data a little bit high but there were too many PBQs to ignore.

Data was further pared down to only include May-September.

Box plots and interval plots were used to display the data graphically. On boxplots, scales were modified to eliminate obvious outliers from view and make the box and whiskers more interpretable.

WQN data collected by SRBC were not included in these data.

Analysis and Results:

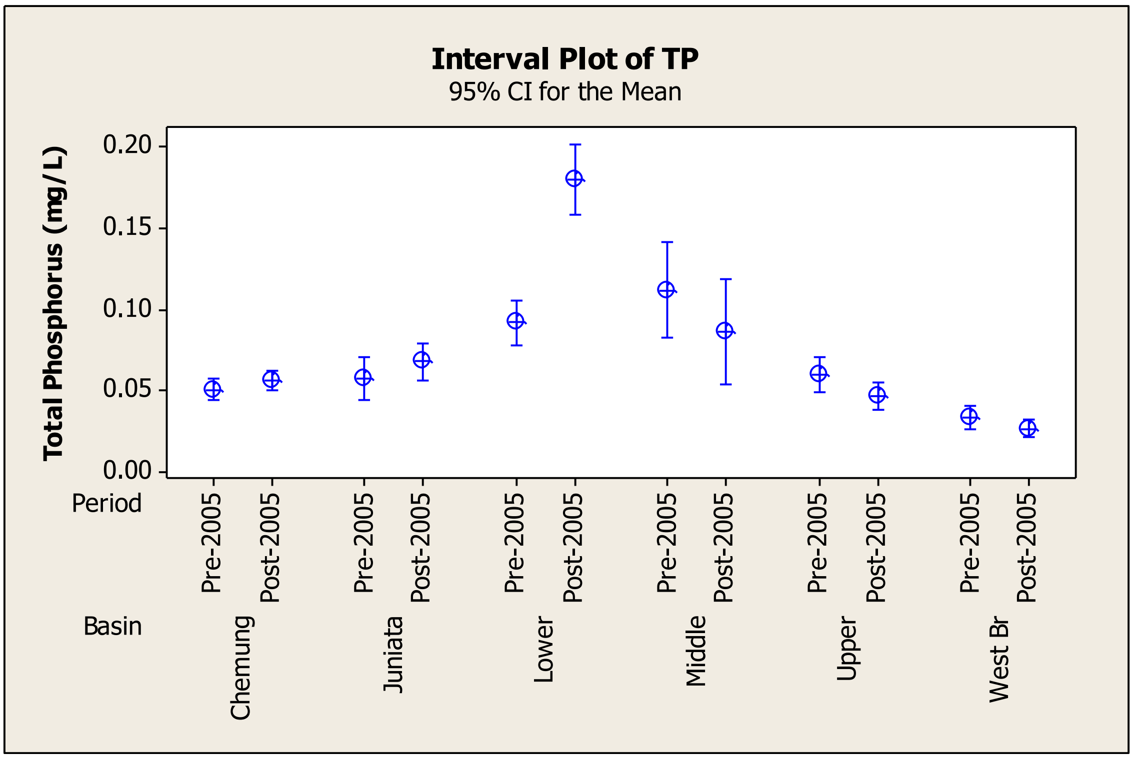
Data sets far from normally distributed so nonparametric tests used.

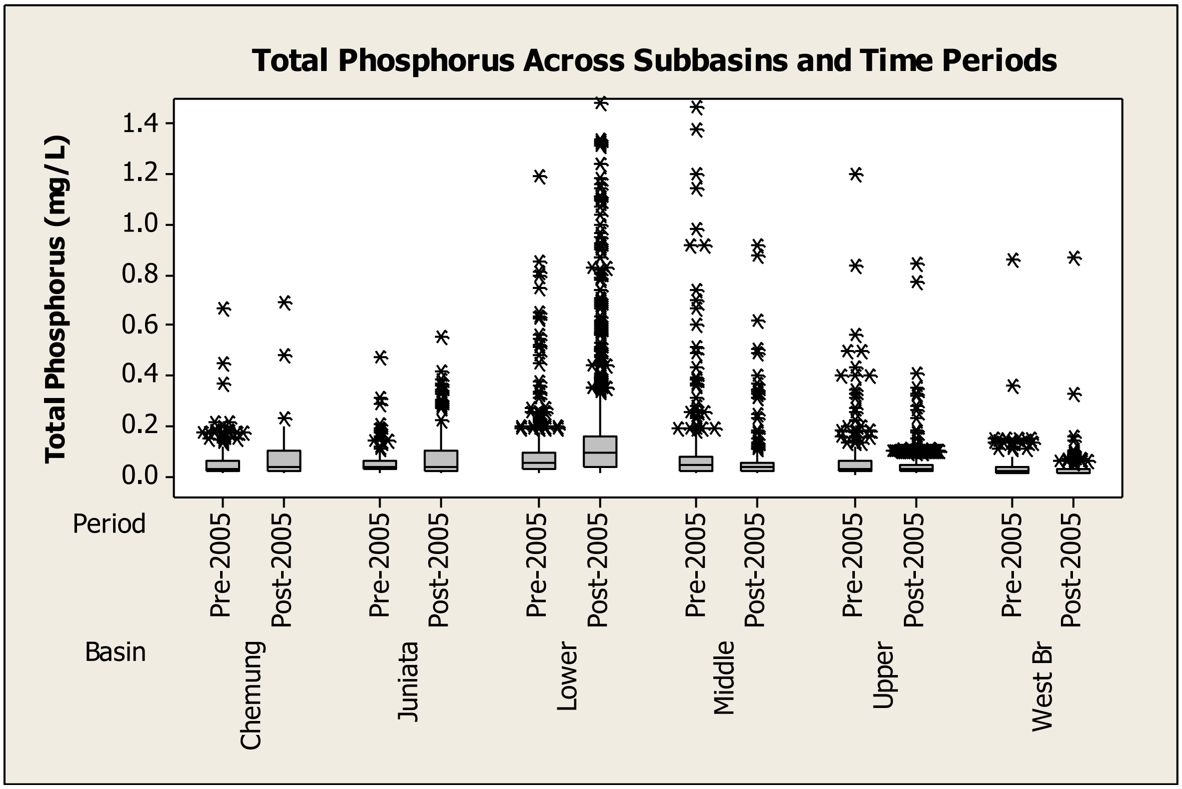
Table 1. Mann –Whitney Output for TP Differences Between pre and post 2005 per subbasin (α=0.05)

	N pre	**N post**	**p value**	**Note**
Chemung	310	367	0.1537	Not significant
Juniata	108	214	0.9747	Not significant

Lower	462	879	**0.0000**	post higher
Middle	356	206	**0.0011**	pre higher
Upper	481	491	**0.0000**	pre higher
West Branch	287	342	0.1741	Not significant

Lower Susquehanna only subbasin where post-2005 TP is significantly higher than pre-2005 TP.




Total Phosphorus Across Subbasins and Time Periods
Total Phosphorus (mg/L)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Period
Pre-2005
Post-2005
Basin
Chemung
Juniata
Lower
Middle
Upper
West Br

Title: Hypothesizing an increase in Total Phosphorus within the Juniata/ Lower Susquehanna Subbasins (WS #17)

Agency: Susquehanna River Basin Commission

Increasing OrthoP in Case Area

Candidate Cause: Low or variable DO either kills YOY directly, or increases YOY susceptibility to disease

Introduction:

This studies the hypothesis that an increase in OrthoP within the Juniata and/or Lower Susquehanna subbasins influence SMB recruitment decline. Pre-2005 data and post-2005 data were compared.**Data:**

SRBC has collected data throughout the Susquehanna River Basin since the mid-1980's at a wide range of sites. All OrthoP data collected anywhere within the SRB was pooled and separated by major subbasin (Chemung, Upper, West Branch, Middle, Lower and Juniata) and into two time period, pre-2005 and post-2005.

All values reported by the lab as "present below detection limit" were included in the analysis by substituting the detection limit (which often varied across years/projects). This likely skews all data a little bit high but there were too many PBQs to ignore.

Data was further pared down to only include May-September.

Box plots and interval plots were used to display the data graphically. On boxplots, scales were modified to eliminate obvious outliers from view and make the box and whiskers more interpretable.

WQN data collected by SRBC were not included in these data.

Analysis and Results:

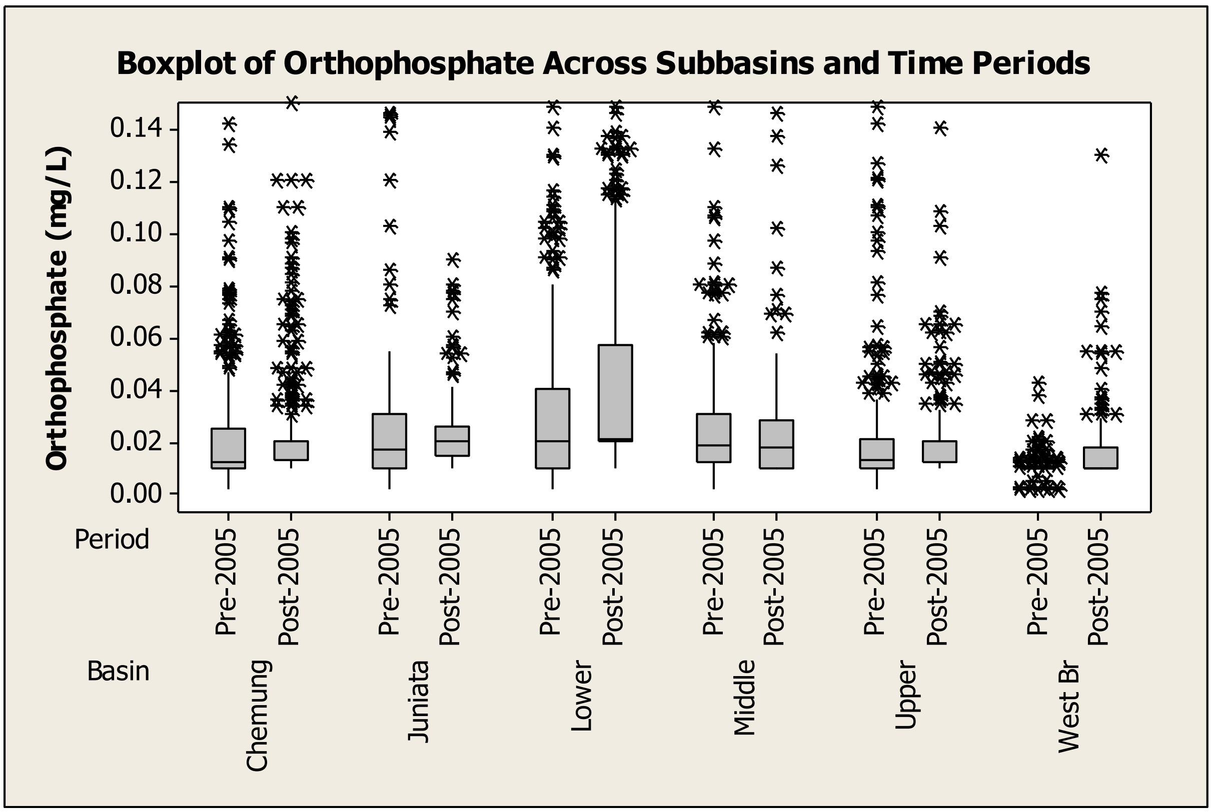
Data sets were far from normally distributed, so nonparametric tests were used.

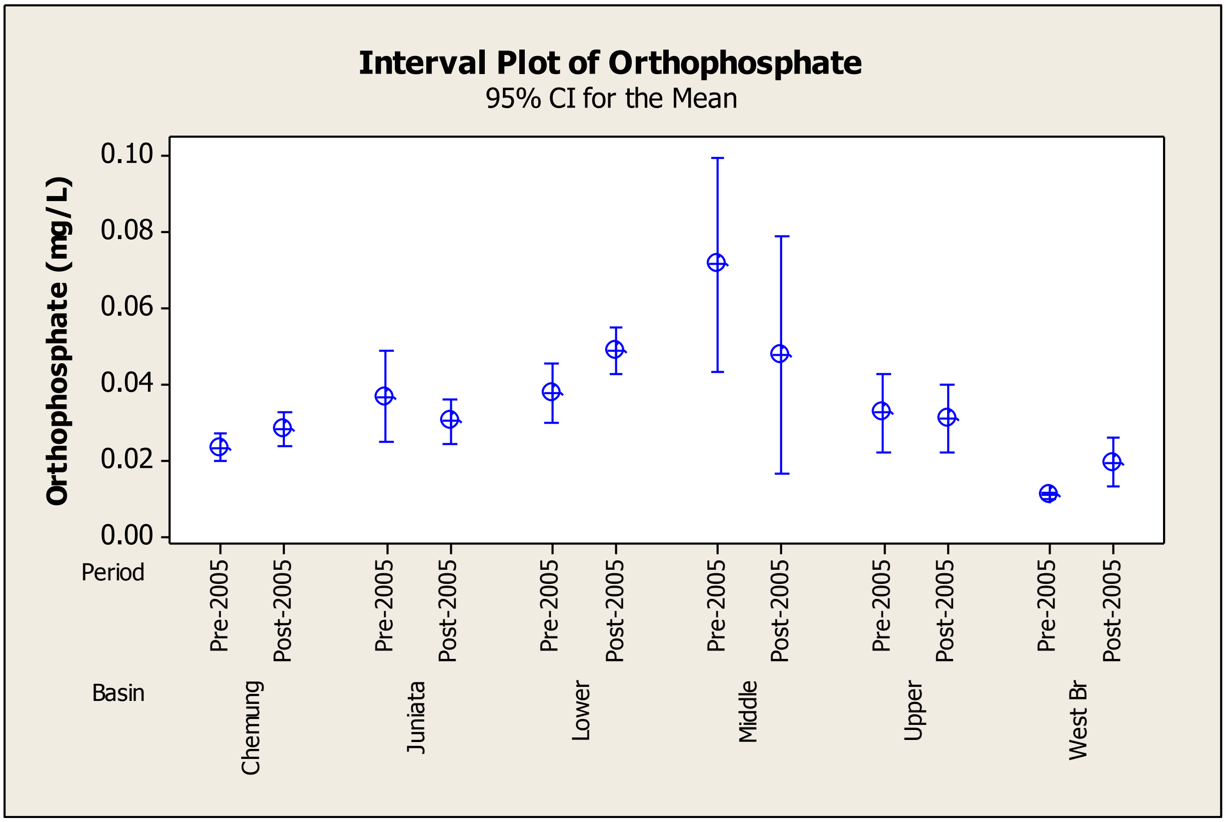
Table 1. Mann –Whitney Output for OrthoP Differences Between pre and post 2005 per subbasin (α=0.05)

	N pre	**N post**	**p value**	**Note**
Chemung	257	331	**0.0000**	post higher
Juniata	105	186	**0.0360**	post higher
Lower	353	508	**0.0000**	post higher
Middle	263	132	0.1050	Not significant

Upper	399	440	**0.0000**	post higher
West Branch	170	215	**0.0000**	same median

Post-2005 OrthoP concentrations are consistently higher across subbasins, not just in case area.





Interval Plot of Orthophosphate
95% CI for the Mean
Orthophosphate (mg/L)
0.10
0.08
0.06
0.04
0.02
0.00
Period
Pre-2005
Post-2005
Basin
Chemung
Juniata
Lower
Middle
Upper
West Br

Title: Cyanobacteria and potential cyanotoxin-producing taxa collected from sites corresponding to the 2014 adult small mouth bass liver tissue toxicology tests (WS #32)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: Algal and bacterial toxins either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

Cyanobacteria are photosynthetic organisms that are common in aquatic environments (Quiblier 2013). Cyanobacteria are also capable of producing toxins, usually referred to as cyanotoxins, that are harmful to fish (Pavagadhi 2013) as well as other organisms. Cyanotoxins are secondary metabolites produced by the cyanobacteria cells (O'Neil 2012) that, in general, may be classified into three broad toxin groups: cyclic peptides, alkaloids, and lipopolysaccharides (IARC 2010).

The more common cyanotoxins include the following (Information from http://www2.epa.gov/nutrient-policy-data/cyanobacteriacyanotoxins and EPA-810F11001 September 2014 unless otherwise stated):

- Microcystins – cyclic peptide (IARC 2010) with at least 80 variants known that primarily affect the liver. Most common variant is Microcystis-LR. Other common variants include MC-YR, RR, LW (Abraxis 2014). Extremely stable and resist chemical breakdown – in typical ambient conditions half-life is 10 weeks (Butler 2009).
- Anatoxin – alkaloid toxin (IRAC 2010) with 2 to 6 variants. Typical variants include Anatoxin-a and homoanatoxin-a. Primarily a neurotoxin.
- Cylindrospermopsons – alkaloid toxin (IRAC 2010). With 3 known variants. Primarily a liver toxin.
- Saxitoxin – alkaloid toxin (IRAC 2010). Commonly associated with marine based Paralytic Shellfish Poisoning (PSP) in humans. However, toxins are also produced in freshwater systems.

Eutrophication and climate change may enhance the possibility of cyanobacteria harmful algal blooms and consequentially cyanotoxin production (O'Neil 2012). Though cyanobacteria blooms are often associated with eutrophication, many cyanobacteria genera are highly competitive in low P (DIP) concentrations and have novel ways to acquire organic P compounds (O'Neil 2012). Additionally, many genera also "display great flexibility in the N sources they exploit to form blooms (O'Neil 2010). Cyanobacteria may also be more resistant to herbicides, thus giving them a competitive advantage over other algal taxa in freshwater systems influenced by agricultural runoff (Quiblier 2013). Though topics of eutrophication, climate change, and herbicide resistance may seem outside the topic of the taxa record at the 2014 small mouth bass tissue collection sites, this taxa record along with appropriately designed future algal collection may be used to demonstrate possible trends in the algal community and cyanotoxin production and may predict a more recent emergence of cyanotoxin production in our natural waters when compared to situations observed some years or decades ago.

Because of the potential role that cyanobacteria and cyanotoxins may play in the health of adult and YOY small mouth bass, **the purpose of this worksheet is to show the cyanobacteria taxa identified at sites where PA DEP collected adult SMB in 2014 for microcystin testing of liver tissue and to suggest possible cyanotoxins present at those sites.** Unfortunately, as discussed below, we are only able to show with this data set a very limited association between the recorded taxa and possible cyanotoxins that may have been present at the 2014 fish tissue collection sites.

Data and Limitations:

The 2014 adult SMB liver tissue collection sites included the following:

- Tuscarora Creek in Perry County
- Juniata River at Newport
- Swatara Creek at Harpers Tavern
- Susquehanna River at Shady Nook
- Allegheny River at Franklin
- Susquehanna River at Harrisburg
- Susquehanna River at Marietta
- Delaware River at Morrisville
- Bald Eagle Creek (Clinton County?)
- W. Branch Susquehanna River
- Loyalsock Creek in Lycoming County
- Pine Creek in Clinton County

Of these sites, PA DEP was able to obtain cyanobacteria data for all except Swatara at Harpers Tavern, Susquehanna River at Shady Nook, and Bald Eagle Creek.

This cyanobacteria taxa data was limited to PA DEP surveys from 2013, 2012, and 2005, as well as various USGS data. 2014 PA DEP algal data, which is not yet available, will eventually supplement this data set. Unfortunately the 2014 algal data will not specifically overlap the fish tissue data temporally or spatially. Temporally, the 2014 algal collections were not designed to specifically correspond to the fish collections. Plus, algal community composition can change dramatically over a period of only a few days, especially during flow events. Also, spatial overlap in collection locations is somewhat of an illusion because adult small mouth bass move. Any algal toxins that may be detected in the adult fish tissue may have been collected by the fish in another location. YOY, because of their residency in nursery areas, may be better suited to correlating *in situ* algal taxa and toxin data with tissue data. Unfortunately, no YOY tissue or nursery algal taxa/toxin data is presently available for Pennsylvania.

The taxa data is also limited in applicability because its collection was not designed with algal toxins and the exposure of small mouth bass, either adult or YOY, in mind.

Consequentially, the limited applicability of this data to the specific issue of small mouth bass decline in the Susquehanna River must be emphasized.

Cyanotoxin associations with particular cyanotaxa were compiled primarily from Quiblier et al. 2010. The benefit of Quiblier versus other references was that this review paper listed “benthic cyanobacteria species in which toxin production has been confirmed through uni-cyanobacterial strain isolation, culturing and toxin testing.” This method is more specific than relying upon toxin observation in conjunction with benthic mats, which contain more than one cyano-species. However, the mat-based toxin observations may provide future analyses. This is because of the possibility that certain cyano-taxa may not necessarily build toxin in a uni-cyanobacterial culture but rather may only build toxin allelopathically in a competitive environment.

Our cyanobacteria taxa data is largely restricted to the genera level. However, not all species within a genera are known to produce toxin (IARC 2010). Even within a species, only certain strains within that species, which possess the appropriate genotype, will produce toxin (IARC 2010). Consequentially, though a so-called toxin producing genera may be present in a sample doesn’t necessarily imply that toxin producing algae were indeed present. The taxa data can only infer the possibility that toxin production capability was present.

Data and Analysis:

Included with this document is an Excel spreadsheet. This spreadsheet indicates all cyanobacteria taxa from 2005, 2012, and 2013 PA DEP algal collections as well as a limited amount of data from USGS – the ”Cyanophyta Data” tab. This spreadsheet also summarizes the potential toxins at various river sites – the “River System Toxin List” tab. Biovolume data for the cyanobacteria found at selected sites is also included – the “Biovolume Data” tab. The spreadsheet is also summarized in Tables 1, 2, & 3 and Chart 1 below.

Labs involved with the identification of PA DEP algal collections included those of Central Michigan University (Hunter Carrick), Phycotech, and The Academy of Natural Sciences. Labs involved with the USGS data referenced appear to be limited to The Academy of Natural Sciences.

Table 1 summarizes all the cyanobacteria genera (and in some cases, the species) present in the mentioned PA DEP and USGS data sets that were similar in location to the 2014 cyanotoxin fish tissue collection sites. Also listed in this table are potential cyanotoxins that may be produced by the genera at those sites. As a whole, the possibility that cyanotoxins such as Microcystin, Anatoxin, Cylindrospermopson, and Saxitoxin were present at the fish collection sites did exist. However, given the limitations of the algal taxa identification, it cannot be said with certainty that the specific toxin producing strains were or were not present at the sites – only that the possibility exists.

In Table 1, several cyanobacteria genera are highlighted in yellow. The reason for this highlighting is that two identification labs – Central Michigan University and Phycotech – reported taxa names that do not exist in the Academy of Natural Science taxa name database. Because the scientists associated with The Academy, along with those who participated in specially designed workshops, were diligent in creating a list of taxa names based upon a consensus in the scientific community, the Academy names list is considered for this work to be the nomenclatural standard. These highlighted names were retained in the data set because their usefulness may be recovered later through a synonym search.

The attached Excel spread sheet lists the various labs used to identify the cyanobacteria taxa. These included The Academy of Natural Sciences (= A in the spreadsheet), Phycotech (= P), and Hunter Carrick (= C). An examination of the taxa present at each site will show that the taxa identified by each laboratory differs. The possible reasons for this are: 1) Labs did not necessarily get the same sample – sometimes multiple samples from a location were collected at different times of the year and each lab got a different sample, 2) Sub-sampling error when the labs did get duplicate samples, and 3) potential identification errors.

Table 2, lists the cyanotoxin potential, based on identified cyano-taxa, for various river sites associated with the fish tissue collection sites. As may be seen in this table, appreciable cyanotoxin potential exists in all of the major river systems sampled for fish tissue – these include the Susquehanna mainstem, the Allegheny at Franklin, and the Delaware at Trenton. It is important to recognize that the presence of taxa potentially capable of producing toxin does not imply that the taxa will produce toxin. The factors that cause benthic taxa to produce toxin are not well defined in the scientific literature (Quiblier 2010). However, preliminary results of the 2014 adult fish tissue toxicology suggests that microcystins were present in the Allegheny River as well as the Susquehanna River.

Based upon their presentation during the CADDIS 2 workshop, West Virginia DNR considered *Phormidium autumnale* to be of particular importance in their SMB decline investigations. Quiblier et al. 2010 confirms that *P. autumnale* does produce Homo/anatoxin-a. Importantly, *P. autumnale* was identified in PA DEP samples associated with this study. These identifications were shown by The Academy, and in one instance was recorded by Carrick at the 7/29/2013 W. Br. Susquehanna River site. Interestingly, Carrick frequently identified *P. retzii* in samples whereas no other lab confirmed the presence of this cyano-taxon. Academy data indicates that *P. autumnale* is very commonly identified in samples throughout the United States compared to *P. retzii,* which is much less frequently found in samples (The Academy 2011 *P. autumnale* and *P. retzii*). Because of the possible importance, this discrepancy between *P. autumnale* and *P retzii* identifications in PA DEP samples will need to be further researched. We can presently find to information that suggests *P. retzii* produces toxin. Undefined species within the genera of *Phormidium* were also found.

Chart 1 shows the percent cyanobacteria biovolume for major river sites used in the fish tissue analysis. The variability between sites is easily recognized in this chart. Even sites from the same approximate river locations demonstrate marked variation.

For example, consider the Juniata at Newport East and West, Susquehanna at Marietta East and West, Allegheny at Franklin East and West, and Delaware at Trenton East and West. This variation confirms why PA DEP has decided to segregate many of its larger rivers into different sections for analysis – tributary influences and non-mixing in the channels segregates each river essentially into "sub rivers."

Visually, Chart 1 is difficult to interpret quantitatively. To institute some level of quantified comparison between algae sampling sites the EPA, in its Rapid Bioassessment Protocol, recommends using a community similarity index computation based on Whittaker and Fairbanks 1958 (Barbour et al 1999). Performing the index calculations –attached Excel spreadsheet, 'Community Similarity – calc" tab – shows some interesting comparisons. First, the greater the CSI (community similarity index) the more similar are the two compared communities and a CSI = 100 would indicate identical communities. The Juniata Newport East and West sites have a CSI = 49.44. Compare this to the two Susquehanna Marietta sites with a CSI = 15.86. Clearly conditions in the Susquehanna at Marietta indicate a marked difference between the "east river' and the "west river" at this site. Comparing between major rivers indicate that the Allegheny at Franklin (8/13/2013) was more similar to the Delaware at Trenton (9/19/2013) than any of the other major river comparisons. However the Susquehanna at Marietta (9/18/2013) compared to the Allegheny at Franklin, with a CSI of 41.21 was only slight less than that of the Allegheny / Delaware couple.

The reason why the algal community comparisons between rivers and within rivers is important to the present topic of toxins found in adult SMB is that it demonstrates the irregularity of the benthic cyanobacteria community between sites that are adjacent. For example, consider those CSI values for adjacent sites at Susquehanna Marietta and Juniata Newport. This irregularity complicates the adult SMB tissue results because the adult bass are capable of easily moving over distances that would substantially change their cyano-algal toxin exposure. Contrast this with YOY while they reside in nursery habitats. We suspect that the exposure of YOY to cyanotoxins, in their nursery habitats, are likely to be much more uniform because their movements are much more restricted. However, variation at a scale as fine as a nursery bed has been noted in the scientific literature (refer to Wood et al. 2010). Unfortunately, based upon past algal sampling design, PA DEP presently lacks the data necessary to test this hypothesis. Moving forward, we would recommend a redesigned algal collection technique to more appropriately sample YOY nursery sites as well as monitor for cyanotoxins in those locations (refer to Wood et al. 2011).

Table 1

	Toxins potentially associated with the PADEP 2014 fish algal toxin tests				
Cyanophya Genera and select species [1] present in Pennsylvania stream sites where PADEP 2014 fish algal toxin tests were collected.	**Microcystin - 80 to 90 known variants. LR, YR, RR, LW common. LR most deadly. Cyclic peptide liver toxin.**	**Anatoxin / Homoanatoxin - 2 to 6 known variants. Anatoxin-a most common. Alkaloid nervous system toxin.**	**Cylindrospermopsin - 3 known variants. Alkaloid liver toxin.**	**Saxitoxin - Alkaloid toxin**	**Other toxins - such as novel (yet to be understood), Lyngbyatoxin, Aplysiatoxin**
Anabaena	YES	YES	YES		
Aphanocapsa					
Aphanothece					
Blennothrix					
Calothrix					
Capsosirira					
Chamesiphon					
Chroococcus					
Dactylococopsis					
Geitlerinema					
Heteroleibleinia					
Homoeothrix					
Hydrococcus					
Komvophoron					
Leptolyngbya					
Lyngbya		YES	YES	YES	YES
Merismopedia					
Microcystis	YES				
Oscillatoria	YES	YES	YES		
Oscillaria scanta					
Phormidium	YES	YES			
Phormidium autumnale		YES			
Phormidium retzii					
Planktothrix	YES	YES			
Pleurocapsa					
Pseudanabaena					
Rhabdogloea					
Schizothrix					
Snowella	YES				
Synechococcus					
Synechocystis					
Xenococcus					

1 Genera & species names in agreement with Non-Diatom Taxa Names - ANSP/NAWQA/EPA 2011 at http://diatom.ansp.org/nawqa/ANSPtaxa.aspx?diatom=0&taxonomy_id=26.

Yellow designates genera names not in agreement with Non-Diatom Taxa Names - ANSP?NAWQA/EPA 2011.

Table 2

	Toxins potentially associated with the PADEP 2014 fish algal toxin tests[1]				
2014 Fish Algal Toxin Tissue Test Sites	**Microcystin - 80 to 90 known variants. LR, YR, RR, LW common. LR most deadly. Cyclic peptide liver toxin.**	**Anatoxin / Homoanatoxin - 2 to 6 known variants. Anatoxin-a most common. Alkaloid nervous system toxin.**	**Cylindrospermopsin - 3 known variants. Alkaloid liver toxin.**	**Saxitoxin - Alkaloid toxin**	**Other toxins - such as novel (yet to be understood), Lyngbyatoxin, Aplysiatoxin**
Juniata - Newport	YES	YES	YES	YES	YES
Juniata - Lewistown	YES	YES			
Susquehanna - Harrisburg	YES	YES	YES	YES	YES
Susquehanna - Marietta	YES	YES	YES	YES	YES
Susquehanna - Sunbury	YES	YES	YES	YES	YES
Tuscarora Creek	YES	YES	YES	YES	YES
West Branch Susquehanna River		YES			
Pine Creek					
Loyalsock Creek					
Allegheny - Franklin		YES	YES	YES	YES
Delaware - Trenton	YES	YES	YES		
Chillisquaque Creek					
Kettle Creek		YES	YES	YES	YES

1. Based upon a historical (any record) presence of cyanotoxin producing taxa at the site.

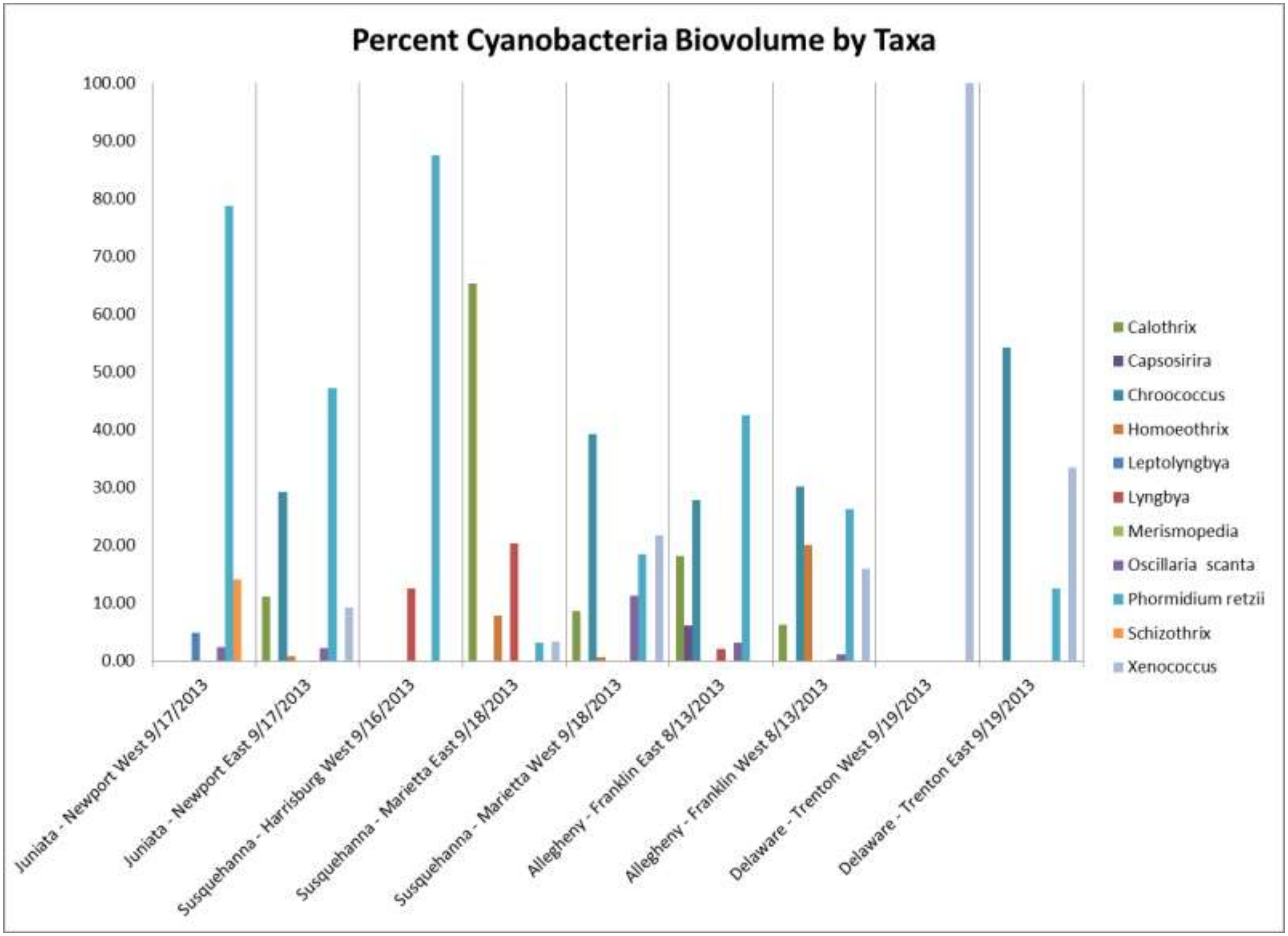
Table 3

Compared Rivers		Community Similarity Index [1]
Juniata Newport East 9/18/2013	Juniata Newport West 9/18/2013	49.44
Susquehanna Harrisburg West 9/16/2013	Susquehanna Marietta West 9/18/2013	18.39
Susquehanna Marietta East 9/18/2013	Susquehanna Marietta West 9/18/2013	15.86
Susquehanna Marietta Combined [2] 9/18/2013	Allegheny Franklin Combined [2] 8/13/2013	41.21
Susquehanna Marietta Combined [2] 9/18/2013	Delaware Trenton Combined [2] 9/19/2013	24.35
Allegheny Franklin Combined [2] 8/13/2013	Delaware Trenton Combined [2] 9/19/2013	50.62

1. Index based upon Percent Communit Similarity as used by Whittaker and Fairbanks (1958) and recommended for comparing algal communities by the the EPA Rapid Bioassessment Protocols, Chapter 6.

2. Combined data for the site derived by adding the biovolumes of taxa together.

Chart 1



Percent Cyanobacteria Biovolume by Taxa
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Juniata - Newport West 9/17/2013
Juniata - Newport East 9/17/2013
Susquehanna - Harrisburg West 9/16/2013
Susquehanna - Marietta East 9/18/2013
Susquehanna - Marietta West 9/18/2013
Allegheny - Franklin East 8/13/2013
Allegheny - Franklin West 8/13/2013
Delaware - Trenton West 9/19/2013
Delaware - Trenton East 9/19/2013
Calothrix
Capsosirira
Chroococcus
Homoeothrix
Leptolyngbya
Lyngbya
Merismopedia
Oscillaria scanta
Phormidium retzii
Schizothrix
Xenococcus

Conclusions:

Cyanobacteria taxa identification data exists for many stream sites around the Commonwealth of Pennsylvania. From that data, sites corresponding to 2014 PA DEP cyanotoxin fish tissue collection were extracted and analyzed for the potential that cyanotoxin producing cyanobacteria may have been present at those sites. The result of that analysis suggested that cyanobacteria genera known to produce toxins historically existed at many sites. The Susquehanna, Delaware, and Allegheny River systems apparently have harbored several genera capable of producing cyanotoxins. This is also true for creeks such as the Tuscarora and Kettle. Whereas streams such as the West Branch Susquehanna River, Loyalsock Creek, and Chillisquaque Creek contained fewer cyanotoxin-producing taxa.

The presence of toxin producing cyano-taxa does not necessarily imply that toxins are being produced. This is because only certain strains within a taxa group possess the genotype necessary for toxin production. Also the environmental factors that stimulate algal toxin production are not well understood for benthic cyanobacteria.

The irregularity in the cyanobacteria community at fine scales makes predicting adult small mouth bass exposure to cyantoxins difficult. However, young-of-the-year small mouth bass while they reside in their nursery habitats move much less than the adults. This reduced movement of YOY during critical periods of their development may expose them to cyanotoxins at a rate much different than that of the adults. Unfortunately, given present PA DEP data limitations, it is not presently possible to confirm this possible YOY cyanotoxin exposure.

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Title: Spatial comparison of Microcystin levels in smallmouth bass liver tissue (WS # 33)

Agency (Pennsylvania Department of Environmental Protection

Candidate Cause: Algal and bacterial toxins either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

Algal and bacterial toxins may directly affect recruitment of young-of-year (YOY) smallmouth bass (SMB) into age class 1+. Microcystin (MC) in particular is known to bioaccumulate in fish (Martin and Vasconcelos, 2009). Chronic exposure to MCs can cause serious health problems in animals and humans such as hepatic, gastric and epidermic diseases; neurological impairment; and death (Babica et al., 2006; Leao et al., 2009; Sivonen and Jones, 1999). There is also potential for a negative relationship between condition factor and increasing MC concentrations (Acuna et al. 2012).

If MC is affecting recruitment of SMB into age class 1+, then it's possible that there is a greater frequency of positive detections or higher MC values within the defined CADDIS area compared to other sites.

Data:

PA DEP collected liver tissue from adult female SMB during the spring of 2014. Stations within the defined CADDIS area included Juniata River at Newport, Susquehanna River at Harrisburg and Susquehanna River at Sunbury. Comparison sites within the Susquehanna basin included Swatara Creek at Harper's Tavern, Tuscarora Creek near Port Royal, Pine Creek near Jersey Shore, Loyalsock Creek near Montoursville, Bald Eagle Creek near Unionville and West Branch Susquehanna River near Jersey Shore. Comparison sites outside the Susquehanna basin included the Allegheny River at Franklin and Delaware River at Morrisville.

Analysis and Results:

Liver samples were analyzed using the enzyme-linked immunosorbent assay (ELISA) method at the PA DEP laboratory. Sample results were not confirmed using mass spectrometry so the dataset potentially contains false positive readings. Consequently, these samples must be evaluated qualitatively. Results were graphed using dot plots for spatial comparisons.

Overall, results were mixed between the sites (Figure 1). The West Branch Susquehanna River had the single highest result for MC; however, many of the Susquehanna basin comarison sites had at least one or two high results with respect to the whole dataset. Sites within the defined CADDIS area generally had similar or lower MC levels than either the in basin or out of basin comparison sites. The Juniata River tended to have the lowest amount of MC, with many samples falling below the dection limit.

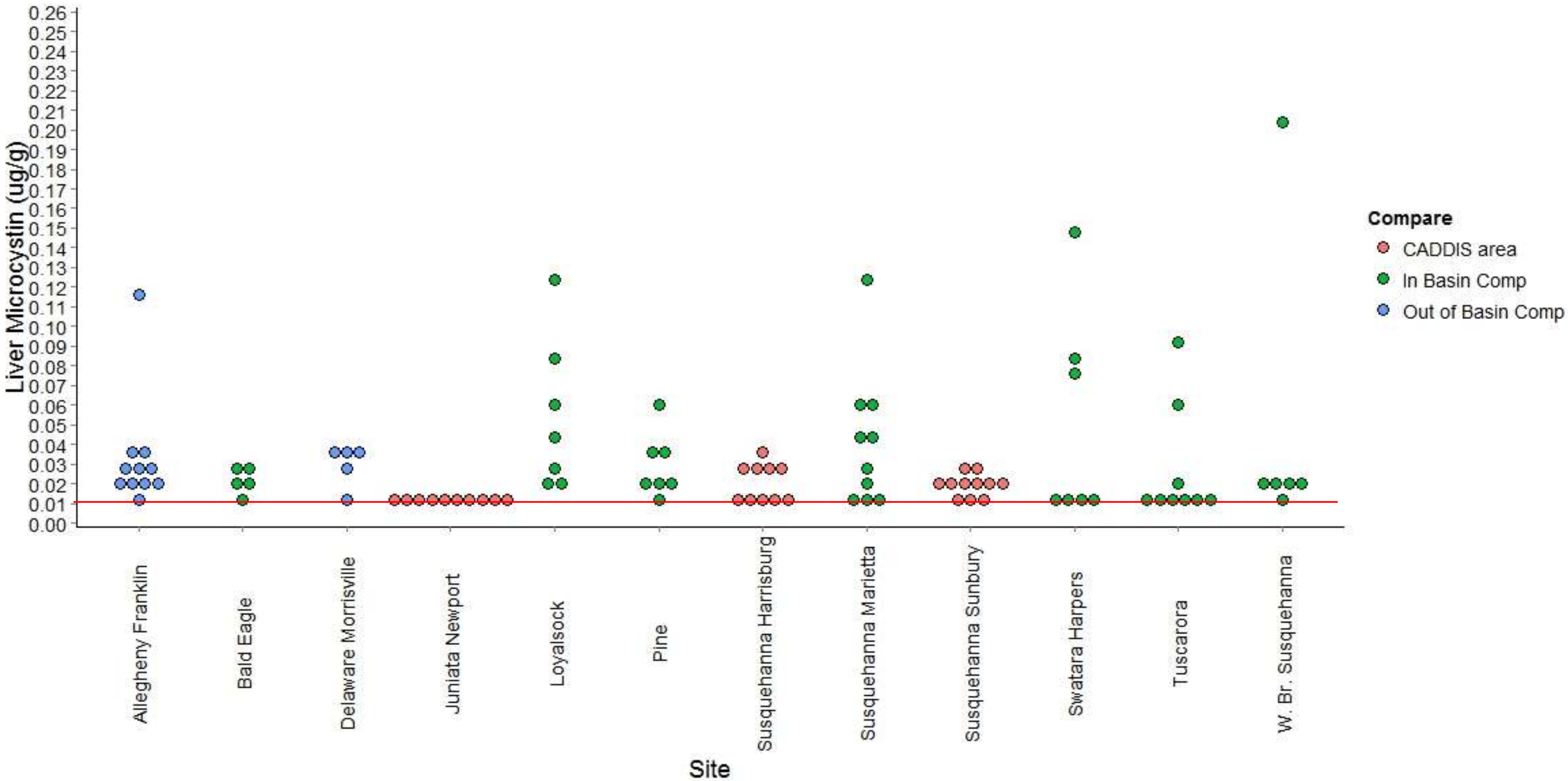

Figure 1. Smallmouth Bass results for liver microcystin at each site. The red line indicates the lower detection limit for the ELISA method.

Conclusion:

All sites had at least some positive detection for MC in SMB liver tissue. Sites within the defined CADDIS area were typically similar or lower in MC than all comparison sites. There does seem to be some pattern with sporadically higher results occuring in tributaries, but without more data, it is difficult to validate this pattern. These data suggest there is no clear spatial pattern relating to MC and its affect on SMB recruitment into age class 1+, yet more data are needed to confirm these results in YOY and adult male SMB.

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Title: Nutrients and Cyanobacteria (WS #50)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: Algal and bacterial toxins either kill YOY directly, or increase YOY susceptibility to disease

Introduction:

Cyanobacteria are very common in aquatic environments and may typically be found growing in the water column, on the water surface, on other algae and macrophytes, or on benthic substrates (Quiblier 2013).

Conventional thinking regarding cyanobacteria blooms, nutrients, and PAR (based on lakes):

Proliferation of certain cyanobacteria species have been known to be associated with warm weather conditions, high incident photosynthetically active radiation (PAR), increased organic matter loading (DOC & POC), increased inorganic nitrogen and phosphorous loading, among other chemical and biologic factors (Paerl 1988). In particular, eutrophication is generally considered a major driver of planktonic cyanobacteria blooms (Quiblier 2013). These associations hold for lake planktonic forms of cyanobacteria as characterized by those studied in lakes and reservoirs (Quiblier 2013).

Conventional thinking may not apply to rivers and streams:

Prolific benthic cyanobacteria mats have been observed in a variety of water conditions ranging from oligotrophic to eutrophic environments in rivers (Quiblier 2013). For example, in New Zealand, the greatest *Phormidium* mat coverage in rivers was found in conjunction with high total nitrogen to phosphorous ratios (with low reactive P). Whereas in Spanish rivers, cyanobacteria blooms were found associated with low total nitrogen to total phosphorous ratios but also in anything ranging from oligiotrophic to hypertrophic conditions (Quiblier 2013). Consequentially, based on any one or a few measured parameters, it seems difficult to predict the emergence of a river benthic cyanobacteria bloom. The current state of cyanobacteria research shows that in contrast with planktonic environments, much less is understood regarding the conditions that produce growth and toxin production in benthic riverine environments (Quiblier 2013).

Challenging conventional thinking regarding algal blooms and nutrients:

Authors suggest that cyanobacteria often possess novel nitrogen and phosphorus acquisition capability (Quiblier 2013). The ability of certain cyanobacteria species to fix nitrogen is well known. The ability of certain cyanobacteria species to acquire phosphorous when such may be limited as judged by conventional measures of bio-available phosphorous, may provide cyanobacteria with a competitive advantage (Quiblier 2013). Because of these novel uptake capacities, relying upon the supposition that elevated phosphorous will produce cyanobacteria blooms will miss the possibility that cyanobacteria may be very capable of blooming when phosphorous, by conventional measures, appears limited. Barbara Pawlik-Skowronska (2013) suggests another nutrient measure, that being DIN:DIP ratio, that may serve as an emergence predictor of cyanotoxin producing cyanobacteria.

Predicting cyanobacteria blooms and cyanotoxin production from DIN:DIP ratios based on Pawlik (2013):

Given the state of knowledge regarding the benthic cyanobacteria community, it may be useful, at least as a starting point, to rely upon that which is known regarding lake and planktonic cyanobacteria communities (Pawlik-Skowronska 2013):

- A decrease in phosphate concentration as well as that of total phosphorous, driving an increase of DIN:DIP ratio is correlated with an increase in cyanobacteria species richness.
- An increase in cyanobacteria biomass correlates with an increase of anatoxin-a production.
- Phosphorous concentration is negatively correlated with microcystin concentration.
- An increase in DIN:DIP ratio, corresponding with a decrease in biomass, seems to increase the competitive advantage of efficient microcystin and anatoxin-a producers.
- In low DIN:DIP ratio (order of 2.3 to 3.0), anatoxin-a production prevails.
- In high DIN:DIP ratio (order of 23 to 30), microcystin production prevails.

With this guidance from lake / planktonic cyanobacteria and cyanotoxin production knowledge, we will examine existing cyano-taxa and nutrient data from three river systems to explore if any inferences may be discovered. However, **the purpose of this**

<u>worksheet is to analyze cyano-taxa and nutrient data from three Pennsylvanian river sites – those being the Juniata at Newport, Susquehanna at Harrisburg, and the Delaware at Morrisville – and associate that data with likely cyanotoxin production at various nutrient conditions as suggested by the scientific literature.</u>

Data and Analysis:

Charts 1 and 2, below, show discrete DIN:DIP ratios in the Juniata at Newport and the Susquehanna at Harrisburg at various times in 2012 and 2013 taken from WQN data. Chart 3 shows the discrete TN:TP ratios in the Delaware at Morrisville (DIN:DIP was not available at the time this paper was written) at various times in 2012 and 2013 (WQN data).

Readily apparent from these charts is the seasonal variation in these ratios. Given what Pawlik-Skowronska 2013 suggests – that certain cyanobacteria may gain a competitive advantage at various DIN:DIP ratios – we may infer that seasonally certain cyanobacteria taxa may gain competitive advantage and consequentially may produce toxins during those periods of advantage. Chart 4, which demonstrates the cyanobacteria taxa present at various sites, does seem to indicate that the cyanotaxa community may shift seasonally, perhaps as a consequence of these seasonal DIN:DIP ratio changes.

Summarizing the DIN:DIP and TN:TP ratio data we see that:

- WQN data at Susquehanna Harrisburg recorded a low DIN:DIP ratio of 8.67:1 and all other ratios greater than 23:1.
- WQN data at Juniata Newport recorded a low DIN:DIP ratio of 24.9:1 and all other ratios greater than 23:1.
- WQN data at Delaware Morrisville recorded a low TN:TP ratio of 10.3:1 with ratios frequently below 23:1.
- PA DEP data at Susquehanna Harrisburg show values generally near 23:1 and above.
- PA DEP data at Juniata Newport show values of 40:1.
- PA DEP data at Delaware Morrisville show values ranging from 17.4:1 to 21.8:1.

Clearly, WQN and PA DEP data suggest that with regard to DIN:DIP ratios, the Delaware River is noticeably different from that of the Juniata and Susquehanna Rivers

Based upon the DIN:DIP ratios suggested by Pawlik-Skowronska (2013), and the taxa / toxin list from Chart 4 and Table 2, microcystin toxin production in the Susquehanna and Juniata Rivers is likely. This is because the microcystin toxin producing taxa of *Phormidium* and Microcystis are present at these sites and the DIN:DIP ratios are usually above the 23:1 threshold suggested by Pawlik-Skowronska (2013) to favor the production microcystin. Though *Phormidium* was also identified at the Delaware Morrisville sites, the DIN:DIP ratio threshold suggested by Pawlik-Skowronska (2013) is likely met less often there.

Chart 1 – Juniata Newport DIN:DIP from WQN

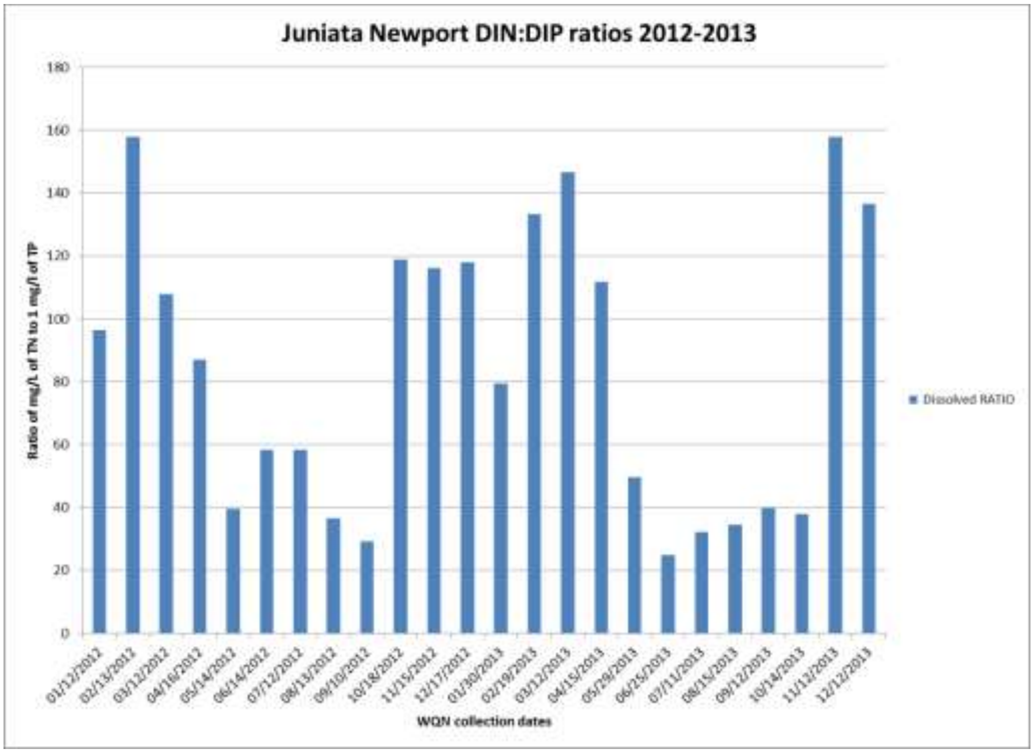


Chart 2 - Susquehanna Harrisburg DIN:DIP from WQN

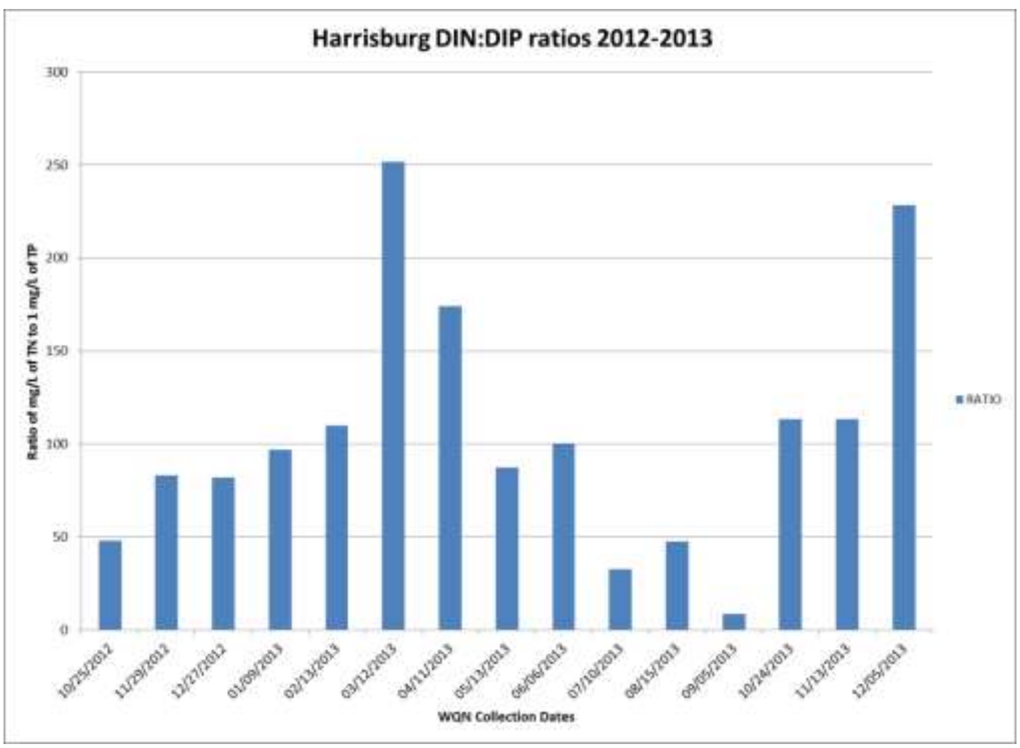


Chart 3 – Delaware Morrisville TN:TP from WQN

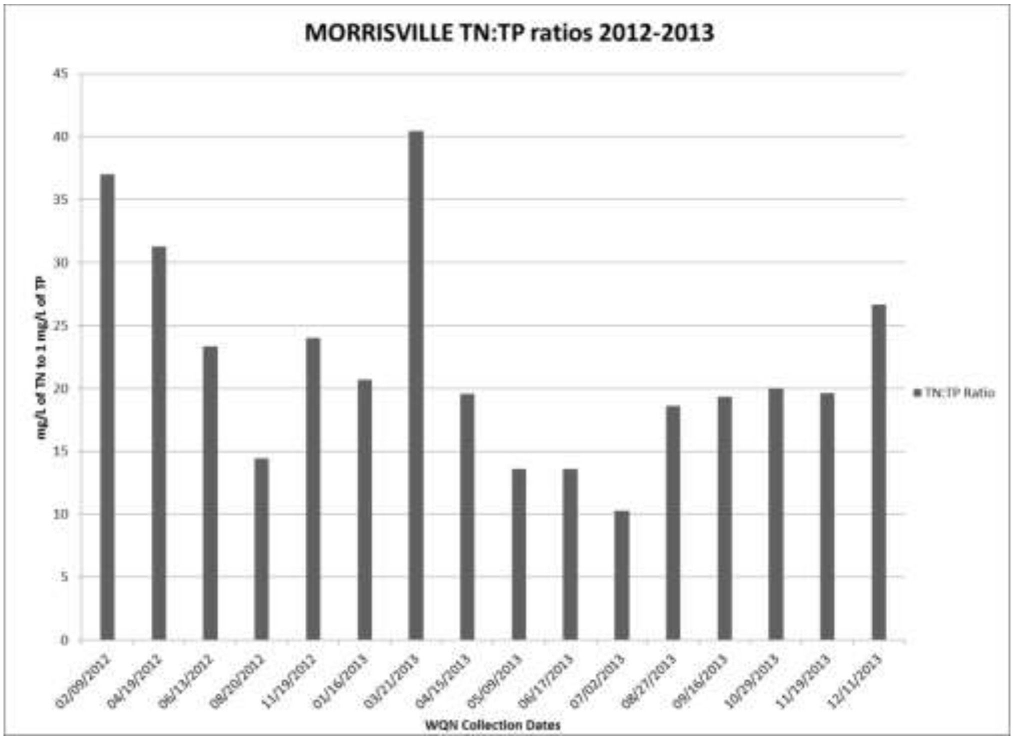


Table 1 Discrete DIN:DIP from PA DEP water Chemistry

Site	Date	DIN:DIP Ratio [1]
Susquehanna - Harrisburg East	7/9/2012	22.0 : 1
Delaware - Morrisville East	7/11/2012	17.4 : 1
Susquehanna - Harrisburg East	9/16/2013	44 .7 : 1
Susquehanna - Harrisburg Middle	9/16/2013	39.4 : 1
Susquehanna - Harrisburg West	9/16/2013	51.3 : 1
Juniata - Newport West	9/17/2013	40.9 : 1
Juniata - Newport East	9/17/2013	40.0 : 1
Delaware - Morrisville East	9/19/2013	18.8 : 1
Delaware - Morrisville West	9/19/2013	21.8 : 1

1. Based upon Nitrogen as dissolved ammonia, nitrate, and nitrite, and as Phosphorous a dissolved ortho phosphorous.

Chart 4 – Cyanobacteria Taxa at Three River Sites

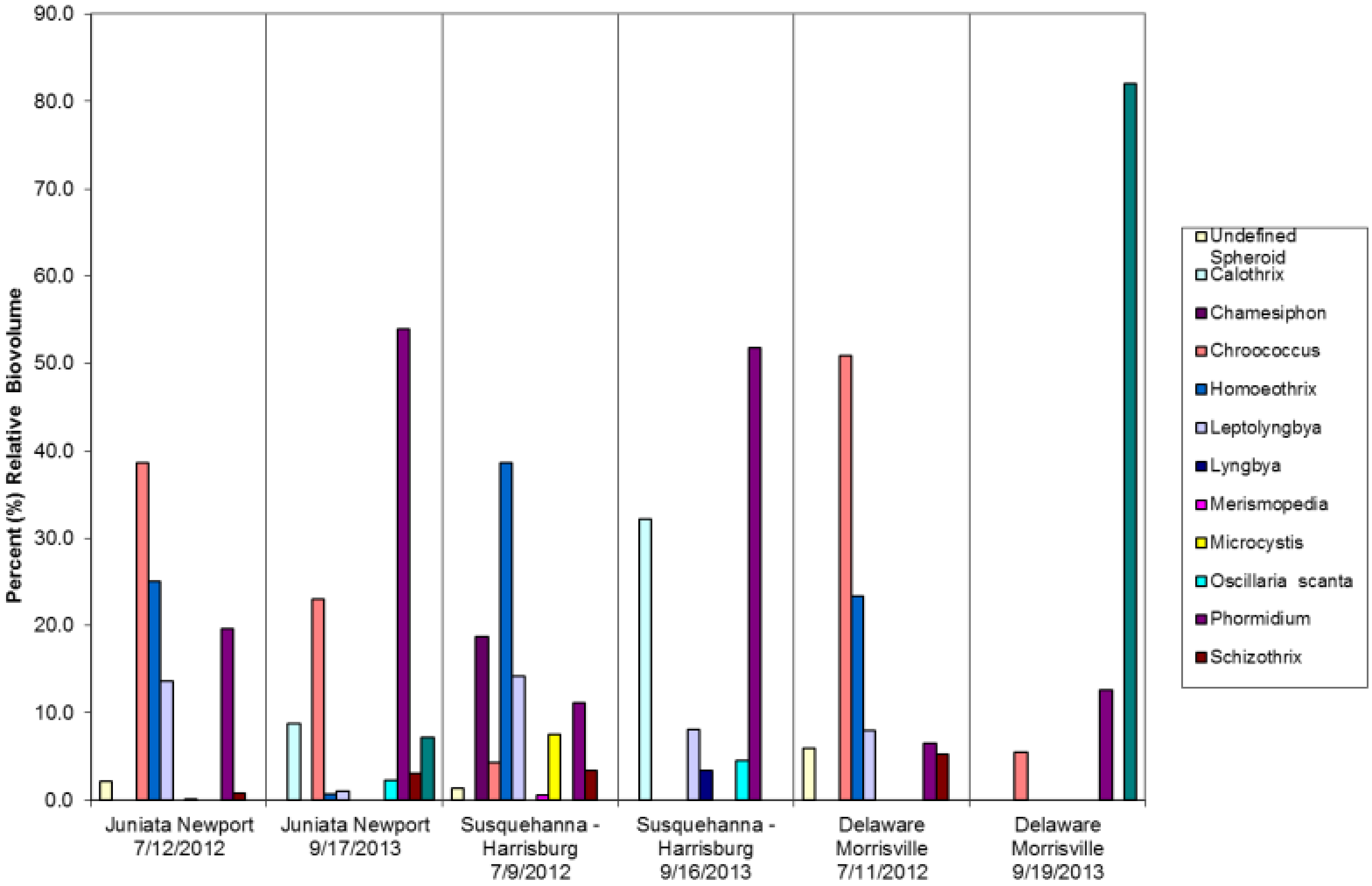


Table 2 – Cyanobacteria Taxa Biovolume Corresponding to Chart 4 with Possible Cyanotoxin Produced

		Cyanobacteria Taxa - Percent Relative Biovolume					
Cyanophya Genera	Possible Toxin Produced	Juniata Newport 7/12/2012	Juniata Newport 9/17/2013	Susquehanna - Harrisburg 7/9/2012	Susquehanna - Harrisburg 9/16/2013	Delaware Morrisville 7/11/2012	Delaware Morrisville 9/19/2013
Undefined Spheroid		2.163	0.000	1.432	0.000	5.912	0
Calothrix		0.000	8.696	0.000	32.184	0.000	0.00
Chamesiphon		0.000	0.000	18.716	0.000	0.000	0.00
Chroococcus		38.634	23.018	4.385	0.000	50.922	5.42
Homoeothrix		25.085	0.767	38.593	0.000	23.395	0.00
Leptolyngbya		13.586	1.023	14.180	8.046	7.978	0.00
Lyngbya	**Anatoxin-a & Homoanatoxin-a**	0.000	0.000	0.000	3.448	0.000	0.00
Merismopedia		0.076	0.000	0.618	0.000	0.000	0.00
Microcystis	**Microcystin**	0.000	0.000	7.535	0.000	0.000	0.00
Oscillaria scanta		0.000	2.302	0.000	4.598	0.000	0.00
Phormidium	**Anatoxin-a, Homoanatoxin-a, & Microcystin**	19.583	53.964	11.136	51.724	6.516	12.61
Schizothrix		0.873	3.069	3.406	0.000	5.277	0.00
Xenococcus		0.000	7.161	0.000	0.000	0.000	81.97

As an aside, it is helpful to review the various cyanobacteria communities in the three rivers we have analyzed in this worksheet for similarities these communities may have to each other. Table 2 summarizes the Community Similarity Index values of various site pair. These pair demonstrate not only similarity between river sites but also a seasonal similarity, of a sort, at individual sites. The CSI (Community Similarity Index) values were computed from that described by Whittaker and Fairbanks in 1958 and as recommended by U.S. EPA Rapid Bioassessment Protocols, Chapter 6. U.S EPA suggested that a CSI value of 75 or greater implies an equivalent taxa composition in the compared communities.

Noticeable in similarity are the 2012 Juniata and Delaware sites as well as the 2013 Juniata and Harrisburg sites. Equally of note is the dissimilarity between the Delaware in July of 2012 and that from September 2013. The degree of similarity, or dissimilarity, between sites and its biological meaning is still being researched by PA DEP. What is clear is the dissimilarity at a site that may have been produced by a seasonal transition. However, this dissimilarity may also be a consequence of differences in flow from one year to the next – refer to any of the site comparisons at same site but different date. Unfortunately, not enough data exists at this time to discern variation due to seasonality or flow.

The reason why the cyanobacteria community composition is considered here is because it helps to emphasize the multitude of variables that may affect the cyano-taxa composition and the associated possible cyanotoxin production. Simply, there is much that is not yet understood by researchers concerning the response of the many cyanobacteria taxa to various stimuli in benthic river environments.

Table 2 – Cyanobacteria Community Comparison

Compared Rivers		Community Similarity Index [1]
Juniata Newport 7/12/2012	Juniata Newport 9/17/2013	45.3
Delaware Morrisville 7/11/2012	Delaware Morrisville 9/19/2013	11.9
Susquehanna Harrisburg 7/9/2012	Susquehanna Harrisburg 9/16/2013	19.2
Juniata Newport 7/12/2012	Delaware Morrisville 7/11/2012	79.6
Juniata Newport 9/17/2013	Delaware Morrisville 9/19/2013	25.2
Juniata Newport 7/12/2012	Susquehanna Harrisburg 7/9/2012	56.6
Juniata Newport 9/17/2013	Susquehanna Harrisburg 9/16/2013	63.7
Susquehanna Harrisburg 7/9/2012	Delaware Morrisville 7/11/2012	47.1
Susquehanna Harrisburg 9/16/2013	Delaware Morrisville 9/19/2013	12.6

1. Index based upon Percent Communit Similarity as used by Whittaker and Fairbanks (1958) and recommended for comparing algal communities by the the EPA Rapid Bioassessment Protocols, Chapter 6.

Conclusions:

The purpose of this worksheet was to analyze cyano-taxa and nutrient data from three Pennsylvanian river sites and associate that data with likely cyanotoxin production at various nutrient conditions as suggested by the scientific literature. Cyanobacteria taxa data from 2012 and 2013 PA DEP algal sampling at the Juniata Newport, Susquehanna Harrisburg, and Delaware Morrisville sites indicate the presence of taxa known to potentially produce cyanotoxin. These taxa included the possible Anatoxin-a/homoanatoxin-a producing genera of *Lyngbya, Anabaena, Oscillatoria, Phormidium,* and *Planktothrix*. Taxa found at thes sites also included the possible Microcystin producing genera of *Anabaena, Microcystis, Phormidium, Planktothrix, Oscillatoria,* and *Snowella*. Based upon DIN:DIP ratios suggested by Pawlik (2013), microcystin producing genera at these sites would likely gain competitive advantage and may consequentially produce the microcystin toxin. However, among these three sites, the Delaware generally showed N:P ratios that were suppressed when compared to those in the Juniata or Susquehanna. Consequentially, as suggested by Pawlik-Skowronska (2013), microcystin production in the Delaware may be curtailed when compared to that which may be produced in the Susquehanna and Juniata.

Because much of what is understood by researchers about cyanobacteria blooms and their associated cyanotoxins is based upon work done in lake or reservoir systems, predicting responses of cyano-taxa, and their possible cyanotoxin production, in benthic river systems is currently problematic. This knowledge gap, when taken in tandem with the incompleteness of the PA DEP data record, make predicting or understanding cyanobacteria blooms and cyanotoxin releases in Pennsylvania river systems particularly difficult.

Literature Cited:

Paerl, Hans W. 1988. Nuisance phytoplankton blooms in costal, estuarine, and inland waters. Limnology and Oceanography 33 (823-847).

Pawlik-Skowronska, B., R. Kalinowska, & T, Skowronski. 2013. Cyanotoxin diversity and food web bioaccumulation in a reservoir with decreasing phosphorus concentrations and perennial cyanobacterial blooms. Harmful Algae 28 (118-125).

Quiblier C., S Wood, I. Echenique-Subiabre, M. Heath, A. Villeneuve, JF. Humbert. 2013. A review of current knowledge on toxic benthic freshwater cyanobacteria – Ecology, toxin production and risk management. Water Research 47 (5464 – 5479).

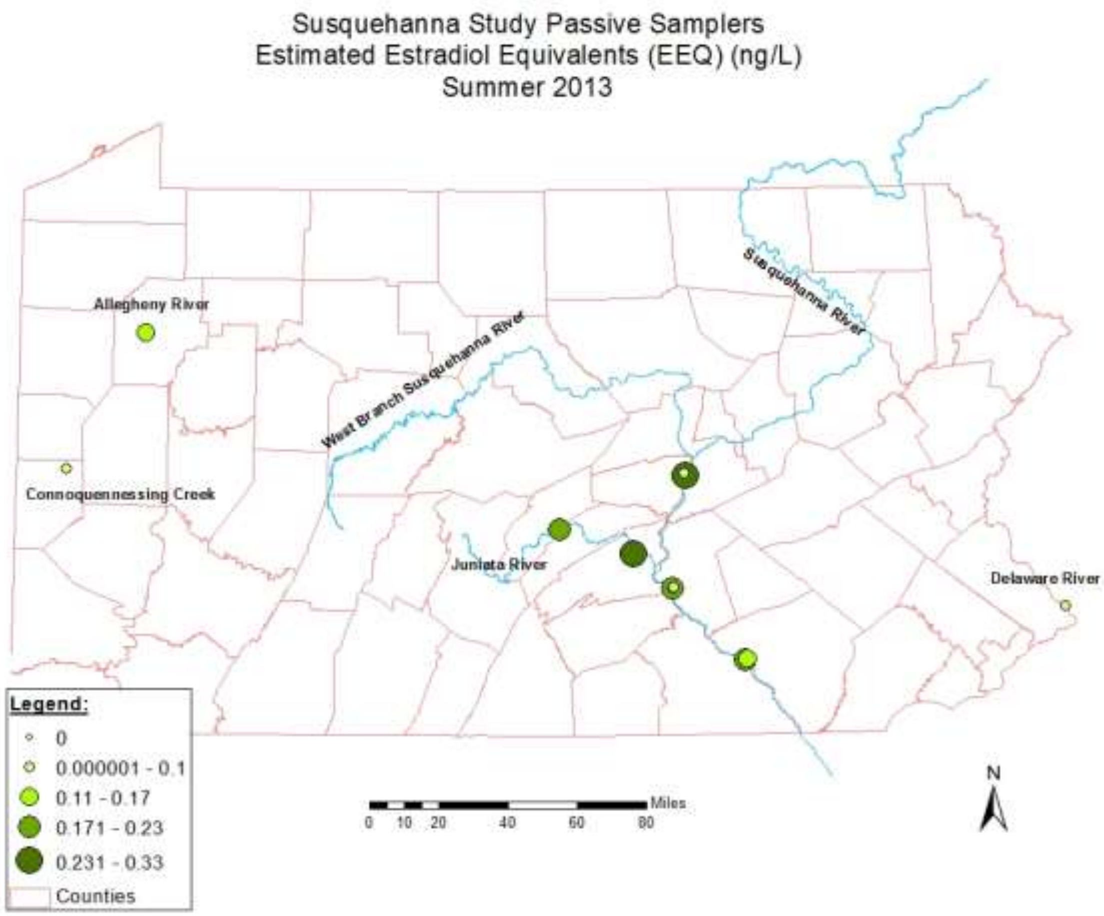
Candidate Cause 12: Toxic Chemicals

Worksheets: 37.1, 38, 39, 40, 42, 44, 45, and 46

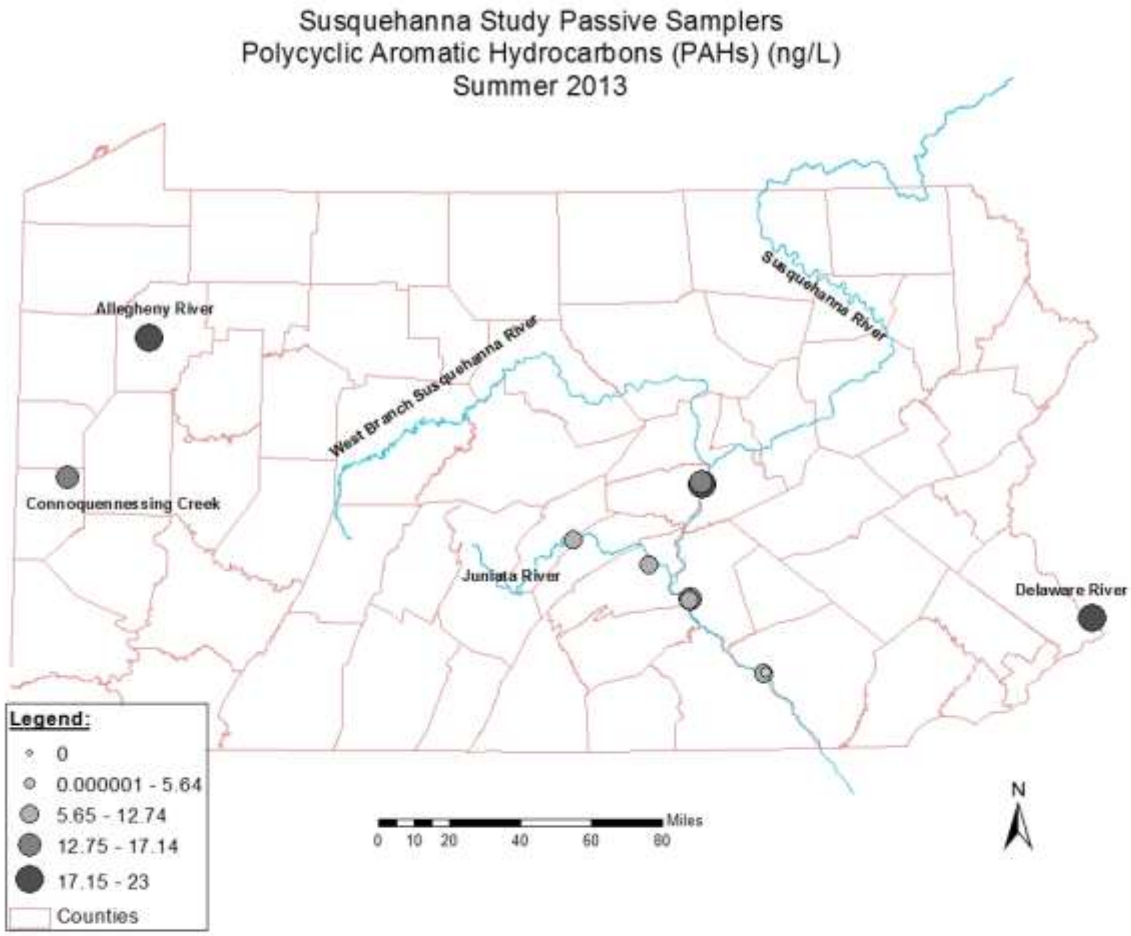
Title: Passive Sampler and Sediment Results Maps (Select Results); 2013-2014 Data (WS # 37.1)

Agency: Pennsylvania Department of Environmental Protection

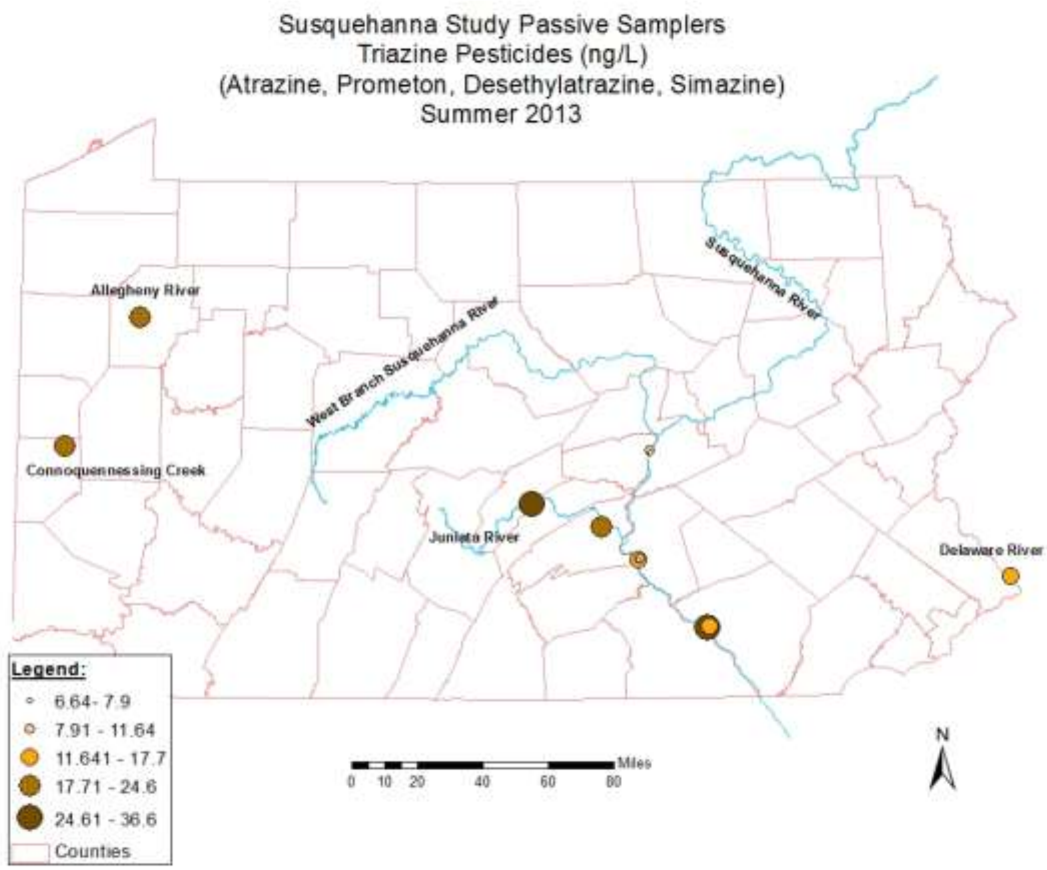
Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease. Samples were collected by PA DEP and analyzed by USGS.



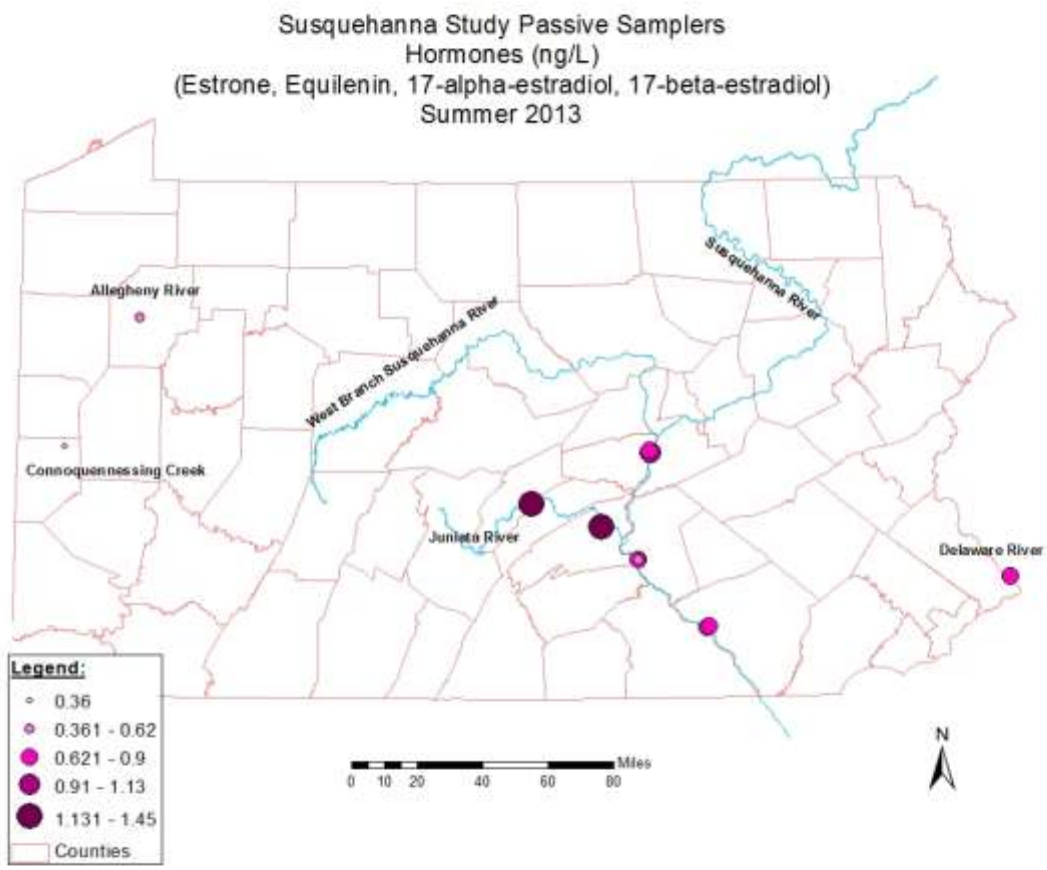
Highest total estrogenicity found in passive samplers was around Sunbury, Juniata, and Harrisburg/Marietta sites.



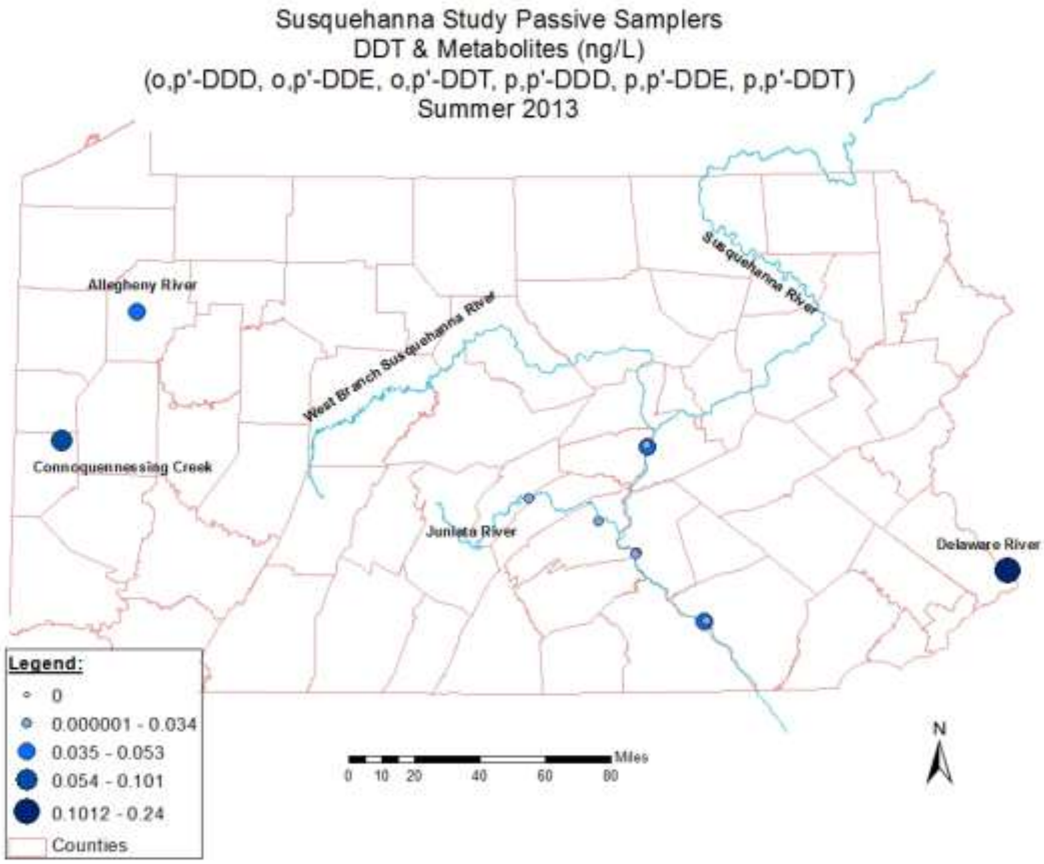
PAH concentrations were summed for this map. They are ubiquitous and high all over the state.



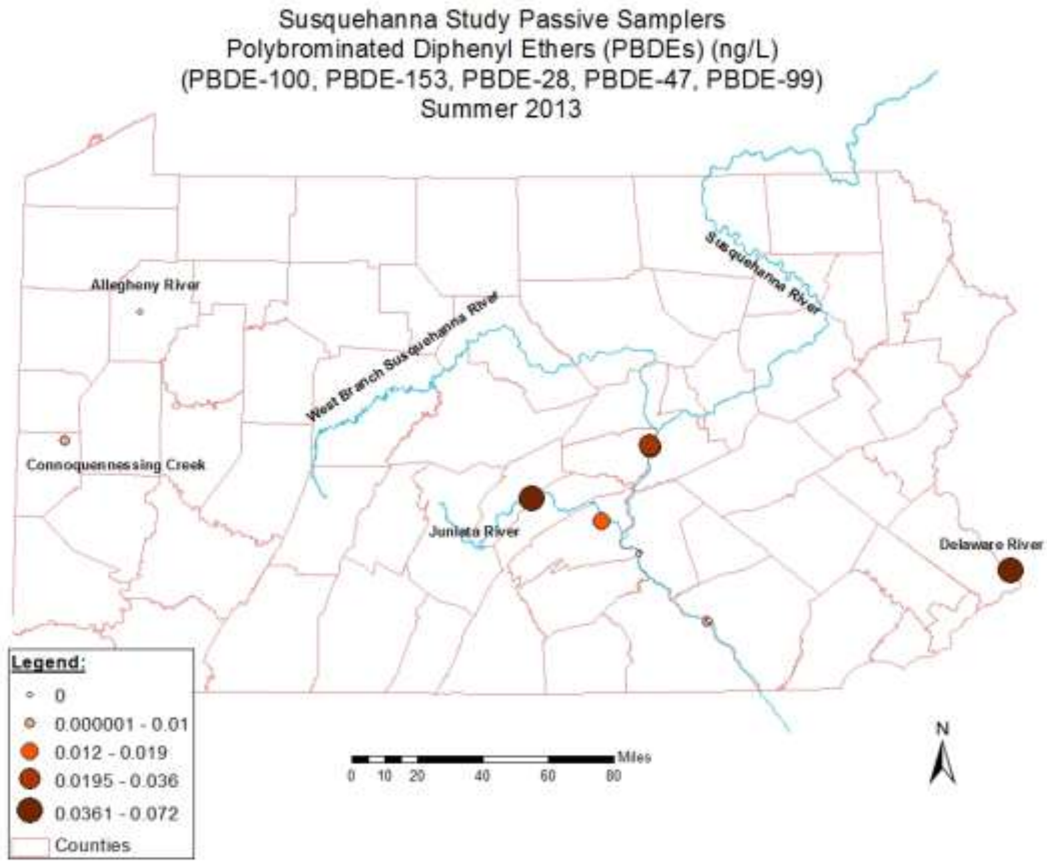
Triazine pesticide concentrations were also summed.
They are also ubiquitous across the state, with highest concentrations at Juniata @ Lewistown and Susquehanna @ Marietta.



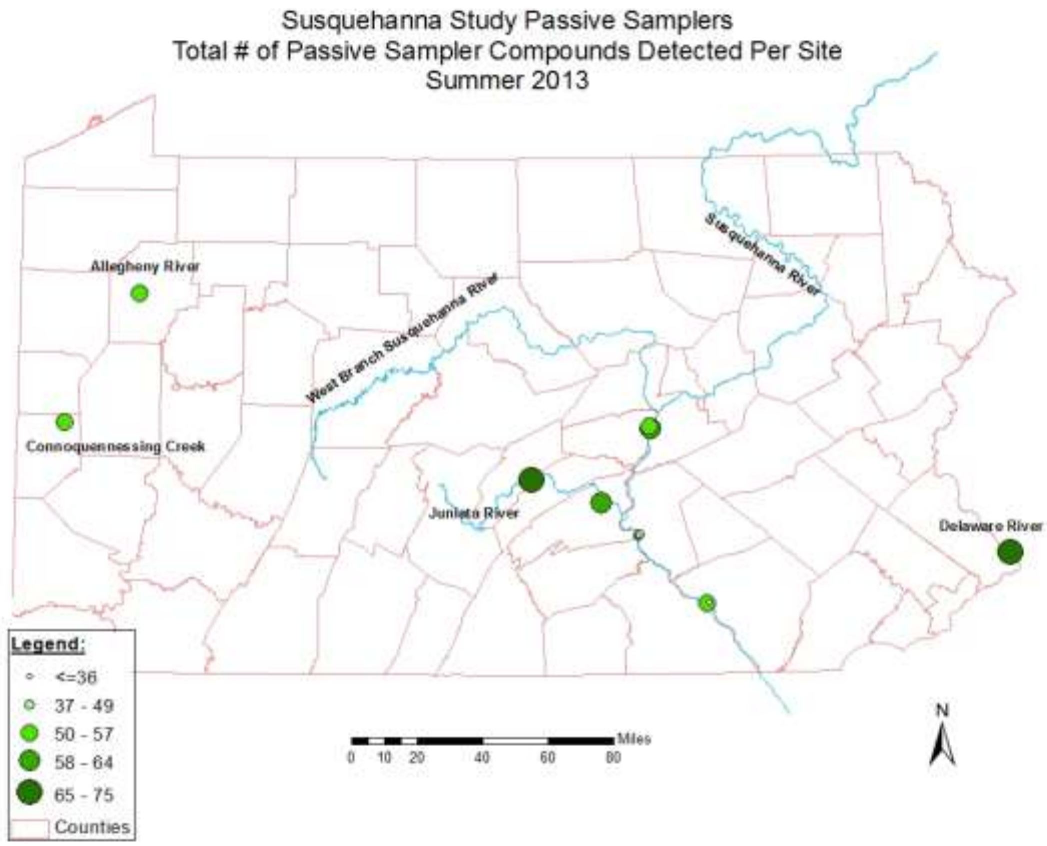
Hormones concentrations were summed. Hormone concentrations were noticeably higher in the Juniata River than other parts of the state.



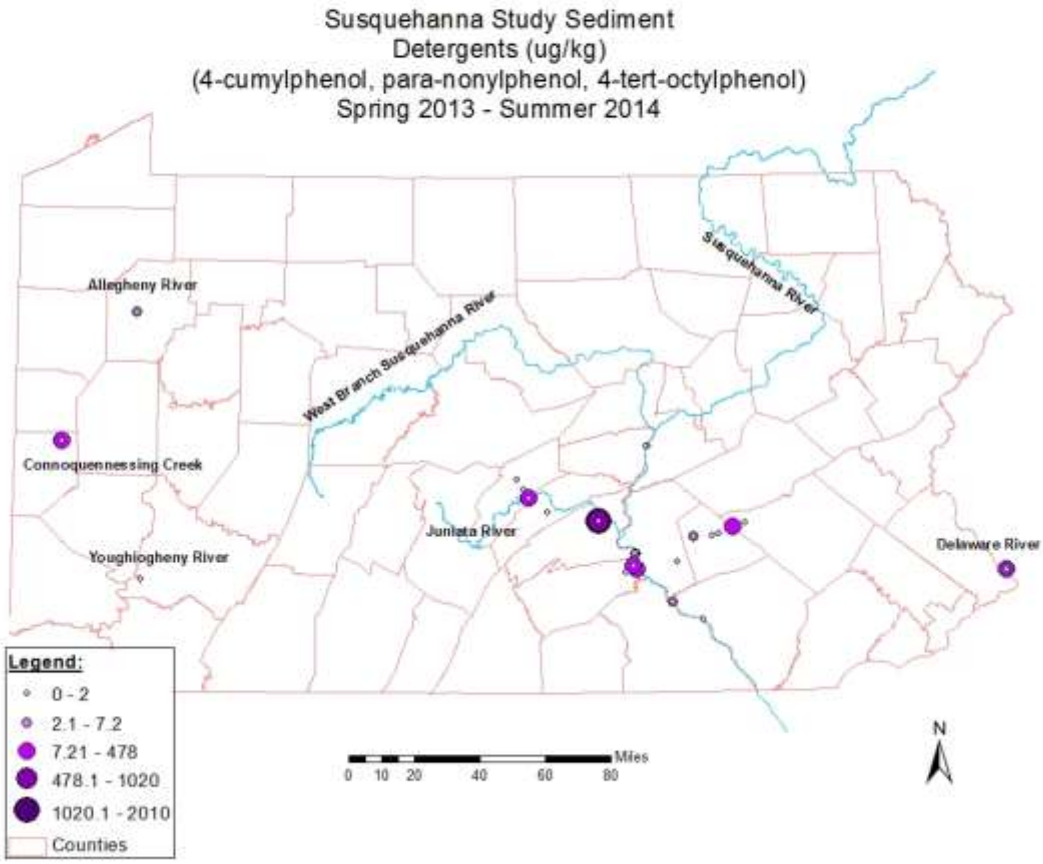
DDT & its metabolites were summed.
Highest concentrations were in the control sites, although all were quite low.



PBDE concentrations were summed.
Highest total concentrations were in the Delaware River & Juniata @ Lewistown, although all concentrations were low.

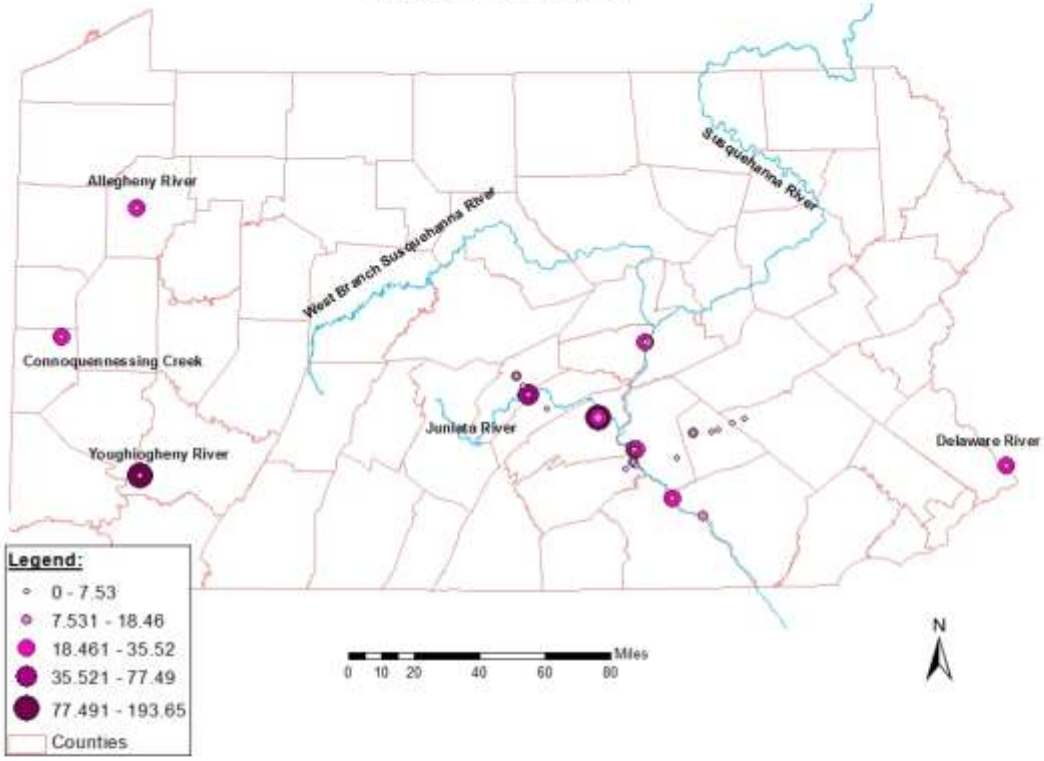


Total # of compounds detected in passive samplers per site are shown. Highest numbers were detected at Delaware River and Juniata @ Lewistown.

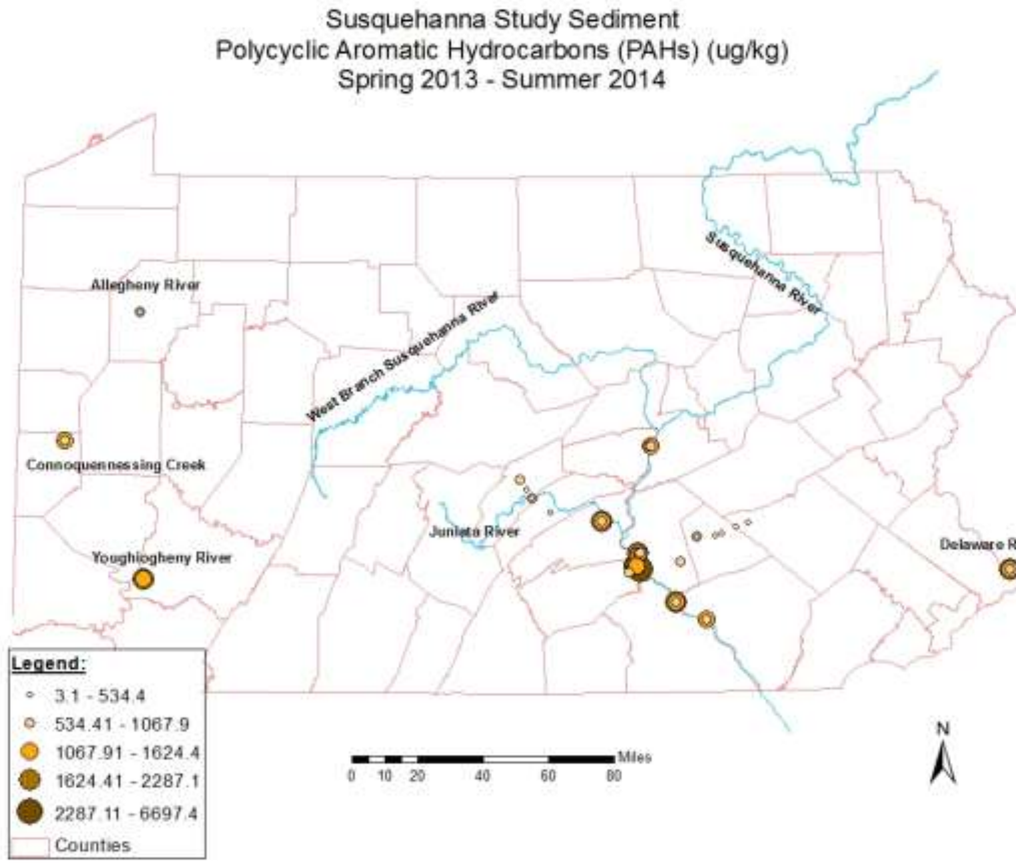


Detergent compound concentrations detected in streambed sediment were summed. Highest concentrations were at Juniata @ Newport.

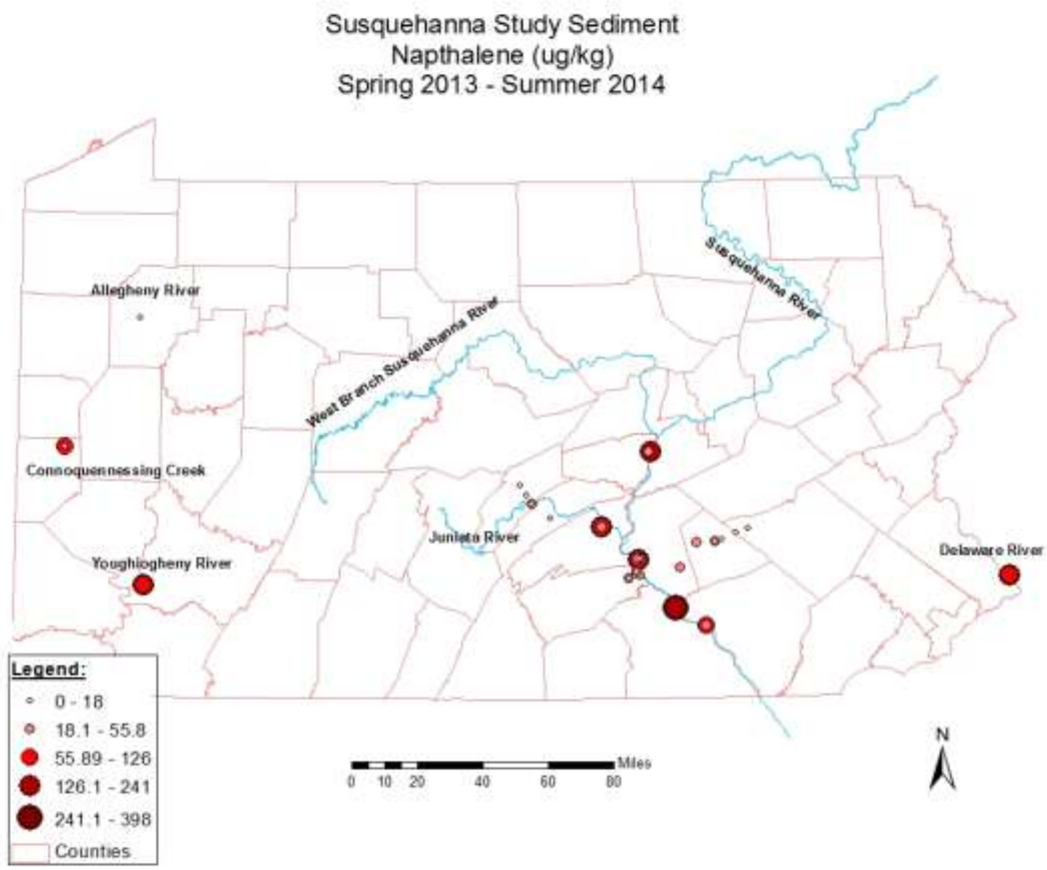
Susquehanna Study Sediment
Hormones & similar (ug/kg)
(BPA, BPF, estrone, 17-beta-estradiol, 17-alpha-estradiol, 17-alpha-ethynyl estradiol, estriol, mestranol)
Spring 2013 - Summer 2014



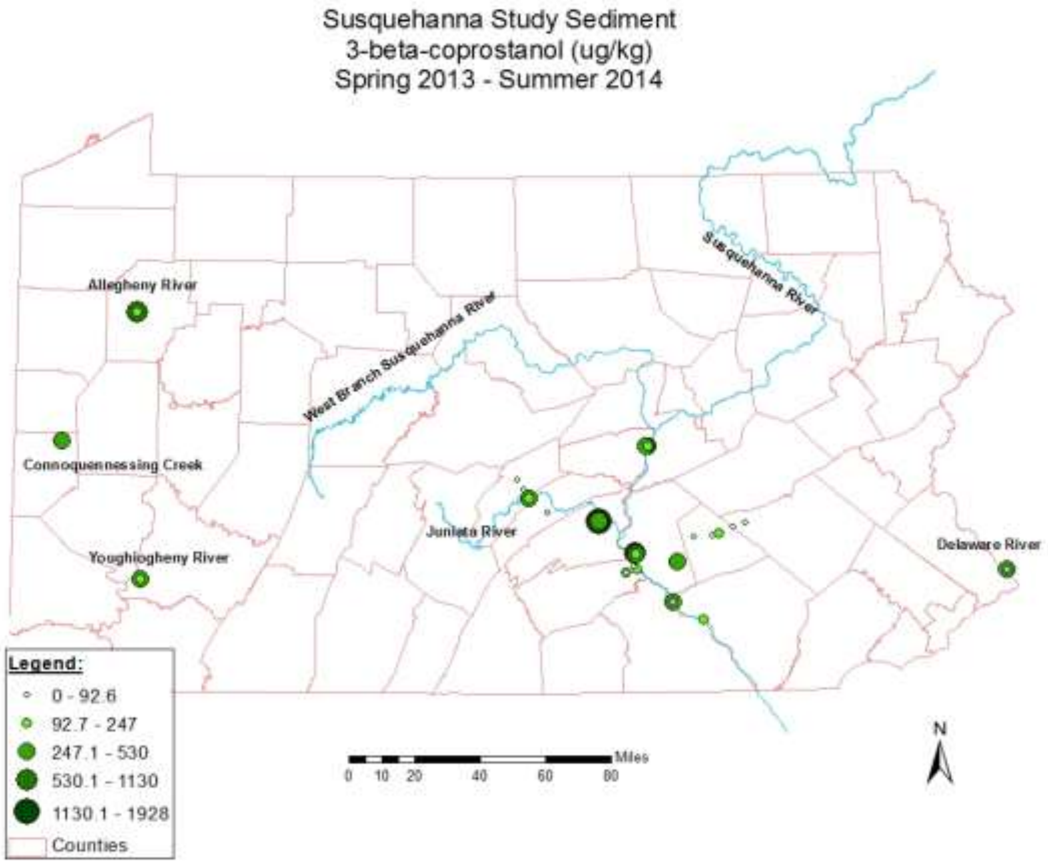
Hormone concentrations were summed. Highest concentrations were at Youghiogheny River, Juniata @ Newport, and Juniata @ Lewistown.



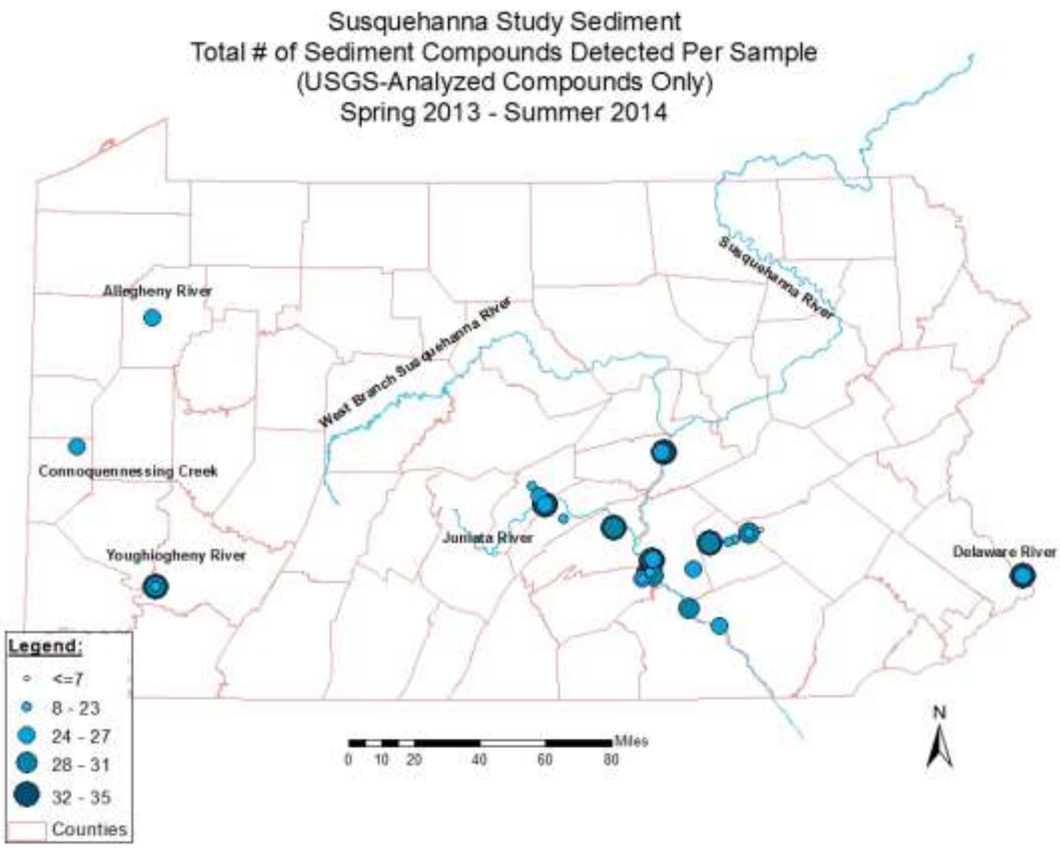
PAH concentrations were summed. Highest concentrations were on the Susquehanna @ Wormleysburg and Conodoguinet Creek.



Highest concentrations of the PAH naphthalene were at Susquehanna @ Marietta.



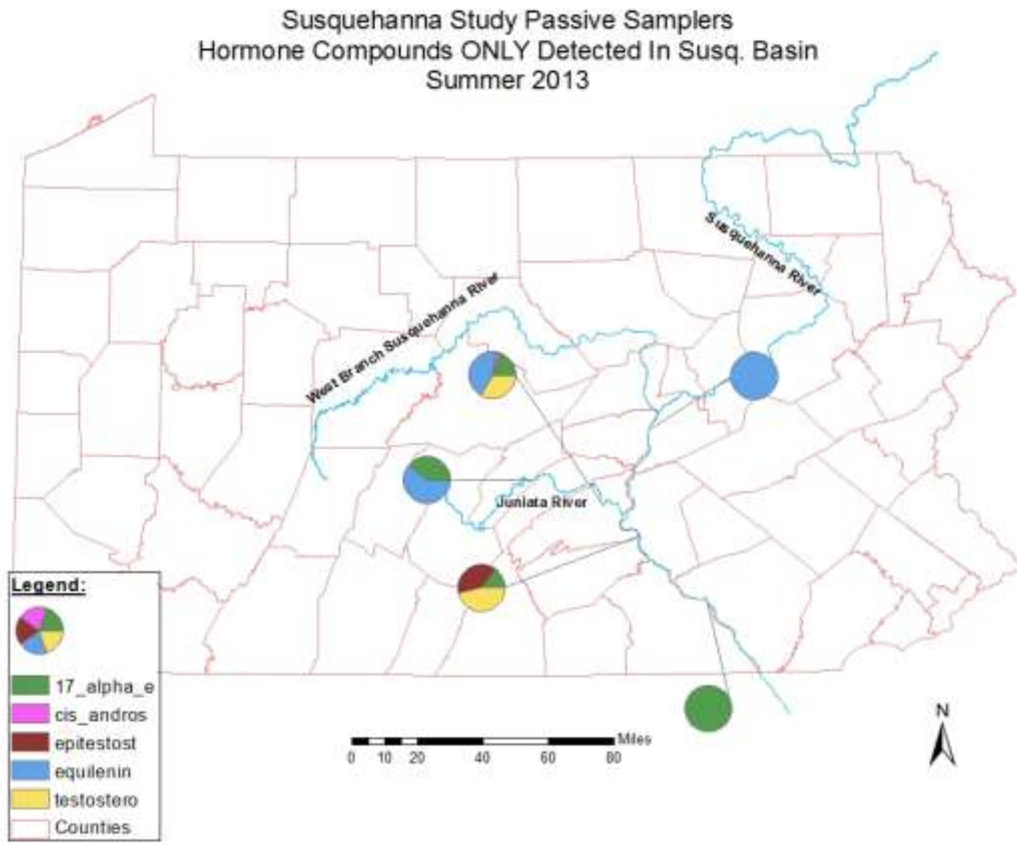
Highest concentrations of 3-beta-coprostanol were at Juniata @ Newport.



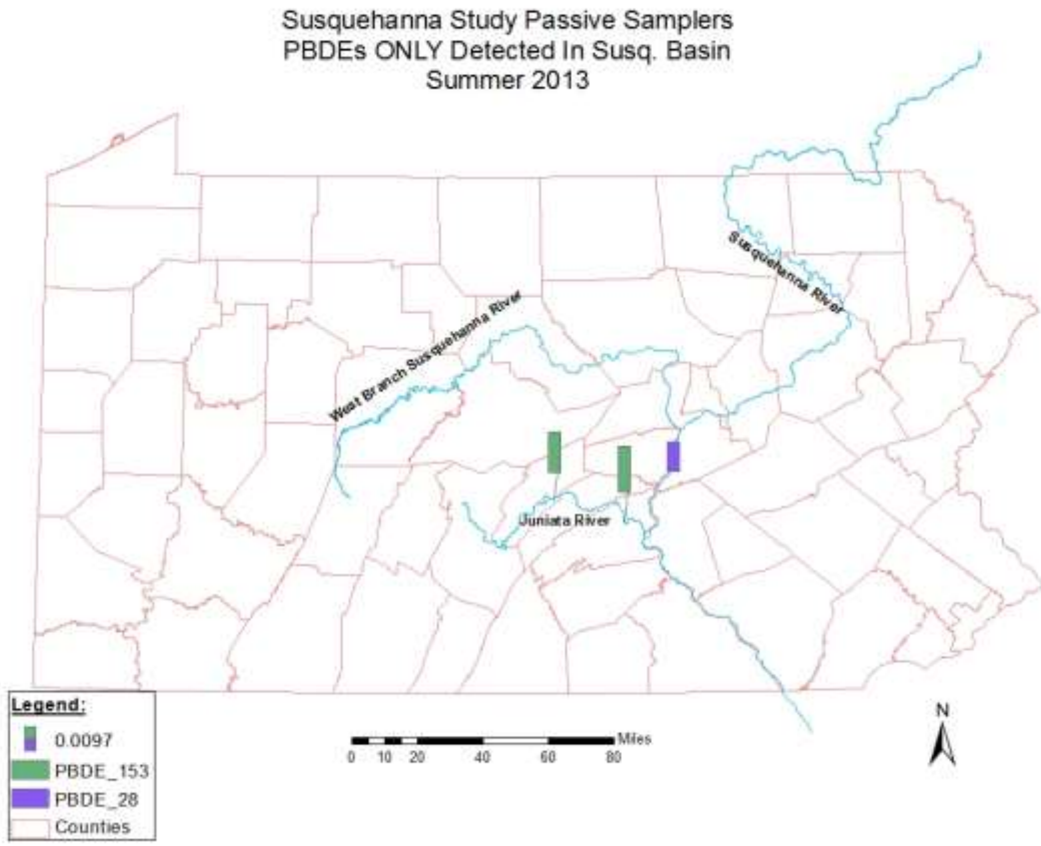
Total # of sediment compounds detected per sample are shown. Highest numbers were found at Youghiogheny River, Delaware River, Juniata @ Lewistown and Newport, Susquehanna @ Sunbury and Harrisburg, Conodoguinet Creek, and Swatara Creek.

Maps only of parameters found in Susquehanna basin/higher in basin:

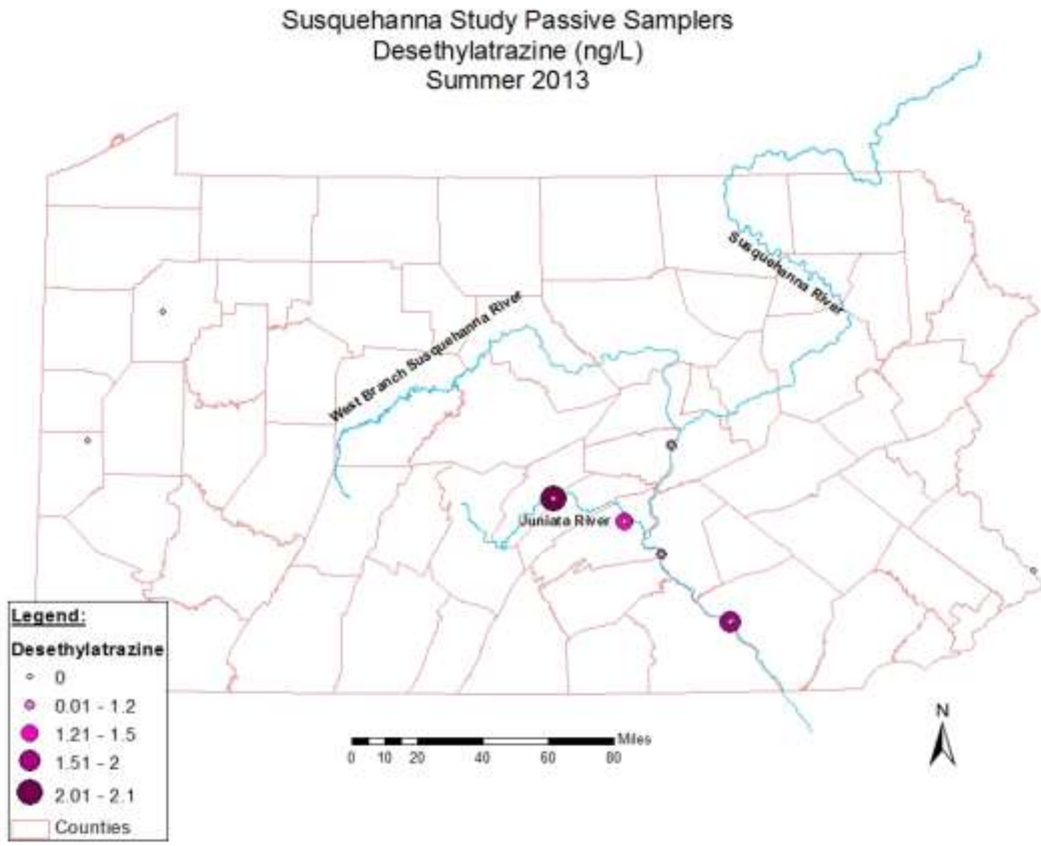
PASSIVE SAMPLERS:		
Only At Basin Sites, Not Controls:		**In Controls, But Mostly Higher In Basin:**
1,2-dimethylnaphthalene		**atrazine**
17-alpha-estradiol		**endosulfan-II**
bromoform		**para-nonylphenol-total**
cis-androsterone		
cumene		
desethylatrazine		
epitestosterone		
equilenin		
PBDE-153		
PBDE-28		
perylene		
prometon		
testosterone		



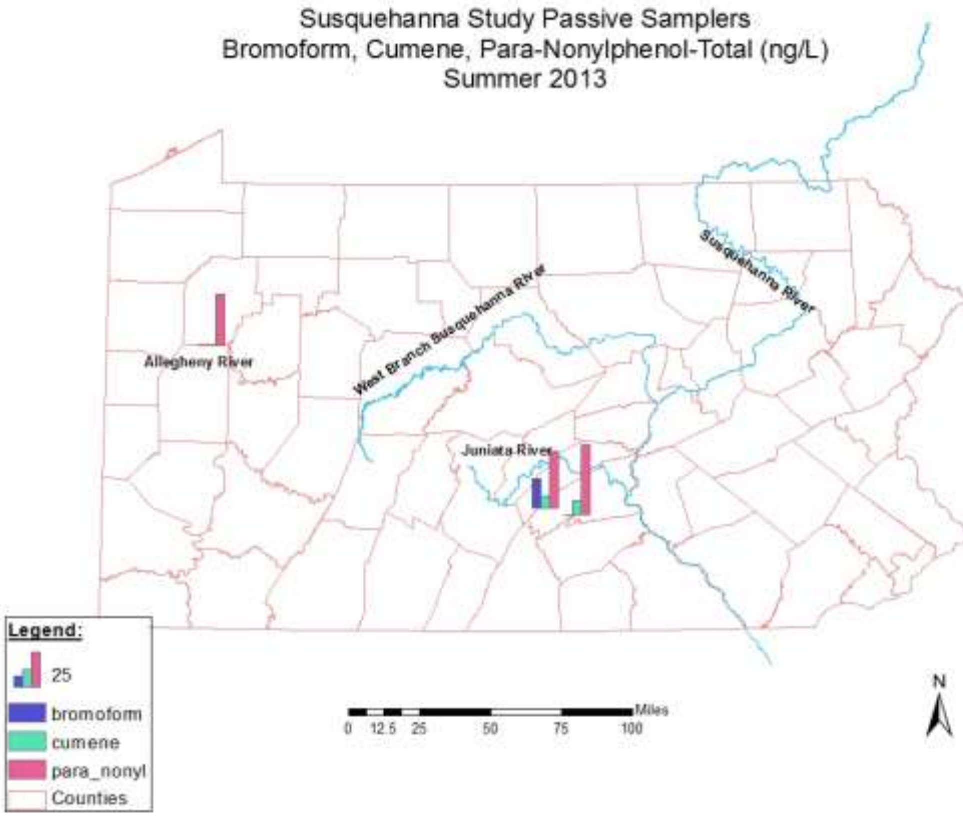
Some hormones only detected in Susquehanna basin are centered on the Juniata and downstream.



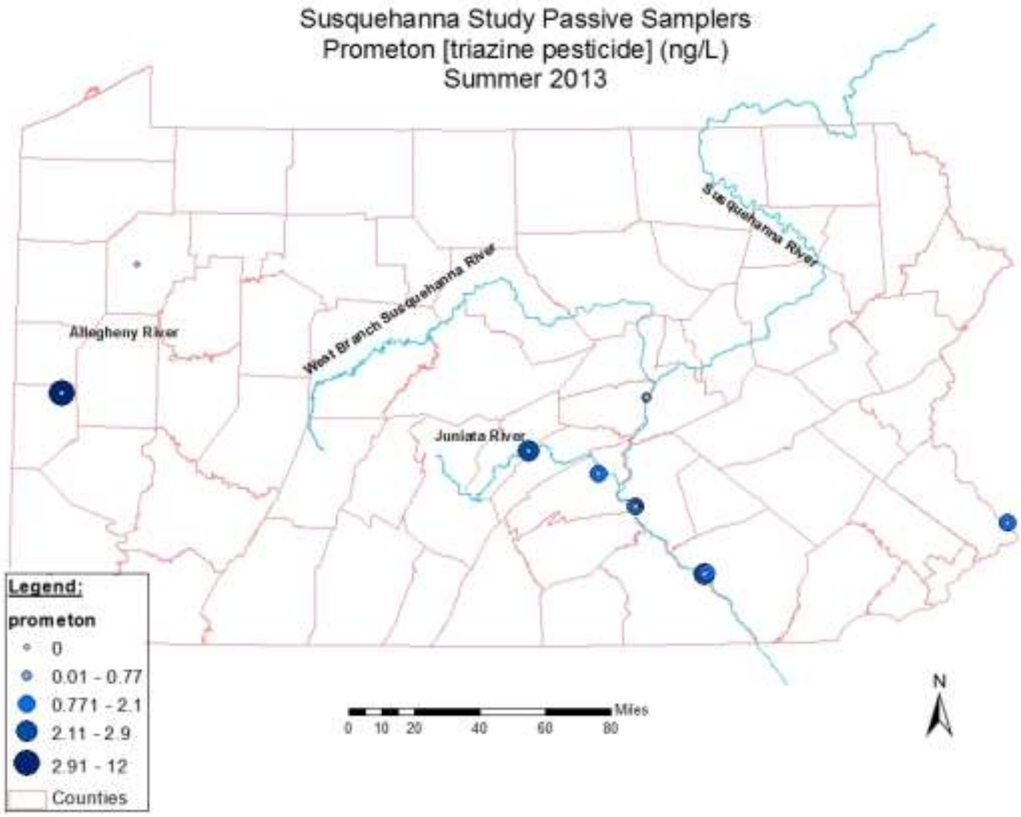
A couple PBDE varieties only detected on the Juniata/Susquehanna.



Metabolite of the pesticide atrazine only detected in the basin, particularly on the Juniata and downstream.

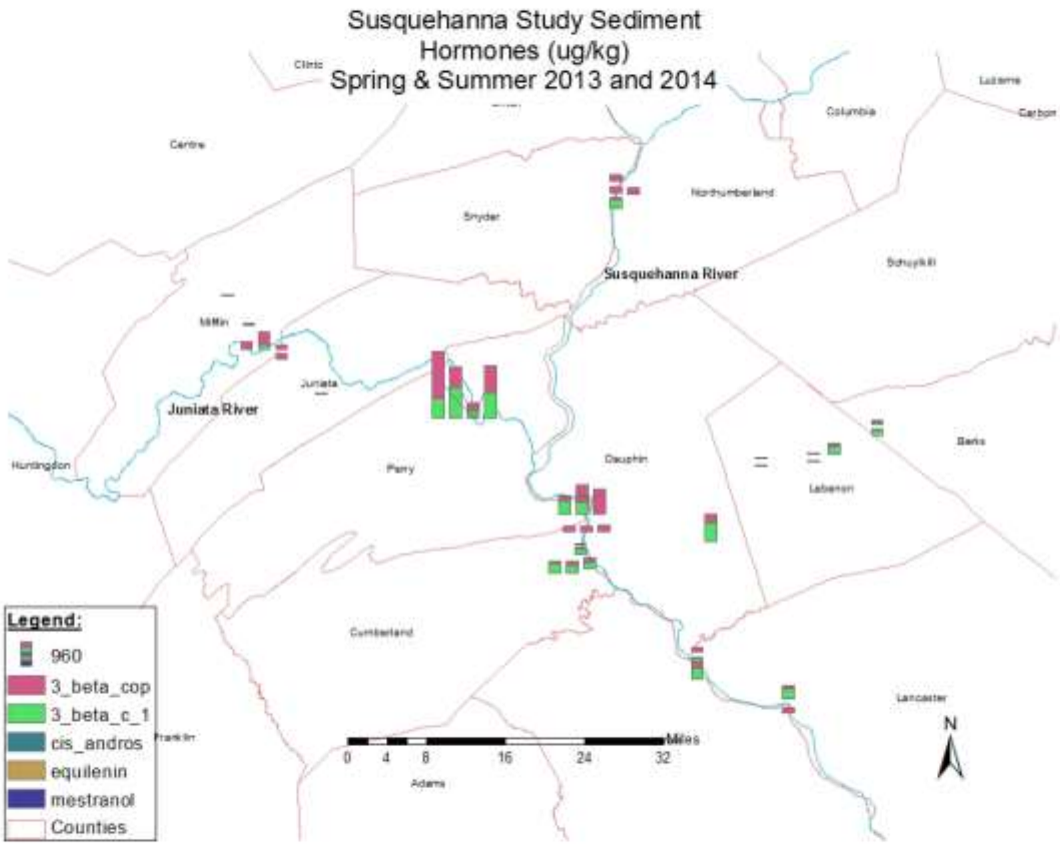


Various other compounds detected largely on Juniata (also on Allegheny).

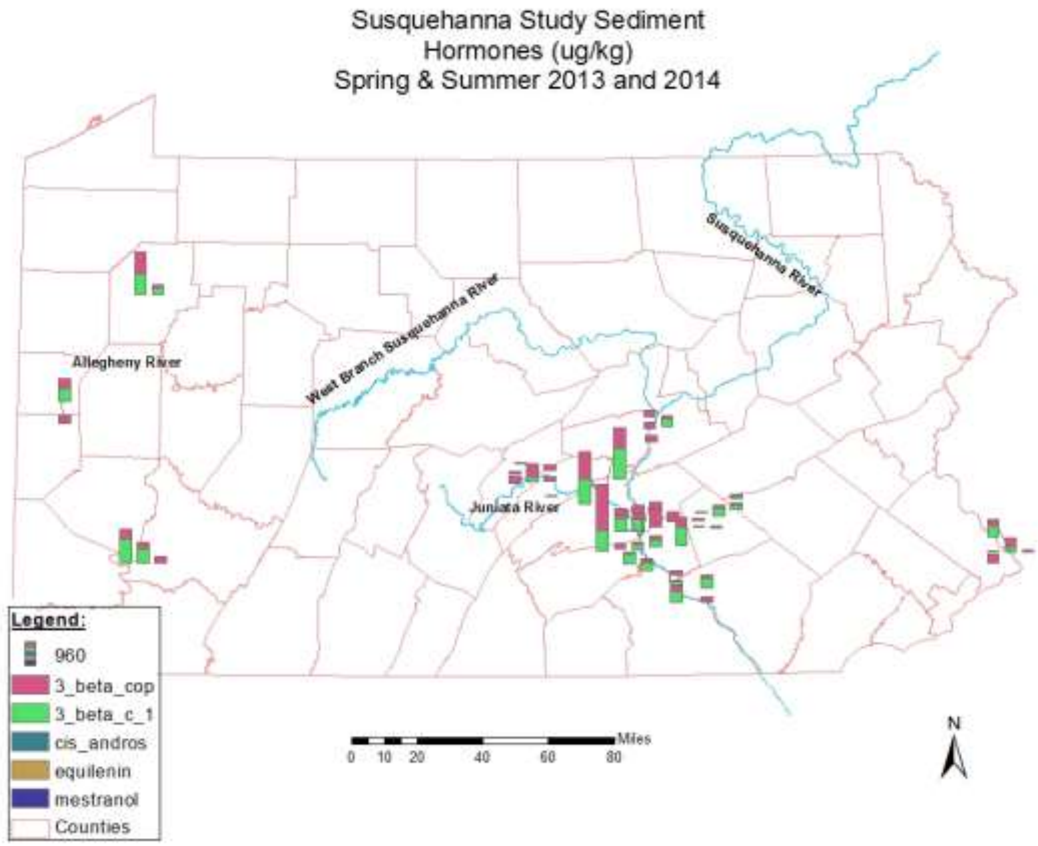


Prometon detected at control sites, but also to a large extent on the Juniata & downstream.

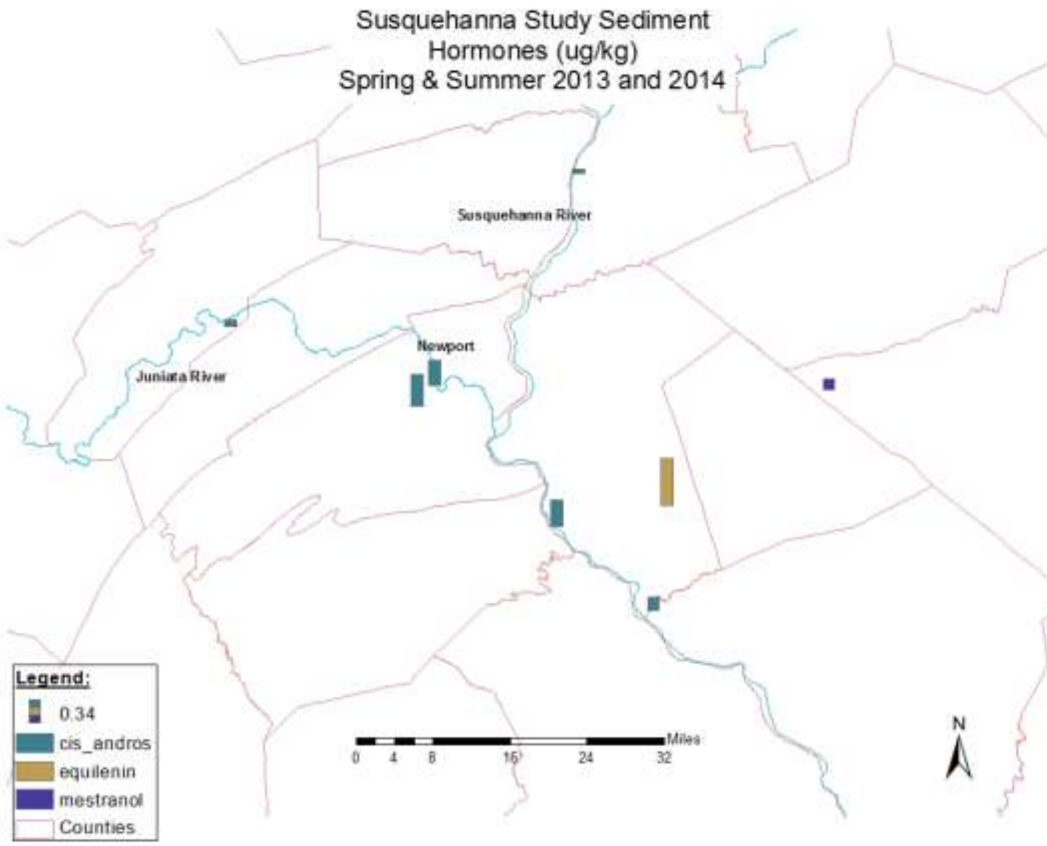
SEDIMENT:		
Only At Basin Sites, Not Controls:		**In Controls, But Mostly Higher In Basin:**
4,4-bisphenol f		**3,4-dichloroaniline**
4-octylphenol diethoxylate-(op2eo)		**3-beta-coprostanol**
atrazine		**bisphenol a**
benzophenone		**indole**
camphor		**para-cresol**
cis-androsterone		**para-nonyl-phenol (total)**
cis-permethrin		**triclosan**
dacthal		
equilenin		
mestranol		
metolachlor		
nonylphenol, diethoxy-(total,np2eo)		
pendimethalin		
trans-permethrin		
tributyl phosphate		
triphenyl phosphate		
tris (dichloroisopropyl) phosphate		
DEET		



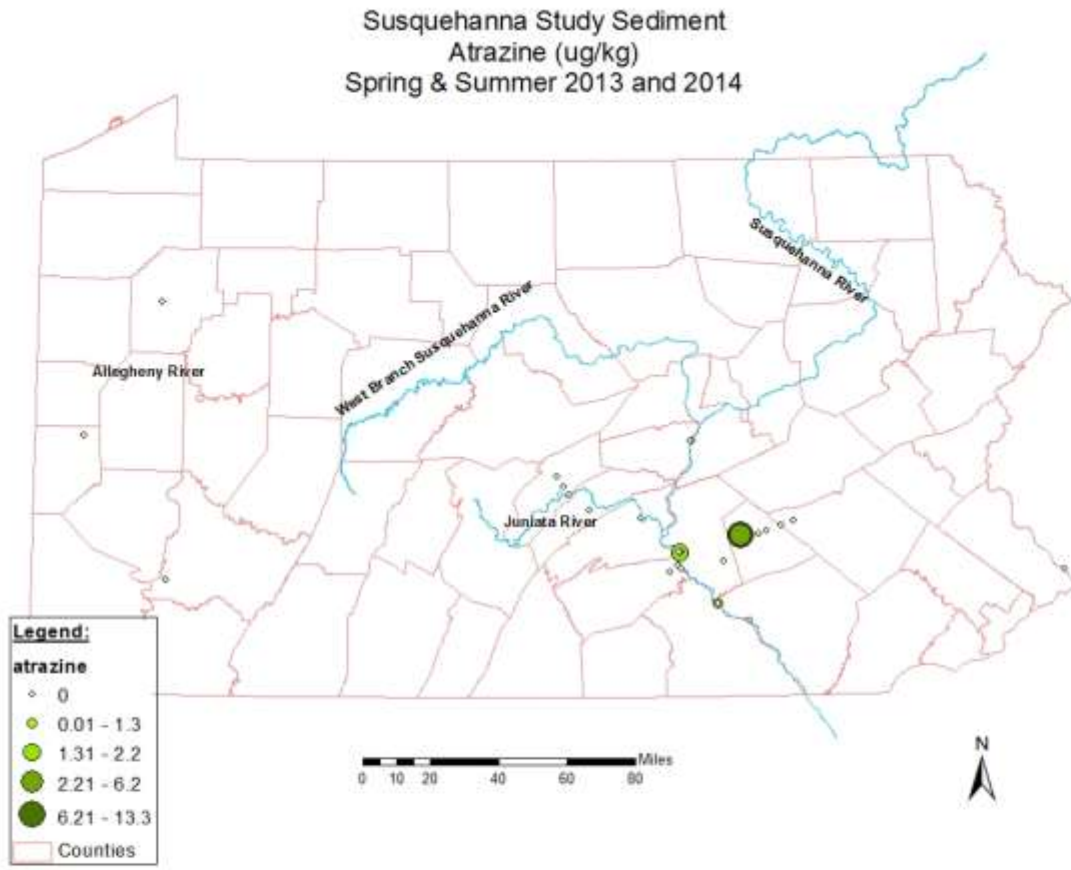
Hormones that were only detected in the Susquehanna basin or at higher levels in the basin than controls.



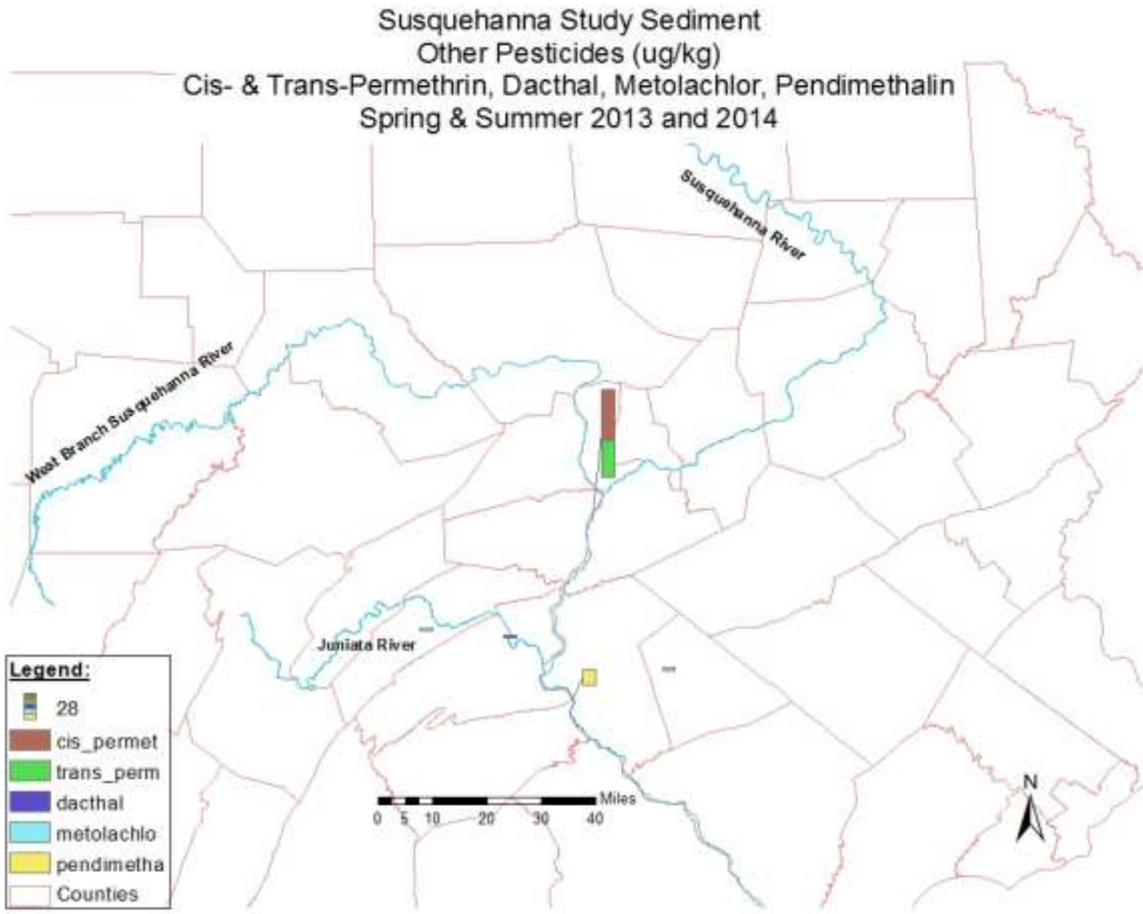
Hormones that were only detected in the Susquehanna basin or at higher levels in the basin than controls.



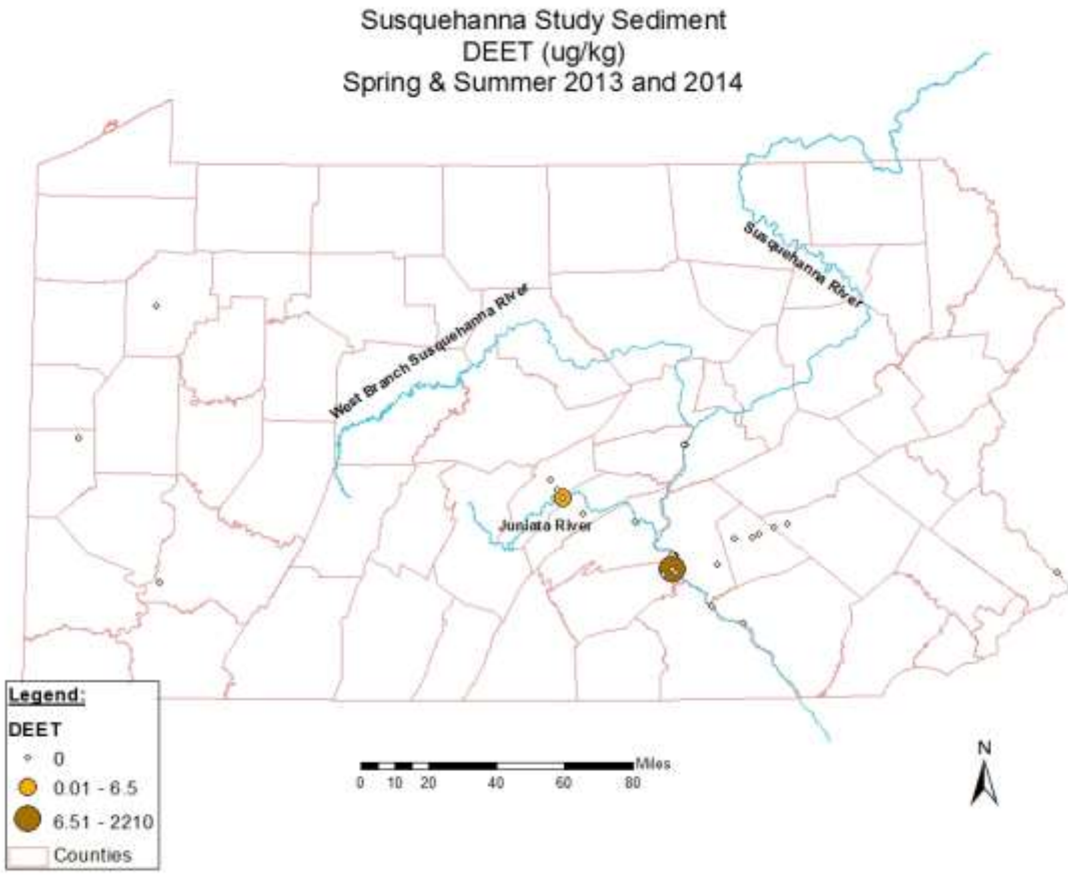
For better visualization, hormones only detected in the basin other than 3-beta-coprostanol were mapped separately.



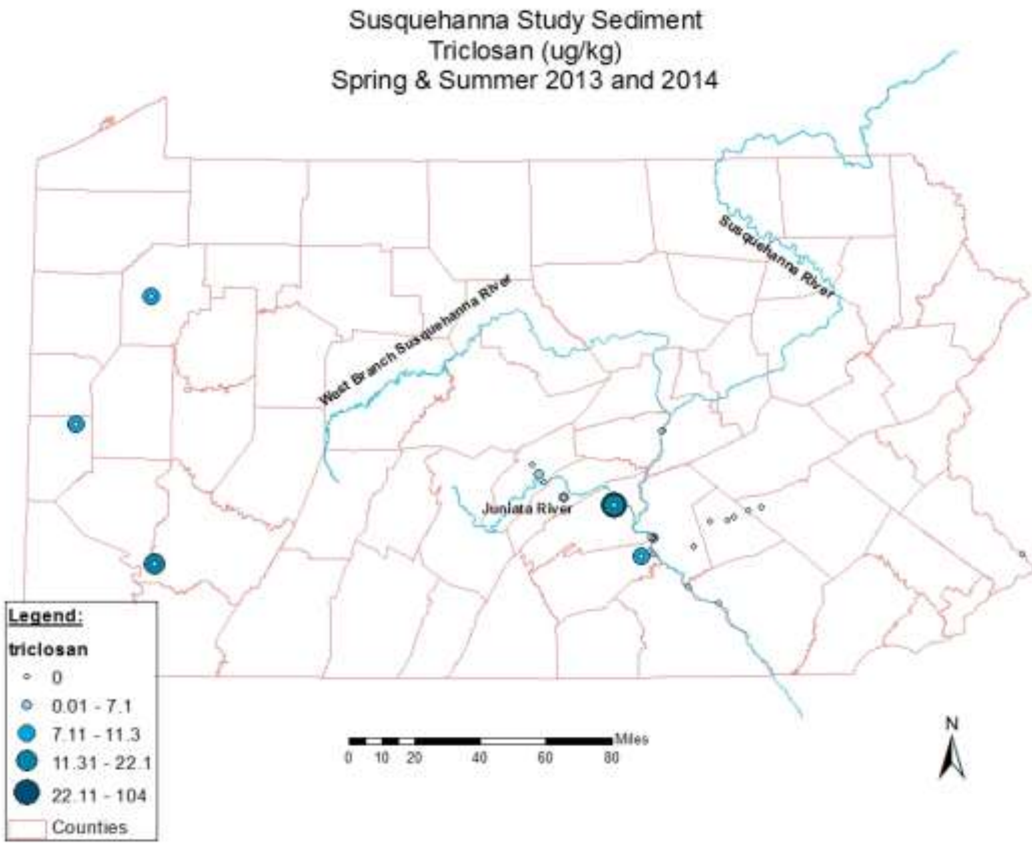
Atrazine detected in sediment only in the Susquehanna basin (Swatara Creek, Susquehanna @ Harrisburg, & Susquehanna @ Marietta).



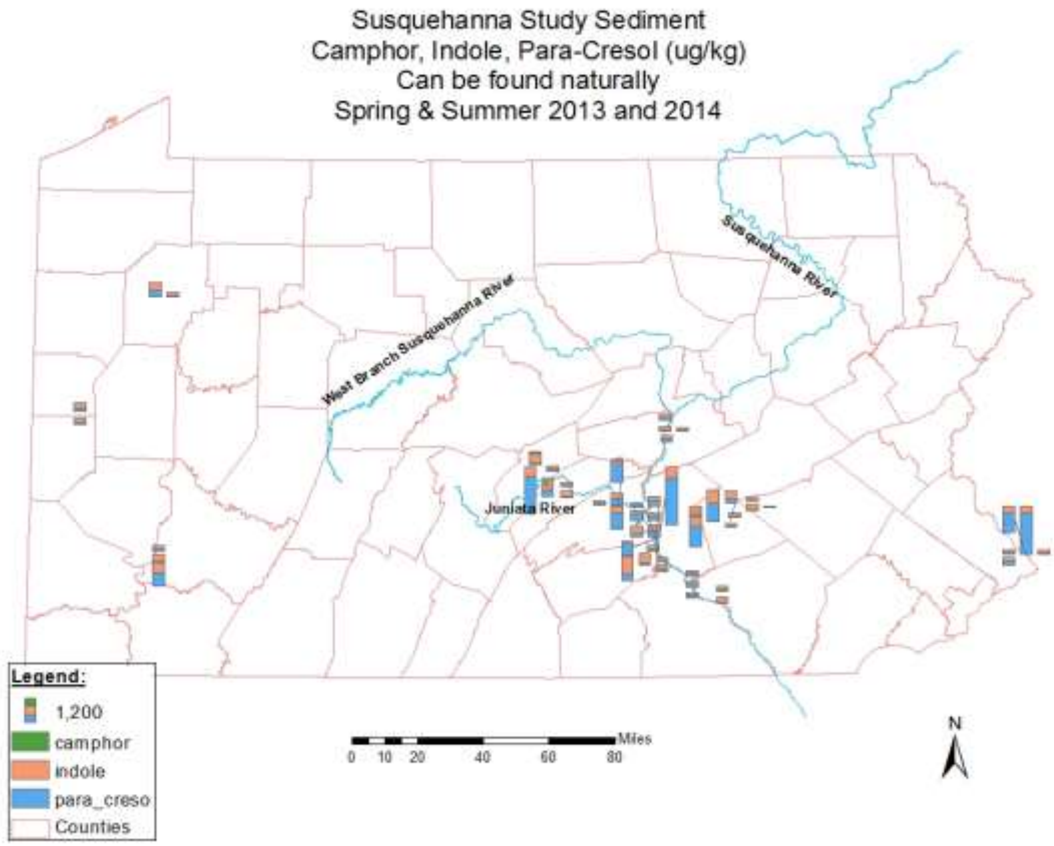
Other pesticides that were detected only in the Susquehanna basin.



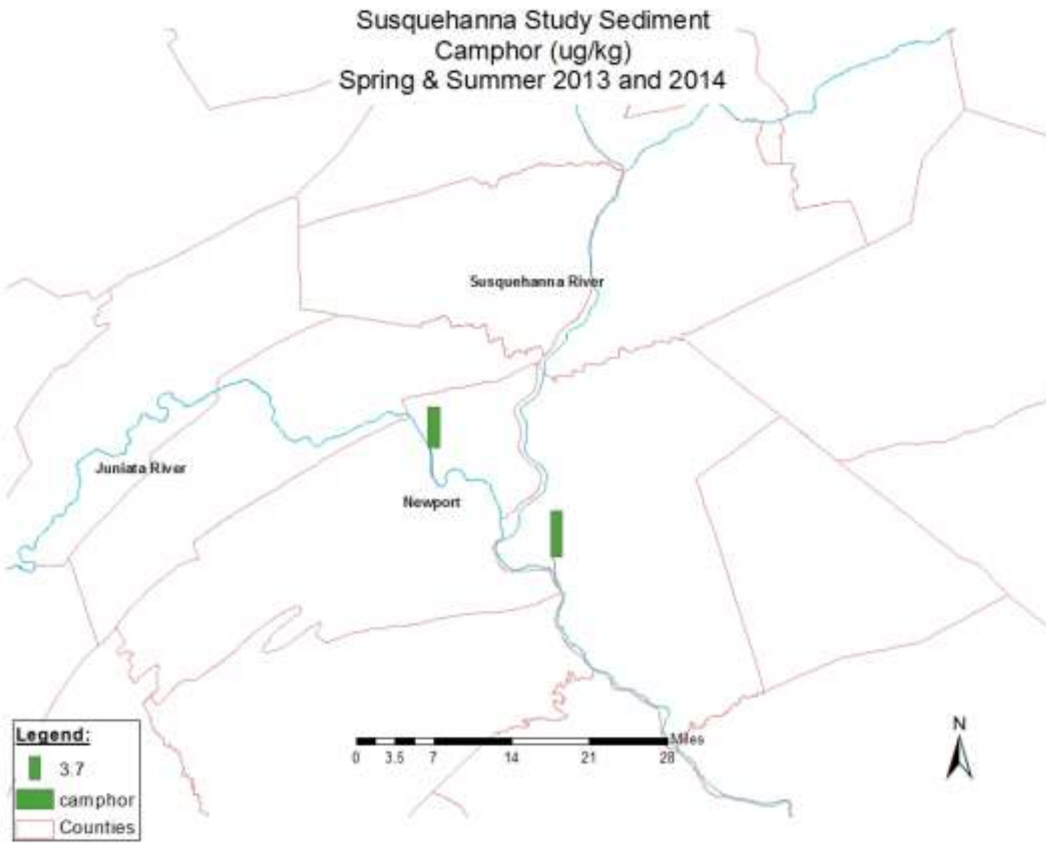
DEET was only detected in the Susquehanna basin.



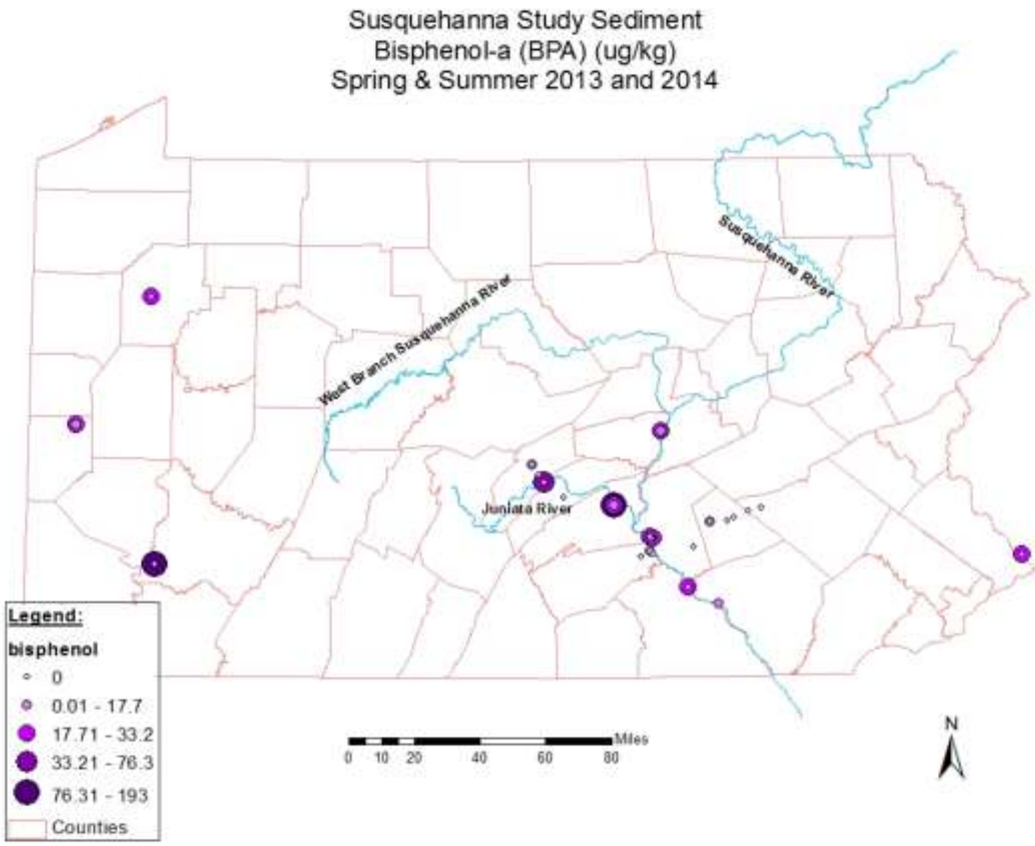
Triclosan was detected in and out of the Susquehanna basin, but highest levels were found on the Juniata.



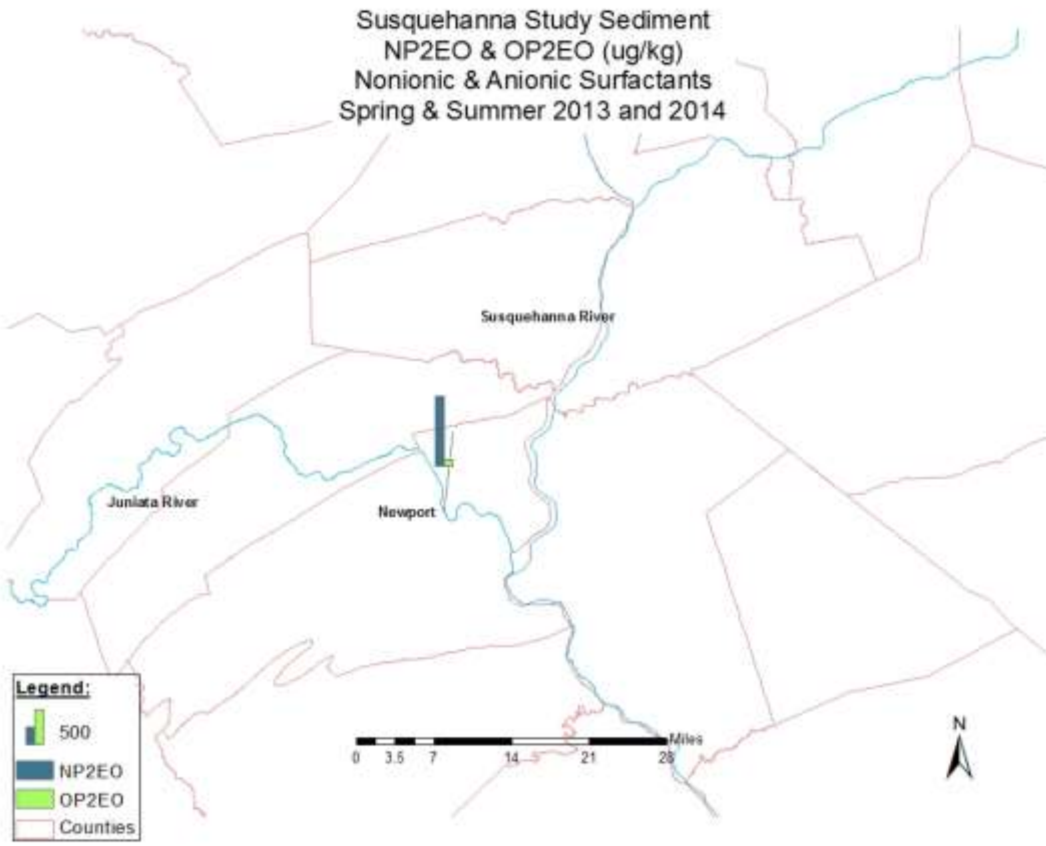
Naturally-occurring (also can be man-made) materials found generally higher in the Susquehanna basin.



Camphor mapped separately for better visualization (naturally occurring, used as aromatic).



BPA was detected all over, but generally highest in the Susquehanna basin.



The surfactants NP2EO and OP2EO were found only on the Juniata.

Below are listed all compounds analyzed for with passive samplers (not all of these are mapped above; many compounds were not detected or were in similar concentrations inside and outside the Susquehanna River basin, so were not considered a cause of the YOY SMB recruitment decline):

Organochlorine Pesticides
alpha-Benzenehexachloride (a-BHC)
beta-Benzenehexachloride (b-BHC)
Chlorpyrifos
cis-Chlordane
cis-Nonachlor
cis-Permethrin
delta-Benzenehexachloride (d-BHC)
Endosulfan
Endosulfan Sulfate
Endosulfan-II
Endrin
Heptachlor
Heptachlor Epoxide
Hexachlorobenzene (HCB)
Mirex
o,p'-DDD
o,p'-DDE
o,p'-DDT
Oxychlordane
p,p'-DDD
p,p'-DDE
p,p'-DDT
p,p'-Methoxychlor
Pentachloroanisole (PCA)
Tefluthrin
trans-Chlordane
trans-Nonachlor
trans-Permethrin

Total PCBs
Total PCBs

PBDEs
PBDE-100
PBDE-153
PBDE-28

PBDE-47
PBDE-99

PAHs
1,2-dimethylnaphthalene
1-ethylnaphthalene
1-methylfluorene
1-methylnaphthalene
2,3,5-trimethylnaphthalene
2-methylfluoranthene
2-methylnaphthalene
2-methylphenanthrene
3,6-dimethylphenanthrene
4-methylbiphenyl
9-methylanthracene
Acenaphthene
Acenaphthylene
Anthracene
Benz[a]anthracene
Benzo[a]pyrene
Benzo[b]fluoranthene
Benzo[b]naphtho[2,1-d]thiophene
Benzo[b]thiophene
Benzo[e]pyrene
Benzo[g,h,i]perylene
Benzo[k]fluoranthene
Biphenyl
Chrysene
Dibenz[a,h]anthracene
Dibenzothiophene
Fluoranthene
Fluorene
Indeno[1,2,3-c,d]pyrene
Naphthalene
Perylene
Phenanthrene
Pyrene

Waste Indicator Compounds
1,4-dichlorobenzene
3,4-dichlorophenyl isocyan

4-cumylphenol
4-n-octylphenol
4-tert-octylphenol
5-methyl-1H-benzotriazole
acetophenone
anthraquinone
benzophenone
beta-sitosterol
BHA
bisphenol A
bromacil
bromoform
camphor
carbazole
cholesterol
cumene
dichlorvos
diethyl phthalate
diethylhexyl phthalate
d-limonene
ethanol,2-butoxy-,phosphat
ethyl citrate
galaxolide (HHCB)
indole
isoborneol
isophorone
isoquinoline
menthol
metalaxyl
methyl salicylate
N,N-diethyltoluamide (DEET
NPEO1-total
NPEO2-total
OPEO1
OPEO2
para-cresol
para-nonylphenol-total
pentachlorophenol
phenol
prometon
skatol

stigmastanol
tetrachloroethylene
tonalide (AHTN)
tri(2-chloroethyl)phosphat
tri(dichlorisopropyl)phosp
tributylphosphate
triphenyl phosphate

Current Use Pesticides
2,6 - diethylanaline
acetochlor
alachlor
alpha hch
atrazine
benfluralin
butylate
carbaryl
carbofuran
cis-permethrin
cyanazine
dacthal
dde
desethylatrazine
desulfinylfipronil
desulfinylfipronil amide
diazinon
dieldrin
disulfoton
eptam
ethalfluralin
ethoprop
fipronil
fipronil sulfide
fipronil sulfone
fonofos
lindane
linuron
malathion
methyl azinphos
methyl parathion
metolachlor

metribuzin
molinate
napropamide
parathion
pebulate
pendimethilan
phorate
prometon
pronamide
propachlor
propanil
propargite (1&2)
simazine
tebuthiuron
terbacil
terbufos
thiobencarb
triallate
trifluralin

Pharmaceutical Compounds
1,7-dimethylxanthine
Acetaminophen
Albuterol
Aspirin
Azithromycin
Bupropion
Caffeine
Carbamazapine
Cimetidine
Citalopram
Clofibric Acid
Codeine
Cotinine
Dehydronifedipine
Diclofenac
Diltiazem
Diphenhydramine
Duloxetine
Enaliprilat
Erythromycin

Fluoxetine
Fluvoxamine
Furosemide
Gemfibrozil
HCTZ
Ibuprofen
Ketoprofen
Miconazole
Naproxen
Norfluoxetine
Norsertraline
Paroxetine
Ranitidine
Sertraline
Simvastatin
Sulfamethoxazole
Thiabendazole
Triclocarban
Triclosan
Trimethoprim
Venlafaxine
Warfarin

Hormones
11-ketotestosterone
17-alpha-estradiol
17-alpha-ethynylestradiol
17-beta-estradiol
3-beta-coprostanol
4-androstene-3,17-dione
cis-androsterone
diethylstilbestrol
dihydrotestosterone
epitestosterone
equilenin
equilin
estriol
estrone
mestranol
norethindrone
progesterone

testosterone

Yeast Estrogen Screen
Estimated Estradiol Equivalents (EEQ)

Below are listed compounds tested for in sediment. Metals and historical pesticides/PCBs are listed in other Worksheets in this Appendix (not all of these are mapped above; many compounds were not detected or were in similar concentrations inside and outside the Susquehanna River basin, so were not considered a cause of the YOY SMB recruitment decline):

Current Pesticides		
1-Naphthol	Disulfoton	Metribuzin
2-Chloro-2,6-diethylacetanilide	Disulfoton sulfone	Molinate
2-Ethyl-6-methylaniline	alpha-Endosulfan	Myclobutanil
3,4-Dichloroaniline	Endosulfan sulfate	Oxyfluorfen
3,5-Dichloroaniline	EPTC	Parathion-methyl
4-Chloro-2-methylphenol	Ethion	Pendimethalin
Acetochlor	Ethion monoxon	Phorate
Alachlor	Ethoprophos	Prometon
2,6-Diethylaniline	Fenamiphos	Prometryn
Atrazine	Fipronil	Propanil
Azinphos-methyl	Desulfinylfipronil amide	Propargite
Benfluralin	Fipronil sulfide	Propyzamide
Carbaryl	Fipronil sulfone	Simazine
Chlorpyrifos	Desulfinylfipronil	Tebuconazole
cis-Permethrin	Fonofos	Tefluthrin
cis-Propiconazole	*alpha-HCH-d6 (surrogate)*	Terbufos
Cyfluthrin	Iprodione	Terbuthylazine
Cypermethrin	Isofenphos	Thiobencarb
Dacthal	lambda-Cyhalothrin	trans-Permethrin
Diazinon	Malathion	trans-Propiconazole
Diazinon-d10 (surrogate)	Methidathion (Supracide)	Tribufos
Dieldrin	Metolachlor	Trifluralin

Wastewater Compounds	
1,4-Dichlorobenzene	Carbazole
1-Methylnaphthalene	Chlorpyrifos
2,2',4,4'-Tetrabromodiphenylether (PBDE 47)	Cholesterol
2,6-Dimethylnaphthalene	d-Limonene

2-Methylnaphthalene	*Decafluorobiphenyl (surrogate)*
3-beta-Coprostanol	Diazinon
3-Methyl-1(H)-indole (Skatol)	Diethyl phthalate
3-tert-Butyl-4-hydroxyanisole (BHA)	Fluoranthene
4-Cumylphenol	*Fluoranthene-d10 (surrogate)*
4-n-Octylphenol	Hexahydrohexamethylcyclopentabenzopyran (HHCB)
4-Nonylphenol (sum of all isomers)	Indole
4-Nonylphenol diethoxylate, (sum of all isomers) aka NP2EO	Isoborneol
4-Nonylphenol monoethoxylate, (sum of all isomers) aka NP1EO	Isophorone
4-tert-Octylphenol	Isopropylbenzene
4-tert-Octylphenol diethoxylate, aka OP2EO	Isoquinoline
4-tert-Octylphenol monoethoxylate, aka OP1EO	Menthol
Acetophenone	Metolachlor
Acetyl hexamethyl tetrahydronaphthalene (AHTN)	N,N-diethyl-meta-toluamide (DEET)
Anthracene	Naphthalene
Anthraquinone	p-Cresol
Atrazine	Phenanthrene
Benzo[a]pyrene	Phenol
Benzophenone	Prometon
beta-Sitosterol	Pyrene
beta-Stigmastanol	Tributyl phosphate
bis(2-Ethylhexyl) phthalate	Triclosan
Bisphenol A	Triphenyl phosphate
Bisphenol A-d3 (surrogate)	Tris(2-butoxyethyl)phosphate
Bromacil	Tris(2-chloroethyl)phosphate
Camphor	Tris(dichloroisopropyl)phosphate

Hormones	
11-Ketotestosterone	Equilenin
16-Epiestriol-2,4-d2 (surrogate)	Equilin
17-alpha-Estradiol	Estriol
17-alpha-Ethynylestradiol	*Estriol-2,4,16,17-d4 (surrogate)*
17-alpha-Ethynylestradiol-2,4,16,16-d4 (surrogate)	Estrone
17-beta-Estradiol	*Estrone-13,14,15,16,17,18-13C6 (surrogate)*
17-beta-Estradiol-13,14,15,16,17,18-13C6 (surrogate)	*Medroxyprogesterone-d3 (surrogate)*
3-beta-Coprostanol	Mestranol
4-Androstene-3,17-dione	*Mestranol-2,4,16,16-d4 (surrogate)*

Bisphenol A	*Nandrolone-16,16,17-d3 (surrogate)*
Bisphenol-A-d16 (surrogate)	Norethindrone
Cholesterol	Progesterone
Cholesterol-d7 (surrogate)	*Progesterone-2,3,4-13C3 (surrogate)*
cis-Androsterone	Testosterone
cis-Androsterone-2,2,3,4,4-d5 (surrogate)	*trans-Diethyl-1,1,1',1'-d4-stilbesterol-3,3',5,5'-d4 (surrogate)*
Dihydrotestosterone	trans-Diethylstilbestrol
Epitestosterone	

Title: Historical Pesticides/PCB's in Sediment (WS #38)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Sediment could directly affect YOY because the eggs are laid directly on it and the young are in close contact with it. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected sediment samples from 13 sample sites within and out of the Susquehanna basin in 2013 to be tested for historical pesticides and PCBs:

Waterbody	Location Name
Susquehanna River	Marietta West
Susquehanna River	Marietta-Falmouth East
Susquehanna River	Rockville West
Susquehanna River	Rockville Middle
Susquehanna River	Rockville East
Juniata River	Lewistown Narrows
Juniata River	Newport South
Susquehanna River	Sunbury West
Susquehanna River	Sunbury East
Youghiogheny River	West Newton-Sutersville
Connequenessing Creek	Zelienople
Allegheny River	Franklin
Delaware River	Morrisville East

Analysis and Results:

PCBs were detected only once (0.0602 mg/kg arochlor 1254 at Susquehanna River @ Marietta-Falmouth East).

Only six historically-used pesticides were detected, with no obvious patterns:

- **4,4’-DDD**: Susq @ Marietta East (12.6 ug/kg) [AUG]
- **Chlorneb**: Susq @ Harrisburg Middle (23.4 ug/kg); Yough (19.9 ug/kg) [AUG]
- **cis-Permethrin**: Susq @ Harrisburg West (14.7 ug/kg); Delaware (18.6 ug/kg); Yough (24.7 ug/kg) [MAY]
- **Metolachlor**: Susq @ Sunbury West (34.7 ug/kg); Juniata @ Lewistown (54.9 ug/kg) [MAY]
- **Propachlor**: Susq @ Harrisburg West (28.9 ug/kg) [AUG]
- **Trifluralin**: Susq @ Marietta East (16.1 ug/kg) [MAY]
- **Hexachlorobenzene**: Susq @ Marietta West (20.51 ug/kg) [MAY]
- Additionally, **chloraneb, metolachlor, propachlor, trans-permethrin, & trifluralin** all had detects but the QA results were not satisfactory (i.e., analyte was probably there but concentration may not be accurate)

Conclusion:

The evidence weakens the argument for historically-used pesticides and/or PCBs as the cause of low recruitment of YOY SMB.

Title: Historical Pesticides (PCBs) (WS #39)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Sediment could directly affect YOY because the eggs are laid directly on it and the young are in close contact with it. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected sediment samples to be tested for historical pesticides and PCBs from multiple sites within and out of the basin in 2013 and 2014. Basin sites were generally sampled twice (two times per one of the years), although a few were sampled four times (twice per year). Samples were analyzed by PA DEP Bureau of Laboratories:

Waterbody	Type
Conodoguinet Creek @ East Pennsboro Park	Basin
Conodoguinet Creek @ Good Hope	Basin
Crosskill Creek	Basin
Elizabeth Run	Basin
Juniata River @ Lewistown	Basin
Juniata River @ Newport	Basin
Kishacoquillas Creek @ Highland Park	Basin
Kishacoquillas Creek @ Reedsville	Basin
Little Swatara Creek	Basin
Little Swatara Creek @ Mill Road	Basin
Susquehanna River @ Fairview	Basin
Susquehanna River @ Harrisburg East	Basin

Susquehanna River @ Harrisburg Middle	Basin
Susquehanna River @ Harrisburg West (Rockville)	Basin
Susquehanna River @ Marietta East	Basin
Susquehanna River @ Marietta West	Basin
Susquehanna River @ Sunbury East	Basin
Susquehanna River @ Sunbury West	Basin
Swatara Creek @ Harpers Tavern	Basin
Swatara Creek @ Hershey	Basin
Tuscarora Creek	Basin
Allegheny River @ Franklin	Control
Connoquenessing @ Zelienople	Control
Delaware River @ Morrisville	Control
Youghiogheny River @ Sutersville	Control

Parameter Name
4,4'-DDD
4,4'-DDE
4,4'-DDT
Alachlor
Aldrin
alpha-BHC
alpha-Chlordane
beta-BHC
Chloroneb
Chlorobenzilate
Chlorothalonil
Chlorpyriphos
cis-Permethrin
Cyanazine
delta-BHC
Dieldrin
Dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate
Endosulfan I
Endosulfan II
Endosulfan Sulfate
Endrin
Endrin Aldehyde
Endrin Ketone
Etridiazole
gamma-BHC (Lindane)
gamma-Chlordane
Heptachlor

Heptachlor Epoxide
Methoxychlor
Metolachlor
Metribuzin
Propachlor
trans-Permethrin
Trifluralin
Hexachlorobenzene
Hexachlorocyclopentadiene

Parameter Name
Arochlor 1260
Arochlor 1221
Arochlor 1232
Arochlor 1248
Arochlor 1016
Arochlor 1242

Analysis and Results:

Historical pesticides detected were 4,4'-DDD, 4,4'-DDE, chlorneb, cis-permethrin, trans-permethrin, hexachlorobenzene, metolachlor, propachlor, and trifluralin. PCBs were detected a total of six (6) times, at very low concentrations. The sites PCBs were detected at was Crosskill Creek, Elizabeth Run, Conodoguinet Creek @ East Pennsboro Park, Swatara Creek @ Hershey, and Susquehanna River @ Marietta East. Historical pesticides were detected at many sites, with one or two detections per site per sample, where they were detected. There were little discernable patterns in the data; Juniata River sites tended to have higher concentrations of detections than other sites, but had very few parameters (one or two).

Conclusion:
The evidence weakens the argument for historically-used pesticides and/or PCBs as the cause of low recruitment of YOY SMB.

Title: Metals in Sediment (WS #40)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Sediment could directly affect YOY because the eggs are laid directly on it and the young are in close contact with it. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected sediment samples to be tested for metals within and out of the Susquehanna basin from 13 sample sites in 2013 and 18 sample sites in 2014 (analyzed at DEP Bureau of Laboratories):

Site	Year	Type
Allegheny River Franklin	2013	Control
Connoquenessing Creek	2013	Control
Delaware River	2013	Control
Juniata River Lewistown	2013	Basin
Juniata River Newport	2013	Basin
Susquehanna River Harrisburg E	2013	Basin
Susquehanna River Harrisburg M	2013	Basin
Susquehanna River Harrisburg W	2013	Basin
Susquehanna River Marietta E	2013	Basin
Susquehanna River Marietta W	2013	Basin
Susquehanna River Sunbury E	2013	Basin
Susquehanna River Sunbury W	2013	Basin

Youghiogheny River	2013	Control
Conodoguinet Creek E Pennsboro	2014	Basin
Conodoguinet Creek Good Hope	2014	Basin
Crosskill Creek	2014	Basin
Delaware River	2014	Control
Elizabeth Run	2014	Basin
Juniata River Lewistown	2014	Basin
Juniata River Newport	2014	Basin
Kishacoquillas Creek Highland Park	2014	Basin
Kishacoquillas Creek Reedsville	2014	Basin
Little Swatara Creek	2014	Basin
Little Swatara Creek Mill Rd	2014	Basin
Susquehanna River Fairview	2014	Basin
Susquehanna River Harrisburg W	2014	Basin
Susquehanna River Marietta E	2014	Basin
Swatara Creek Harpers Ferry	2014	Basin
Swatara Creek Hershey	2014	Basin
Tuscarora Creek	2014	Basin
Youghiogheny River	2014	Control

Metals (mg/kg)
Aluminum
Arsenic
Barium
Bromide
Cadmium
Calcium
Chloride
Chromium
Copper
Iron
Lead
Magnesium
Manganese
Mercury
Nickel
Potassium
Selenium
Strontium
Zinc

Analysis and Results:

For ease of viewing the data, 2013 and 2014 samples were analyzed separately. In 2013, the Youghiogheny River tended to have more elevated levels of metals than other sites. Iron, magnesium, zinc, barium, bromide, copper, selenium, and chromium were all high at Youghiogheny. Chromium in particular had a large spike there; it's unclear whether this was a error or actual occurrence.

Youghiogheny River had the highest levels of aluminum, iron, magnesium, manganese, barium, strontium, zinc, and chromium. Connoquenessing Creek had the highest levels of chloride and arsenic. Juniata River (either Newport or Lewistown, but most often Lewistown) had the highest levels of lead, bromide, cadmium, mercury, and selenium. Susquehanna River @ Marietta East had the highest levels of copper. Susquehanna River @ Harrisburg Middle had the highest levels of nickel. Overall, the trends were Youghiogheny River and Juniata River @ Lewistown having the highest levels of metals (Figures 1-4).

In 2014, mostly new sites were sampled (Figures 5-7). These sites did not have the consistency of the 2013 sites; i.e. the same sites were not coming in high for many metals. However, there were a few trends.

Youghiogheny River had the highest concentrations of manganese, chloride, and nickel. Conodoguinet Creek @ Good Hope displayed some of the highest, if not the highest, concentrations of many metals, such as aluminum, iron, barium, chloride, strontium, bromide, cadmium, chromium, mercury, and selenium.

Although Pennsylvania does not have published sediment metals aquatic life criteria, many states and/or references have published background levels or benchmarks for various metals. We sampled for every metal in the following tables, except for silver:

Parameter	Reference Range (mg/kg)
Aluminum	28,000 – 53,000
Arsenic	11 - 25
Barium	170 - 360
Cadmium	0.3 - 0.96
Calcium	21,000 - 120,000
Chromium	29 - 53
Copper	25 - 42
Iron	31,000 - 51,000
Lead	47
Magnesium	7,100 - 35,000
Manganese	780 - 3,000
Mercury	0.12

Nickel	33 - 61
Potassium	5,900 - 14,000
Silver	0.43
Strontium	62 - 390
Zinc	100 – 190

Adapted from Ohio EPA (2008).

Parameter	Freshwater Screening Benchmark (mg/kg)*	Lowest Effect Level (ug/g)**	Severe Effect Level (ug/g)**	Lowest Effect Level (ug/g)[#]	Severe Effect Level (ug/g)[#]	Threshold Effect Concentration (mg/kg) [+]	Midpoint Effect Concentration (mg/kg) [+]	Probable Effect Concentration (mg/kg) [+]	Lowest Effect Level (mg/kg) [@]	Severe Effect Level (mg/kg) [@]
Arsenic	9.8	6	33	6	33	9.8	21.4	33	9.979	33
Cadmium	0.99	0.6	9	0.6	10	0.99	3	5	0.6 / 0.990	10
Chromium	43.4	26	110	26	110	43	76.5	110	26 / 43.4	110
Copper	31.6	16	110	16	110	32	91	150	16 / 31.6	110
Iron	20,000					20,000	30,000	40,000		
Lead	35.8	31	110	31	250	36	83	130	31 / 35.8	250
Manganese	460	460	1,100	460	1,100	460	780	1,100	630	1,100
Mercury	0.18	0.15	1.3	0.2	2	0.18	0.64	1.1	0.2 / 0.174	2
Nickel	22.7	16	50	16	75	23	36	49	16 / 22.7	75
Silver	1	1	2.2			1.6	1.9	2.2		
Zinc	121	120	270	120	820	120	290	460	120 / 121	820

** Adapted from US EPA 2012*
*** Adapted from NY DEC 1999 - from Persuad et al. (1992) and Long & Morgan (1990); ug/g = mg/kg*
[#] *Adapted from Persuad et al. 1992 - from Long & Morgan (1990); ug/g = mg/kg*
[+] *Adapted from Wisconsin Department of Natural Resources 2003*
[@] *Adapted from NJ DEP 1998*

Several metals – arsenic, cadmium, copper, iron, lead, manganese, mercury, nickel, and zinc – were elevated in study samples. Most had elevated levels within and outside the Susquehanna basin, with the exception of lead, which had elevated levels in the basin.

Conclusion:

There is no consistent pattern between prevalence of metals and low recruitment of YOY SMB.

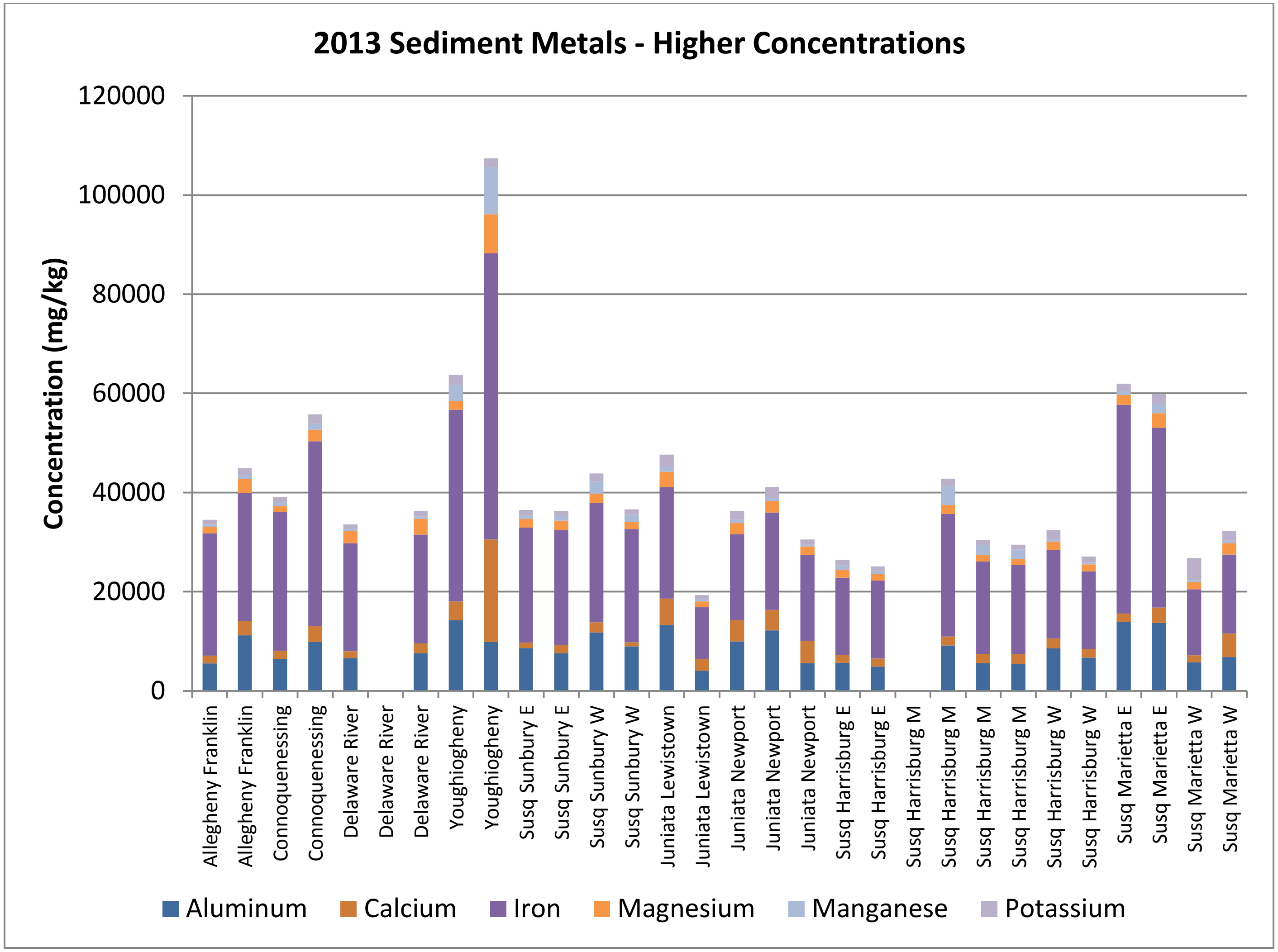


Figure 1: 2013 sediment metals – highest concentration metals

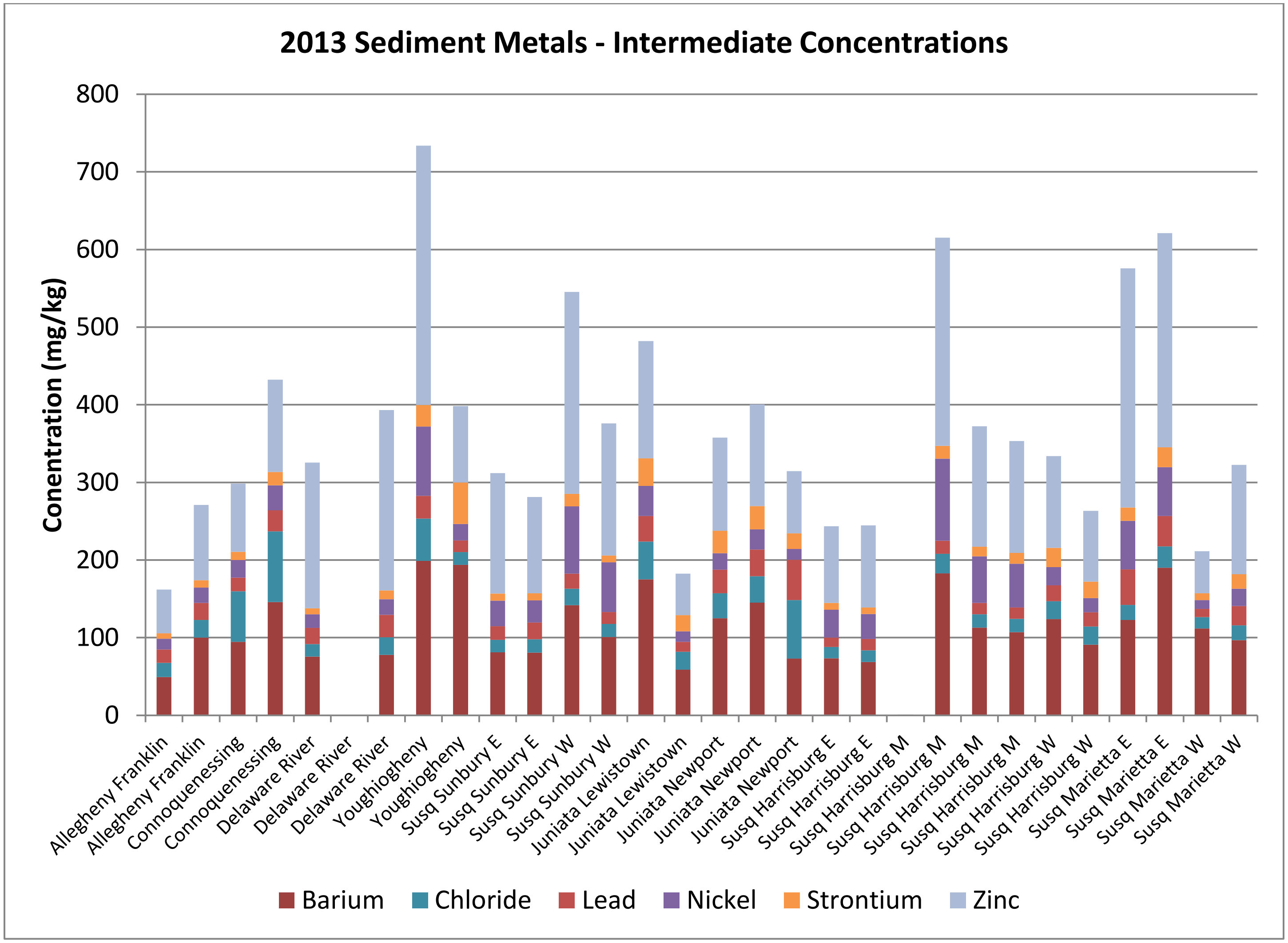


Figure 2: 2013 sediment metals – metals with intermediate concentrations

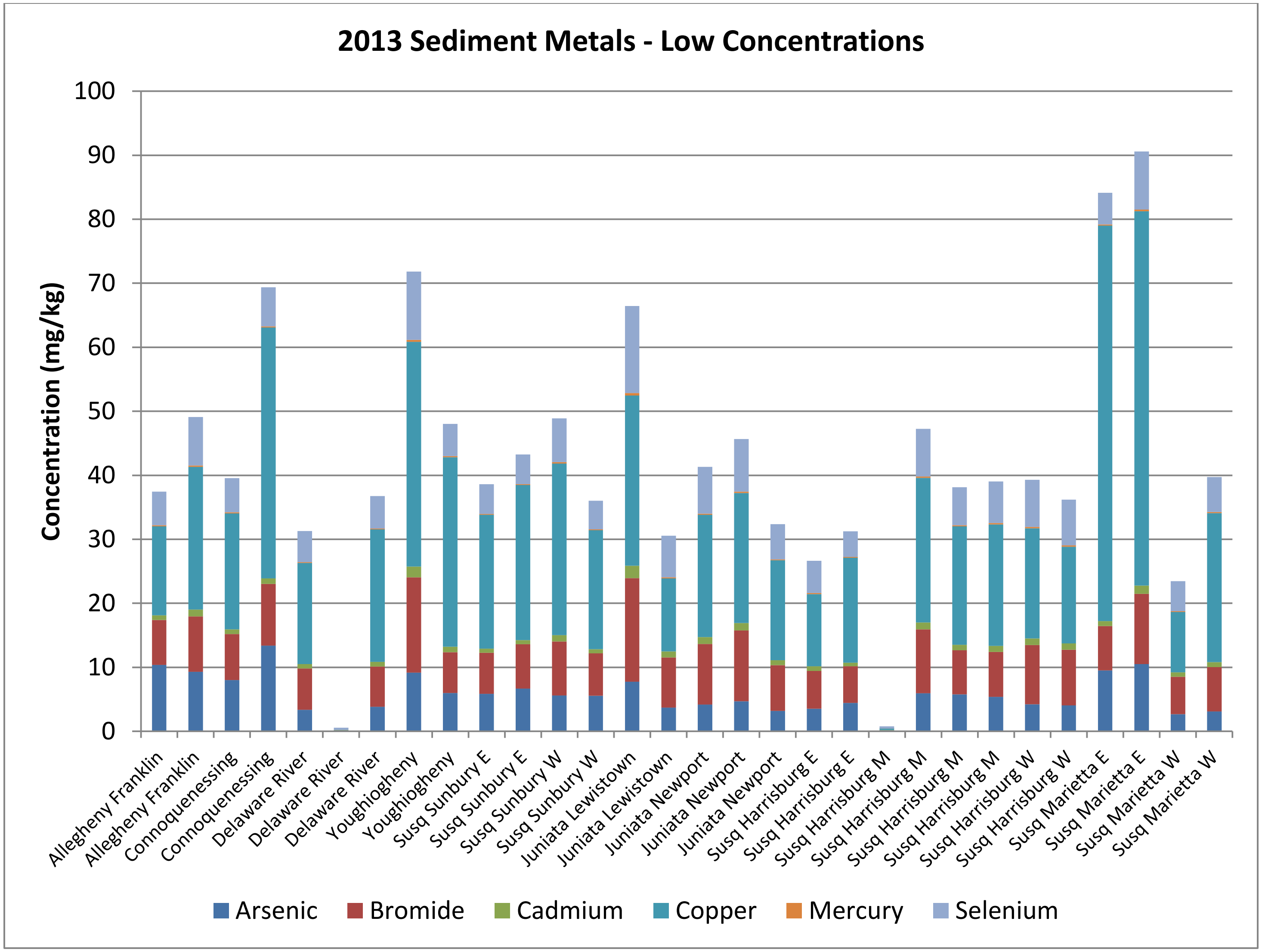


Figure 3: 2013 sediment metals – low concentration metals

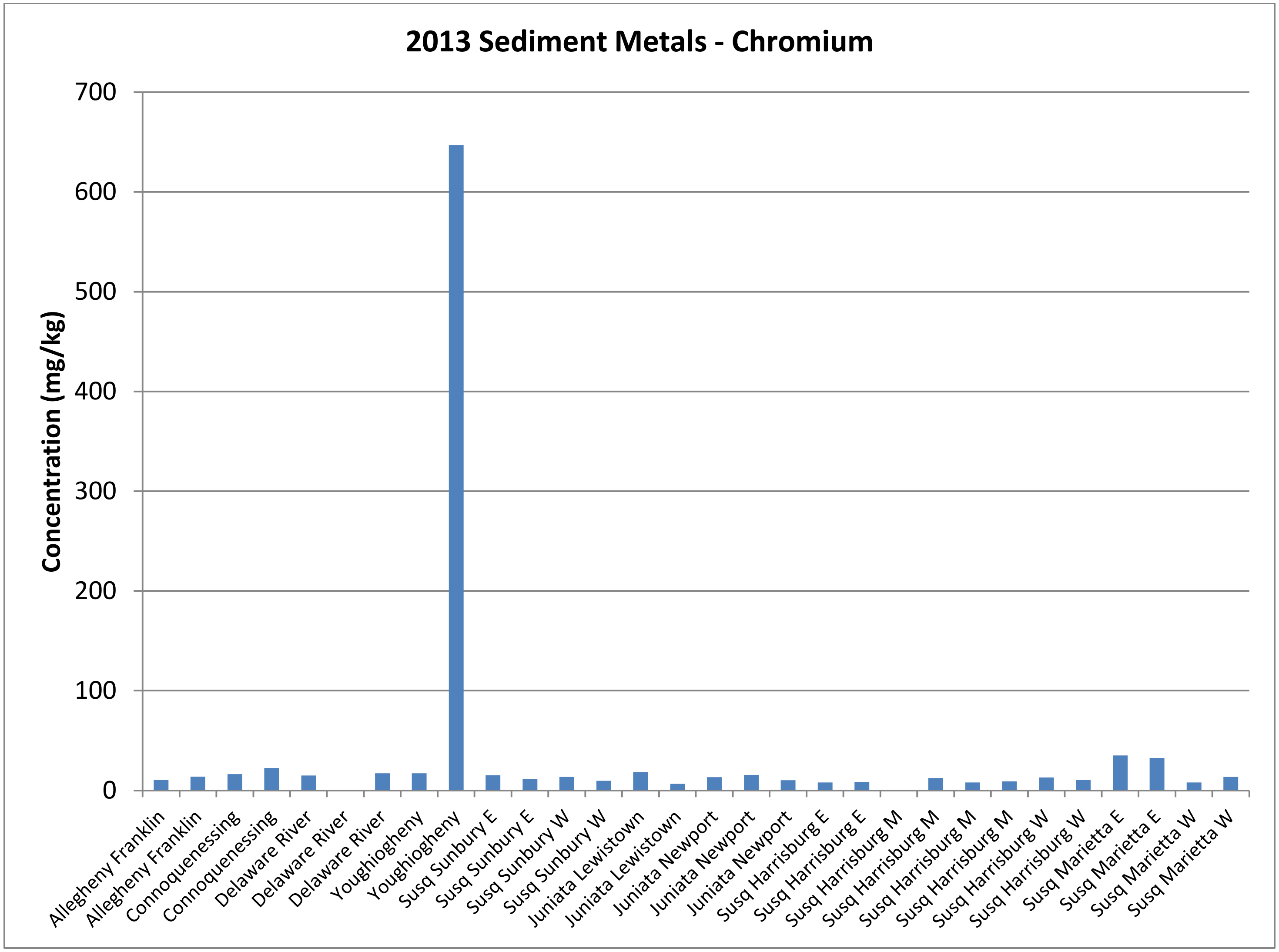


Figure 4: 2013 sediment metals – chromium data (separated out due to outlier at Youghiogheny River)

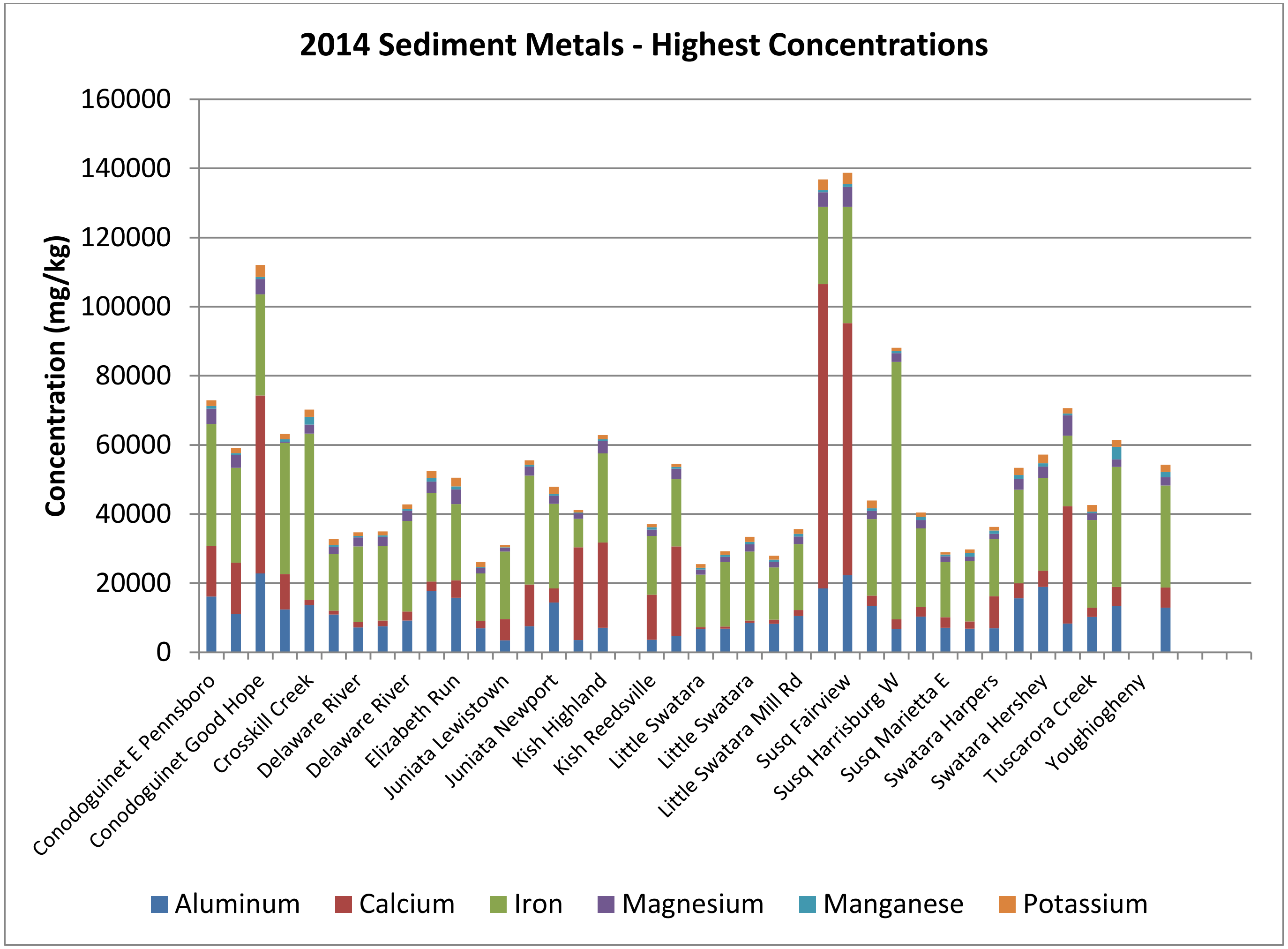


Figure 5: 2014 sediment metals – highest concentration metals

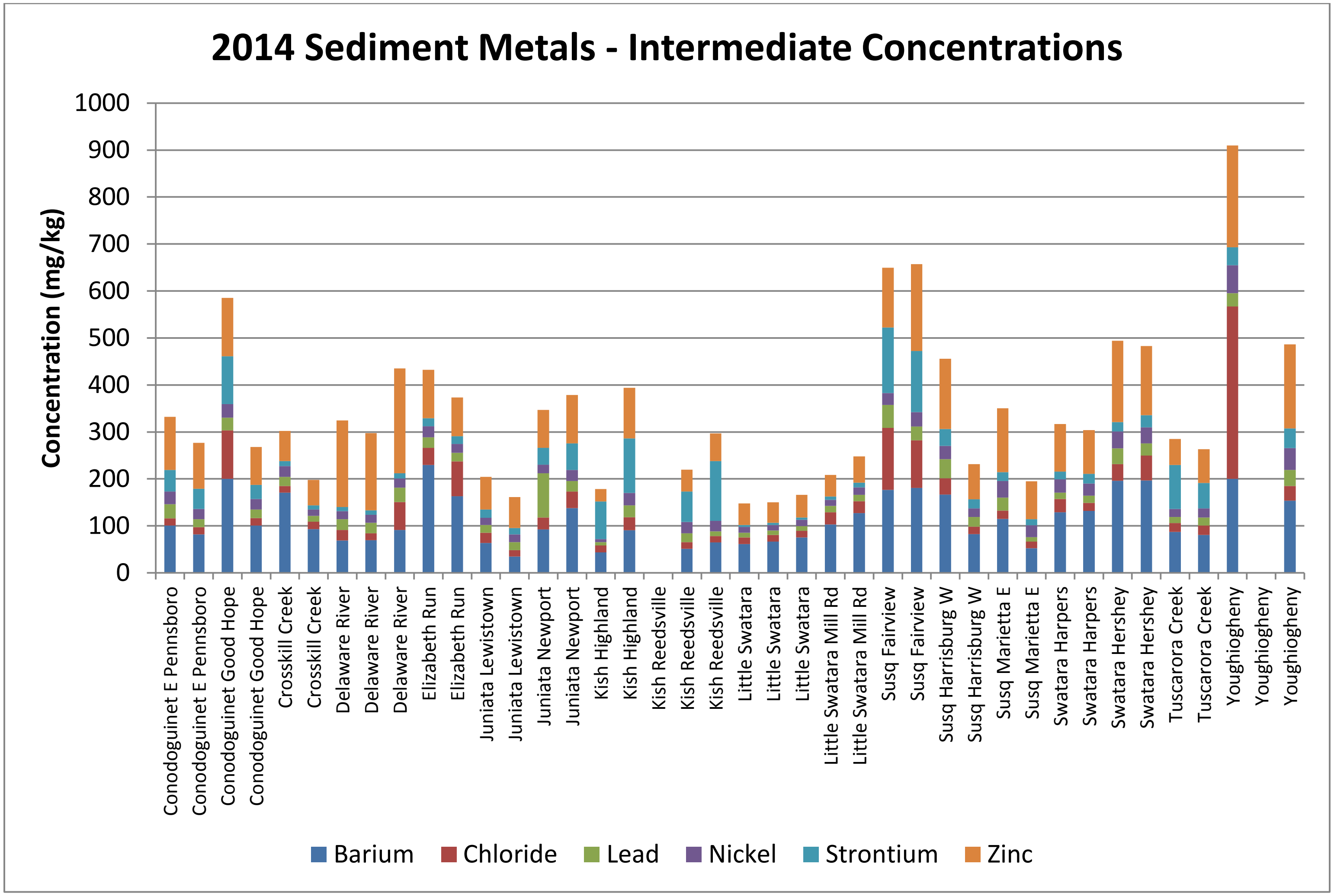


Figure 6: 2014 sediment metals – metals with intermediate concentrations

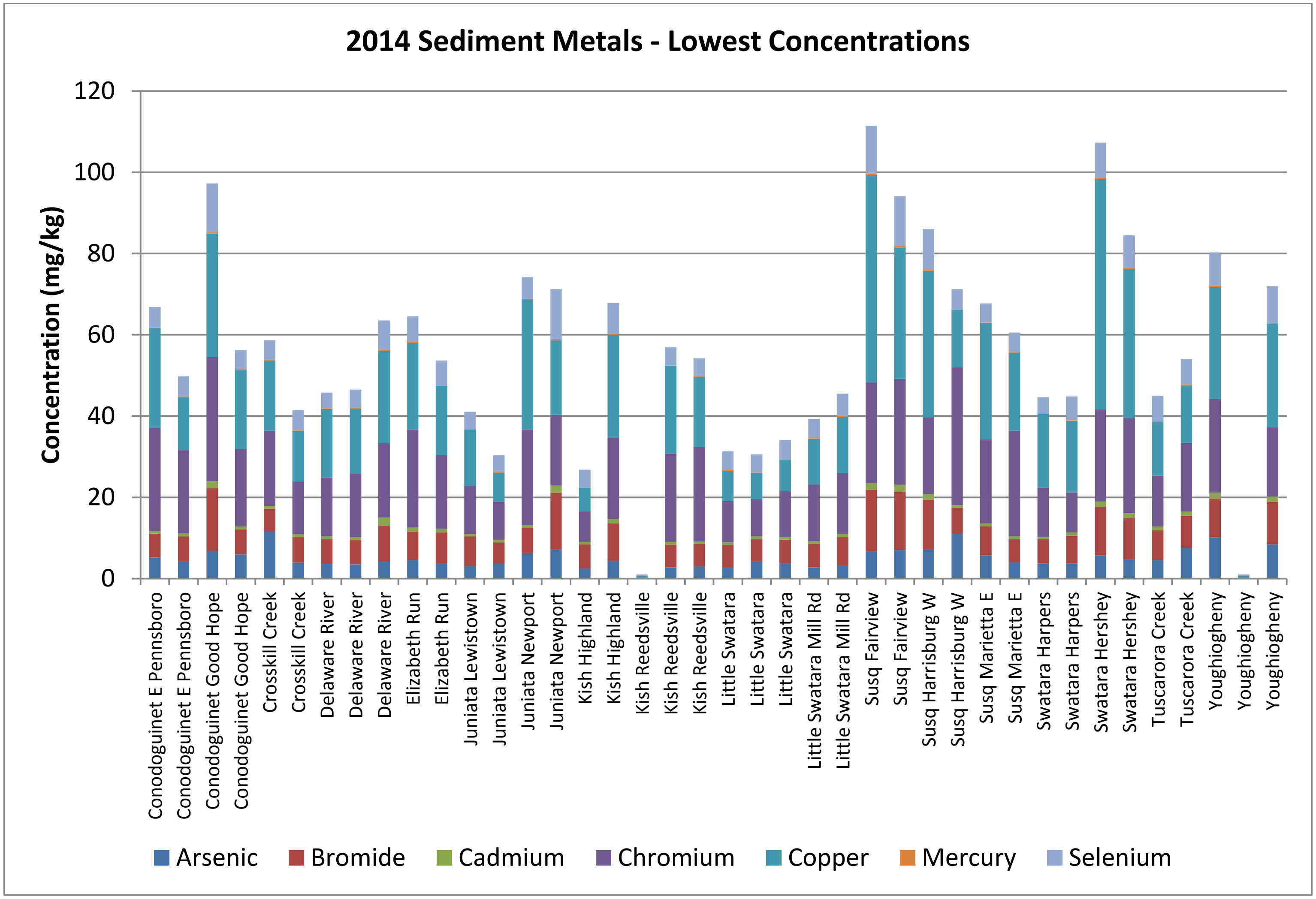


Figure 7: 2014 sediment metals – low concentration metals

Literature Cited:

Long, E.R. & L.G. Morgan. 1990. The Potential for Biological Effects of Sediment-Sorbed Contaminants Tested in the National Status and Trends Program. NOAA Tech Memo. NOS OMA 52. 175 pp.

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Ohio EPA, Division of Emergency and Remedial Response. 2008. Ecological Risk Assessment Guidance Document. Table 2: Sediment Reference Values (mg/kg), pg. 3-32.

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Wisconsin Department of Natural Resources. 2003. Consensus-Based Sediment Quality Guidelines - Recommendations for Use & Application - Interim Guidance. WT-732 2003. pg. 17. Accessed on the web at http://dnr.wi.gov/topic/Brownfields/documents/cbsqg_interim_final.pdf on 6/5/2012.

Title: Chemical Compounds in the Susquehanna River Basin (WS # 42)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Water concentrations of these compounds could come into direct contact with YOY fish. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected passive water samples from 12 sample sites within and out of the Susquehanna basin in 2013 to be tested for a variety of different compounds (organochlorine & currently used pesticides, PCBs, PBDEs, hormones, wastewater indicators, and PAHs – pharmaceutical data is currently pending). Samples were analyzed by USGS:

Waterbody	Location Name	Type
Susquehanna River	Marietta West	Basin
Susquehanna River	Marietta-Falmouth East	Basin
Susquehanna River	Rockville West	Basin
Susquehanna River	Rockville Middle	Basin
Susquehanna River	Rockville East	Basin
Juniata River	Lewistown Narrows	Basin
Juniata River	Newport South	Basin
Susquehanna River	Sunbury West	Basin
Susquehanna River	Sunbury East	Basin
Connequenessing Creek	Zelienople	Control
Allegheny River	Franklin	Control
Delaware River	Morrisville East	Control

Analysis and Results:

Many compounds were analyzed for, which made data analysis very difficult. In order to facilitate data analysis, control site results (Connoquenessing Creek, Allegheny River, and Delaware River) were compared to basin site results, and those compounds appearing in both basin and control sites were removed. Those compounds appearing only in basin sites were retained; compounds that were present at control site(s) but were mainly higher in concentrations in the basin were also retained:

Only At Basin Sites, Not Controls
1,2-dimethylnaphthalene
17-alpha-estradiol
bromoform
cis-androsterone
cumene
desethylatrazine
epitestosterone
equilenin
PBDE-153
PBDE-28
perylene
prometon
testosterone

In Controls, But Mostly Higher In Basin
atrazine
Endosulfan-II
para-nonylphenol-total

This substantially decreased the list of compounds that needed to be analyzed. Two figures were produced to visually look at compounds at the various sites in the basin only (Figures 1 & 2). Juniata River @ Newport and Lewistown had the highest number of basin-only compounds detected, at nine and seven, respectively. Susquehanna River @ Harrisburg West had the next highest number at four compounds, with Susquehanna River @ Marietta East and Susquehanna River @ Sunbury East having three. The remaining locations did not have any of these basin-only compounds detected or only had one or two of them. While Susquehanna River @ Sunbury and Susquehanna River @ Harrisburg West had some of the highest concentrations of the low-concentration compounds, Juniata River @ Newport and Lewistown had the only detections of some of the higher concentration compounds (bromoform, cumene). Juniata @ Lewistown also had the highest concentration of desethylatrazine (2.1 ng/L). Of particular interest in this group of compounds is prometon, an herbicide. Prometon was detected at only Juniata River @ Newport in the wastewater suite, but was detected at many sites (controls and basin) in the pesticide suite. However, these detections are still very, very low (ng/L) and spread out over a one-month period of time.

Of the lower-concentration compounds, there were two flame retardants (PBDE-28 and PBDE-153), five hormones (cis-androsterone, epitestosterone, 17-alpha-estradiol, testosterone, and equilenin), a metabolite of the pesticide atrazine (desethylatrazine), and two polycyclic aromatic hydrocarbons (1,2-dimethylnaphthalene and perylene). Of the higher concentration compounds – cumene is a component of crude oil and fuels, bromoform is an organic solvent, and, as already mentioned, prometon is an herbicide.

Compounds that were in the controls but noticeably higher in the basin included atrazine (triazine pesticide and known endocrine disrupting compound), endosulfan-II (an organochlorine insecticide and acaricide), and para-nonylphenol (used as oil additives, detergents, etc.). Endosulfan-II was only found in the control Allegheny River (0.34 ng/L), but was found at Susquehanna River @ Marietta East (0.77 ng/L) and Juniata River @ Newport (0.72 ng/L) at slightly higher concentrations. Atrazine was found in all controls, but was also found at higher concentrations (and lower concentrations) in the basin, and was particularly high at both Juniata River sites (40 ng/L & 18 ug/L at Newport and 33 ng/L & 30 ug/L at Lewistown). Atrazine was tested in two different suites. Para-nonylphenol was found at Allegheny River (36 ng/L), but also at both Newport (49 ng/L) and Lewistown (40 ng/L) at higher concentrations. Regardless of biological effect, it is apparent that the Juniata River sites had higher numbers of detected compounds and often had higher concentrations overall; however, some of the other basin sites had higher concentrations of certain compounds.

Conclusion:

Compounds found only in the basin sites, or in significantly higher levels than control sites, remain parameters of concern.

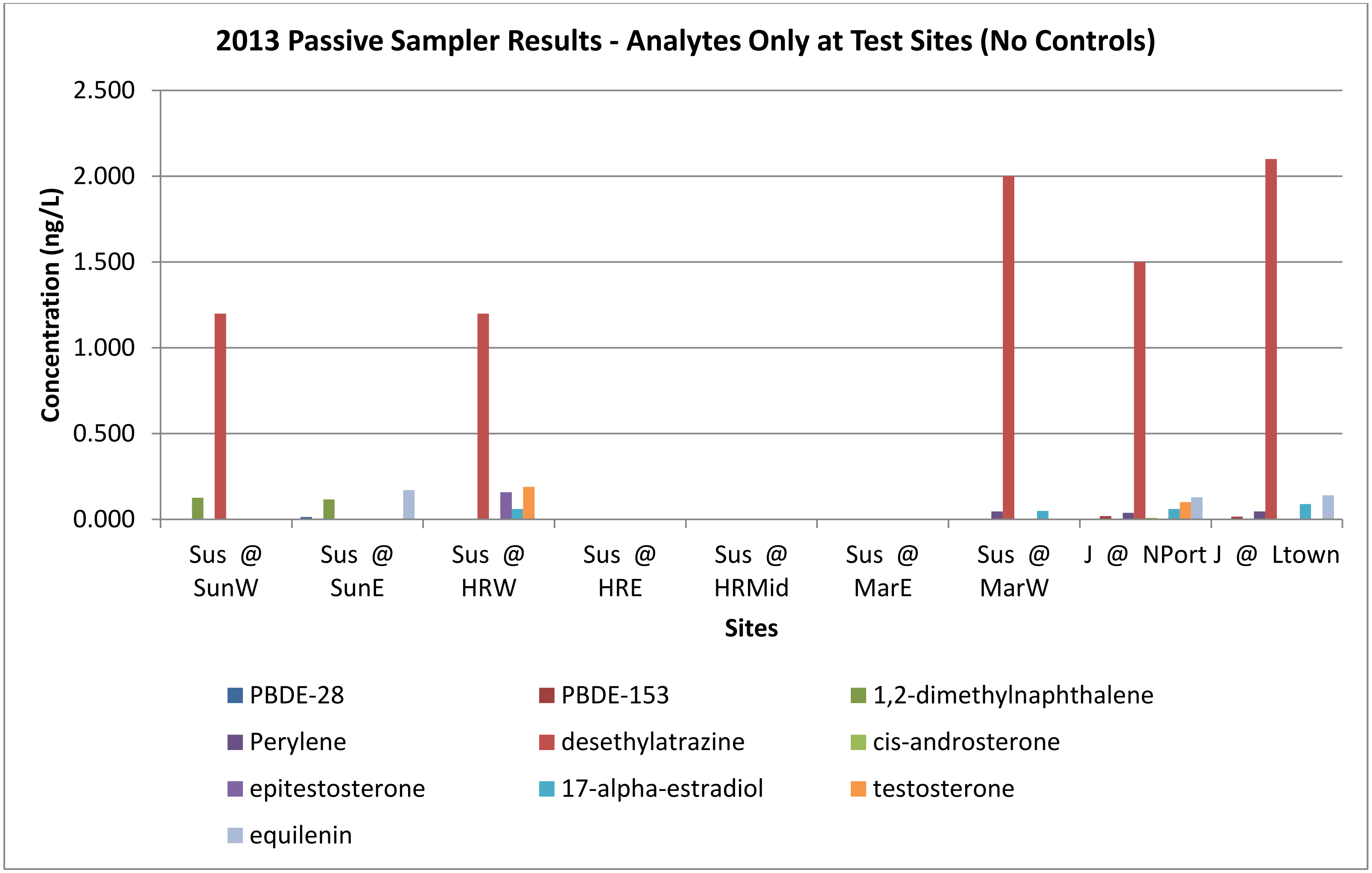


Figure 1: 2013 passive sampler results – compounds found only in the Susquehanna basin; compounds present at low concentrations

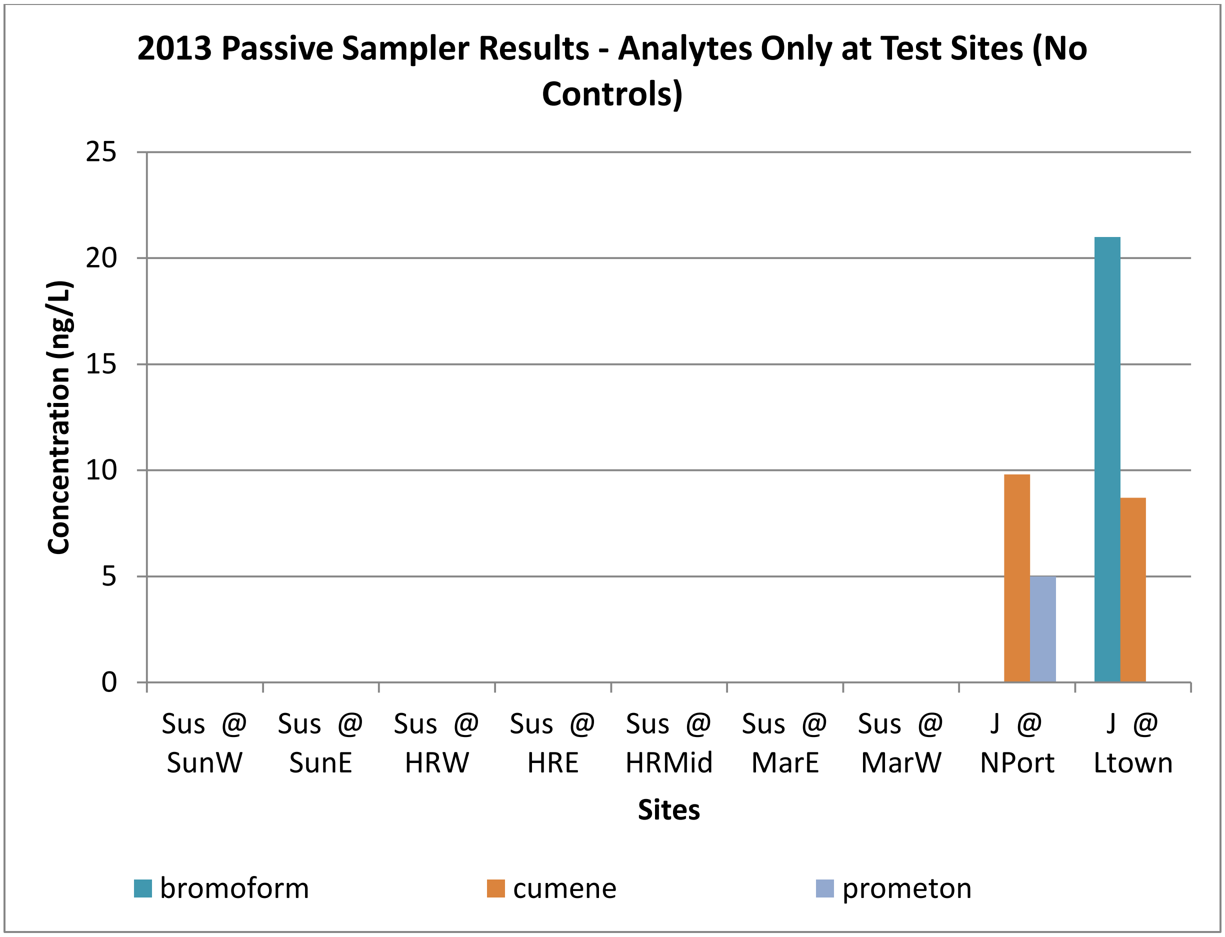


Figure 2: 2013 passive sampler results – compounds found only in the Susquehanna basin; compounds that were present at higher concentrations

Title: WQN Pesticides (WS# 44)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Water concentrations of these compounds could come into direct contact with YOY fish. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

USGS and SRBC staff collected regular flow and stormwater pesticide samples at the following five WQN stations from March through August 2013 and 2014. Samples were analyzed by USGS:

Waterbody	Location Name	WQN	Type
Susquehanna River	Marietta (Columbia)	201	Basin
Susquehanna River	Harrisburg	202	Basin
Juniata River	Newport	214	Basin
Susquehanna River	Sunbury	203	Basin
Delaware River	Trenton	101	Control

Grab Water Samples (ug/L)
propachlor
simazine
prometon
2-chloro-4-isopropylamino-6-amino-s-triazine (CIAT)
cyanazine
fonofos
alpha-hch
p,p'-DDE
chlorpyrifos
gamma-hch (lindane)
dieldrin
metolachlor
malathion

parathion
diazinon
atrazine
alachlor
acetochlor
fipronil
fipronil sulfide
fipronil sulfone
desulfinylfipronil amide
desulfinylfipronil
metribuzin
2,6-diethylaniline
trifluralin
ethalfluralin
phorate
terbacil
linuron
methyl parathion
eptc
pebulate
tebuthiuron
molinate
ethoprophos
benfluralin
carbofuran
terbufos
propyzamide
disulfoton
triallate
propanil
carbaryl
thiobencarb
dacthal (dcpa)
pendimethalin
napropamide
propargite
azinphos-methyl
1rs cis-permethrin
diazinon-d10 (surrogate)
alpha-hch-d6 (surrogate)
butylate

Analysis and Results:

Twenty-four pesticides were detected. In general, all stations had the same number of average compounds detected (Figure 1). Many detections had lab comments/qualifiers attached to them, such as estimated value, highly variable compound, or below laboratory reporting level but above long-term method detection level. These values were kept. Prometon had a few detections that were also in the laboratory blanks. These detections were very low, so they were also kept in the data analysis.

The most commonly detected compounds were graphed as a group (Figure 2). In general, these compounds were highest at Juniata River @ Newport and Susquehanna River @ Marietta/Columbia. The compounds also trended with each other; i.e. when one was high they all tended to be high.

The remaining pesticides were very infrequently detected (Figure 3). Some were more elevated at Delaware River, Juniata River @ Newport, and Susquehanna River @ Marietta/Columbia.

Compounds tended to be detected more frequently at lower discharges (Figure 4). There were a few detections at much higher discharges.

Conclusion:

Current pesticides were rarely detected in water grab samples under normal flow conditions. Occasional spikes were seen under presumed high flow conditions and appeared to be of short duration.

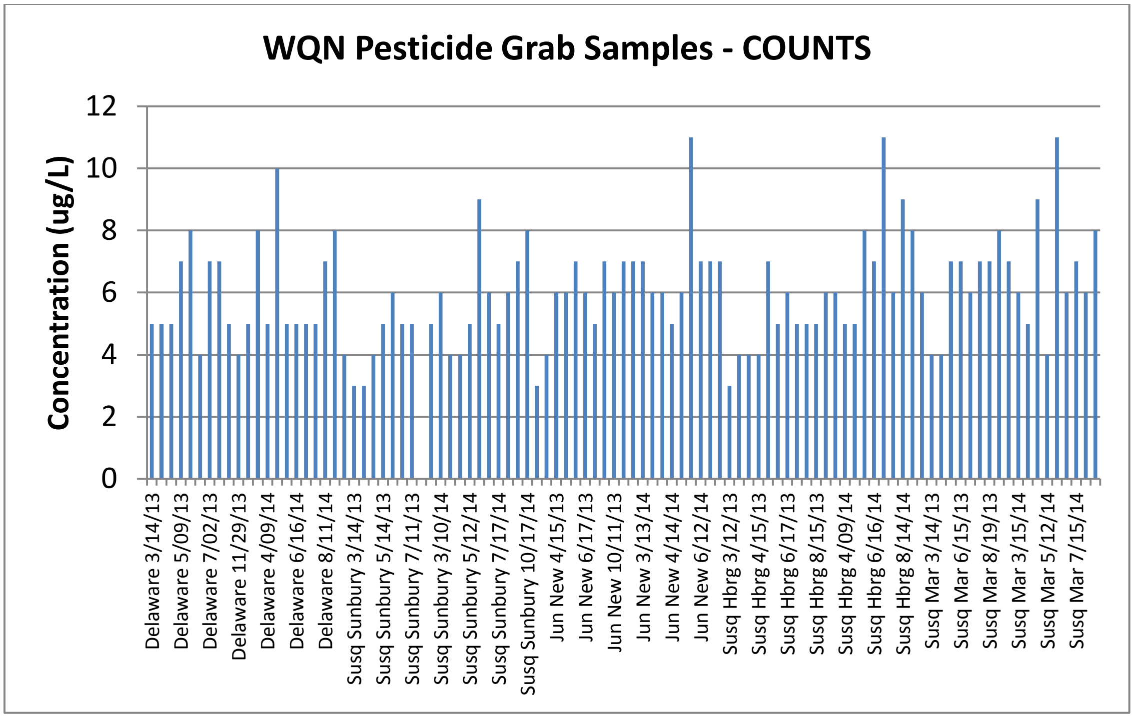


Figure 1: WQN pesticide grab samples – number of compounds detected per sample.

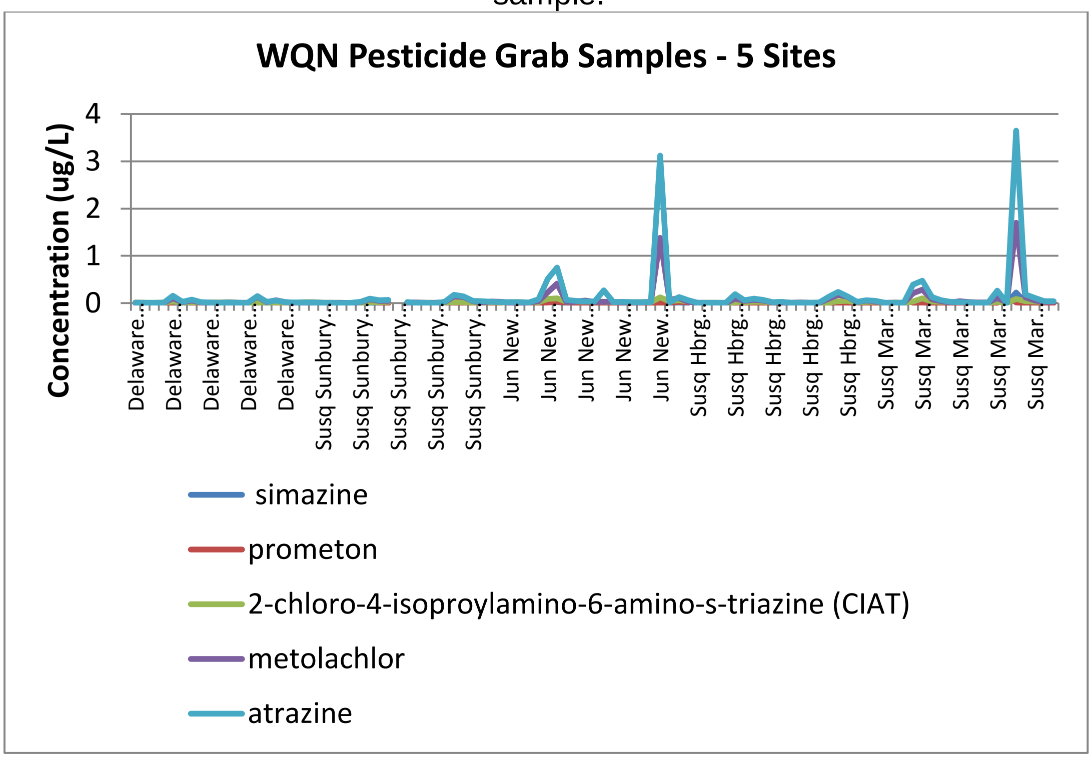


Figure 2: WQN pesticide grab samples – most commonly detected compounds

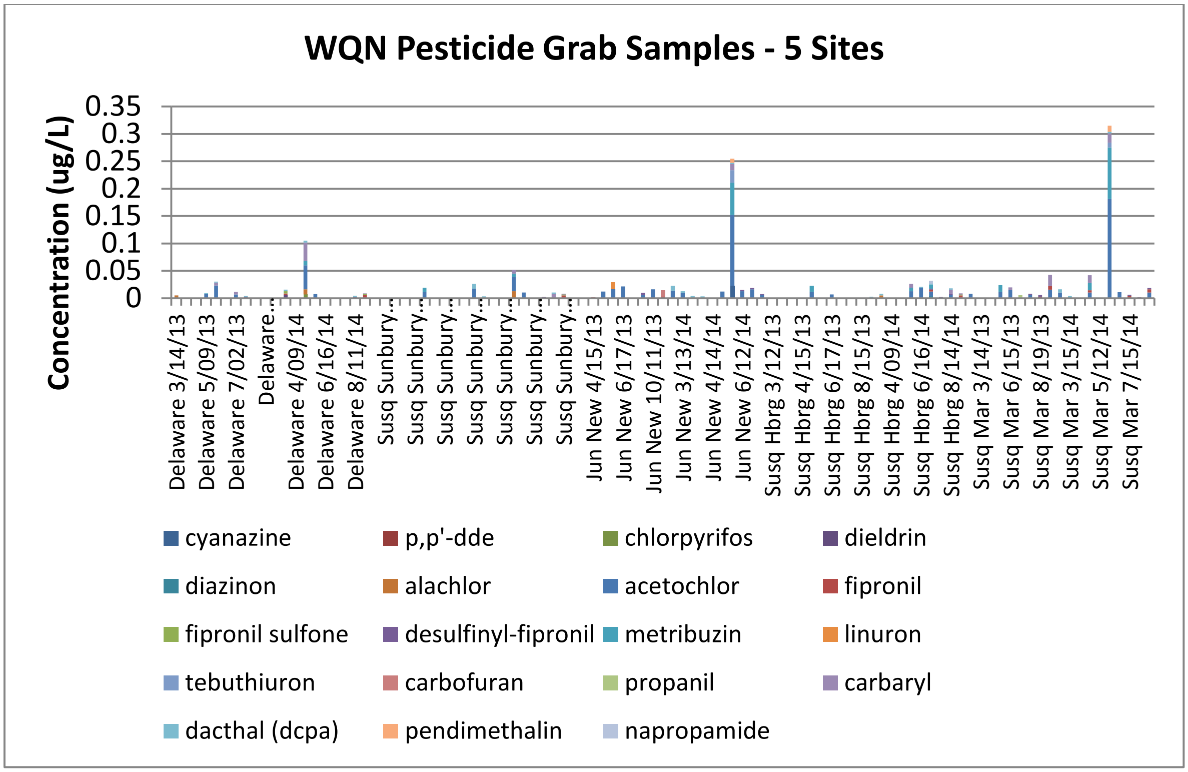


Figure 3: WQN pesticide grab samples – remaining pesticides, infrequently detected

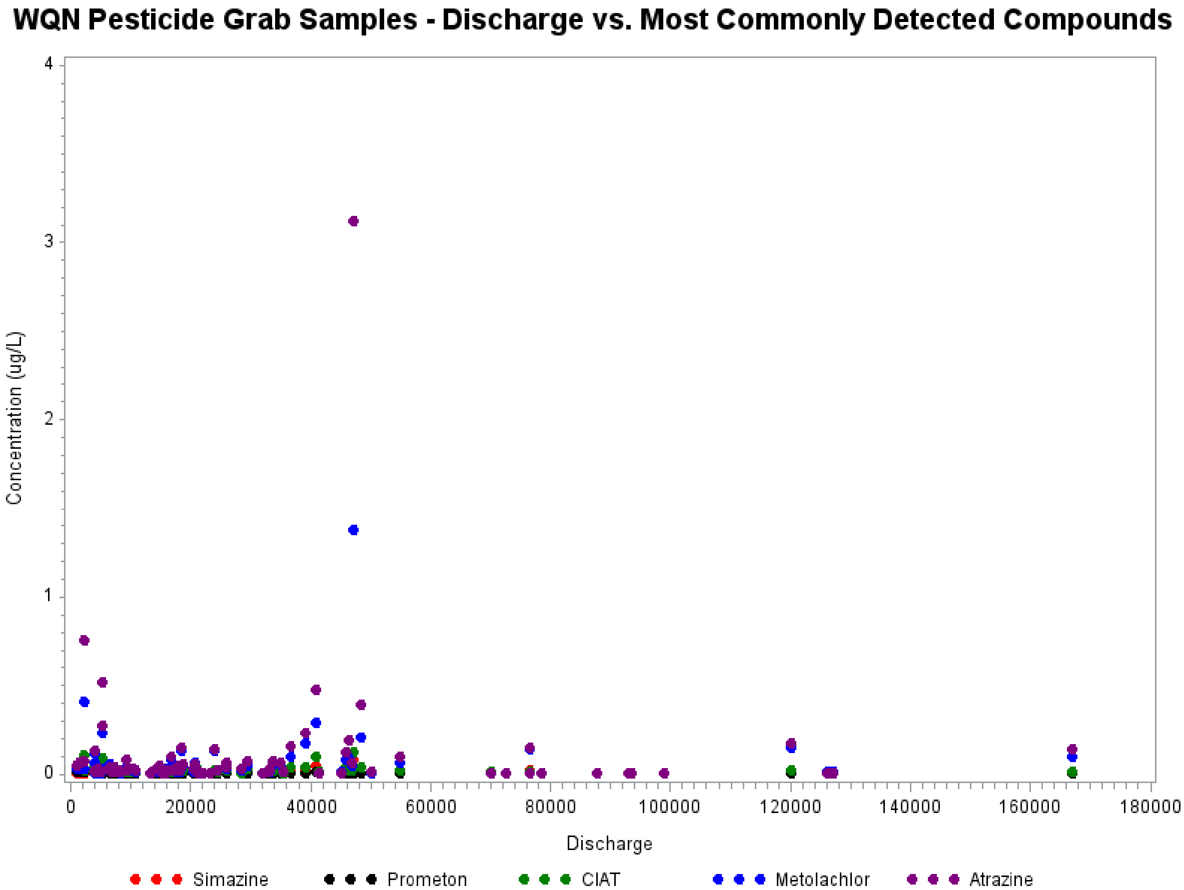


Figure 4: Discharge (cfs) versus concentration of pesticide (ug/L)

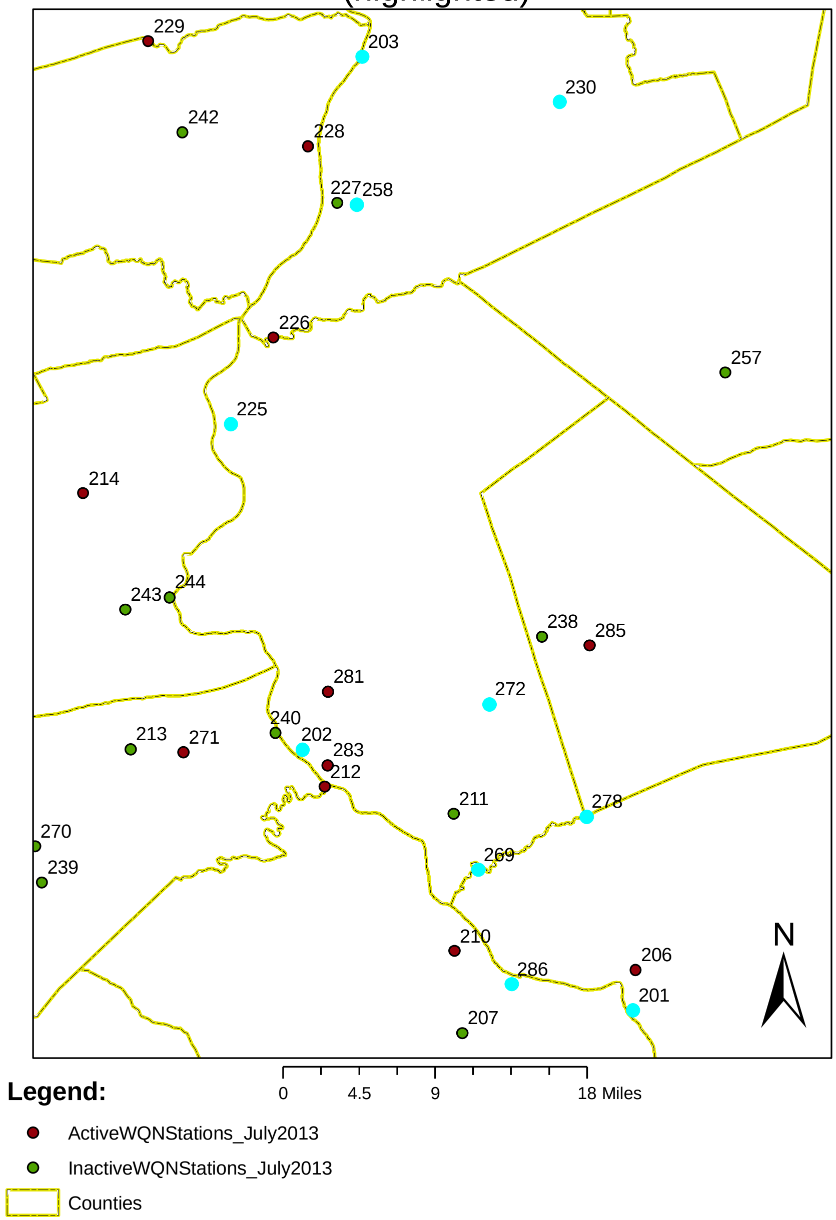
Title: Susquehanna @ Marietta Metals vs. Upstream Sites November 2013 Analysis (WS #45)

Agency: Pennsylvania Department of Environmental Protection

Candidate cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease

Several metals exceeded aquatic life criteria on the Susquehanna River at Marietta (water quality network station (WQN) 201) from 2006 through 2013. Several upstream WQNs were also analyzed for possible influences to the Susquehanna @ Marietta: WQN286 (Codorus Creek, data from 11/2012 onwards): WQN278 (Conewago Creek, data from 10/2011 onwards), WQN269 (Conewago Creek, data from 5/2011 onwards), WQN272 (Swatara Creek, data from 10/04 onwards), WQN202 (Susquehanna @ Harrisburg, 1976 – 1995 data), WQN203 (Susquehanna @ Sunbury), WQN258 (Mahanoy Creek, 1975-1987), WQN230 (Shamokin Creek, 1962-1987), and WQN225 (Wiconisco Creek, 1962-1987). Except for very small streams, any WQN samples collected after 2002 are composites both horizontally (at several locations across channel) and vertically (depth-integrated samples):

WQN Stations Analyzed
(highlighted)

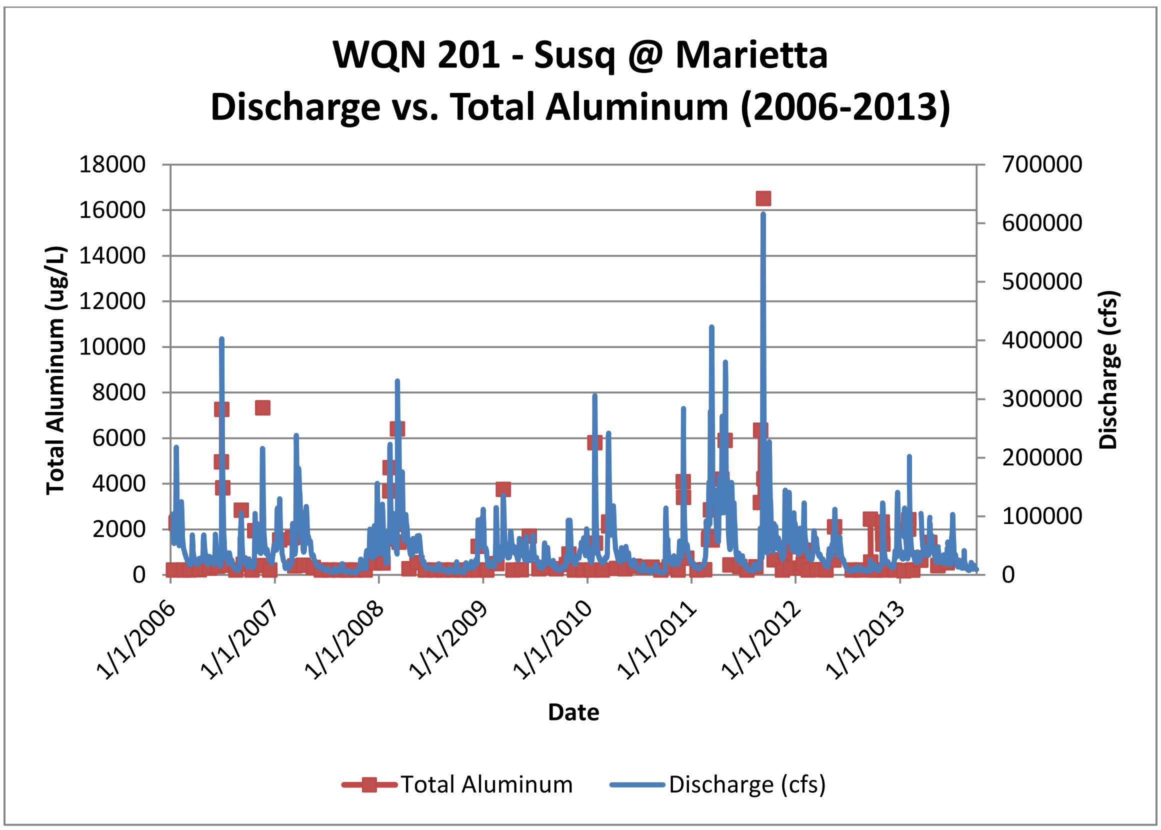


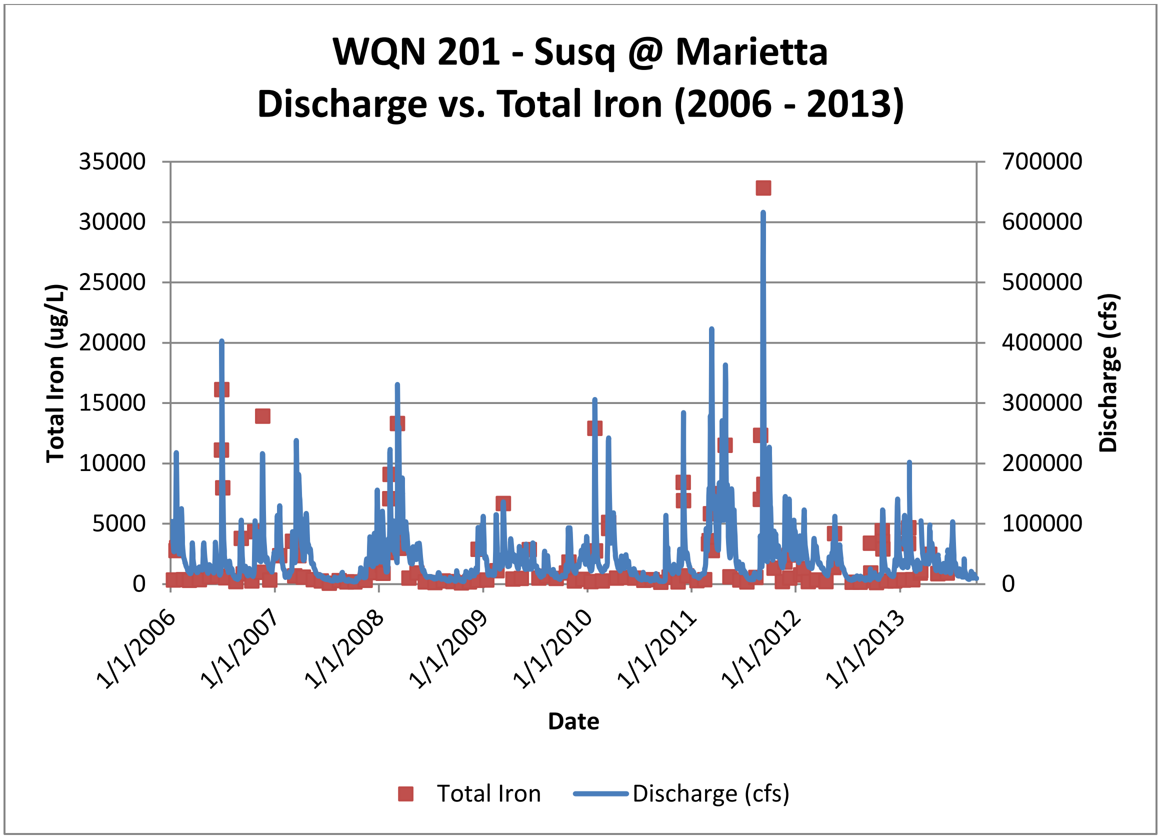
The Susquehanna River @ Marietta WQN has had many exceedances of metals criteria over the years:

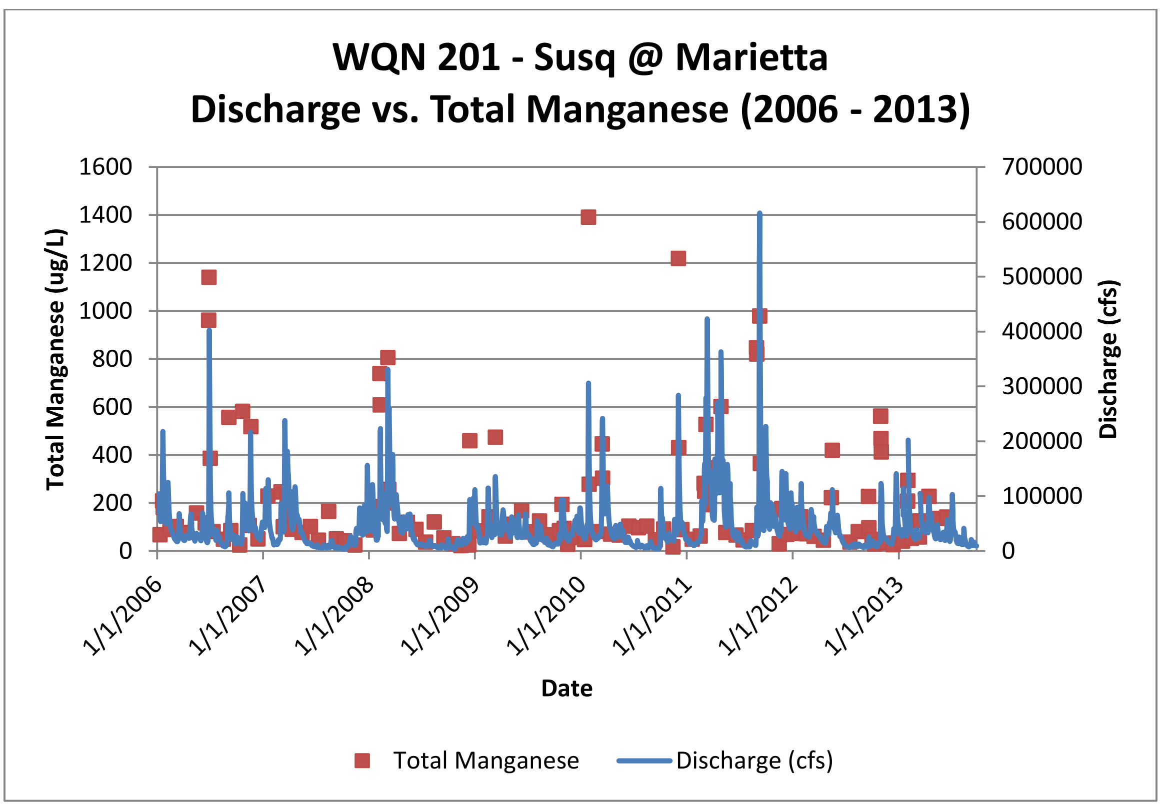
Iron aquatic life criterion is the 30-day average cannot exceed 1.5 mg/L (1,500 ug/L); it must remain below this at least 99% of the time. Iron showed multiple exceedances per calendar year, with samples ranging from 63 ug/L to 32,800 ug/L.

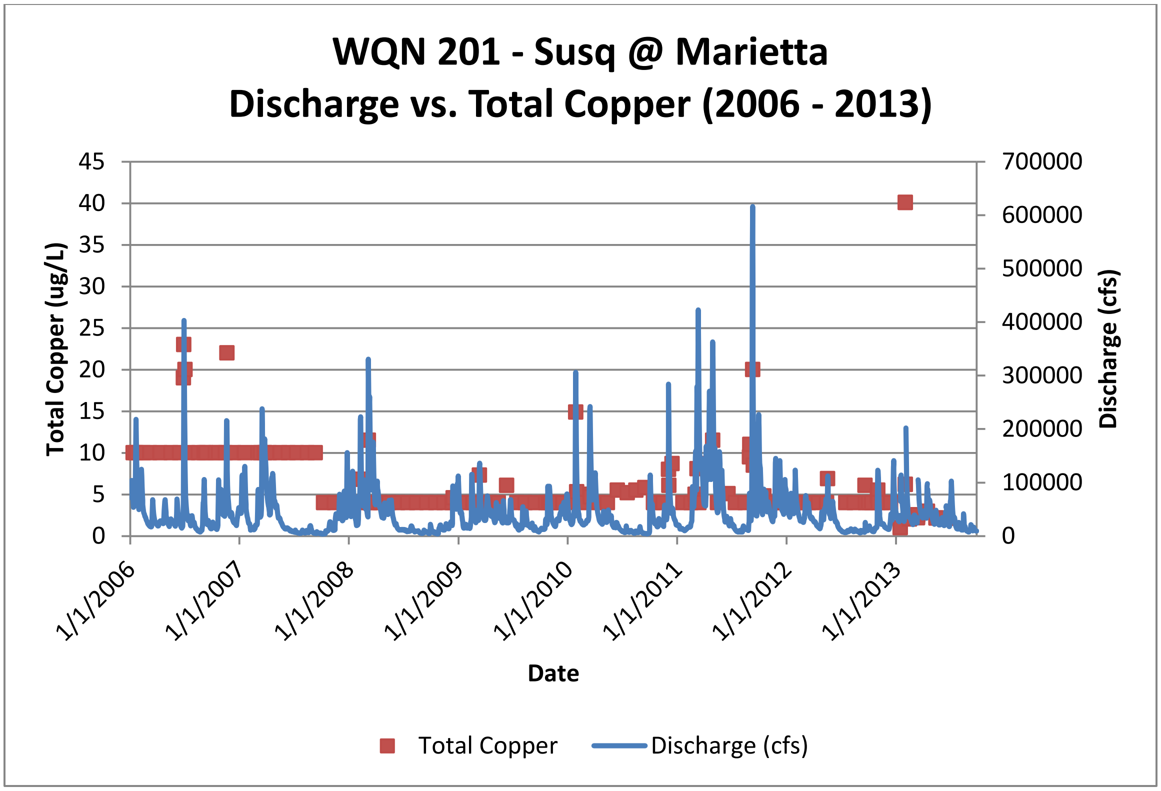
According to PA DEP "Water Use Assessment Decision-Making Based on Physicochemical and Bacteriological Sampling" (2013), "CMC [criteria maximum concentration] pollutants violating criteria more than once in a given 3-year period constitute an impairment; CCC [criteria continuous concentration] pollutants with either a 30-day mean concentration violating a criterion more than once in a 3-year period or a 4-day mean concentration exceeding twice the CCC in any 4-week period constitute an impairment". Many of these criteria are calculated from hardness-based equations. Aluminum criteria (CMC = 750 ug/L, not to be exceeded more than once in a 3 year period) was exceeded at WQN201 many times from 2006 – 2013. Lead criteria (also hardness-based equations for criteria CCC and CMC) was exceeded several times per year (CCC only). The aquatic life criteria for nickel are also hardness-based equations, and were not exceeded at WQN201 except for one date (2/1/13, 552 ug/L), which was an anomaly (most other results were less-than-detect). Copper criteria (based on equations for CCC and CMC) were exceeded several times from 2006 onwards. Zinc criteria (based on equations for CCC and CMC) were exceeded several times from 2006 onwards, although not recently (only one exceedance so far in 2013).

WQN201 – Susquehanna @ Marietta graphs of metals are below. There is definitely a correlation between stream flow and metals concentrations (i.e. high discharge = increased metals concentrations):

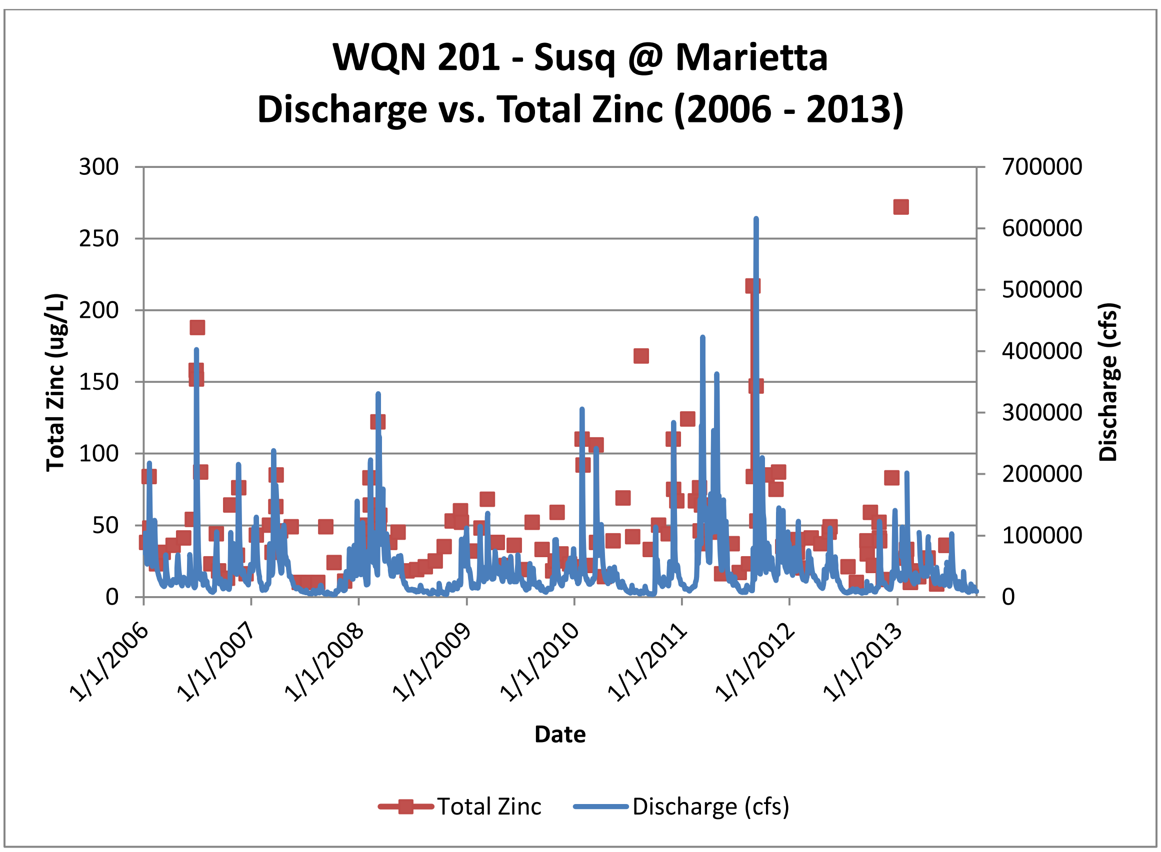



WQN 201 - Susq @ Marietta
Discharge vs. Total Iron (2006 - 2013)
Total Iron (ug/L)
Discharge (cfs)
Date
Total Iron
Discharge (cfs)

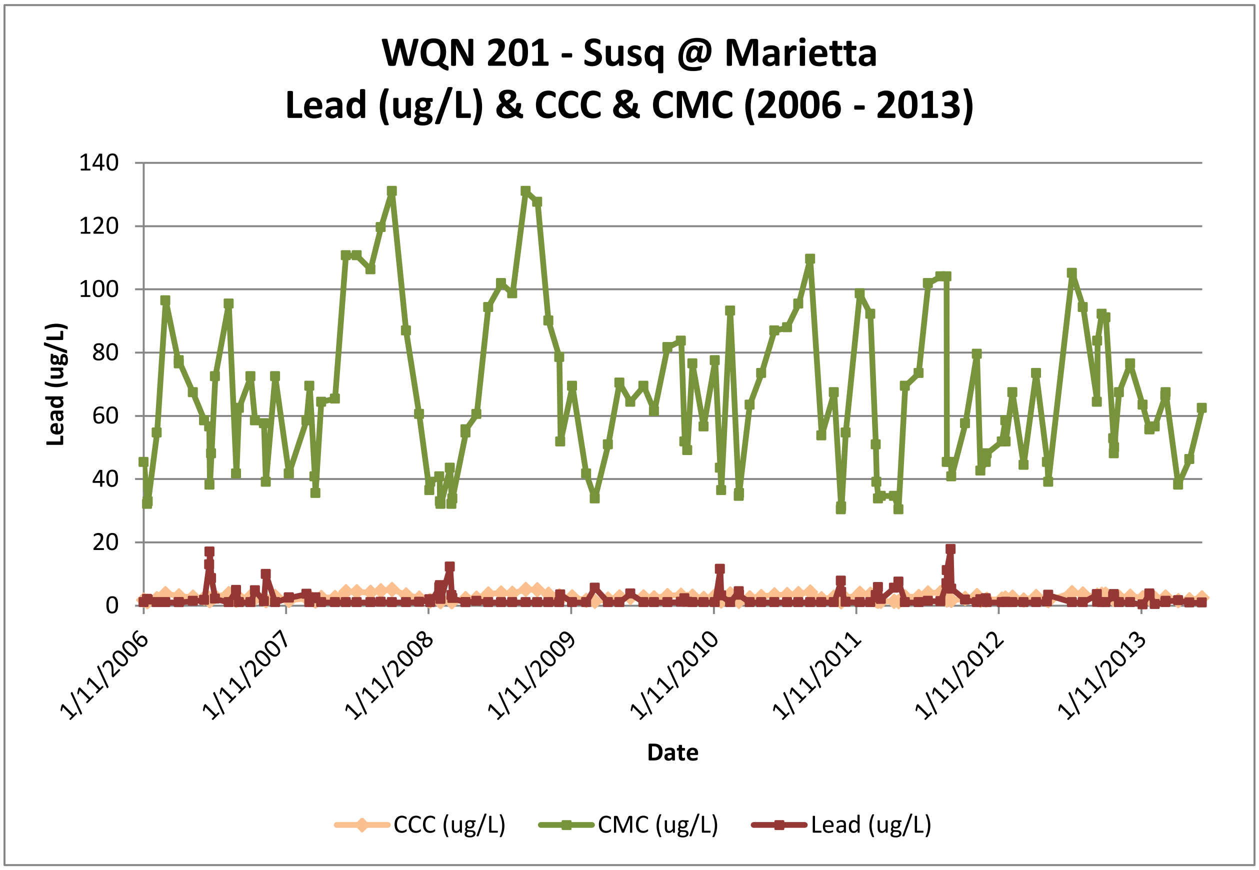

WQN 201 - Susq @ Marietta
Discharge vs. Total Manganese (2006 - 2013)
Total Manganese (ug/L)
Discharge (cfs)
Date
Total Manganese
Discharge (cfs)



WQN 201 - Susq @ Marietta
Discharge vs. Total Copper (2006 - 2013)
Total Copper (ug/L)
Discharge (cfs)
45
40
35
30
25
20
15
10
5
0
700000
600000
500000
400000
300000
200000
100000
0
1/1/2006
1/1/2007
1/1/2008
1/1/2009
1/1/2010
1/1/2011
1/1/2012
1/1/2013
Date
Total Copper
Discharge (cfs)



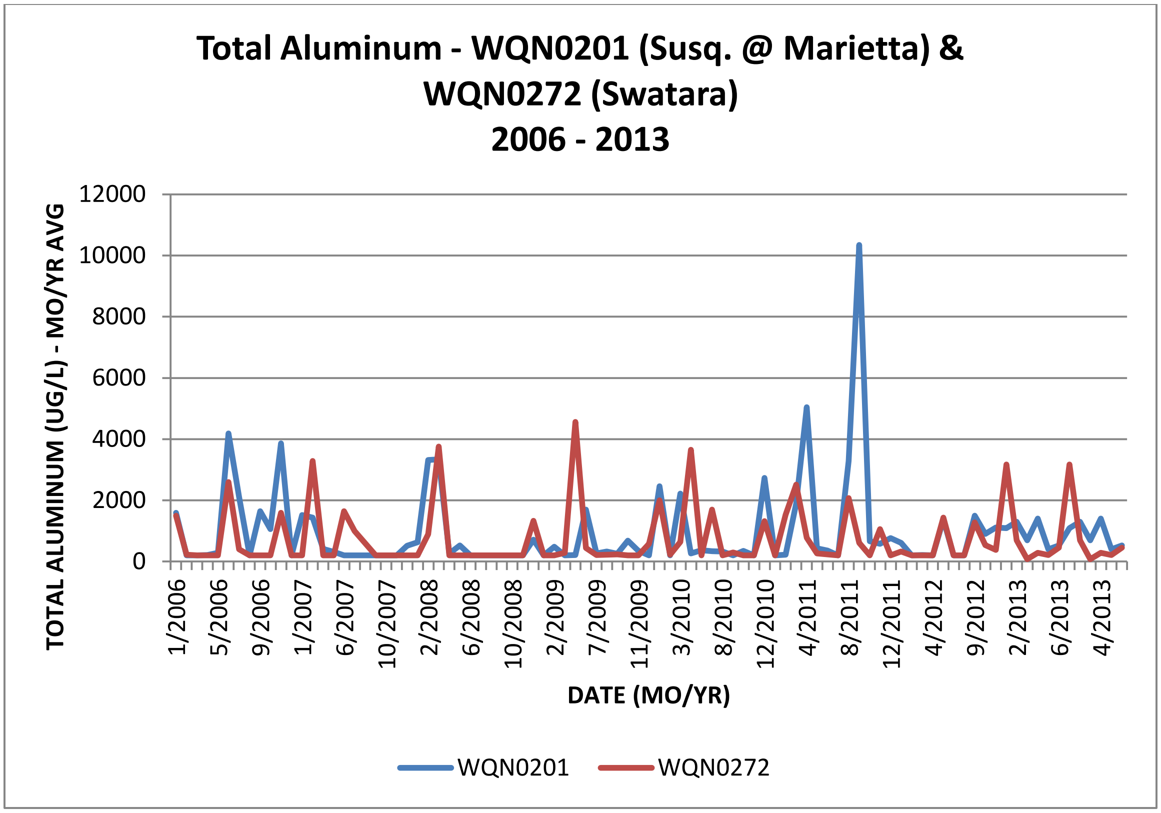
WQN 201 - Susq @ Marietta
Discharge vs. Total Zinc (2006 - 2013)
Total Zinc (ug/L)
Discharge (cfs)
300
250
200
150
100
50
0
700000
600000
500000
400000
300000
200000
100000
0
1/1/2006
1/1/2007
1/1/2008
1/1/2009
1/1/2010
1/1/2011
1/1/2012
1/1/2013
Date
Total Zinc
Discharge (cfs)

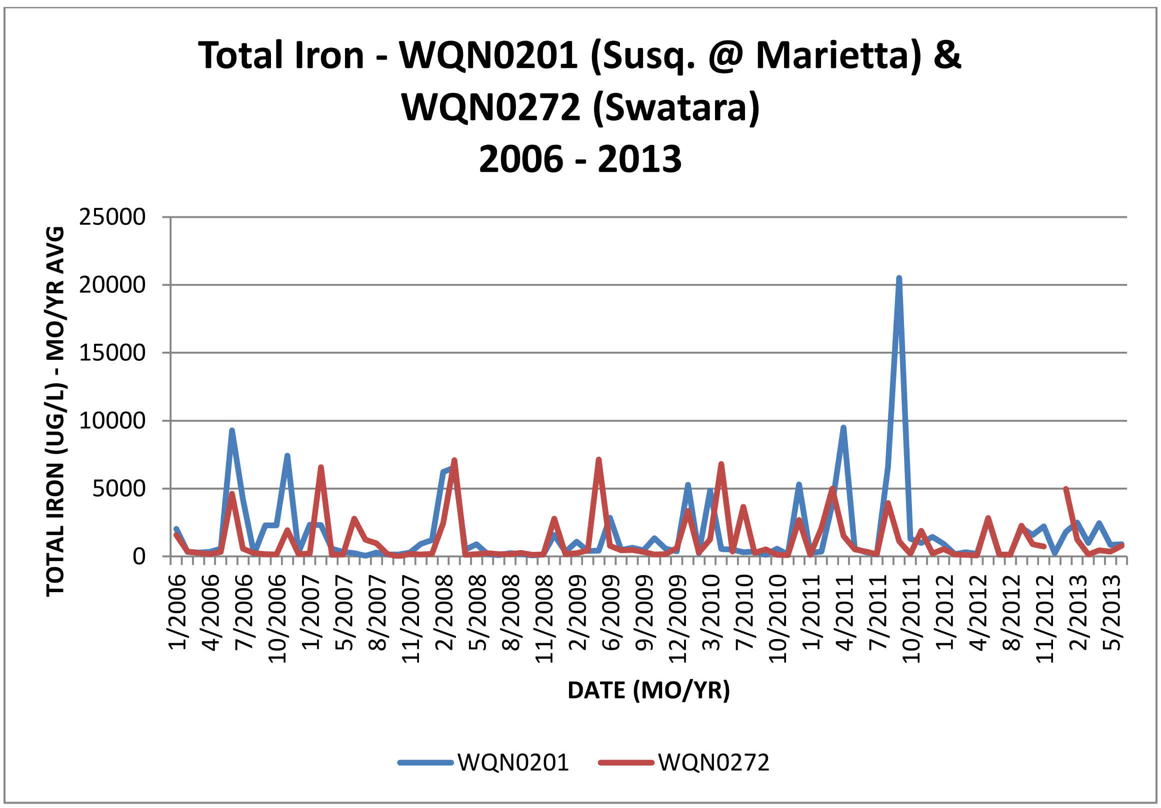


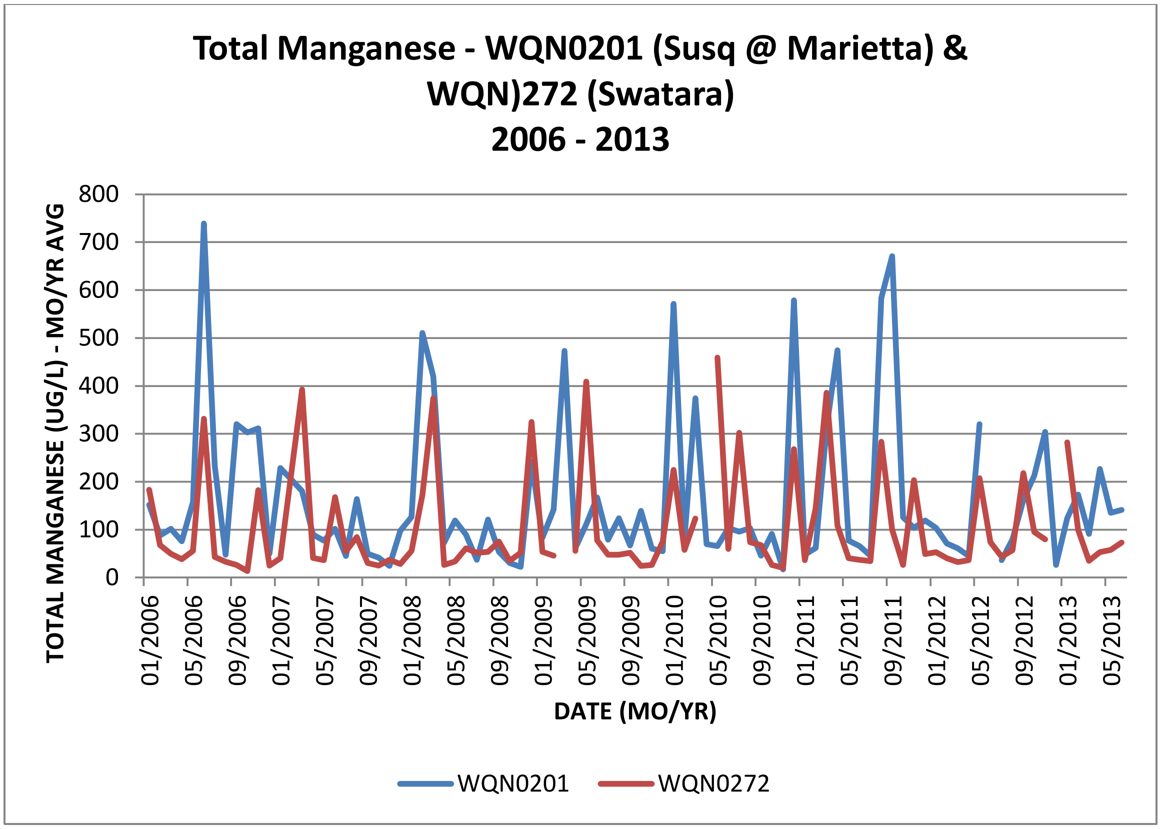
- Exceedences based on CCC; CMC never exceeded.

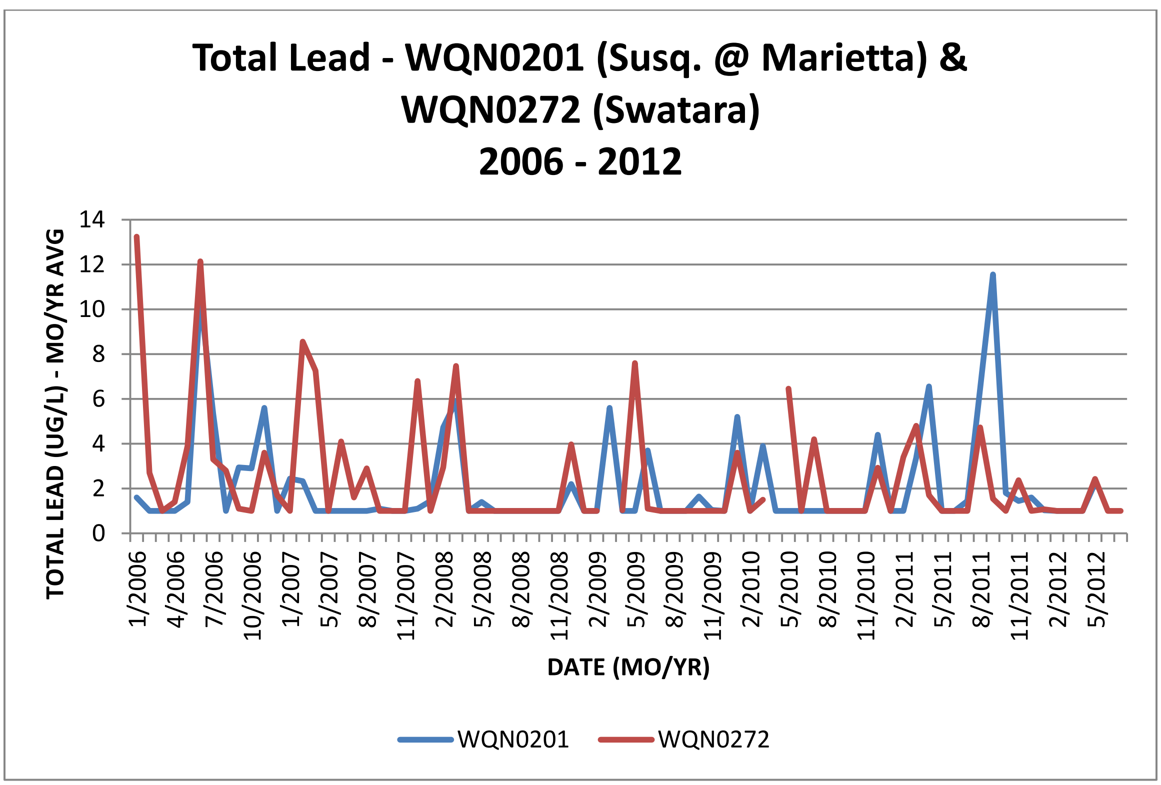
While it is difficult to determine the source of the metals contamination, it is most likely due to non-point source influences from many locations upstream. Some examples of possible upstream influences are graphed below.

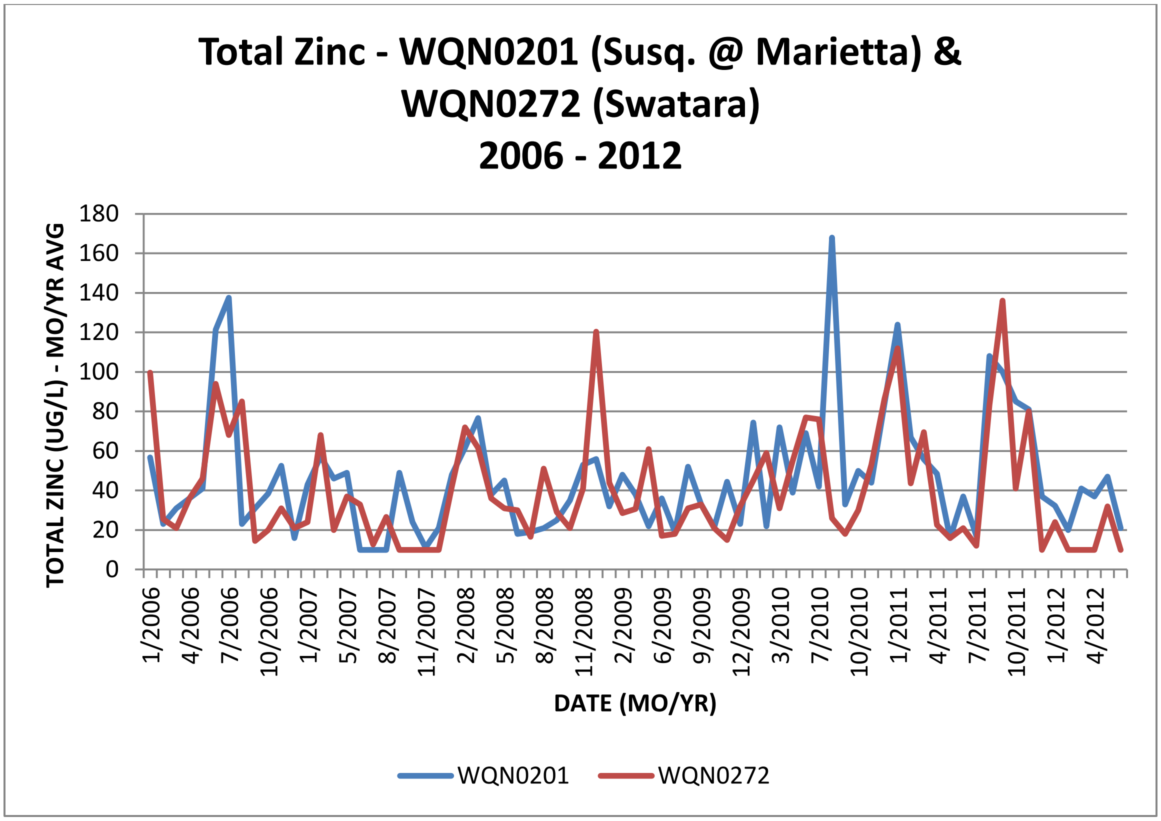
WQN272 (Swatara Creek) is likely influencing the Susquehanna @ Marietta, which is shown in the following graphs of total aluminum, iron, lead, and zinc (averaged by month/year) for both the Swatara and Susquehanna @ Marietta. However, it is not assumed that the Swatara is the only source for the elevated metals, as there are many more possible upstream influences:


Total Aluminum - WQN0201 (Susq. @ Marietta) &
WQN0272 (Swatara)
2006 - 2013
TOTAL ALUMINUM (UG/L) - MO/YR AVG
12000
10000
8000
6000
4000
2000
0
1/2006
5/2006
9/2006
1/2007
6/2007
10/2007
2/2008
6/2008
10/2008
2/2009
7/2009
11/2009
3/2010
8/2010
12/2010
4/2011
8/2011
12/2011
4/2012
9/2012
2/2013
6/2013
4/2013
DATE (MO/YR)
WQN0201
WQN0272

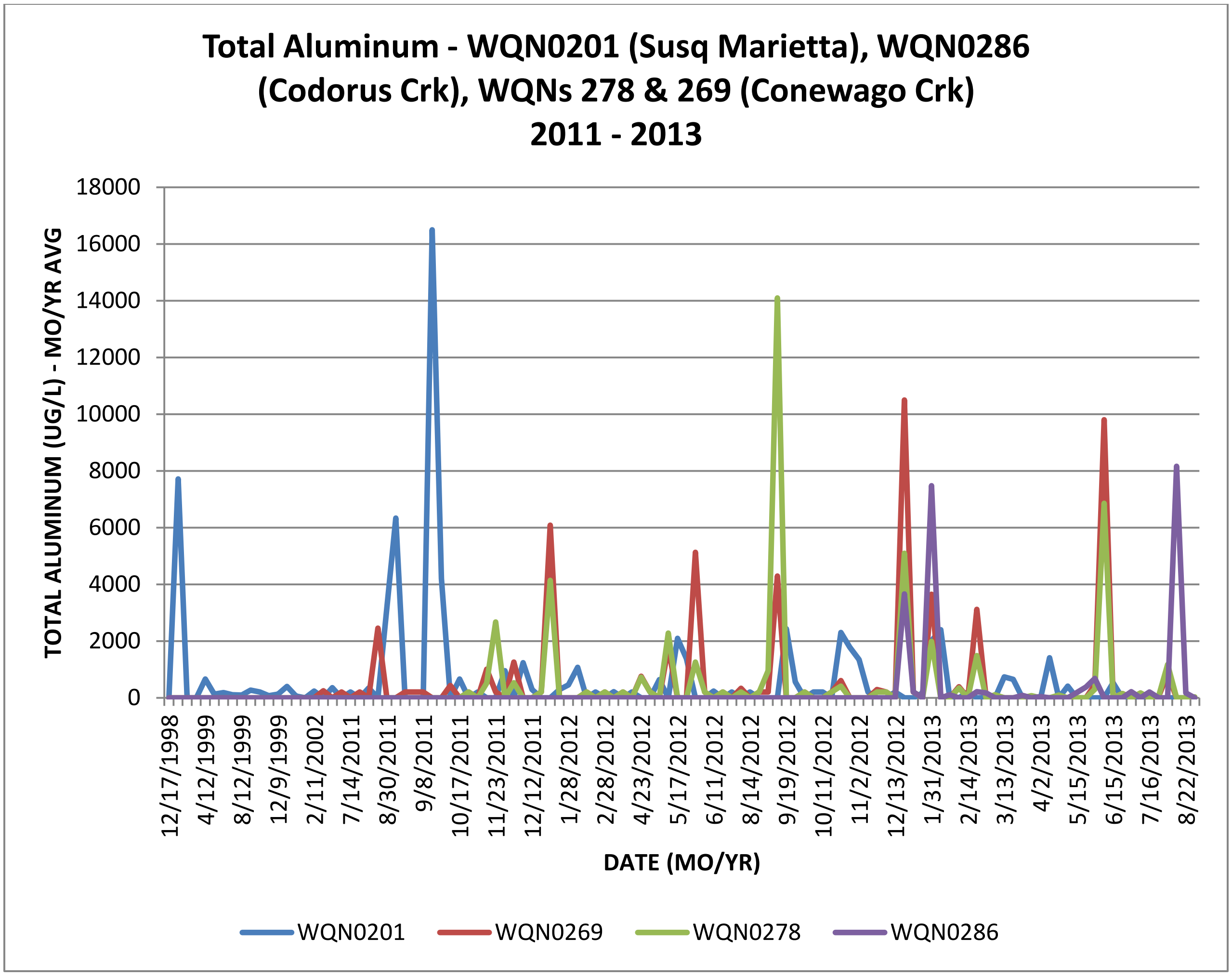

Total Iron - WQN0201 (Susq. @ Marietta) &
WQN0272 (Swatara)
2006 - 2013
TOTAL IRON (UG/L) - MO/YR AVG
25000
20000
15000
10000
5000
0
1/2006
4/2006
7/2006
10/2006
1/2007
5/2007
8/2007
11/2007
2/2008
5/2008
8/2008
11/2008
2/2009
6/2009
9/2009
12/2009
3/2010
7/2010
10/2010
1/2011
4/2011
7/2011
10/2011
1/2012
4/2012
8/2012
11/2012
2/2013
5/2013
DATE (MO/YR)
WQN0201
WQN0272


Total Manganese - WQN0201 (Susq @ Marietta) &
WQN)272 (Swatara)
2006 - 2013
TOTAL MANGANESE (UG/L) - MO/YR AVG
800
700
600
500
400
300
200
100
0
01/2006
05/2006
09/2006
01/2007
05/2007
09/2007
01/2008
05/2008
09/2008
01/2009
05/2009
09/2009
01/2010
05/2010
09/2010
01/2011
05/2011
09/2011
01/2012
05/2012
09/2012
01/2013
05/2013
DATE (MO/YR)
WQN0201
WQN0272

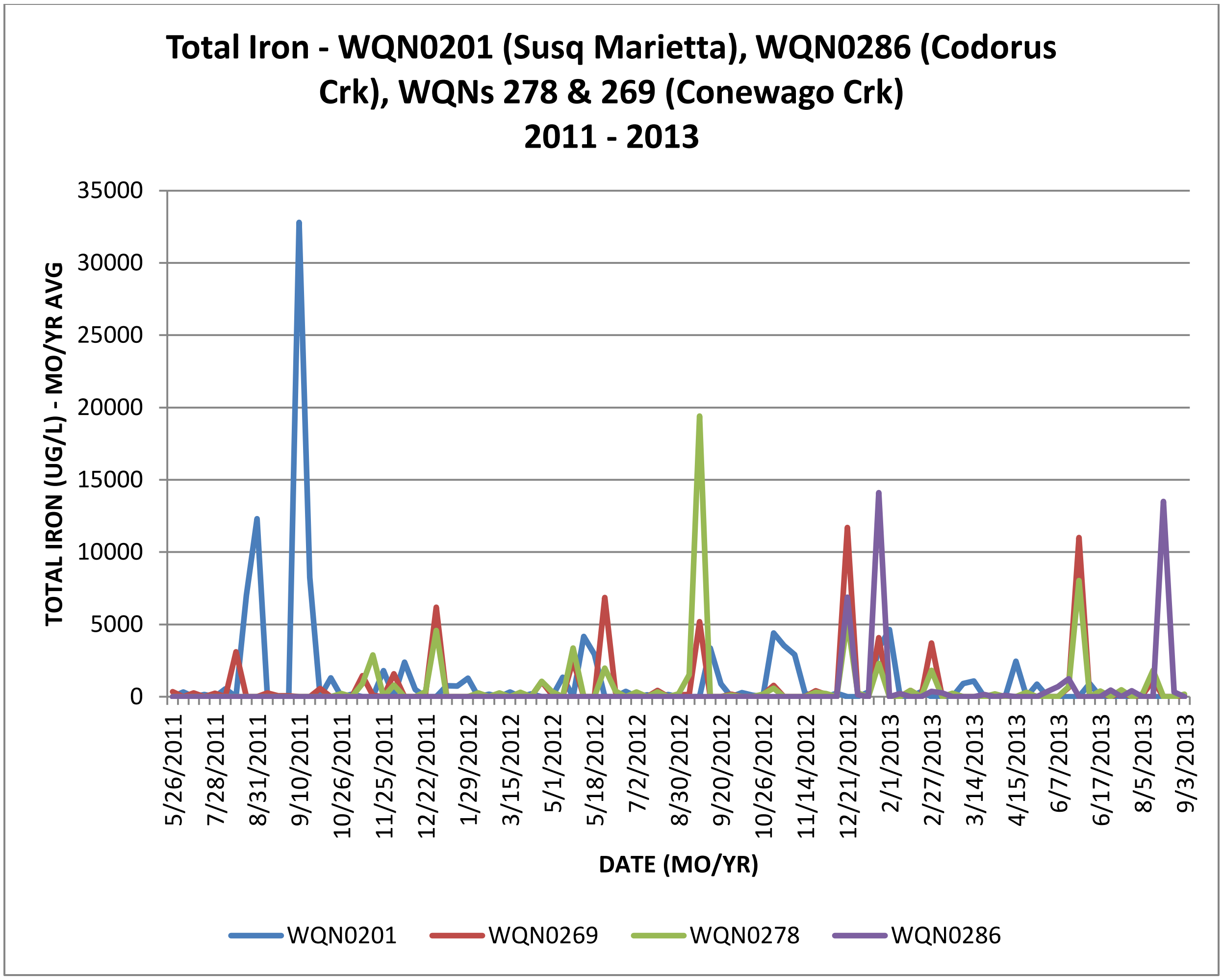

Total Lead - WQN0201 (Susq. @ Marietta) &
WQN0272 (Swatara)
2006 - 2012
TOTAL LEAD (UG/L) - MO/YR AVG
14
12
10
8
6
4
2
0
1/2006
4/2006
7/2006
10/2006
1/2007
5/2007
8/2007
11/2007
2/2008
5/2008
8/2008
11/2008
2/2009
5/2009
8/2009
11/2009
2/2010
5/2010
8/2010
11/2010
2/2011
5/2011
8/2011
11/2011
2/2012
5/2012
DATE (MO/YR)
WQN0201
WQN0272

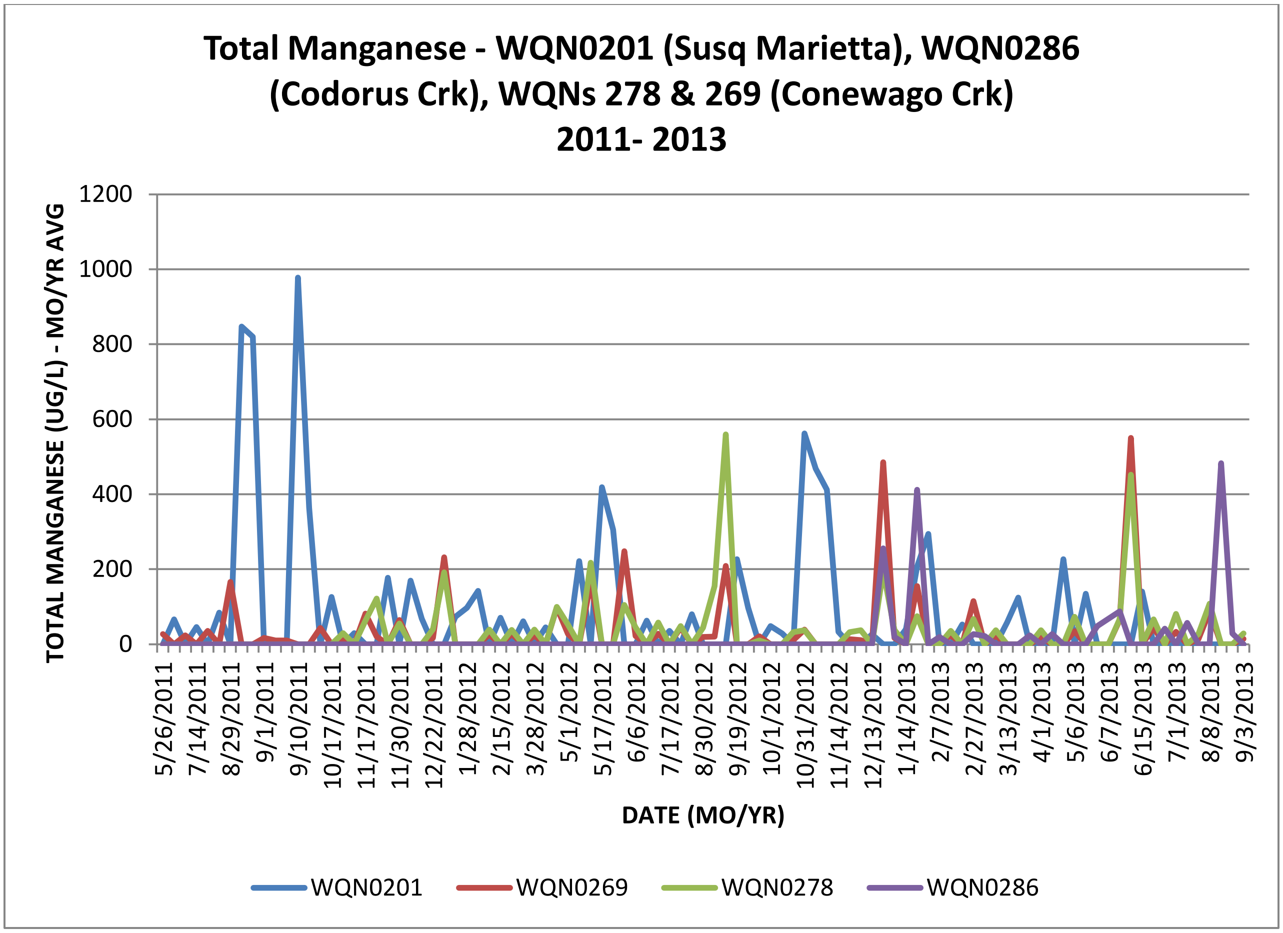


Alternatively, data for Codorus Creek (WQN286) and Conewago Creek (WQNs 278 and 269) were also graphed to view comparisons between those WQNs and WQN 201:



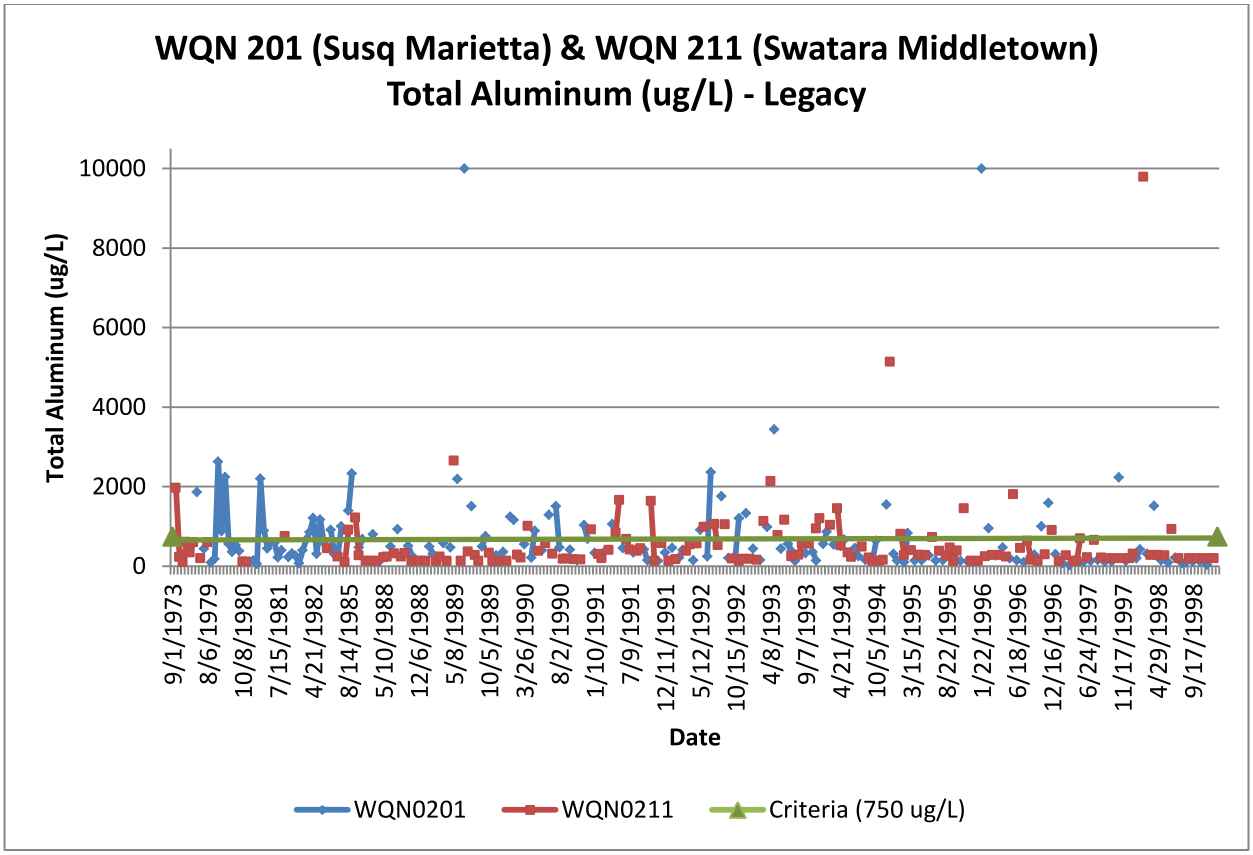
Total Aluminum - WQN0201 (Susq Marietta), WQN0286 (Codorus Crk), WQNs 278 & 269 (Conewago Crk)
2011 - 2013
TOTAL ALUMINUM (UG/L) - MO/YR AVG
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
12/17/1998
4/12/1999
8/12/1999
12/9/1999
2/11/2002
7/14/2011
8/30/2011
9/8/2011
10/17/2011
11/23/2011
12/12/2011
1/28/2012
2/28/2012
4/23/2012
5/17/2012
6/11/2012
8/14/2012
9/19/2012
10/11/2012
11/2/2012
12/13/2012
1/31/2013
2/14/2013
3/13/2013
4/2/2013
5/15/2013
6/15/2013
7/16/2013
8/22/2013
DATE (MO/YR)
WQN0201
WQN0269
WQN0278
WQN0286


Total Iron - WQN0201 (Susq Marietta), WQN0286 (Codorus Crk), WQNs 278 & 269 (Conewago Crk)
2011 - 2013
TOTAL IRON (UG/L) - MO/YR AVG
35000
30000
25000
20000
15000
10000
5000
0
5/26/2011
7/28/2011
8/31/2011
9/10/2011
10/26/2011
11/25/2011
12/22/2011
1/29/2012
3/15/2012
5/1/2012
5/18/2012
7/2/2012
8/30/2012
9/20/2012
10/26/2012
11/14/2012
12/21/2012
2/1/2013
2/27/2013
3/14/2013
4/15/2013
6/7/2013
6/17/2013
8/5/2013
9/3/2013
DATE (MO/YR)
WQN0201
WQN0269
WQN0278
WQN0286

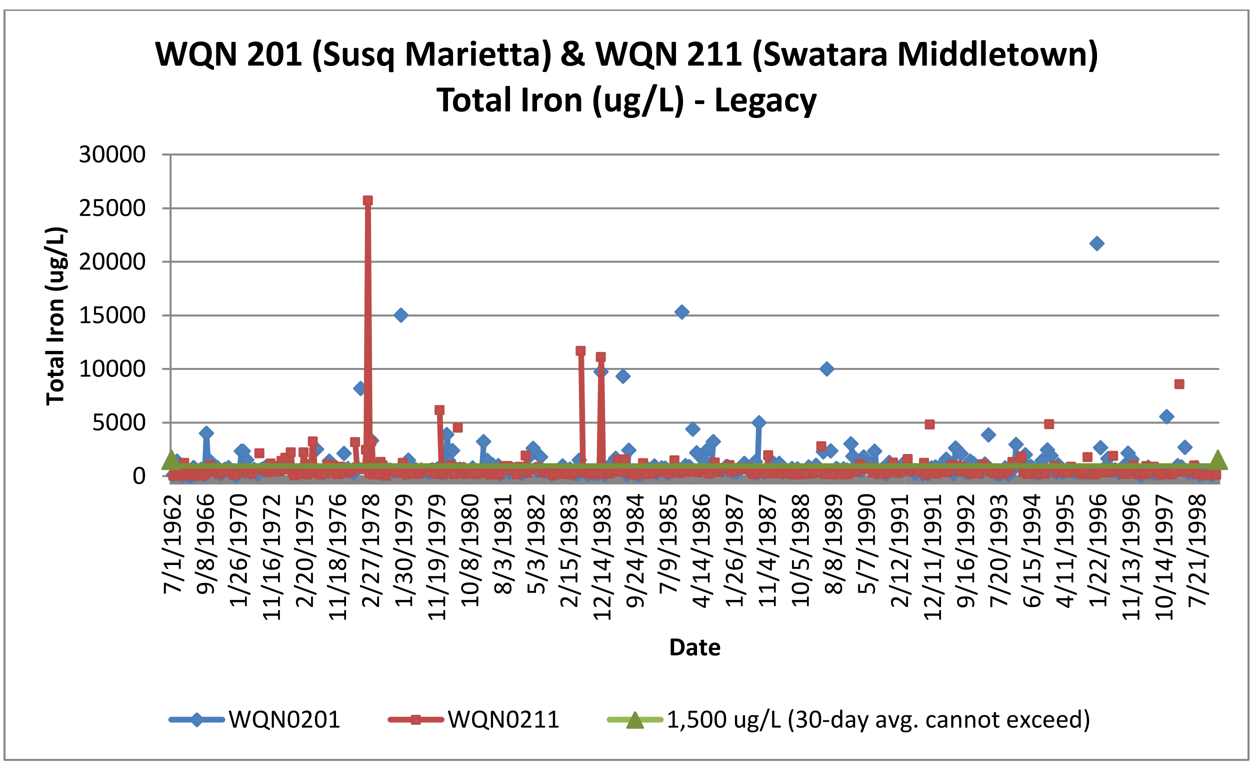


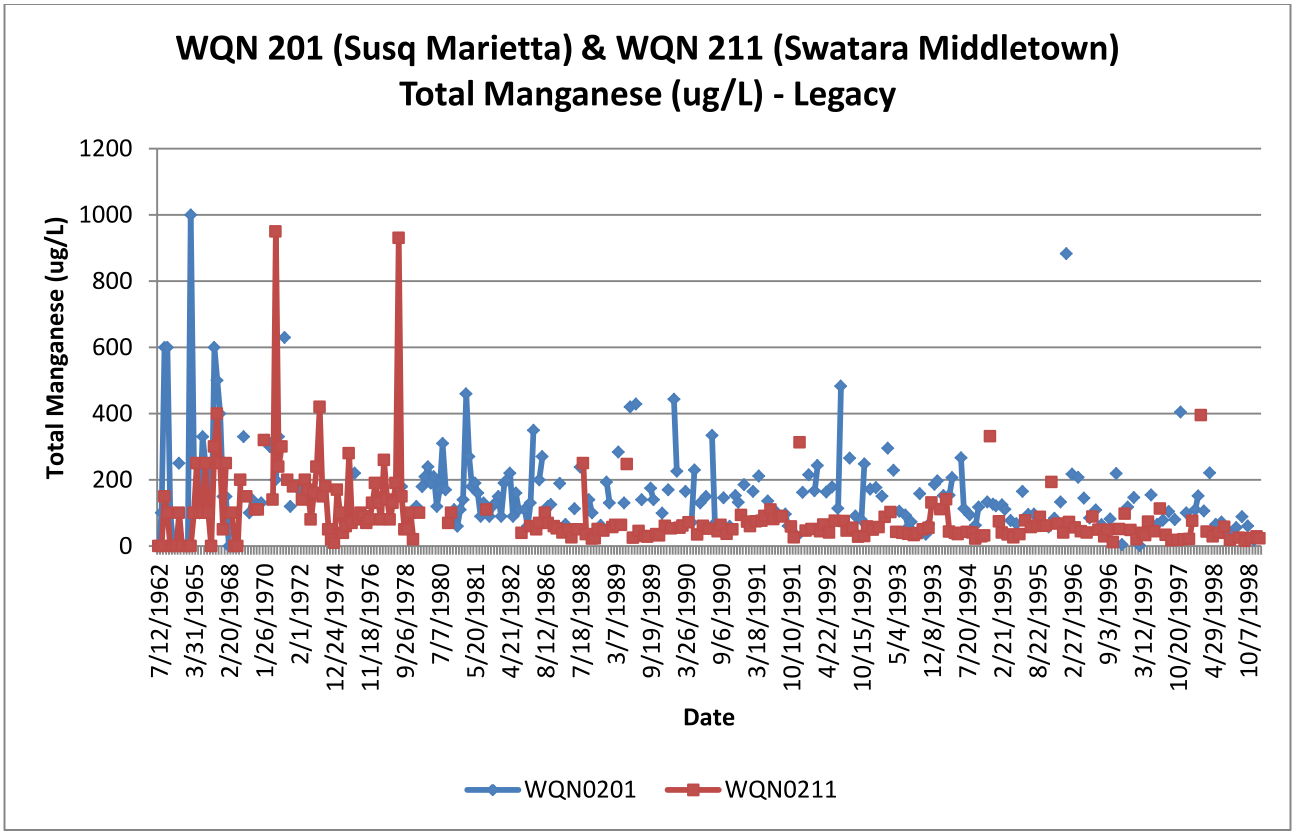
Samples were not collected on the same dates (points have been connected by lines for easier viewing).

Historical data on the Swatara (WQN211 – located further downstream than WQN272) was compared to historical data at Susquehanna River @ Marietta:



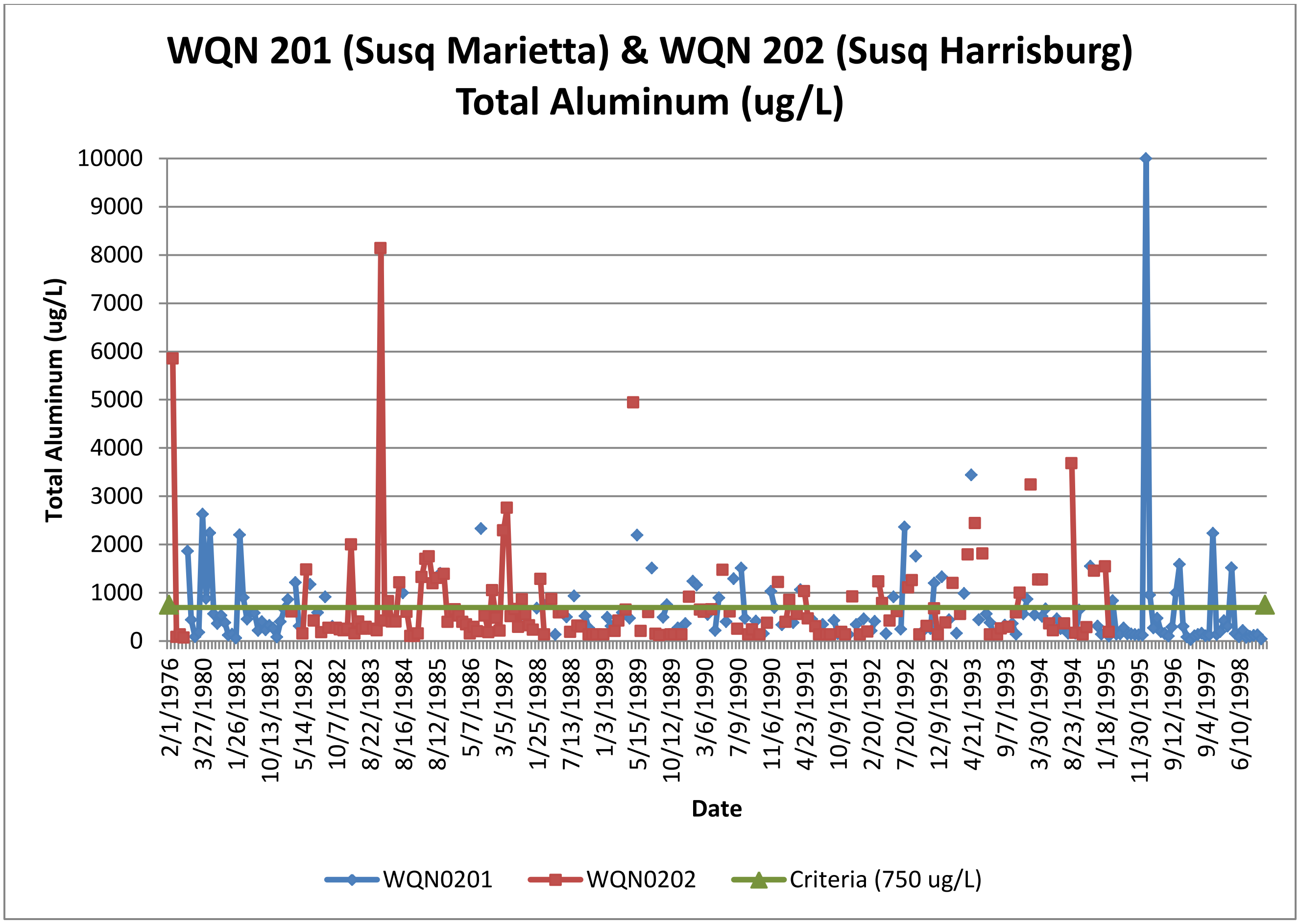
Note: Truncated graph for easier viewing by changing two values (20,000 ug/L on 1/22/1996 & 11,600 ug/L on 6/22/1989 at WQN201) to 10,000 ug/L.



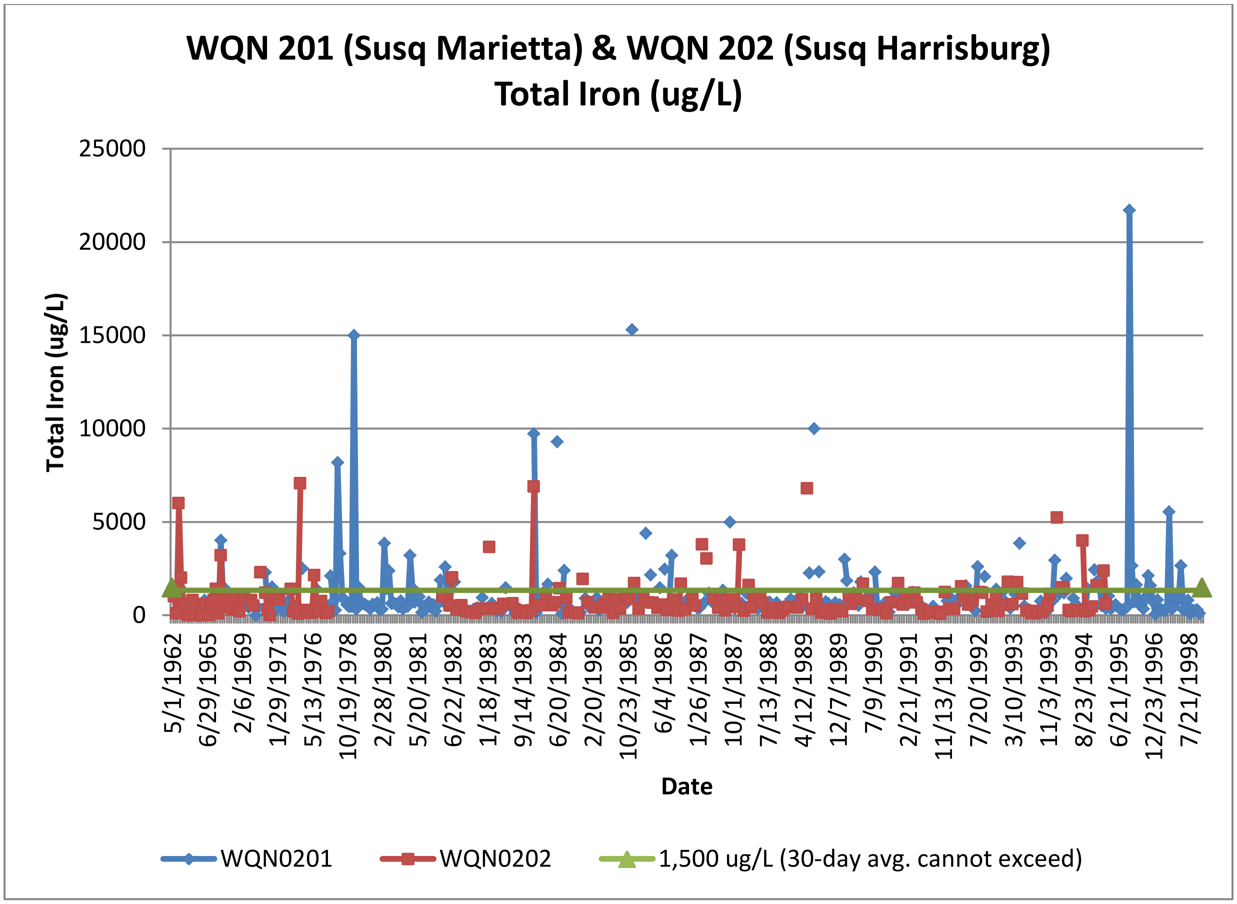


Patterns are difficult to discern from historical data on the Swatara and Susquehanna at Marietta. However, it's apparent that the Swatara has historically exhibited metals exceedances.

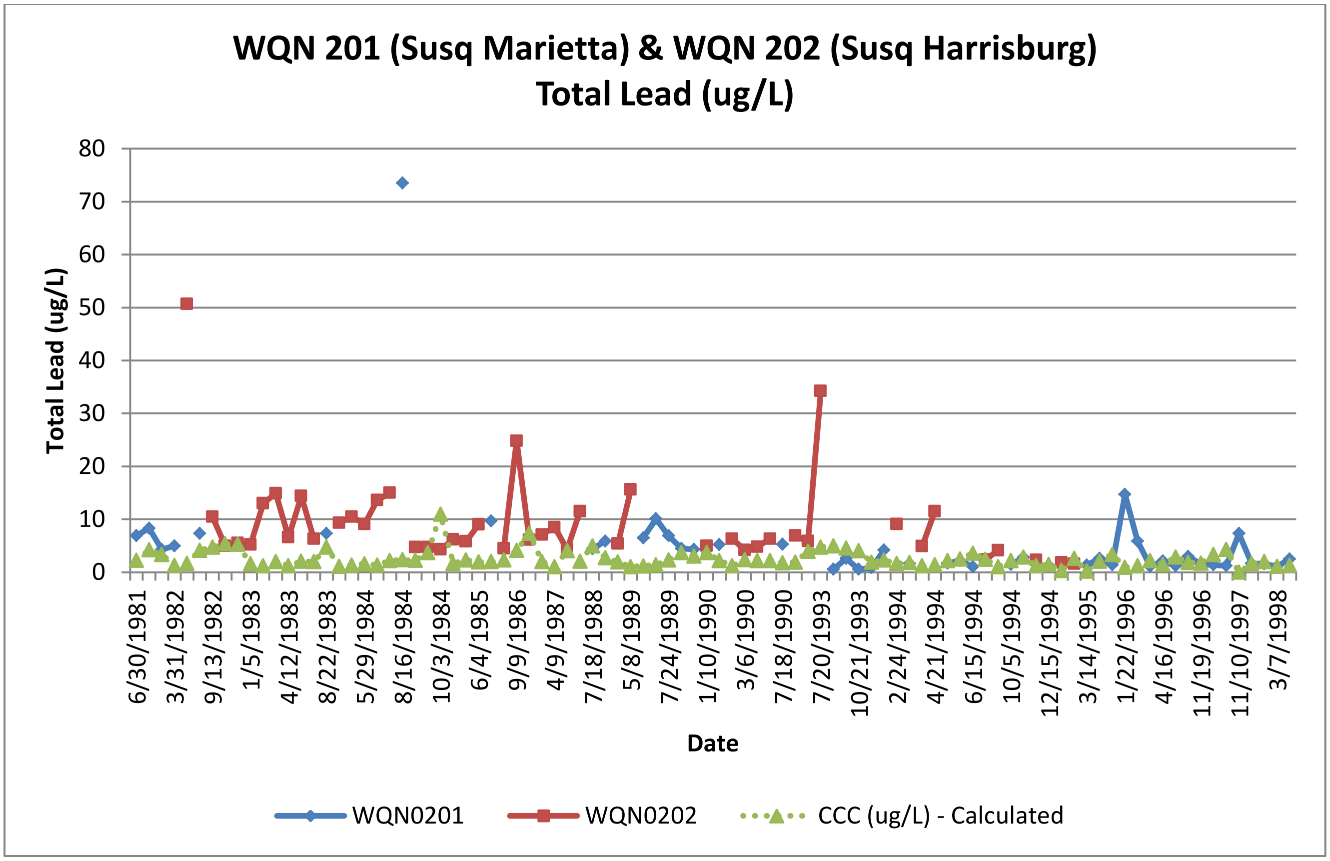
Next, stations further up the watershed were analyzed. WQN202 (Susquehanna River at Harrisburg) contains data from the 1960s – 1995 and was also reactivated in late 2012. Due to the lack of recent data at that station, data from 1976 through 1998 were compared at WQN201 (Susquehanna @ Marietta) and WQN202 (Susquehanna @ Harrisburg) first:



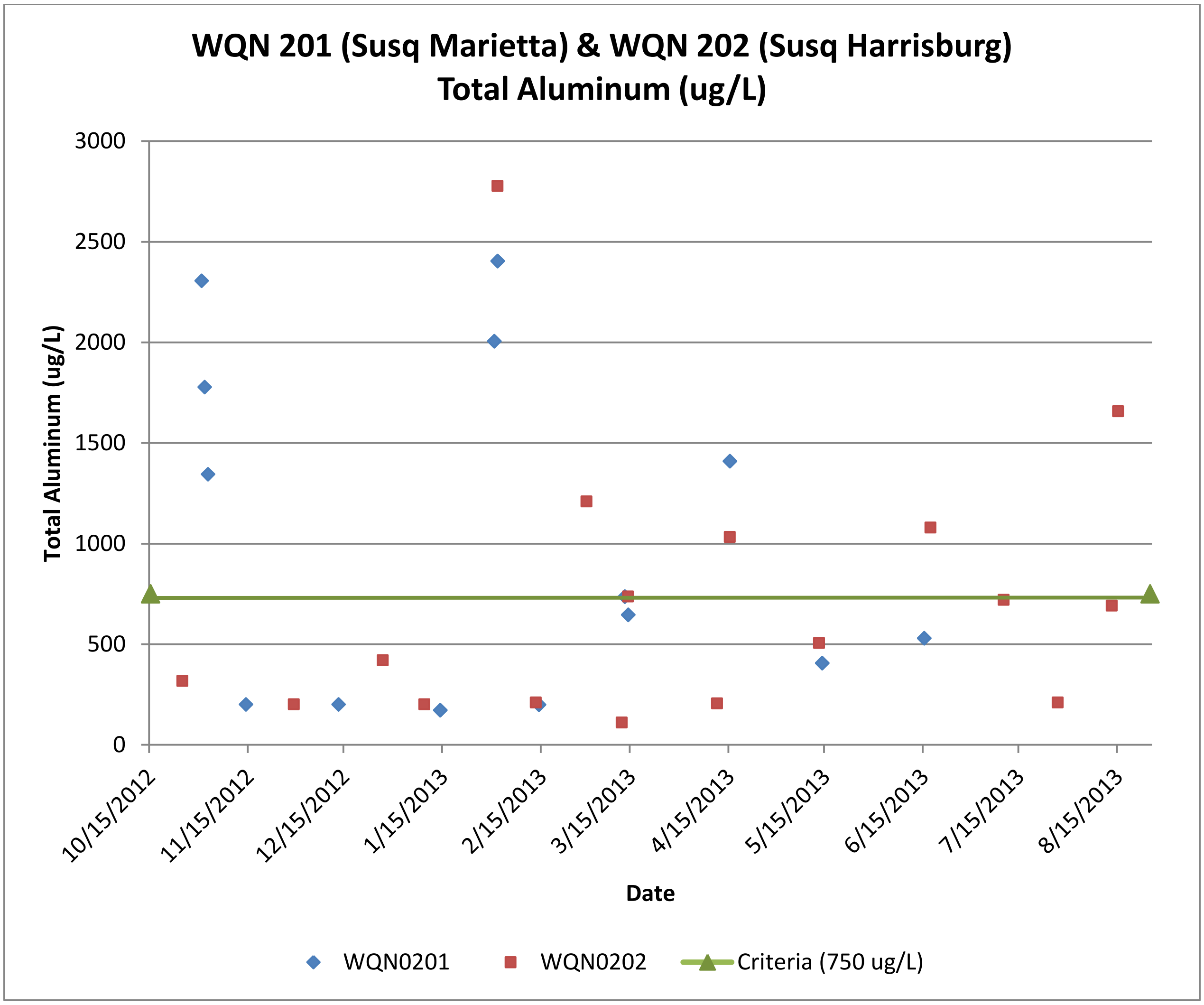
Although samples were often not collected at overlapping times at the two stations, it is apparent that total aluminum criteria (CMC = 750 ug/L, not to be exceeded more than once every 3 years) was exceeded many times at both locations through the years.



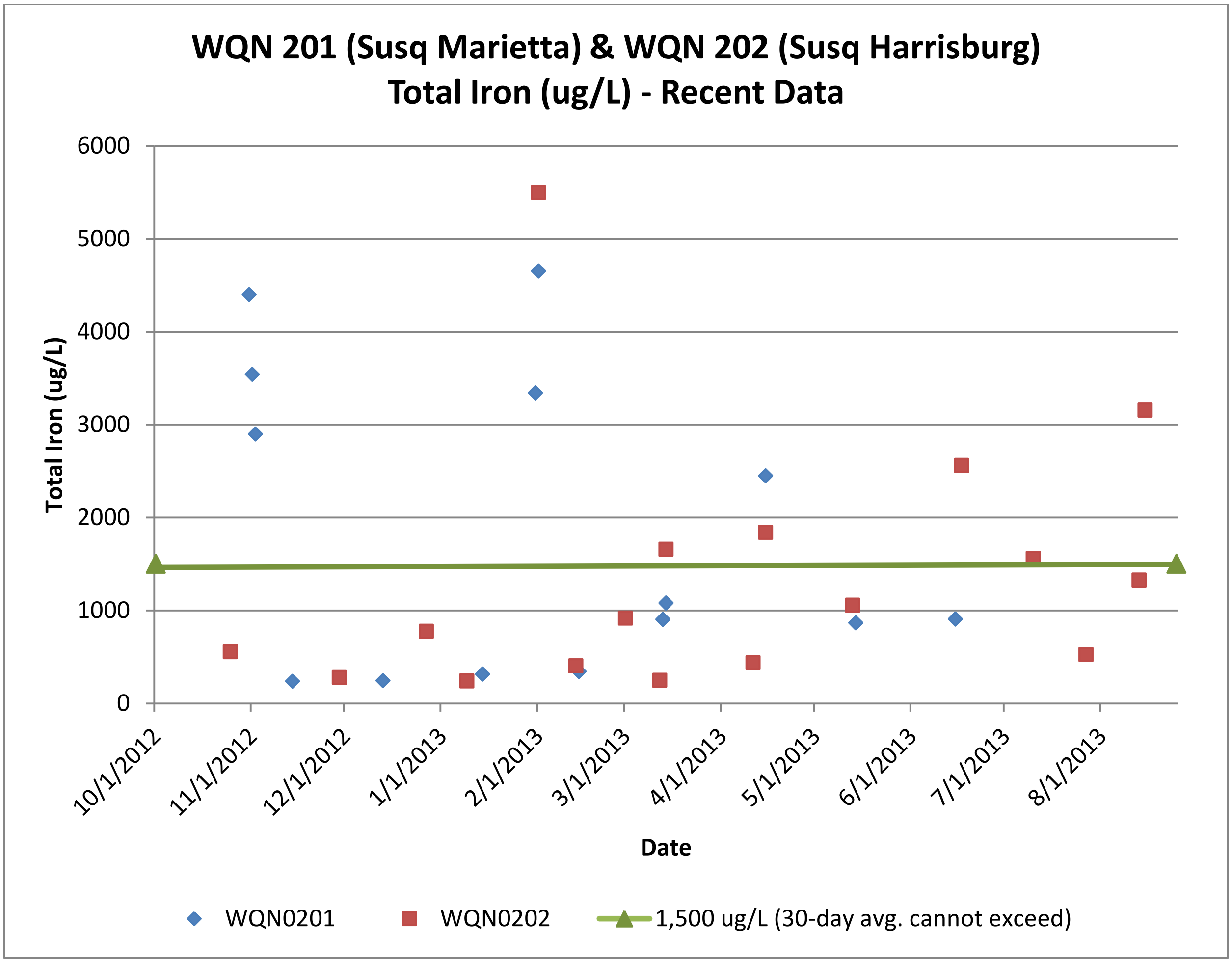
Iron is exceeded when the 30-day average exceeds 1,500 ug/L. Because locations were sampled mainly once per month, this is exceeded multiple times by both stations.



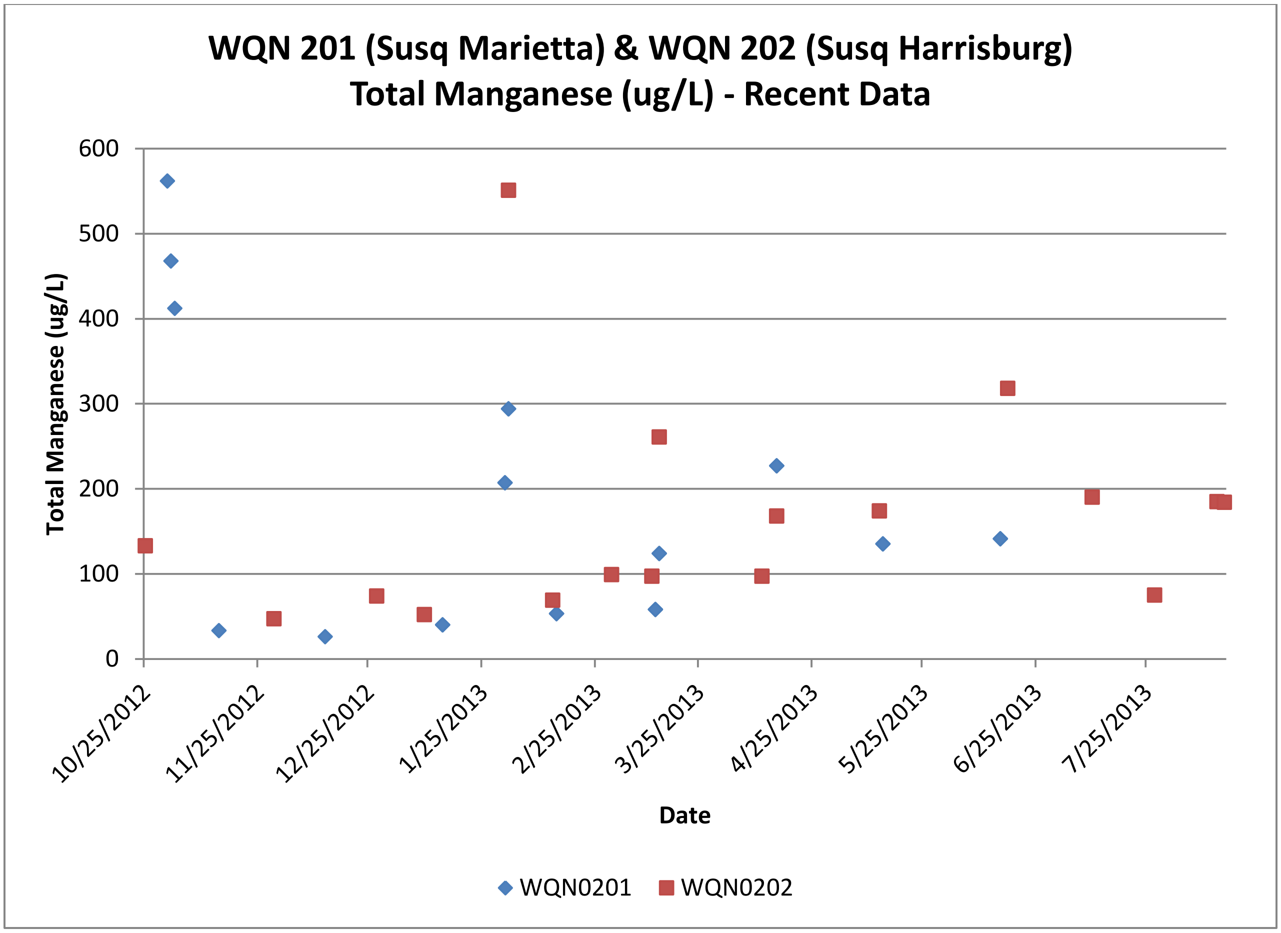
In the graph above, lead criteria continuous concentrations (CCC), calculated based on hardness, are shown with lead results from samples at WQNs 201 and 202. Sample results that were less-than-detect were omitted from the analysis. This was done because some of the method detection limits were quite high (50 ug/L) which would erroneously suggest elevated values based on the calculated criteria. There was at least one time where CCC was exceeded at WQN 201 in that set of data; since samples were largely collected just monthly most of the time at both locations, it is difficult to assume criteria are exceeded more often. Criteria maximum concentrations (CMC) were never exceeded. Therefore, one can speculate that at least some of the metals loading seen at Marietta comes from further upstream than Harrisburg, at least from the period of 1962 – 1995. Next, more recent data are looked at for WQN 202 (Susquehanna River @ Harrisburg), although less is available (late 2012 – present only):



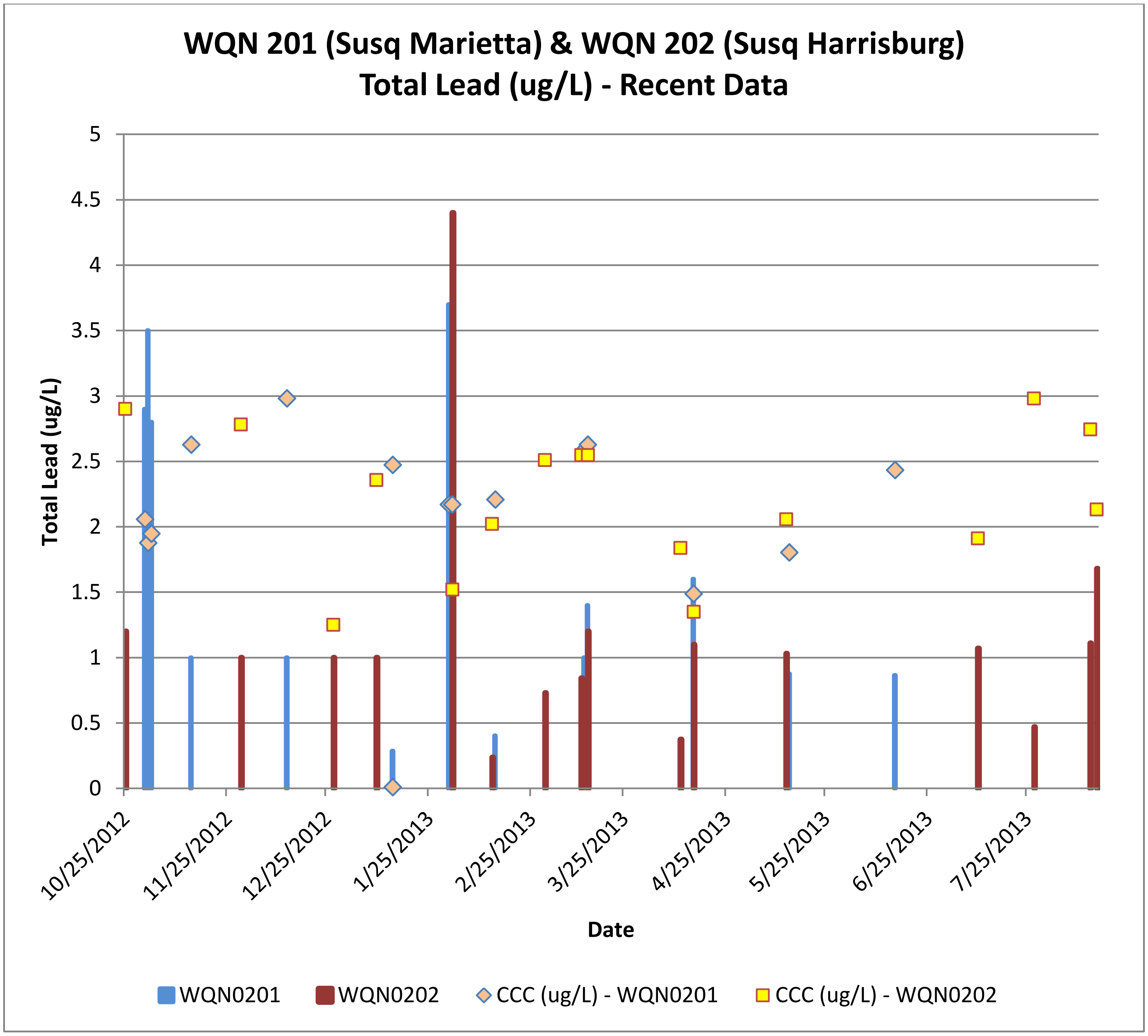
Aluminum spikes occurring in the past year at WQN 201 do not appear to be closely related to WQN 202.



Like aluminum, iron spikes occurring in the past year at WQN 201 do not appear to be closely related to WQN 202.

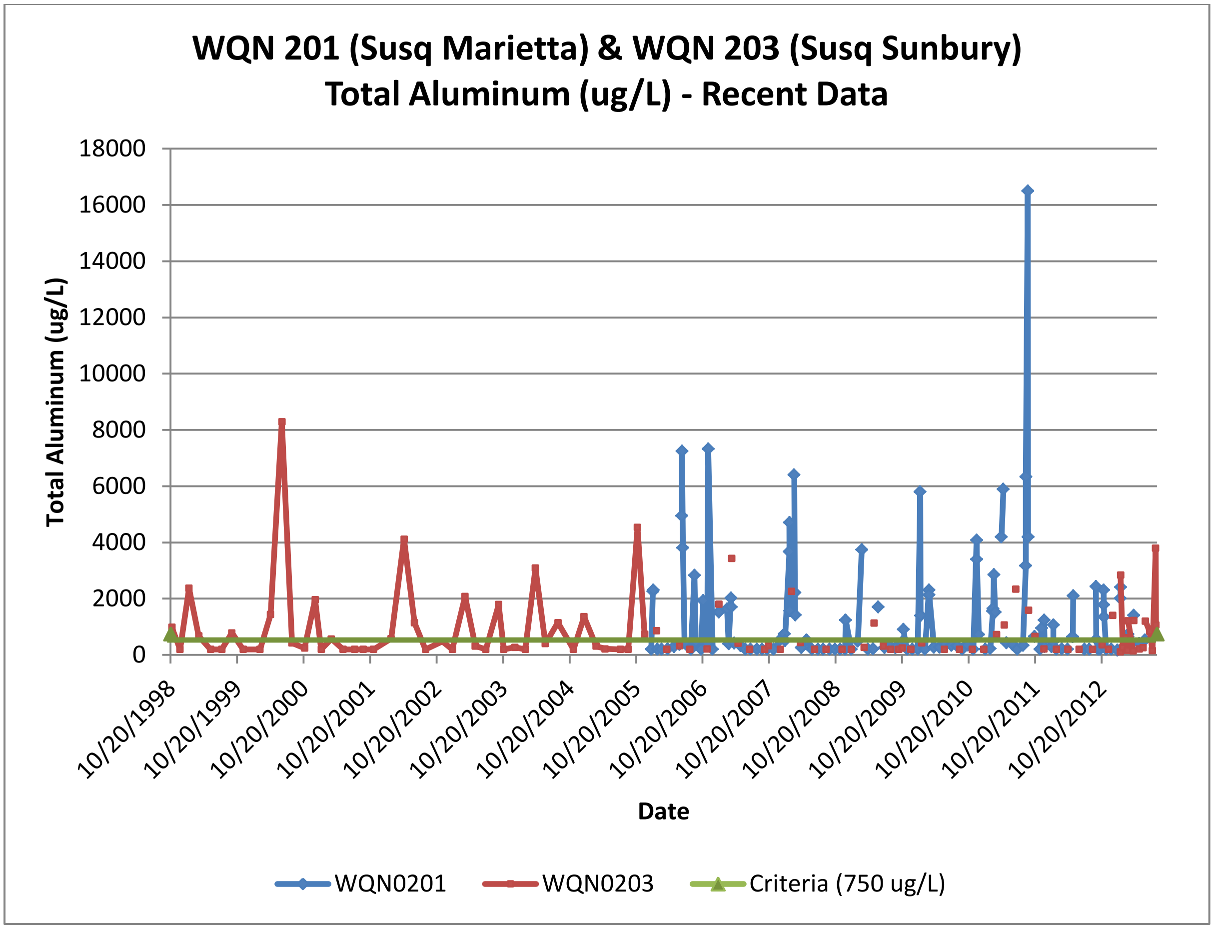


Total manganese had a similar pattern as the previous two graphs.

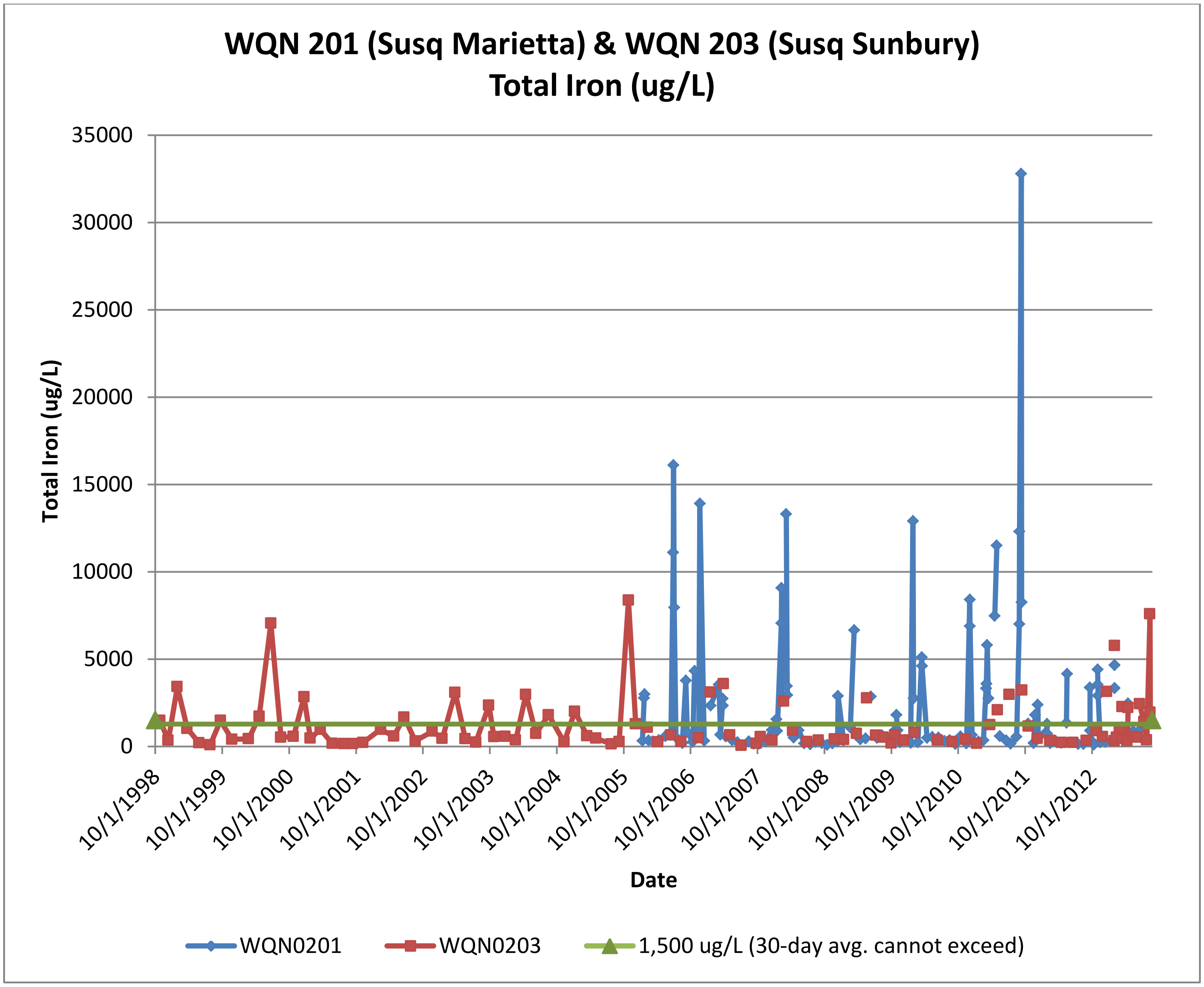


There were more lead CCC exceedances at WQN 201 in the past year than there were at WQN 202.

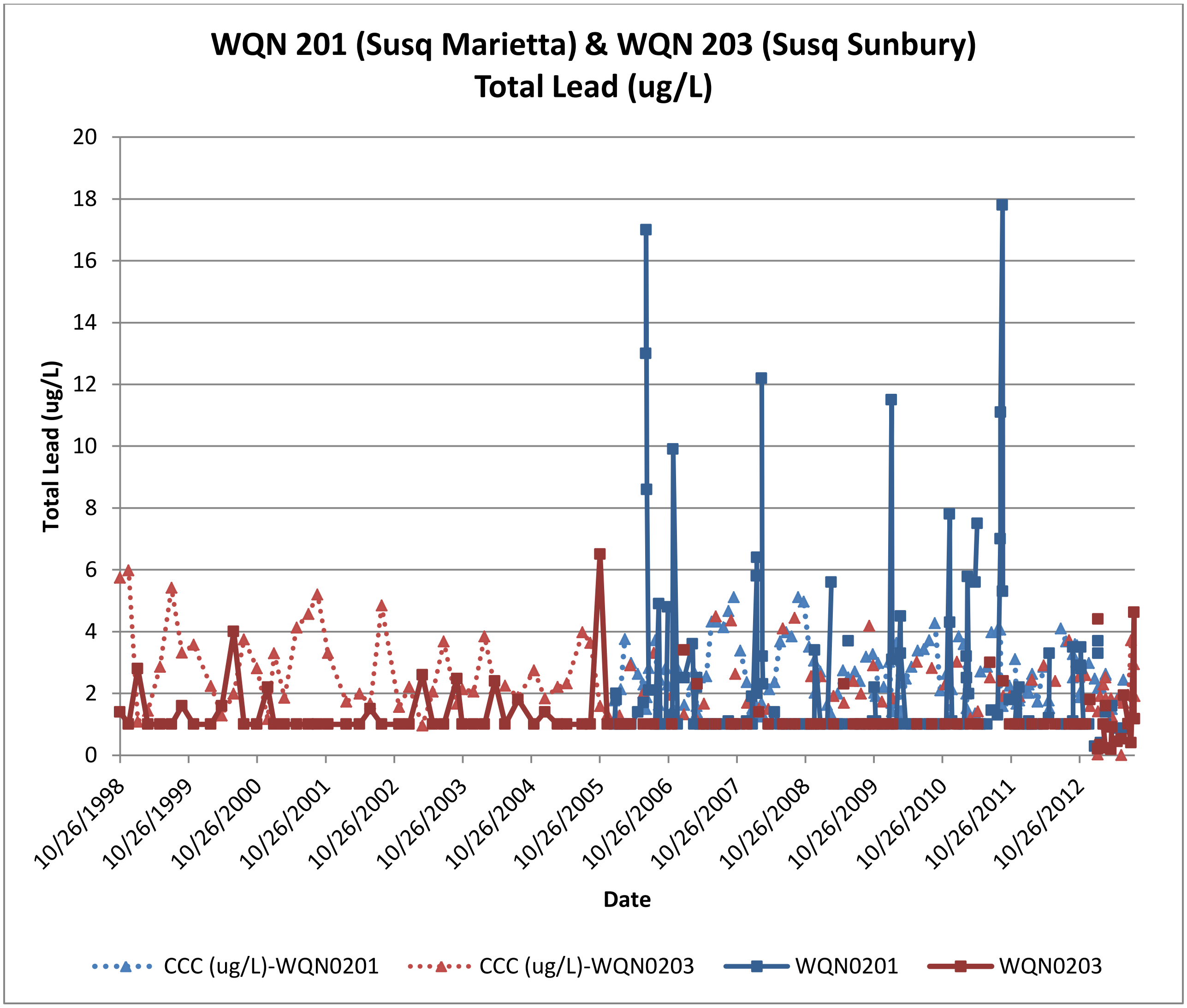
Results from WQN203 (Susquehanna River @ Sunbury), which is even further upstream, are presented in the following four graphs:



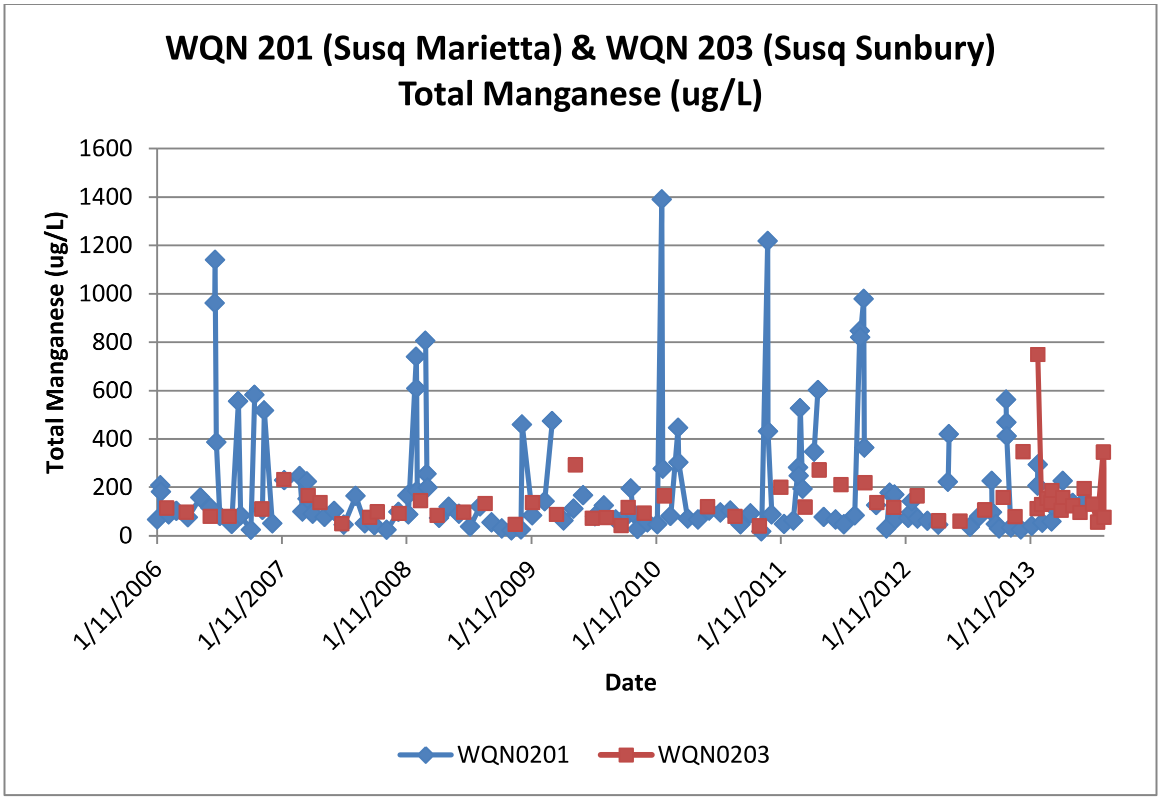
Total aluminum did exceed its 750 ug/L criterion multiple times within 3-year time periods at WQN 203 (Susquehanna @ Sunbury). While samples were collected at different times at WQN 203 and 201, the above graph shows that WQN 203 has a history of exceeding the aluminum criterion.



Total iron was exceeded several times upstream at WQN203; however, there are noticeably higher exceedances downstream at WQN201.

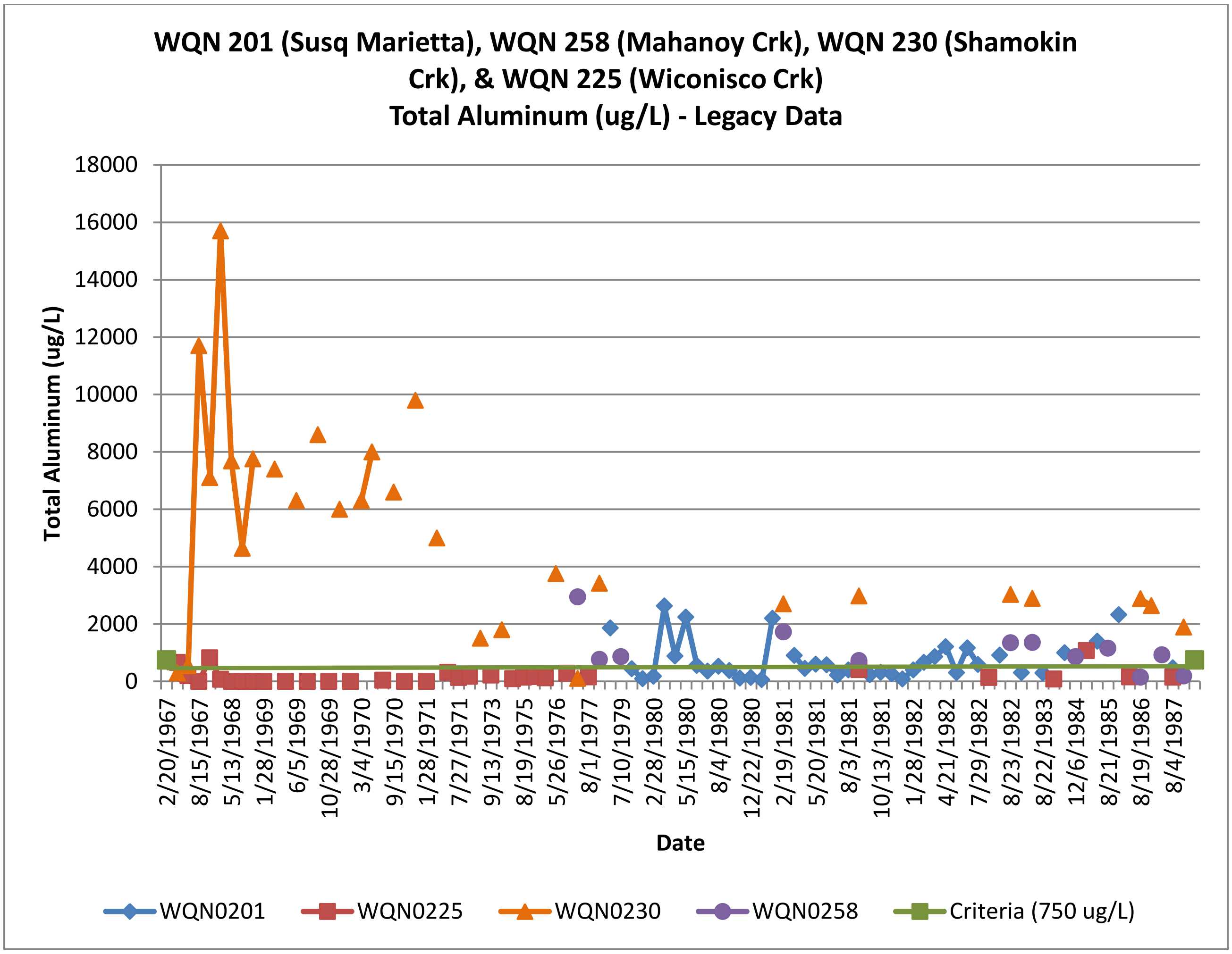


Like iron, total lead was also much higher at WQN201 than at WQN203 and CCC was exceeded more frequently.

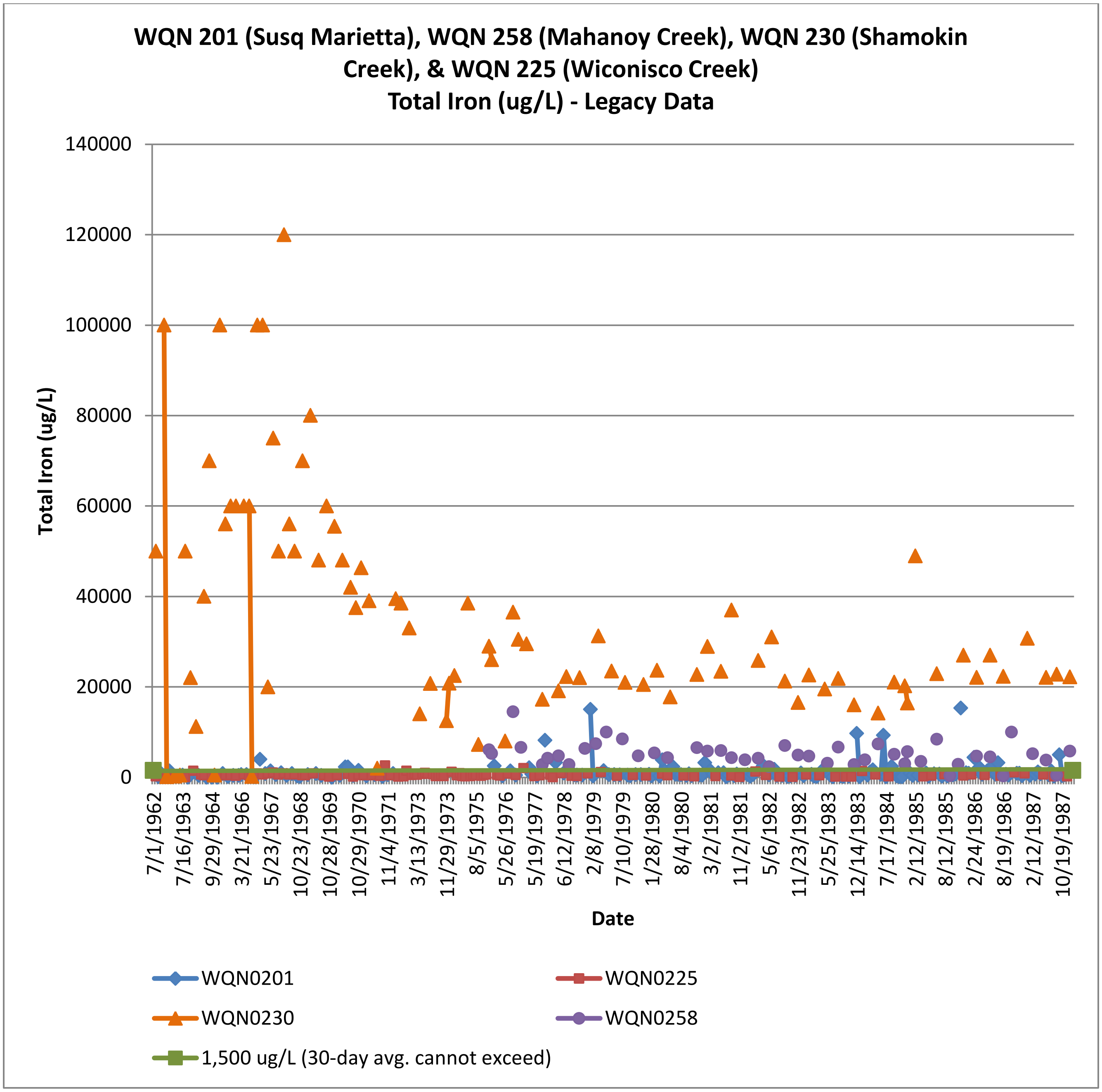


Additionally, manganese showed higher spikes at WQN 201 than at WQN 203 (however, there are no aquatic life criteria for manganese).

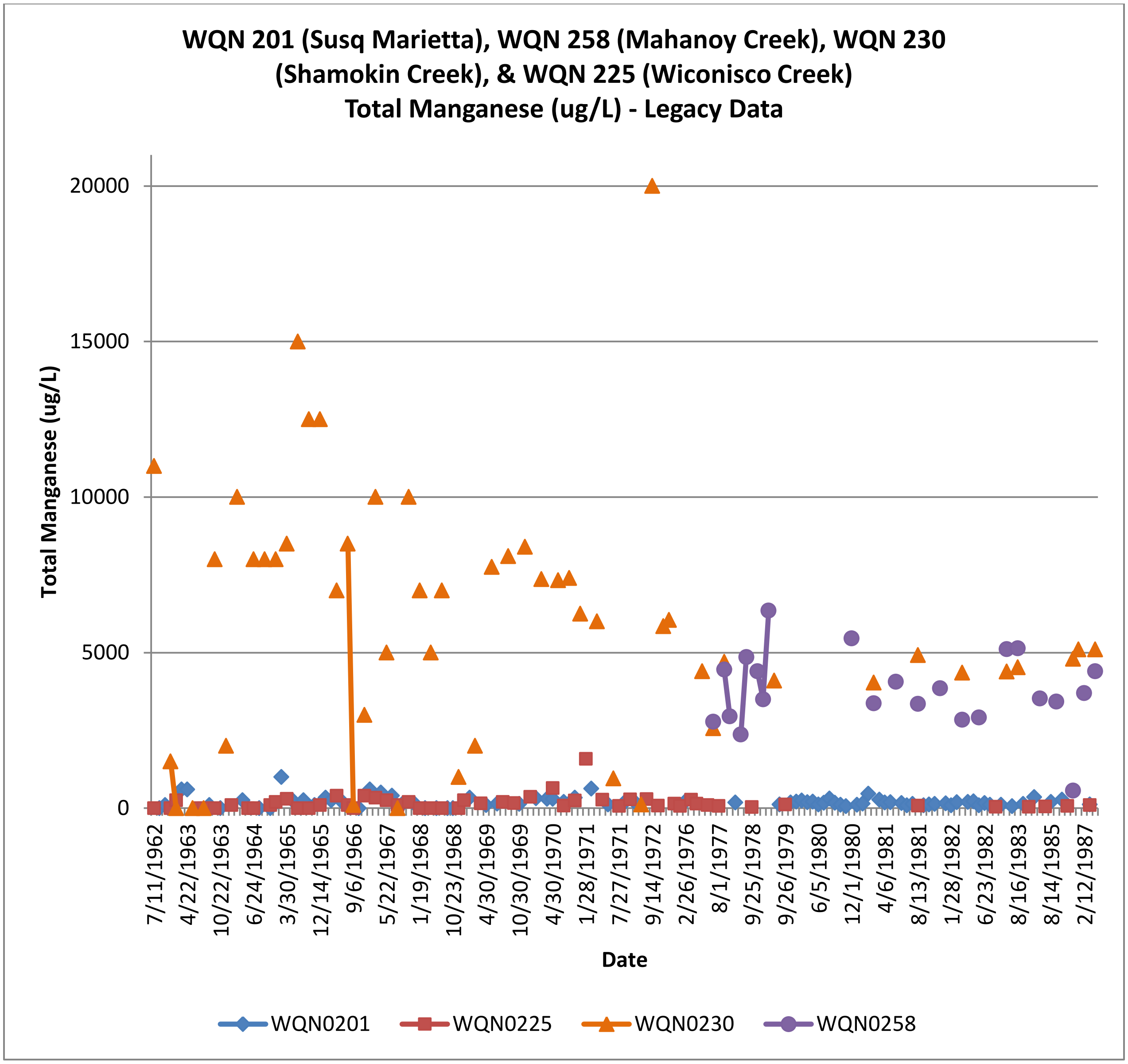
The above graphs of Sunbury data suggest that while elevated metals are in the river upstream, in all likelihood the major sources of the metals at Marietta are located further downstream than Sunbury.



Downstream of Sunbury, but upstream of Harrisburg, many tributaries enter the Susquehanna River. Above is historical total aluminum data in a few of the tribs (WQN258-Mahanoy Creek, WQN230-Shamokin Creek, and WQN225-Wiconisco Creek) compared to Susquehanna River @ Marietta (WQN201). Samples were rarely collected on the same day at multiple stations; however, these graphs show general trends in the various metals through the years. Shamokin Creek has historically had the highest aluminum values.



Likewise, Shamokin Creek has had historically elevated total iron values. Mahanoy Creek (WQN258) had slightly elevated iron values as well.



Note: Truncated graph for easier viewing by changing one value (95,000 ug/L on 9/14/1972 at WQN230) to 20,000 ug/L.

Manganese was also elevated at Shamokin Creek. It has also historically been elevated on Mahanoy Creek (WQN258).

The above data analyses on WQN data suggest that metals loading to WQN201 (Susquehanna River @ Marietta) comes from a variety of sources.

Conclusion: There is no consistent pattern between prevalence of metals and low recruitment of YOY SMB.

Title: Grab Metal Samples (WS #46)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected water grab samples to be tested for metals within and out of the Susquehanna basin from approximately 121 sites in 2013 and 2014 (analyzed at DEP Bureau of Laboratories).

Analysis and Results:

A look at the average of each parameter per site showed consistent sites that had elevated metals (see graphs below for summaries per metal). Youghiogheny River often had elevated metals, particularly aluminum, iron, and zinc. Some smaller sites were consistently elevated in a variety of metals, such as Goose Creek, Indian Creek, Towamencin Creek, Dunkard Creek, and Lower Rausch Creek. W. Br. Susquehanna River at Karthaus consistently had elevated metals, such as boron, manganese, and sulfate. Susquehanna River at Rockville, Clemson Island, and Browns Island also had elevated values of various metals (see table 3 for a summary).

The metals parameters for which Pennsylvania's aquatic life criteria have been established include iron (total), copper (total & dissolved), lead (total & dissolved), nickel (total – although most were non-detect in this study), zinc (total & dissolved), aluminum (total), barium (total), and boron (total) (Table 1):

Compound	Criteria
Iron (Total)	30-day avg. 1.5 mg/L (1,500 ug/L) as total recoverable
Copper (Dissolved)	Equations for CCC and CMC
Copper (Total)	Conversion factors for CCC and CMC
Lead	Equations for CCC and CMC

(Dissolved)	
Lead (Total)	Conversion factors for CCC and CMC
Nickel (Total)	Conversion factors for CCC and CMC
Zinc (Dissolved)	Equations for CCC and CMC
Zinc (Total)	Conversion factors for CCC and CMC
Aluminum (Total)	750 ug/L CMC
Barium (Total)	4,100 ug/L CCC; 21,000 ug/L CMC
Boron (Total)	1,600 ug/L CCC; 8,100 ug/L CMC

Table 1: PA DEP metals aquatic life criteria

Aluminum criteria are exceeded at several sites Susquehanna River @ Rockville, Marietta, Clemson Island, and Browns Island (was often >2,000 ug/L), Youghiogheny River, Chillisquaque Creek, Tohickon Creek, W Br Mahantango Creek, Juniata River at Newport, Buffalo Creek, and Kishacoquillas Creek. Barium and Boron criteria were never exceeded. The iron 30-day average was exceeded on several occasions: Youghiogheny River @ Sutersville, Susquehanna River @ Rockville, Susquehanna River @ Marietta, Susquehanna River @ Clemson Island, and Susquehanna River @ Browns Island.

Dissolved copper criteria (calculated) are never exceeded in any sample. Total copper (calculated) was exceeded in six samples (Table 2):

Location	Date	Copper Concentration (ug/L)	Criteria Maximum Concentration (CMC) (ug/L)	Criteria Continuous Concentration (CCC) (ug/L)
Browns Run	9/10/2013	5.51	1.59	1.3
Kettle Creek	7/31/2014	10.3	3.5	2.66
Neshaminy Creek	4/24/2013	34.9	17	11.13
Perkiomen Creek	7/24/2014	10.8	13.9	9.33
Pine Creek	7/24/2014	4.94	6.59	4.72
Tohickon Creek (Pipersville)	4/24/2013	20.4	8.5	5.94
Tohickon Creek (Pipersville) - Duplicate	4/24/2013	25.2	8.9	6.2

Table 2: Copper criteria exceedances

Dissolved lead criteria (calculated) were never exceeded. Total lead CCC was exceeded in several individual instances, but the CMC was never exceeded – CCC was only exceeded on occassion, not frequently. Most total lead CCC exceedances were on the Susquehanna River and Tohickon Creek.

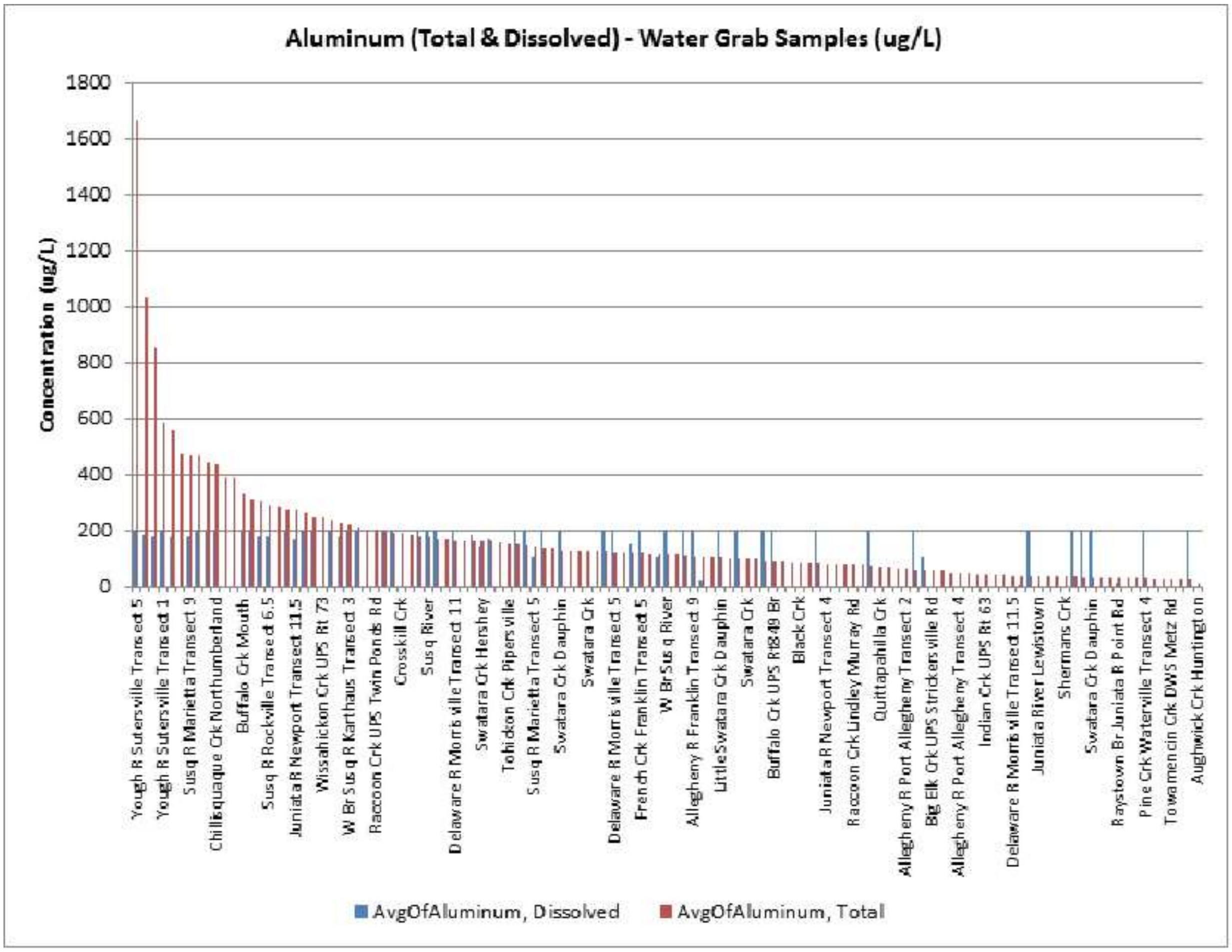
Total nickel (calculated) CCC or CMC were never exceeded.

Dissolved zinc (calculated) CCC or CMC were exceeded only once – 9/15/2014 on the Susquehanna River. Dissolved zinc was 297 ug/L, exceeding both the CCC and CMC. Total zinc (calculated) CCC and CMC were only exceeded three times – Buffalo Creek UPS Route 849 Bridge (5/1/2013 and 5/23/2013) at 71 ug/L and 149 ug/L, respectively; and Raccoon Creek UPS Twin Ponds Road (5/1/2013) at 63 ug/L.

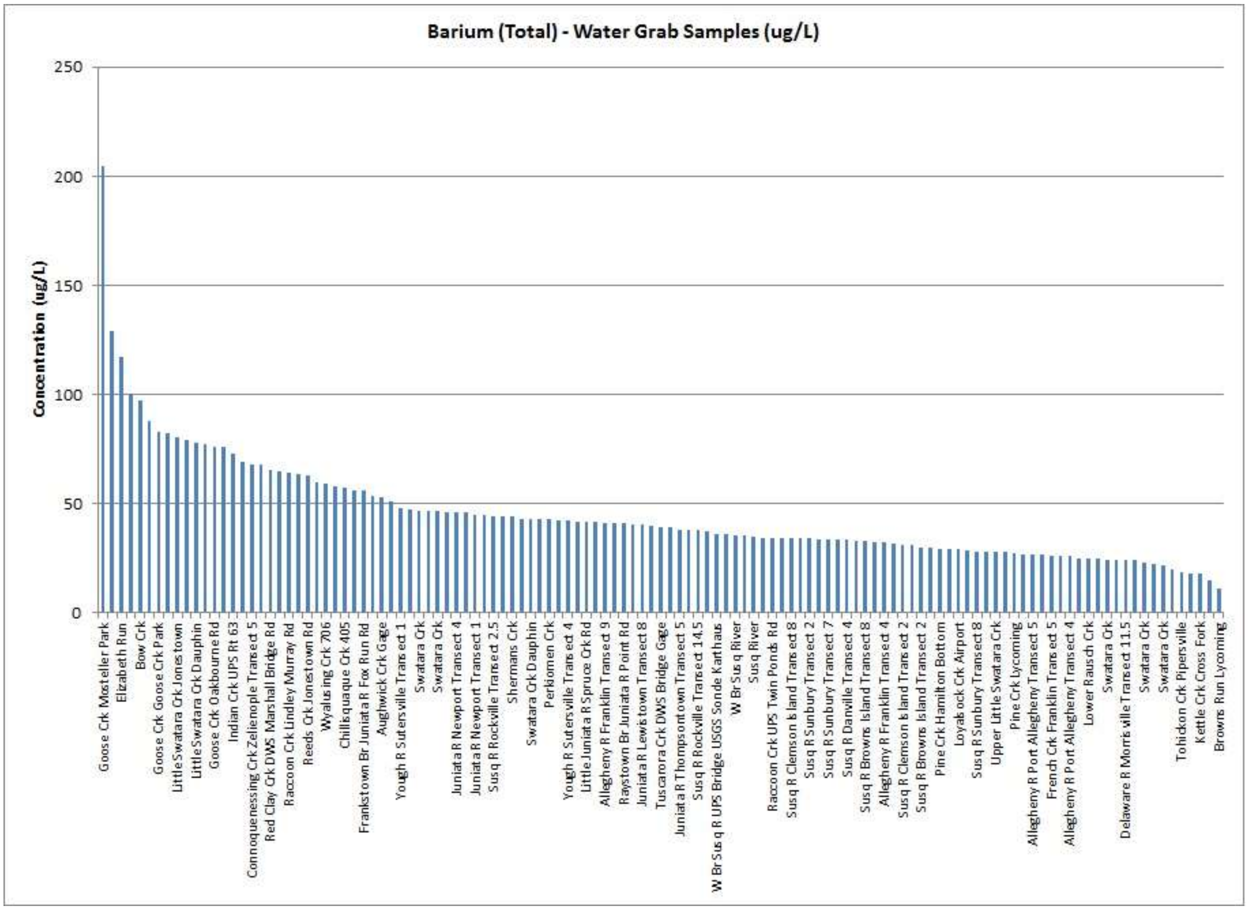
Overall, aluminum, iron, and copper criteria were exceeded most often, although not very frequently. There were no extreme patterns to these exceedances, except that aluminum and iron criteria were most frequently exceeded on the Susquehanna River.

	Listed in order of highest concentrations --> Lowest
Compound	**Summary**
Aluminum (total)	Youghiogheny highest; Susquehanna @ Rockville, Clemson Island, Browns Island next highest (>500 ug/L)
Barium (total)	smaller sites were highest - Goose Creek, Earlakill Run, Elizabeth Run, Towamencin Crk, Bow Crk, Wissahickon Crk >85
Boron (total)	smaller sites were highest - Indian Crk, Goose Crk, W Br Susq River, Indian Crk >70
Copper (total)	relatively low at all sites; highest at Neshaminy Crk, Goose Crk, Kettle Crk (>10)
Iron (total)	>900 at Yough, Susq Rockville, Susq Clemson Island, Susq Browns Island, Susq Marietta, Chillisquaque Crk
Iron (dissolved)	Allegheny, French Crk, Yough had highest levels of dissolved (>150)
Lead (total)	Relatively low at all sites; highest at Pine Crk, Yough, Neshaminy Crk (>2)
Bromide (ug/L)	>100 at S Fork Tenmile Crk, Dunkard Crk, Towamencin Crk, Goose Crk (3 locations)
Magnesium (total)	>19 at Goose Crk (2 locations), Lower Rausch Crk, Quittapahilla Crk
Manganese (total)	>500 at W Br Susq Karthaus, Lower Rausch Crk
Manganese (dissolved)	>100 at Pine Crk Lower Mainstem, W Br Susq Karthaus, Bald Eagle Crk (15,000 at Pine Crk! Mistake?)
Nickel (total)	mainly very low (13) but 18 at W Br Susq Karthaus - Not high at all, not graphing
Selenium (total)	>1 (but not by much) - Delaware R Morrisville PWS intake, Conodoguinet Crk, Susq Sunbury, Delaware R Morrisville Transect 11
Chloride	many are high, >100 - Towamencin Crk, Skippack Crk (both >250), Indian Crk, Goose Crk, Wissahickon Crk, Elizabeth Run, Neshaminy Crk
Sulfate (total)	>100 - Dunkard Crk, W Br Susq Karthaus, Lower Rausch Crk
Strontium (total)	>500 - Doe Run, Shermans Crk, Kishacoquillas Crk, Indian Crk
Zinc (total)	>30 - Indian Crk, Buffalo Crk, Raccoon Crk, Goose Crk, Yough; one dissolved zinc anomoly at Susq River: avg was 105

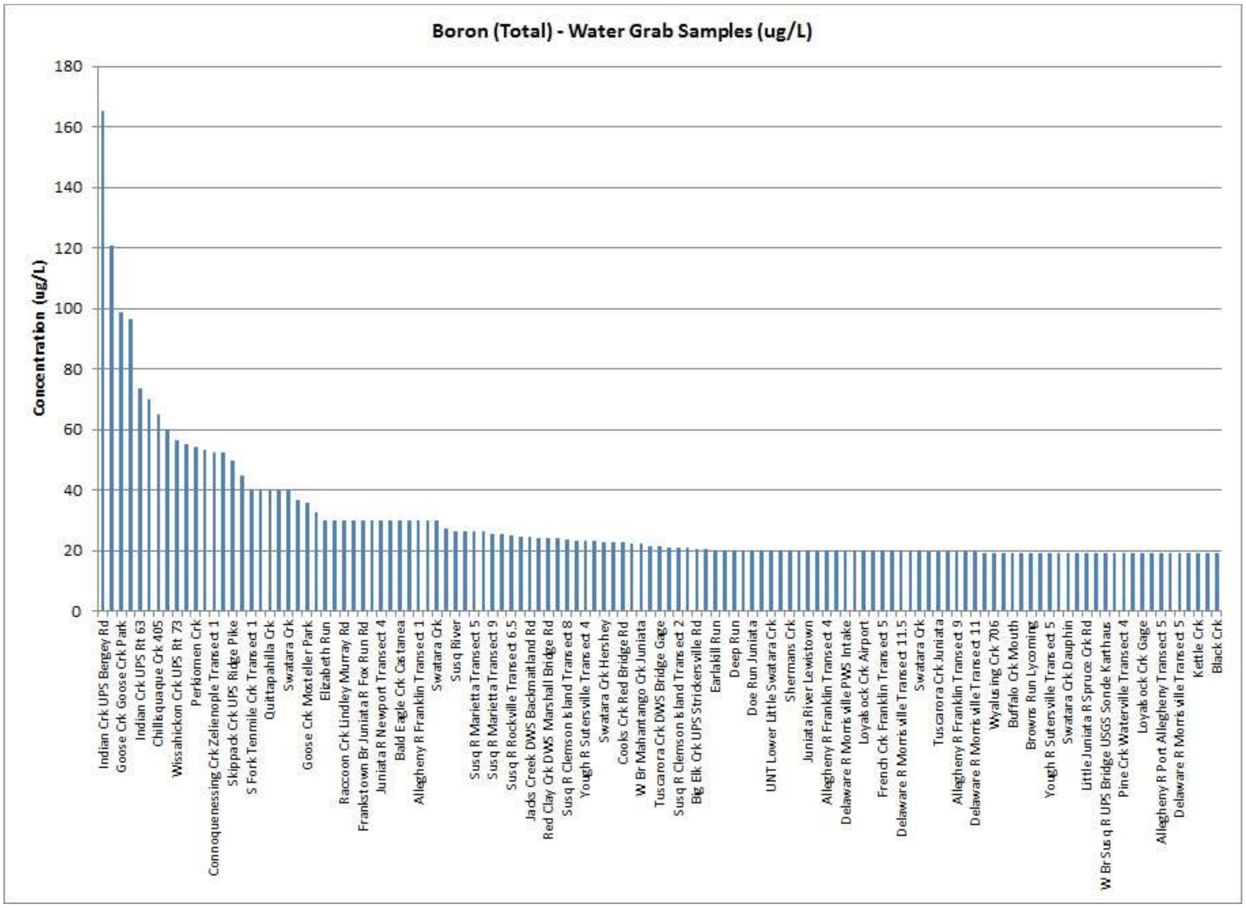
Table 3: Summary of metals results

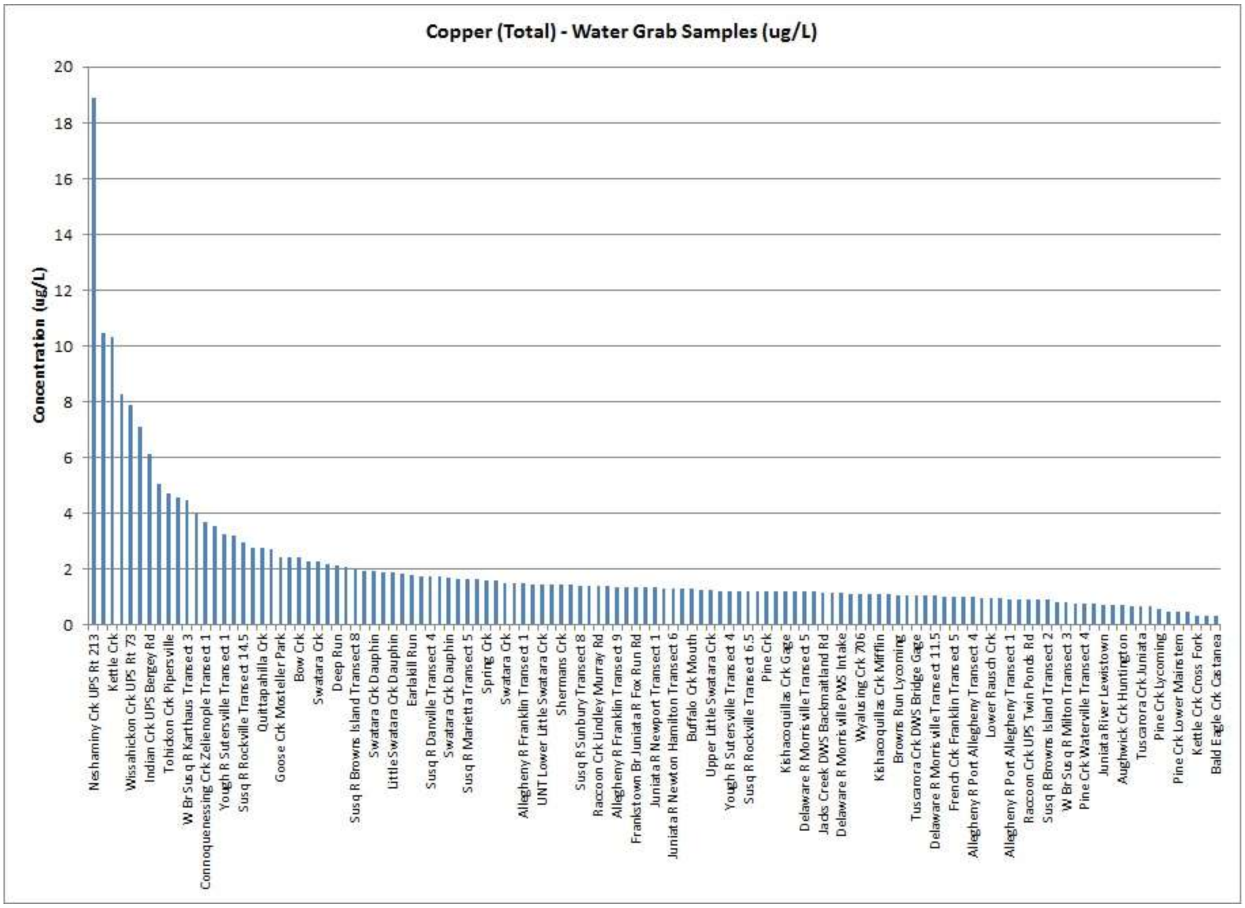


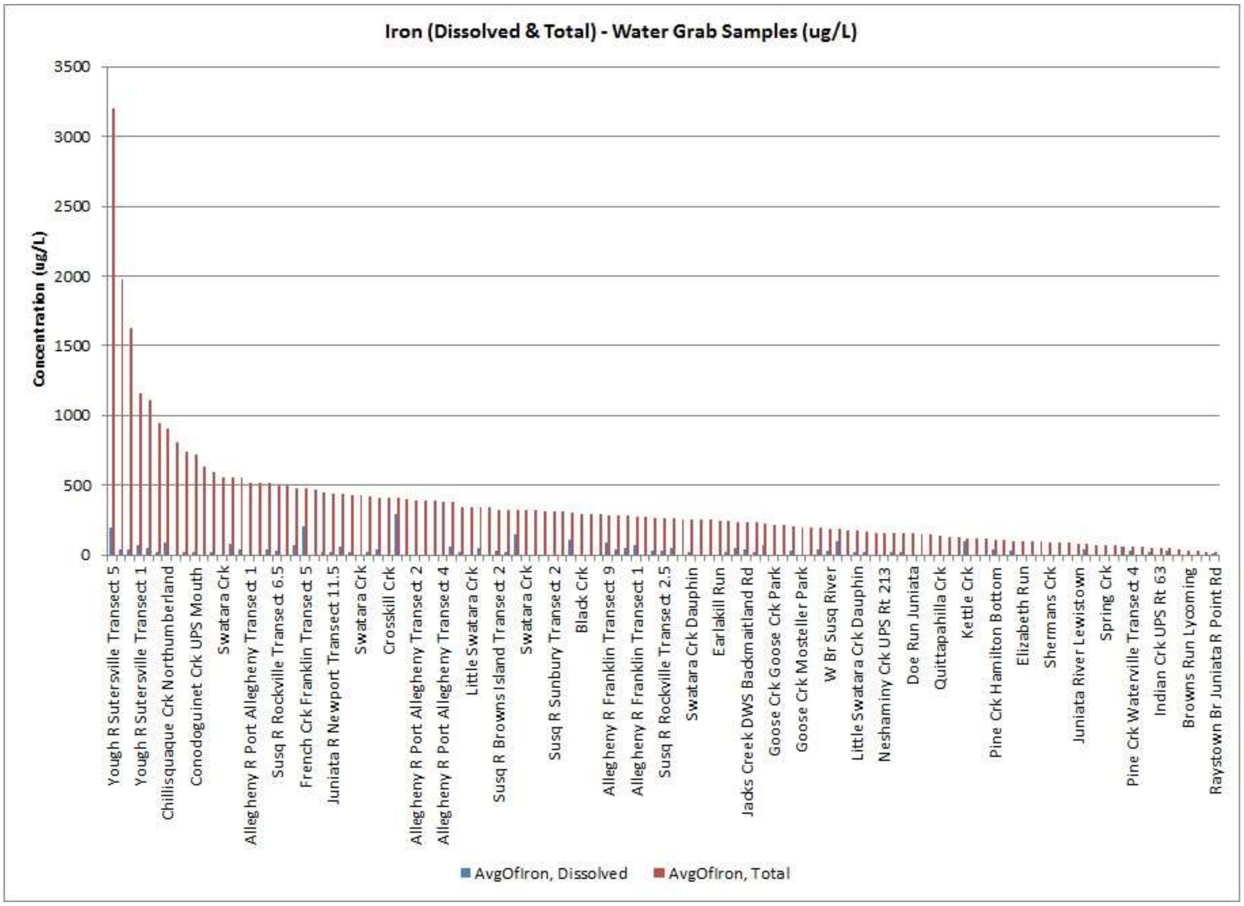
Aluminum (Total & Dissolved) - Water Grab Samples (ug/L)
Concentration (ug/L)
1800
1600
1400
1200
1000
800
600
400
200
0
Yough R Sutersville Transect 5
Yough R Sutersville Transect 1
Susq R Marietta Transect 9
Chillisquaque Crk Northumberland
Buffalo Crk Mouth
Susq R Rockville Transect 6.5
Juniata R Newport Transect 11.5
Wissahickon Crk UPS Rt 73
W Br Susq R Karthaus Transect 3
Raccoon Crk UPS Twin Ponds Rd
Crosskill Crk
Susq River
Delaware R Morrisville Transect 11
Swatara Crk Hershey
Tohickon Crk Pipersville
Susq R Marietta Transect 5
Swatara Crk Dauphin
Swatara Crk
Delaware R Morrisville Transect 5
French Crk Franklin Transect 5
W Br Susq River
Allegheny R Franklin Transect 9
Little Swatara Crk Dauphin
Swatara Crk
Buffalo Crk UPS Rt849 Br
Black Crk
Juniata R Newport Transect 4
Raccoon Crk Lindley Murray Rd
Quittapahilla Crk
Allegheny R Port Allegheny Transect 2
Big Elk Crk UPS Strickersville Rd
Allegheny R Port Allegheny Transect 4
Indian Crk UPS Rt 63
Delaware R Morrisville Transect 11.5
Juniata River Lewistown
Shermans Crk
Swatara Crk Dauphin
Raystown Br Juniata R Point Rd
Pine Crk Waterville Transect 4
Towamencin Crk DWS Metz Rd
Aughwick Crk Huntington
AvgOfAluminum, Dissolved
AvgOfAluminum, Total



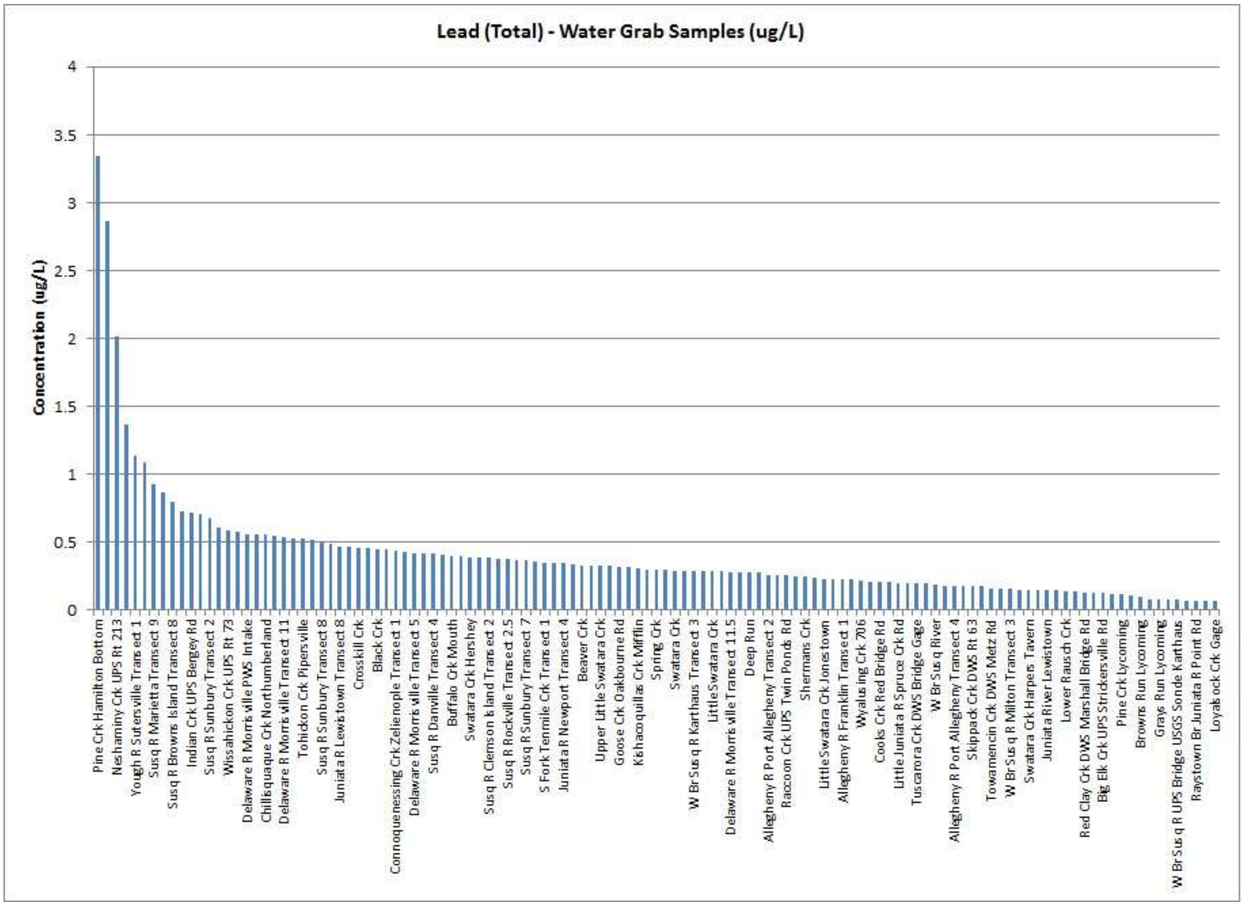
Barium (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
250
200
150
100
50
0
Goose Crk Mosteller Park
Elizabeth Run
Bow Crk
Goose Crk Goose Crk Park
Little Swatara Crk Jonestown
Little Swatara Crk Dauphin
Goose Crk Oakbourne Rd
Indian Crk UPS Rt 63
Connoquenessing Crk Zelienople Transect 5
Red Clay Crk DWS Marshall Bridge Rd
Raccoon Crk Lindley Murray Rd
Reeds Crk Jonestown Rd
Wyalusing Crk 706
Chillisquaque Crk 405
Frankstown Br Juniata R Fox Run Rd
Aughwick Crk Gage
Yough R Sutersville Transect 1
Swatara Crk
Swatara Crk
Juniata R Newport Transect 4
Juniata R Newport Transect 1
Susq R Rockville Transect 2.5
Shermans Crk
Swatara Crk Dauphin
Perkiomen Crk
Yough R Sutersville Transect 4
Little Juniata R Spruce Crk Rd
Allegheny R Franklin Transect 9
Raystown Br Juniata R Point Rd
Juniata R Lewistown Transect 8
Tuscarora Crk DWS Bridge Gage
Juniata R Thompsontown Transect 5
Susq R Rockville Transect 14.5
W Br Susq R UPS Bridge USGS Sonde Karthaus
W Br Susq River
Susq River
Raccoon Crk UPS Twin Ponds Rd
Susq R Clemson Island Transect 8
Susq R Sunbury Transect 2
Susq R Sunbury Transect 7
Susq R Danville Transect 4
Susq R Browns Island Transect 8
Allegheny R Franklin Transect 4
Susq R Clemson Island Transect 2
Susq R Browns Island Transect 2
Pine Crk Hamilton Bottom
Loyalsock Crk Airport
Susq R Sunbury Transect 8
Upper Little Swatara Crk
Pine Crk Lycoming
Allegheny R Port Allegheny Transect 5
French Crk Franklin Transect 5
Allegheny R Port Allegheny Transect 4
Lower Rausch Crk
Swatara Crk
Delaware R Morrisville Transect 11.5
Swatara Crk
Swatara Crk
Tohickon Crk Pipersville
Kettle Crk Cross Fork
Browns Run Lycoming


Boron (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
0
20
40
60
80
100
120
140
160
180

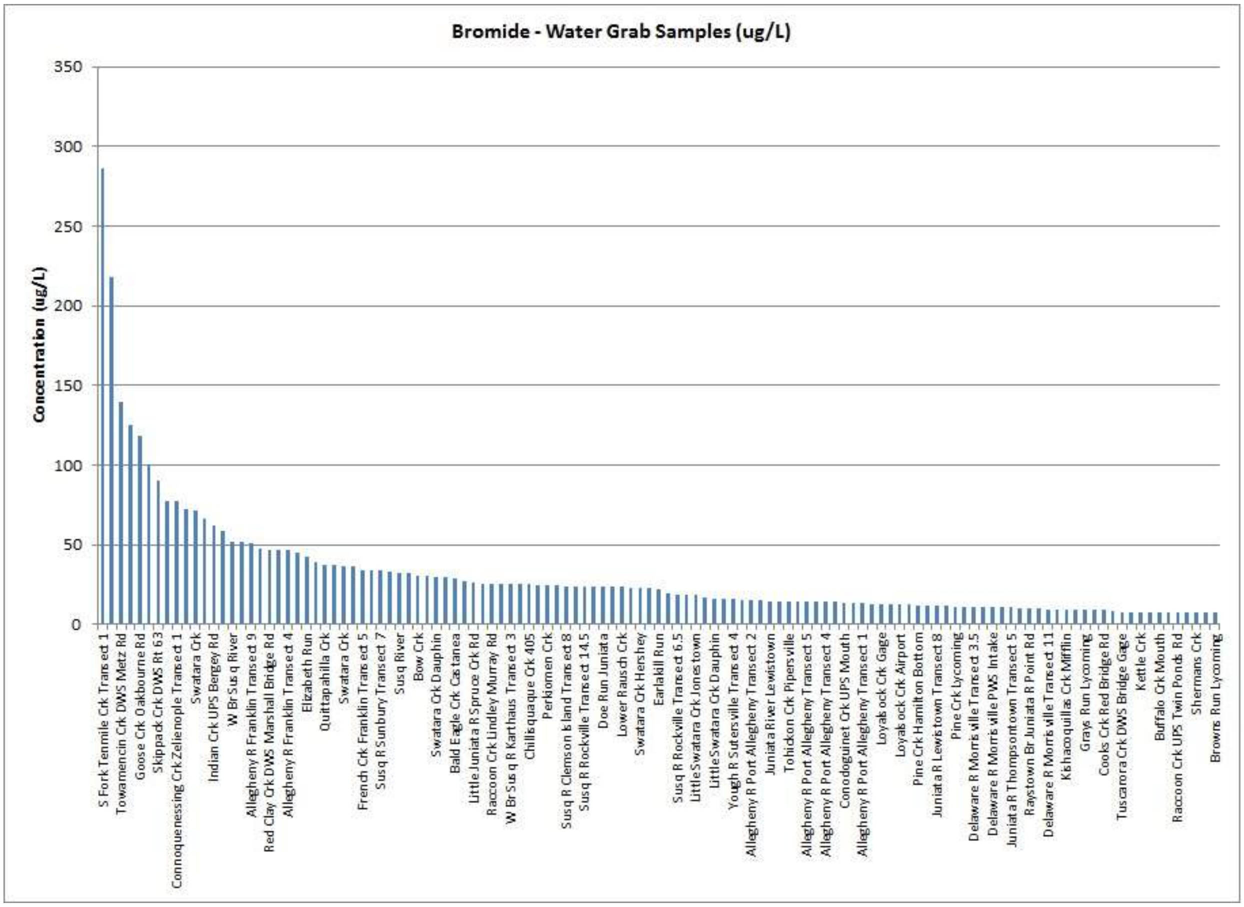

Copper (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
20
18
16
14
12
10
8
6
4
2
0
Neshaminy Crk UPS Rt 213
Kettle Crk
Wissahickon Crk UPS Rt 73
Indian Crk UPS Bergey Rd
Tohickon Crk Pipersville
W Br Susq R Karthaus Transect 3
Connoquenessing Crk Zelienople Transect 1
Yough R Sutersville Transect 1
Susq R Rockville Transect 14.5
Quittapahilla Crk
Goose Crk Mosteller Park
Bow Crk
Swatara Crk
Deep Run
Susq R Browns Island Transect 8
Swatara Crk Dauphin
Little Swatara Crk Dauphin
Earlakill Run
Susq R Danville Transect 4
Swatara Crk Dauphin
Susq R Marietta Transect 5
Spring Crk
Swatara Crk
Allegheny R Franklin Transect 1
UNT Lower Little Swatara Crk
Shermans Crk
Susq R Sunbury Transect 8
Raccoon Crk Lindley Murray Rd
Allegheny R Franklin Transect 9
Frankstown Br Juniata R Fox Run Rd
Juniata R Newport Transect 1
Juniata R Newton Hamilton Transect 6
Buffalo Crk Mouth
Upper Little Swatara Crk
Yough R Sutersville Transect 4
Susq R Rockville Transect 6.5
Pine Crk
Kishacoquillas Crk Gage
Delaware R Morrisville Transect 5
Jacks Creek DWS Backmaitland Rd
Delaware R Morrisville PWS Intake
Wyalusing Crk 706
Kishacoquillas Crk Mifflin
Browns Run Lycoming
Tuscarora Crk DWS Bridge Gage
Delaware R Morrisville Transect 11.5
French Crk Franklin Transect 5
Allegheny R Port Allegheny Transect 4
Lower Rausch Crk
Allegheny R Port Allegheny Transect 1
Raccoon Crk UPS Twin Ponds Rd
Susq R Browns Island Transect 2
W Br Susq R Milton Transect 3
Pine Crk Waterville Transect 4
Juniata River Lewistown
Aughwick Crk Huntington
Tuscarora Crk Juniata
Pine Crk Lycoming
Pine Crk Lower Mainstem
Kettle Crk Cross Fork
Bald Eagle Crk Castanea



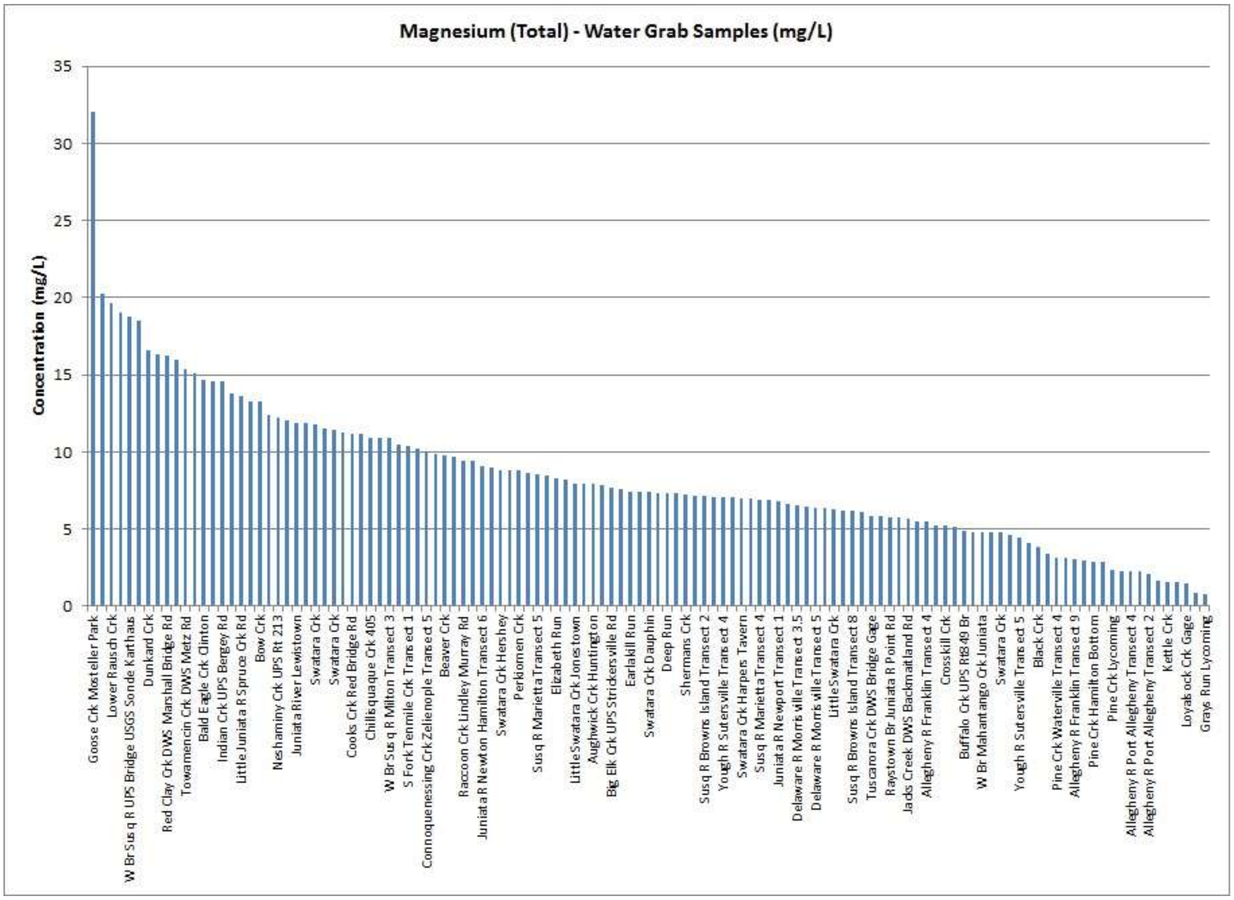
Iron (Dissolved & Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
3500
3000
2500
2000
1500
1000
500
0
Yough R Sutersville Transect 5
Yough R Sutersville Transect 1
Chillisquaque Crk Northumberland
Conodoguinet Crk UPS Mouth
Swatara Crk
Allegheny R Port Allegheny Transect 1
Susq R Rockville Transect 6.5
French Crk Franklin Transect 5
Juniata R Newport Transect 11.5
Swatara Crk
Crosskill Crk
Allegheny R Port Allegheny Transect 2
Allegheny R Port Allegheny Transect 4
Little Swatara Crk
Susq R Browns Island Transect 2
Swatara Crk
Susq R Sunbury Transect 2
Black Crk
Allegheny R Franklin Transect 9
Allegheny R Franklin Transect 1
Susq R Rockville Transect 2.5
Swatara Crk Dauphin
Earlakill Run
Jacks Creek DWS Backmaitland Rd
Goose Crk Goose Crk Park
Goose Crk Mosteller Park
W Br Susq River
Little Swatara Crk Dauphin
Neshaminy Crk UPS Rt 213
Doe Run Juniata
Quittapahilla Crk
Kettle Crk
Pine Crk Hamilton Bottom
Elizabeth Run
Shermans Crk
Juniata River Lewistown
Spring Crk
Pine Crk Waterville Transect 4
Indian Crk UPS Rt 63
Browns Run Lycoming
Raystown Br Juniata R Point Rd
AvgOfIron, Dissolved
AvgOfIron, Total

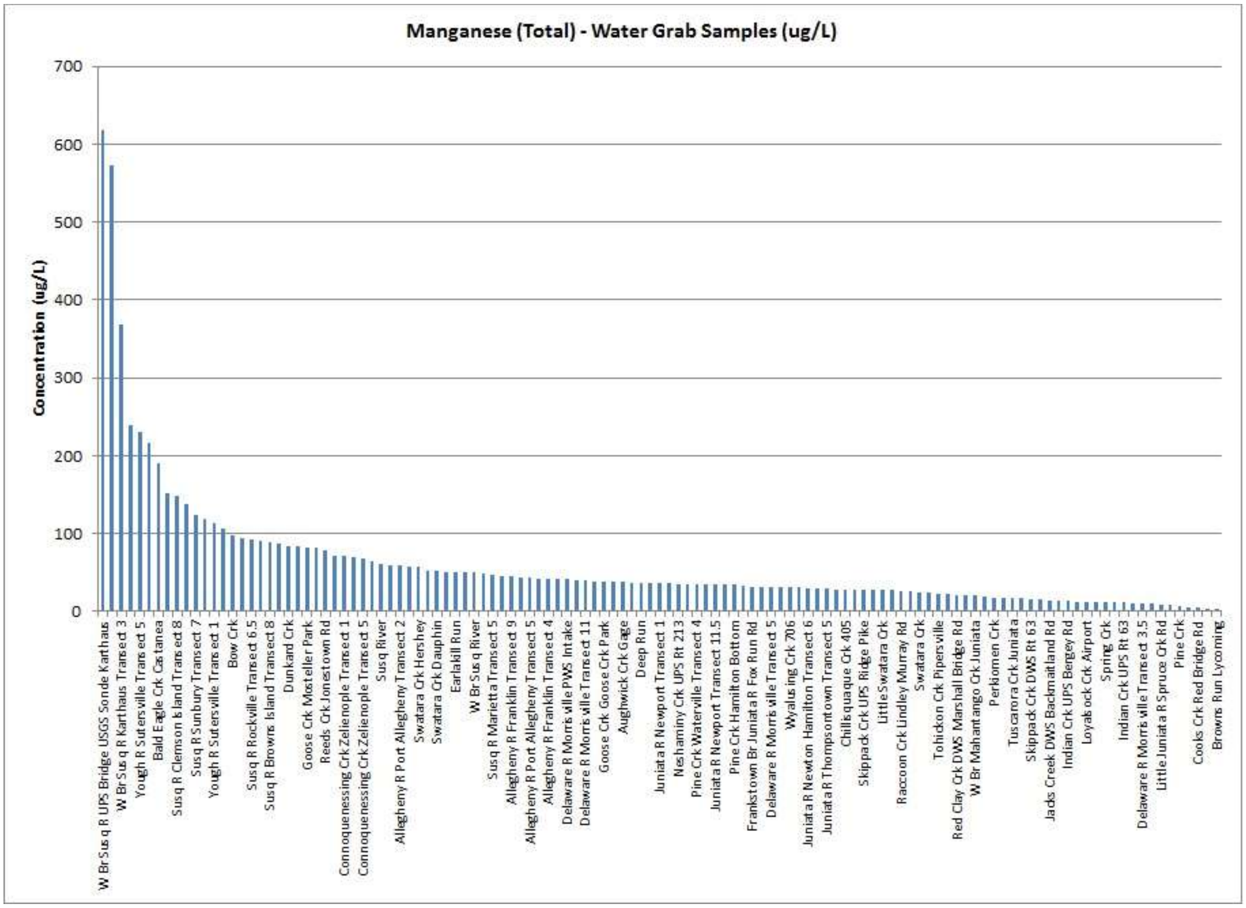


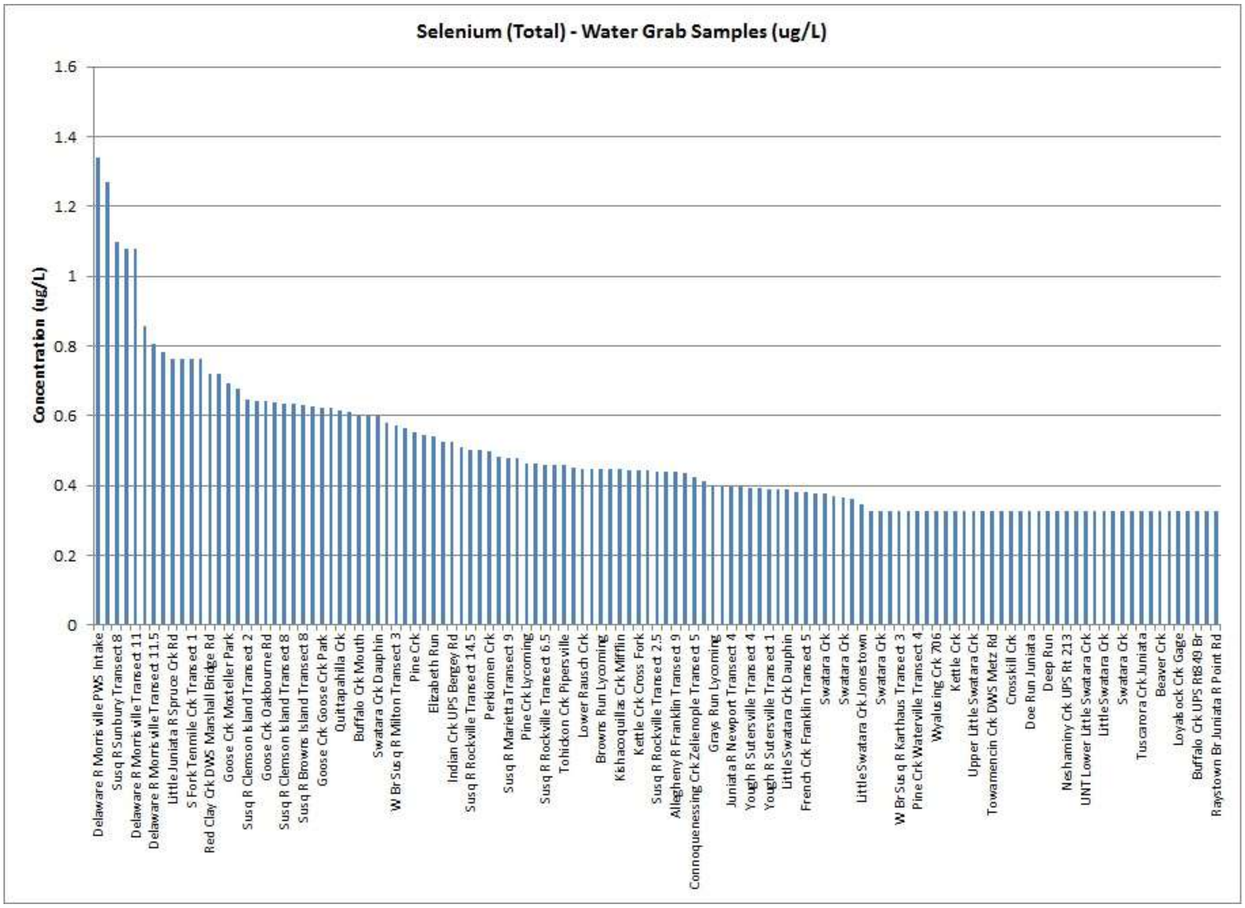
Lead (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)



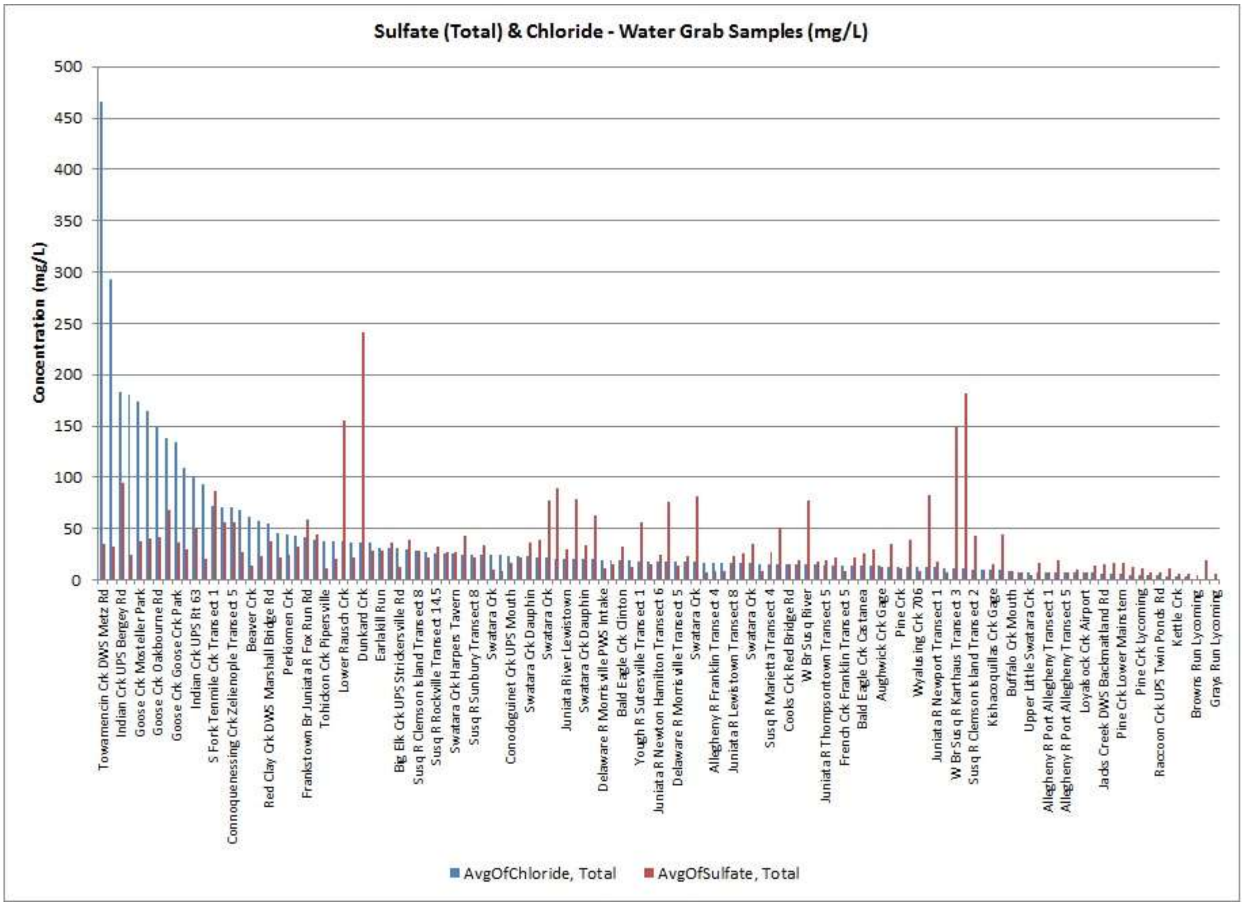
Bromide - Water Grab Samples (ug/L)
Concentration (ug/L)


Magnesium (Total) - Water Grab Samples (mg/L)
Concentration (mg/L)

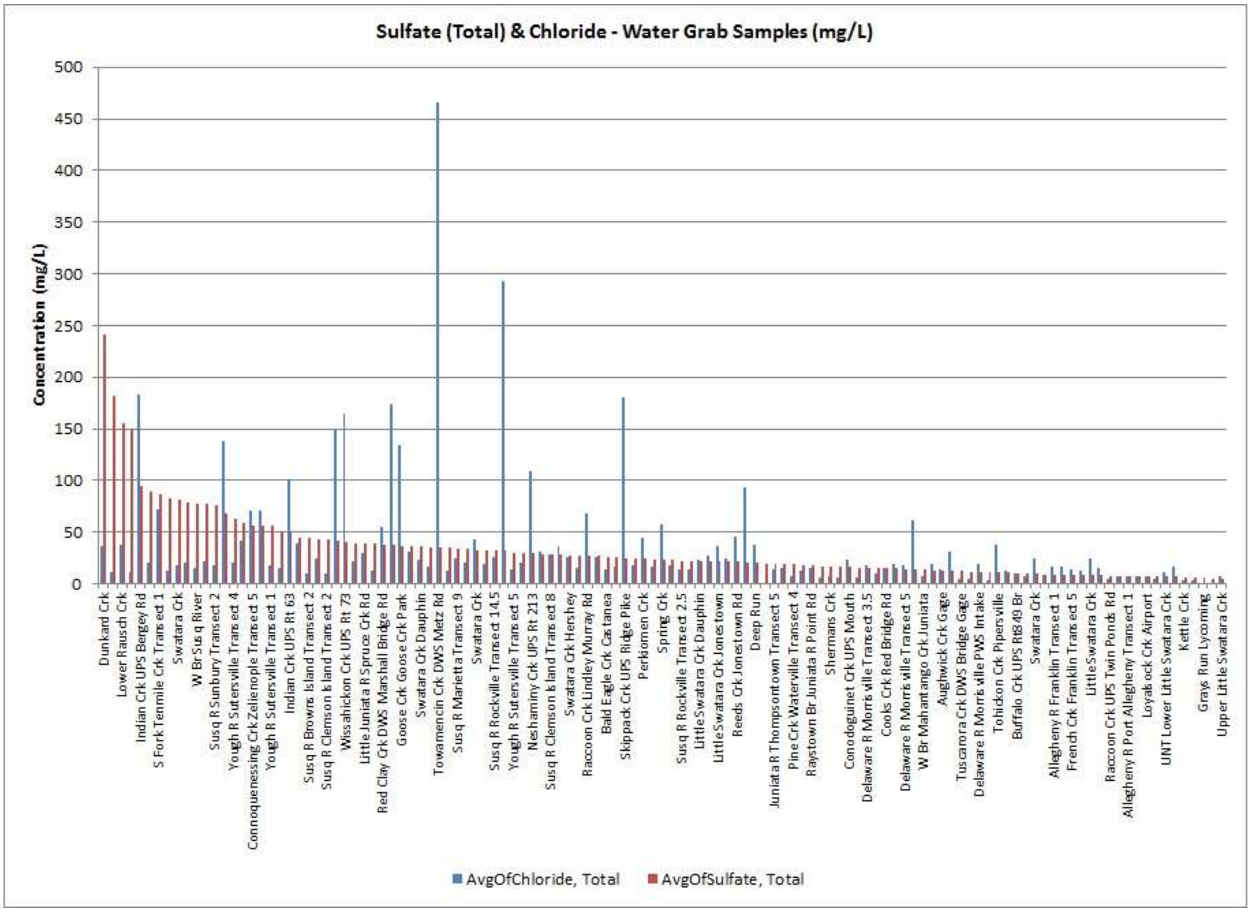

Manganese (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
700
600
500
400
300
200
100
0
W Br Susq R UPS Bridge USGS Sonde Karthaus
W Br Susq R Karthaus Transect 3
Yough R Sutersville Transect 5
Bald Eagle Crk Castanea
Susq R Clemson Island Transect 8
Susq R Sunbury Transect 7
Yough R Sutersville Transect 1
Bow Crk
Susq R Rockville Transect 6.5
Susq R Browns Island Transect 8
Dunkard Crk
Goose Crk Mosteller Park
Reeds Crk Jonestown Rd
Connoquenessing Crk Zelienople Transect 1
Connoquenessing Crk Zelienople Transect 5
Susq River
Allegheny R Port Allegheny Transect 2
Swatara Crk Hershey
Swatara Crk Dauphin
Earlakill Run
W Br Susq River
Susq R Marietta Transect 5
Allegheny R Franklin Transect 9
Allegheny R Port Allegheny Transect 5
Allegheny R Franklin Transect 4
Delaware R Morrisville PWS Intake
Delaware R Morrisville Transect 11
Goose Crk Goose Crk Park
Aughwick Crk Gage
Deep Run
Juniata R Newport Transect 1
Neshaminy Crk UPS Rt 213
Pine Crk Waterville Transect 4
Juniata R Newport Transect 11.5
Pine Crk Hamilton Bottom
Frankstown Br Juniata R Fox Run Rd
Delaware R Morrisville Transect 5
Wyalusing Crk 706
Juniata R Newton Hamilton Transect 6
Juniata R Thompsontown Transect 5
Chillisquaque Crk 405
Skippack Crk UPS Ridge Pike
Little Swatara Crk
Raccoon Crk Lindley Murray Rd
Swatara Crk
Tohickon Crk Pipersville
Red Clay Crk DWS Marshall Bridge Rd
W Br Mahantango Crk Juniata
Perkiomen Crk
Tuscarora Crk Juniata
Skippack Crk DWS Rt 63
Jacks Creek DWS Backmaitland Rd
Indian Crk UPS Bergey Rd
Loyalsock Crk Airport
Spring Crk
Indian Crk UPS Rt 63
Delaware R Morrisville Transect 3.5
Little Juniata R Spruce Crk Rd
Pine Crk
Cooks Crk Red Bridge Rd
Browns Run Lycoming



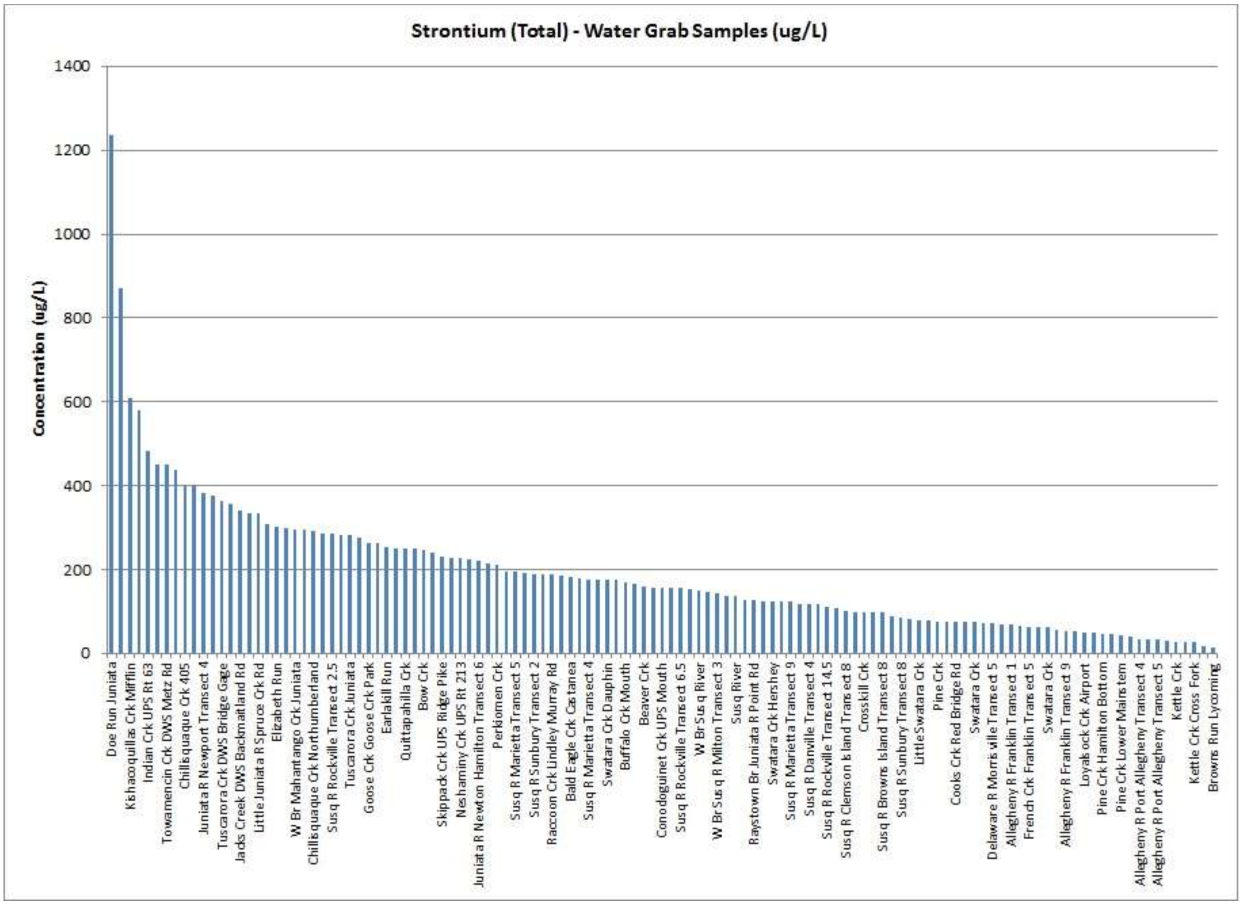
Selenium (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Delaware R Morrisville PWS Intake
Susq R Sunbury Transect 8
Delaware R Morrisville Transect 11
Delaware R Morrisville Transect 11.5
Little Juniata R Spruce Crk Rd
S Fork Tenmile Crk Transect 1
Red Clay Crk DWS Marshall Bridge Rd
Goose Crk Mosteller Park
Susq R Clemson Island Transect 2
Goose Crk Oakbourne Rd
Susq R Clemson Island Transect 8
Susq R Browns Island Transect 8
Goose Crk Goose Crk Park
Quittapahilla Crk
Buffalo Crk Mouth
Swatara Crk Dauphin
W Br Susq R Milton Transect 3
Pine Crk
Elizabeth Run
Indian Crk UPS Bergey Rd
Susq R Rockville Transect 14.5
Perkiomen Crk
Susq R Marietta Transect 9
Pine Crk Lycoming
Susq R Rockville Transect 6.5
Tohickon Crk Pipersville
Lower Rausch Crk
Browns Run Lycoming
Kishacoquillas Crk Mifflin
Kettle Crk Cross Fork
Susq R Rockville Transect 2.5
Allegheny R Franklin Transect 9
Connoquenessing Crk Zelienople Transect 5
Grays Run Lycoming
Juniata R Newport Transect 4
Yough R Sutersville Transect 4
Yough R Sutersville Transect 1
Little Swatara Crk Dauphin
French Crk Franklin Transect 5
Swatara Crk
Swatara Crk
Little Swatara Crk Jonestown
Swatara Crk
W Br Susq R Karthaus Transect 3
Pine Crk Waterville Transect 4
Wyalusing Crk 706
Kettle Crk
Upper Little Swatara Crk
Towamencin Crk DWS Metz Rd
Crosskill Crk
Doe Run Juniata
Deep Run
Neshaminy Crk UPS Rt 213
UNT Lower Little Swatara Crk
Little Swatara Crk
Swatara Crk
Tuscarora Crk Juniata
Beaver Crk
Loyalsock Crk Gage
Buffalo Crk UPS Rt849 Br
Raystown Br Juniata R Point Rd



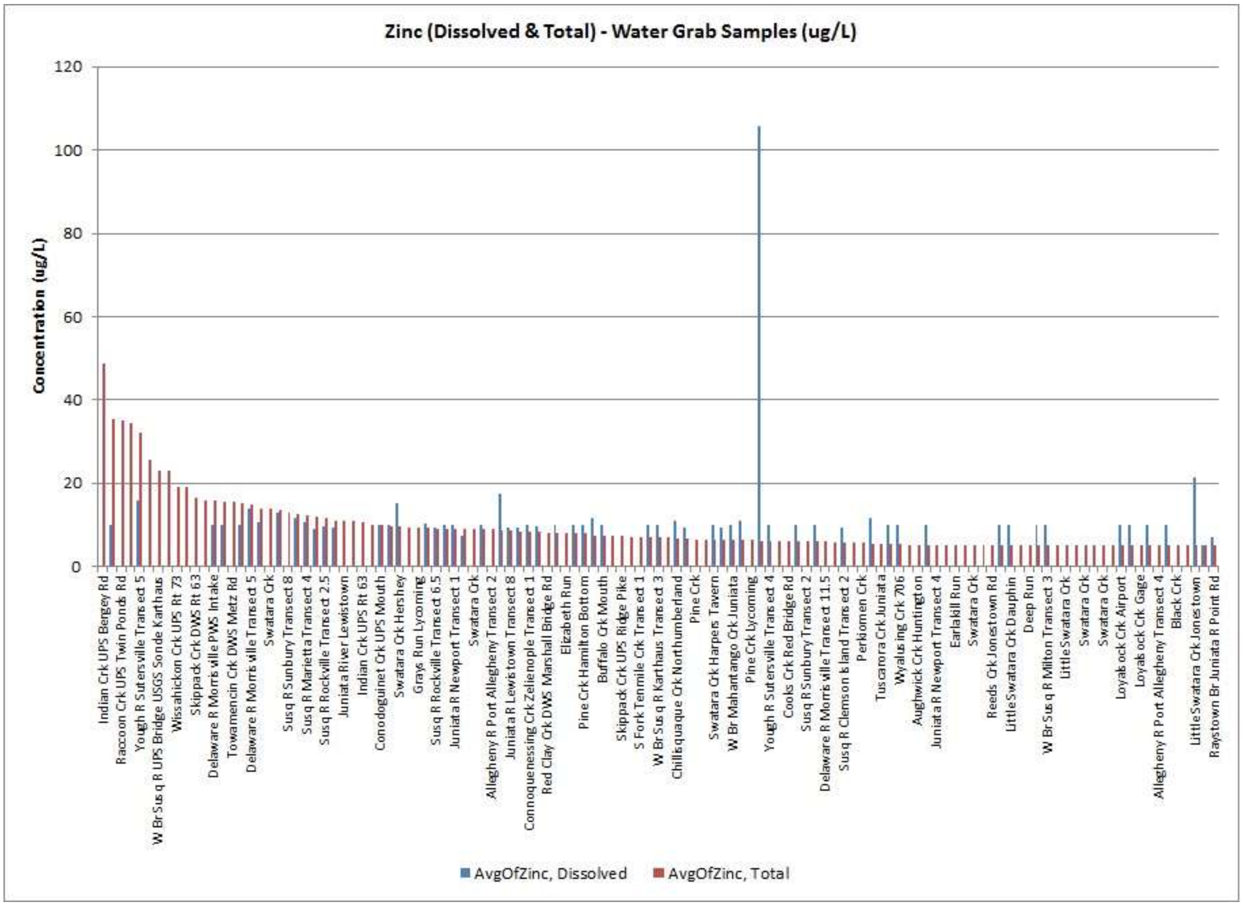
Sulfate (Total) & Chloride - Water Grab Samples (mg/L)
500
450
400
350
300
250
200
150
100
50
0
Concentration (mg/L)
Towamencin Crk DWS Metz Rd
Indian Crk UPS Bergey Rd
Goose Crk Mosteller Park
Goose Crk Oakbourne Rd
Goose Crk Goose Crk Park
Indian Crk UPS Rt 63
S Fork Tenmile Crk Transect 1
Connoquenessing Crk Zelienople Transect 5
Beaver Crk
Red Clay Crk DWS Marshall Bridge Rd
Perkiomen Crk
Frankstown Br Juniata R Fox Run Rd
Tohickon Crk Pipersville
Lower Rausch Crk
Dunkard Crk
Earlakill Run
Big Elk Crk UPS Strickersville Rd
Susq R Clemson Island Transect 8
Susq R Rockville Transect 14.5
Swatara Crk Harpers Tavern
Susq R Sunbury Transect 8
Swatara Crk
Conodoguinet Crk UPS Mouth
Swatara Crk Dauphin
Swatara Crk
Juniata River Lewistown
Swatara Crk Dauphin
Delaware R Morrisville PWS Intake
Bald Eagle Crk Clinton
Yough R Sutersville Transect 1
Juniata R Newton Hamilton Transect 6
Delaware R Morrisville Transect 5
Swatara Crk
Allegheny R Franklin Transect 4
Juniata R Lewistown Transect 8
Swatara Crk
Susq R Marietta Transect 4
Cooks Crk Red Bridge Rd
W Br Susq River
Juniata R Thompsontown Transect 5
French Crk Franklin Transect 5
Bald Eagle Crk Castanea
Aughwick Crk Gage
Pine Crk
Wyalusing Crk 706
Juniata R Newport Transect 1
W Br Susq R Karthaus Transect 3
Susq R Clemson Island Transect 2
Kishacoquillas Crk Gage
Buffalo Crk Mouth
Upper Little Swatara Crk
Allegheny R Port Allegheny Transect 1
Allegheny R Port Allegheny Transect 5
Loyalsock Crk Airport
Jacks Creek DWS Backmaitland Rd
Pine Crk Lower Mainstem
Pine Crk Lycoming
Raccoon Crk UPS Twin Ponds Rd
Kettle Crk
Browns Run Lycoming
Grays Run Lycoming
AvgOfChloride, Total
AvgOfSulfate, Total



Sulfate (Total) & Chloride - Water Grab Samples (mg/L)
Concentration (mg/L)
AvgOfChloride, Total
AvgOfSulfate, Total



Strontium (Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
1400
1200
1000
800
600
400
200
0
Doe Run Juniata
Kishacoquillas Crk Mifflin
Indian Crk UPS Rt 63
Towamencin Crk DWS Metz Rd
Chillisquaque Crk 405
Juniata R Newport Transect 4
Tuscarora Crk DWS Bridge Gage
Jacks Creek DWS Backmaitland Rd
Little Juniata R Spruce Crk Rd
Elizabeth Run
W Br Mahantango Crk Juniata
Chillisquaque Crk Northumberland
Susq R Rockville Transect 2.5
Tuscarora Crk Juniata
Goose Crk Goose Crk Park
Earlakill Run
Quittapahilla Crk
Bow Crk
Skippack Crk UPS Ridge Pike
Neshaminy Crk UPS Rt 213
Juniata R Newton Hamilton Transect 6
Perkiomen Crk
Susq R Marietta Transect 5
Susq R Sunbury Transect 2
Raccoon Crk Lindley Murray Rd
Bald Eagle Crk Castanea
Susq R Marietta Transect 4
Swatara Crk Dauphin
Buffalo Crk Mouth
Beaver Crk
Conodoguinet Crk UPS Mouth
Susq R Rockville Transect 6.5
W Br Susq River
W Br Susq R Milton Transect 3
Susq River
Raystown Br Juniata R Point Rd
Swatara Crk Hershey
Susq R Marietta Transect 9
Susq R Danville Transect 4
Susq R Rockville Transect 14.5
Susq R Clemson Island Transect 8
Crosskill Crk
Susq R Browns Island Transect 8
Susq R Sunbury Transect 8
Little Swatara Crk
Pine Crk
Cooks Crk Red Bridge Rd
Swatara Crk
Delaware R Morrisville Transect 5
Allegheny R Franklin Transect 1
French Crk Franklin Transect 5
Swatara Crk
Allegheny R Franklin Transect 9
Loyalsock Crk Airport
Pine Crk Hamilton Bottom
Pine Crk Lower Mainstem
Allegheny R Port Allegheny Transect 4
Allegheny R Port Allegheny Transect 5
Kettle Crk
Kettle Crk Cross Fork
Browns Run Lycoming



Zinc (Dissolved & Total) - Water Grab Samples (ug/L)
Concentration (ug/L)
AvgOfZinc, Dissolved
AvgOfZinc, Total

Candidate Cause 13: EDCs

Worksheets: 43, 47, 48, and 49

Title: Hormones Analyzed from Passive Water Samplers (WS #43)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Water concentrations of these compounds could come into direct contact with YOY fish. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected passive water samples from 12 sample sites within and out of the Susquehanna basin in 2013 to be tested for 19 hormones. Samples were analyzed at USGS:

Waterbody	Location Name
Susquehanna River	Marietta West
Susquehanna River	Marietta-Falmouth East
Susquehanna River	Rockville West
Susquehanna River	Rockville Middle
Susquehanna River	Rockville East
Juniata River	Lewistown Narrows
Juniata River	Newport South
Susquehanna River	Sunbury West
Susquehanna River	Sunbury East
Connequenessing Creek	Zelienople
Allegheny River	Franklin
Delaware River	Morrisville East

Hormones
bisphenol A
diethylstilbestrol
cis-androsterone
epitestosterone

17-alpha-estradiol
dihydrotestosterone
4-androstene-3,17-dione
estrone
17-beta-estradiol
testosterone
equilin
11-ketotestosterone
norethindrone
mestranol
equilenin
17-alpha-ethynylestradiol
estriol
progesterone
3-beta-Coprostanol

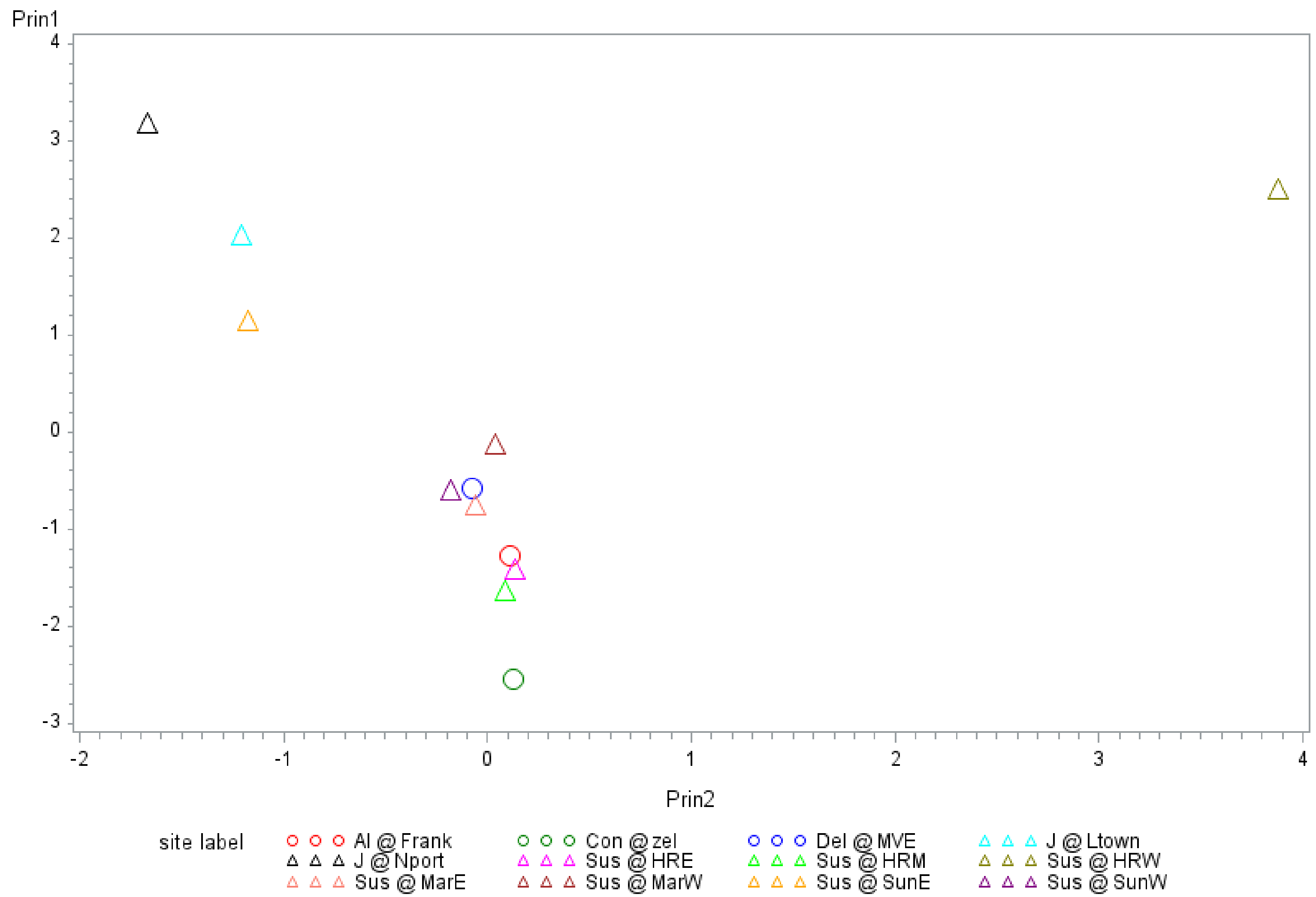
Analysis and Results:

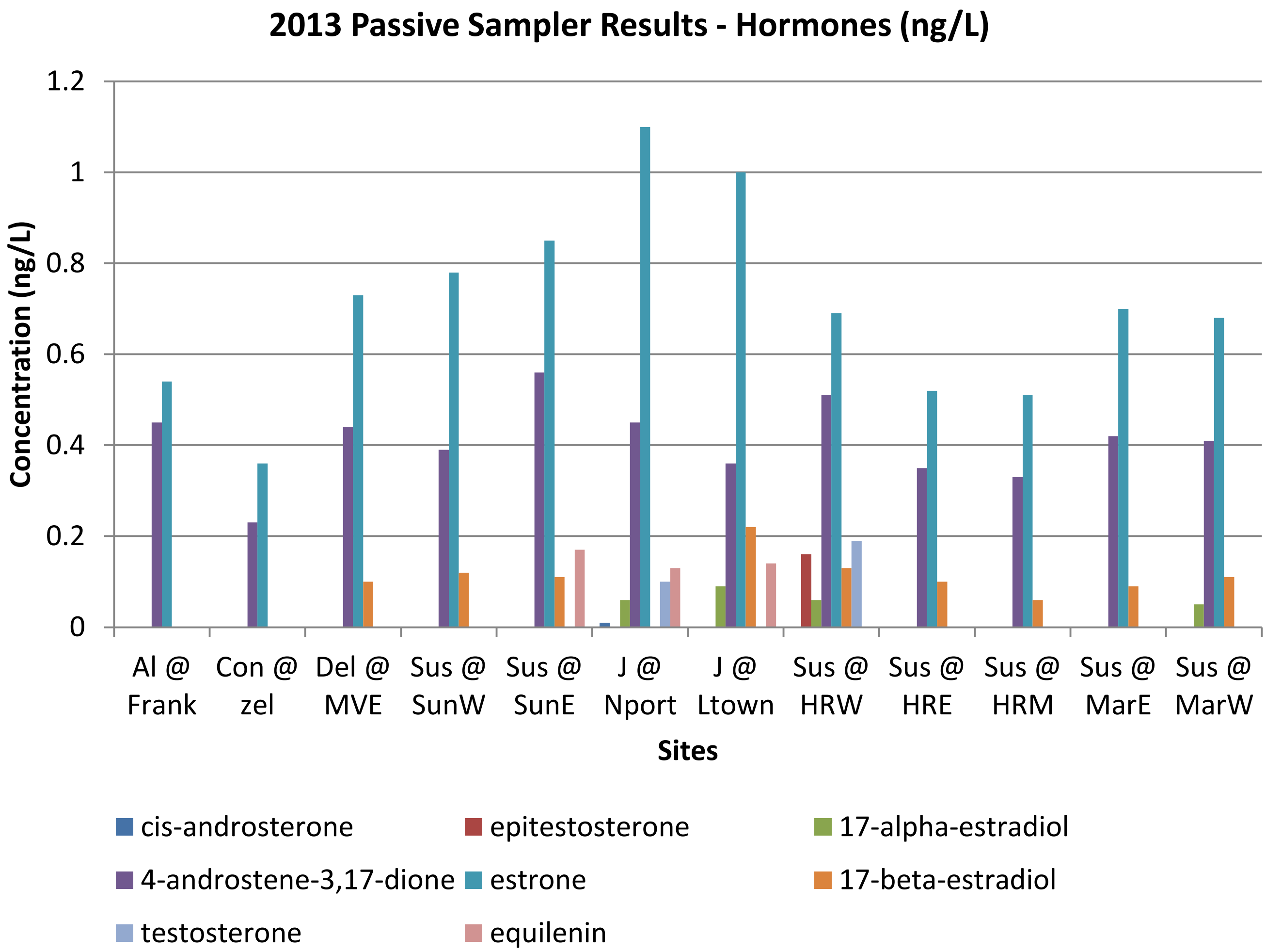
Eight out of nineteen hormones were detected: cis-androsterone; epitestosterone; 17-alpha=estradiol; 4-androstene-3,17-dione; estrone; 17-beta-estradiol; testosterone; and equilenin.

4-androstene-3,17-dione and estrone were found at every site and 17-beta-estradiol was found at nearly every site (except three). Other hormones were found sporadically at different places. Allegheny R and Connoquenessing Crk had the least amount of hormones detected (only 4-androstene-3,17-dione and estrone). Juniata R @ Newport and Lewistown, and Susquehanna R @ Harrisburg West had the highest number of different hormones detected. There definitely seems to be a pattern of the Juniata sites and Harrisburg West site having the highest variety and concentrations of various hormones.

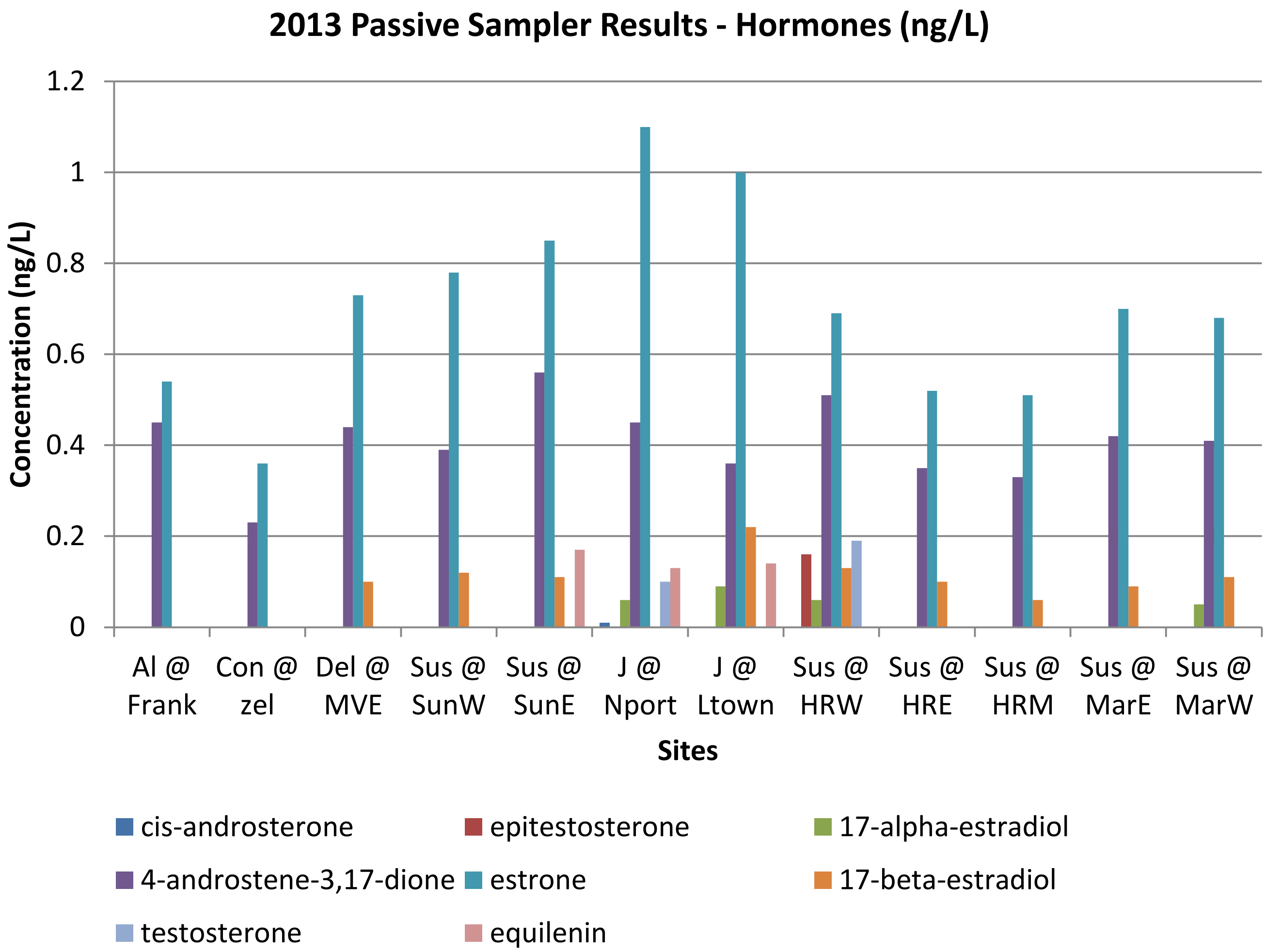
A principal component analysis (PCA) of the hormones at each site indicate that Harrisburg West is actually quite different from the two Juniata sites. The Juniata sites, as well as Susquehanna R @ Sunbury East, are more similar. The remaining sites (shown in the table on the previous page), including the controls, are clustered together.

PCA - 2013 Passive Sampler Results
Hormones (ng/L)





2013 Passive Sampler Results - Hormones (ng/L)
Concentration (ng/L)
1.2
1
0.8
0.6
0.4
0.2
0
Al @ Frank
Con @ zel
Del @ MVE
Sus @ SunW
Sus @ SunE
J @ Nport
J @ Ltown
Sus @ HRW
Sus @ HRE
Sus @ HRM
Sus @ MarE
Sus @ MarW
Sites
cis-androsterone
epitestosterone
17-alpha-estradiol
4-androstene-3,17-dione
estrone
17-beta-estradiol
testosterone
equilenin



2013 Passive Sampler Results - Hormones (ng/L)
Concentration (ng/L)
1.2
1
0.8
0.6
0.4
0.2
0
Al @ Frank
Con @ zel
Del @ MVE
Sus @ SunW
Sus @ SunE
J @ Nport
J @ Ltown
Sus @ HRW
Sus @ HRE
Sus @ HRM
Sus @ MarE
Sus @ MarW
Sites
cis-androsterone
epitestosterone
17-alpha-estradiol
4-androstene-3,17-dione
estrone
17-beta-estradiol
testosterone
equilenin

Title: Hormones in Sediments (2013) (WS # 47)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Sediment could directly affect YOY because the eggs are laid directly on it and the young are in close contact with it. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected sediment samples from 13 sample sites within and out of the Susquehanna basin in 2013 to be tested for 20 hormones. Samples were analyzed by USGS:

Waterbody	Location Name
Susquehanna River	Marietta West
Susquehanna River	Marietta-Falmouth East
Susquehanna River	Rockville West
Susquehanna River	Rockville Middle
Susquehanna River	Rockville East
Juniata River	Lewistown Narrows
Juniata River	Newport South
Susquehanna River	Sunbury West
Susquehanna River	Sunbury East
Youghiogheny River	West Newton-Sutersville
Connequenessing Creek	Zelienople
Allegheny River	Franklin
Delaware River	Morrisville East

11-Ketotestosterone	Equilenin
16-Epiestriol-2,4-d2 (surrogate)	Equilin
17-alpha-Estradiol	Estriol

17-alpha-Ethynylestradiol	*Estriol-2,4,16,17-d4 (surrogate)*
17-alpha-Ethynylestradiol-2,4,16,16-d4 (surrogate)	Estrone
17-beta-Estradiol	*Estrone-13,14,15,16,17,18-13C6 (surrogate)*
17-beta-Estradiol-13,14,15,16,17,18-13C6 (surrogate)	*Medroxyprogesterone-d3 (surrogate)*
3-beta-Coprostanol	Mestranol
4-Androstene-3,17-dione	*Mestranol-2,4,16,16-d4 (surrogate)*
Bisphenol A	*Nandrolone-16,16,17-d3 (surrogate)*
Bisphenol-A-d16 (surrogate)	Norethindrone
Cholesterol	Progesterone
Cholesterol-d7 (surrogate)	*Progesterone-2,3,4-13C3 (surrogate)*
cis-Androsterone	Testosterone
cis-Androsterone-2,2,3,4,4-d5 (surrogate)	*trans-Diethyl-1,1,1',1'-d4-stilbesterol-3,3',5,5'-d4 (surrogate)*
Dihydrotestosterone	trans-Diethylstilbestrol
Epitestosterone	

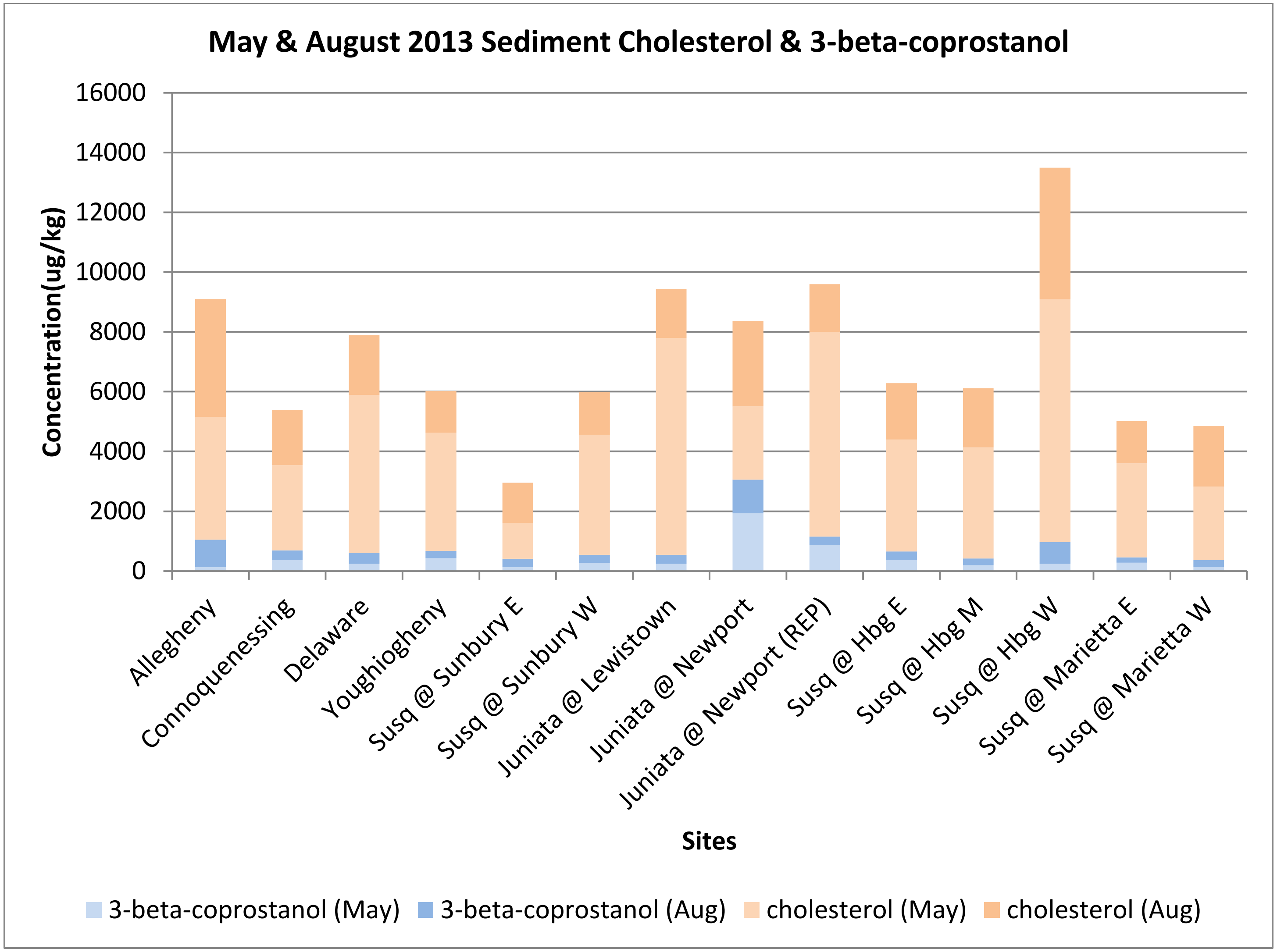
Analysis and Results:

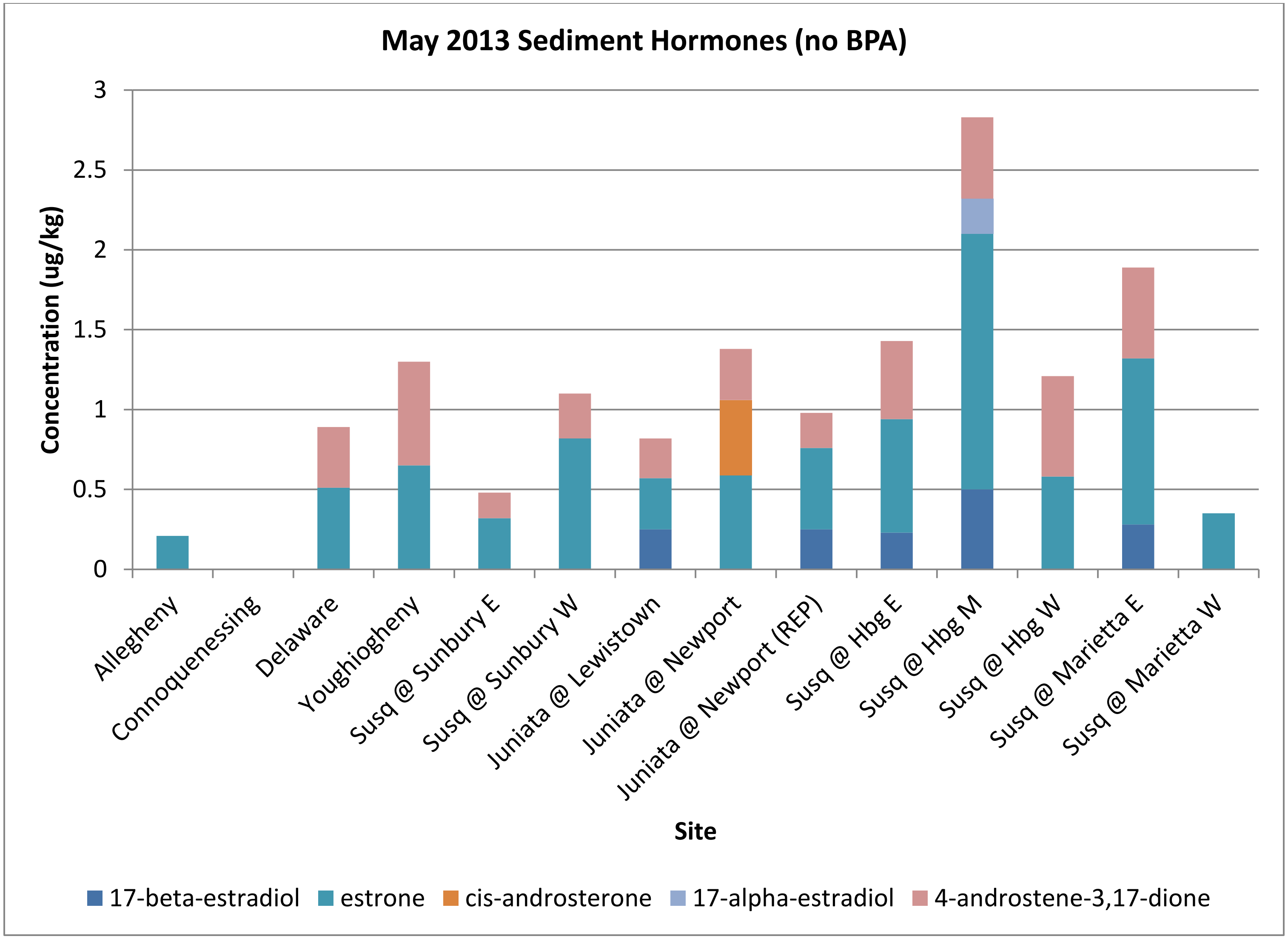
Eleven out of twenty hormones were detected. The most commonly found were bisphenol-a (BPA), 3-beta-coprostanol, and estrone.:

- 17-beta-estradiol: female sex hormone; more potent in estrogenic activity than estrone/estriol (ranged 0.23 – 0.50 ug/kg); most were estimated: (Juniata R @ Newport (E0.25); Juniata R @ Lewistown (0.25); Susq. @ Marietta East (E0.28); Susq Hbg East (E0.23); Susq. @ Hbg Middle (E0.50)
- BPA: used in production of polycarbonate plastics (ranged 15.8 – 48.3 ug/kg; Yough = 193 ug/kg)
- Cholesterol: component of cell membranes, required to establish proper membrane permeability & fluidity (very imprecise, but ranged 1191 – 8124 ug/kg, with Susq @ Hbg Middle highest)
- Estrone: natural estrogenic hormone secreted by ovary & adipose tissue (found at every site except Connoquenessing, ranged 0.21 – 1.60 ug/kg)
- Cis-androsterone: weak androgen (Juniata R @ Newport only – 0.47 ug/kg)
- 17-alpha-estradiol: isomer of 17-beta-estradiol (Susq @ Hbg Middle only – E0.22 ug/kg)
- 4-androstene-3, 7-dione: testosterone precurser (found at every site except Allegheny, Connoquenessing, & Susq @Marietta West; ranged 0.16 – 0.65 ug/kg)
- 3-beta-coprostanol: main sterol in feces produced by reduction of cholesterol by intestinal bacteria; often used as biomarker of human fecal matter in environment (E at every site; ranged 123 – 864 ug/kg; Juniata R @ Newport = E1928 ug/kg; Juniata R @ Newport Rep = E864)

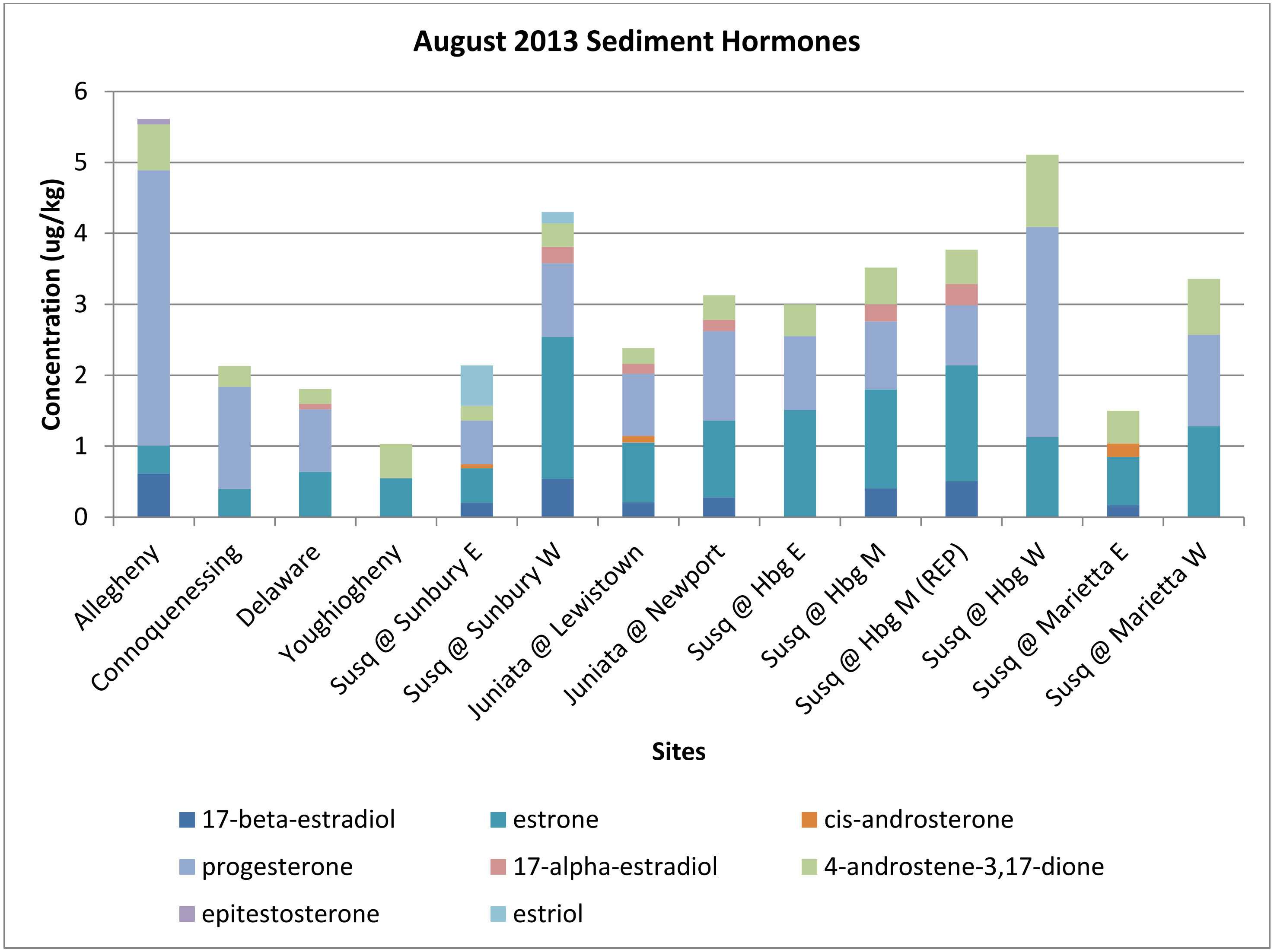
- <u>Progesterone</u>: steroid hormone (found at 11 of 13 sites, August only; this may be factor of lab analysis rather than seasonality)
- <u>Estriol</u>: one of three main estrogens produced by human body (Susq R @ Sunbury West = 0.16 ug/kg; Susq Sunbury East (0.57 ug/kg)
- <u>Epitestosterone</u>: natural steroid (Allegheny R @ Franklin = 0.075 ug/kg)

Few patterns are noticeable in the data. Non-parametric Wilcoxon signed rank sum tests were performed; cholesterol, estrone, and progesterone significantly differed between the May and August samples. Between compounds, 17-beta-estradiol & 17-alpha-estradiol, estrone & 17-alpha-estradiol, and estrone & 4-androstene-3,17-dione show significant correlations.


May & August 2013 Sediment Cholesterol & 3-beta-coprostanol
Concentration(ug/kg)
16000
14000
12000
10000
8000
6000
4000
2000
0
Allegheny
Connoquenessing
Delaware
Youghiogheny
Susq @ Sunbury E
Susq @ Sunbury W
Juniata @ Lewistown
Juniata @ Newport
Juniata @ Newport (REP)
Susq @ Hbg E
Susq @ Hbg M
Susq @ Hbg W
Susq @ Marietta E
Susq @ Marietta W
Sites
3-beta-coprostanol (May)
3-beta-coprostanol (Aug)
cholesterol (May)
cholesterol (Aug)



May 2013 Sediment Hormones (no BPA)
Concentration (ug/kg)
0
0.5
1
1.5
2
2.5
3
Allegheny
Connoquenessing
Delaware
Youghiogheny
Susq @ Sunbury E
Susq @ Sunbury W
Juniata @ Lewistown
Juniata @ Newport
Juniata @ Newport (REP)
Susq @ Hbg E
Susq @ Hbg M
Susq @ Hbg W
Susq @ Marietta E
Susq @ Marietta W
Site
17-beta-estradiol
estrone
cis-androsterone
17-alpha-estradiol
4-androstene-3,17-dione


August 2013 Sediment Hormones
Concentration (ug/kg)
0
1
2
3
4
5
6
Allegheny
Connoquenessing
Delaware
Youghiogheny
Susq @ Sunbury E
Susq @ Sunbury W
Juniata @ Lewistown
Juniata @ Newport
Susq @ Hbg E
Susq @ Hbg M
Susq @ Hbg M (REP)
Susq @ Hbg W
Susq @ Marietta E
Susq @ Marietta W
Sites
17-beta-estradiol
estrone
cis-androsterone
progesterone
17-alpha-estradiol
4-androstene-3,17-dione
epitestosterone
estriol

Conclusion: Few consistent patterns are noticeable in the data, but hormones remain a parameter of concern.

Title: EDCs and Intersex in Adult SMB (WS #48)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: EDCs increase YOY susceptibility to disease and cause intersex among adult small mouth bass resulting in lower fecundity

Introduction:

High chronic exposure to estrogenic chemicals may increase the frequency of intersex among adult male SMB potentially resulting in lower fecundity. If this is the case, adult male SMB would have a higher proportion and severity of intersex at locations with higher concentrations of EDCs. In addition, EDCs could negatively affect YOY survival and/or susceptibility to disease. Water concentrations of these compounds could come into direct contact with YOY fish. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

The content of this worksheet summarizes a portion of the study conducted by Vicki Blazer et. al. from USGS (Blazer et al 2014).

Data:

USGS collected smallmouth bass and white sucker at 16 locations in the basin and outside of the basin from 2007 to 2010 to evaluate the fish for biomarkers of estrogenic exposure including plasma vitellogenin and prevalence and severity of testicular oocyte formation in male fish. In addition, USGS collected grab discrete samples to test for 5 hormones, sediment samples to test for 7 hormones and plant sterols and, in 2010, passive samples to test for a suite of hormones and pesticides.

Sites were selected in the Delaware, Susquehanna, and Ohio drainages and also were selected to bracket WWTP discharges on Brodhead Creek, Susquehanna River at Danville, and Swatara Creek. Upstream and downstream sites were selected on the Allegheny River and Monongahela River but were not focused on specific WWTP’s.

The locations and dates of the sampling are summarized in the table below (Table 1, reproduced from Blazer et al. 2014).

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Table 1 Locations and sampling dates of fish collections in three river drainages within Pennsylvania

Site description	Location		Sampling date
	Latitude	Longitude	
Delaware drainage			
Schuylkill River at Fall Bridge	40.008400	−75.197500	July 11, 2007
Brodhead Creek near East Stroudsburg (upstream)	41.037000	−75.210000	July 19, 2007
Brodhead Creek at Minisink Hills (downstream)	40.998500	−75.143300	July 18, 2007
Susquehanna drainage			
Conodoguinet Creek near Hogestown	40.255200	−77.018900	July 12, 2007
Susquehanna River at Danville (upstream)	40.957700	−76.621200	July 17, 2007
Susquehanna River (downstream)	40.922300	−76.717900	July 16, 2007
Swatara Creek at Harper Tavern (upstream)	40.402500	−76.577400	August 1, 2007
Swatara Creek near Hershey (mid)	40.293321	−76.674794	August 2, 2007
Swatara Creek near Hummelstown (downstream)	40.249900	−76.736200	August 16, 2007
Juniata River at Newport	40.479002	−77.128519	August 14, 2007 April 21, 2010
Yellow Breeches Creek at New Cumberland	40.224186	−76.860560	August 15, 2007
Ohio drainage			
Ohio River at Sewickley	40.533370	−80.187562	July 23, 2008
Monongahela River at North Charleroi (upstream)	40.389900	−79.859200	July 21, 2008
Monongahela River at Braddock (downstream)	40.152010	−79.904123	July 22, 2008
Allegheny River at Kittaning (upstream)	40.812600	−79.522600	August 4, 2008 April 28, 2010
Allegheny River at Oakmont (downstream)	40.527100	−79.846400	August 5, 2008

Table 1. reproduced from Blazer et al. 2014, p.6474

Analysis and Results:

The percentage of male SMB with testicular oocytes was higher at the basin sites than the percentage with testicular oocytes at the out-of-basin sites (Figure 2, from Blazer et al. 2014) The percent intersex at the Schuylkill River site was intermediate and higher than the percent intersex at the Ohio basin sites but lower than the percent intersex at all of the Susquehanna River sites. The Intersex Severity Index for small mouth bass was significantly higher at the basin sites with the exception of the Swatara up and Swatara mid versus the out of basin sites (Table 4, reproduced from Blazer et al 2014). In addition, the Intersex Severity Index for SMB was significantly higher at the Juniata River site at Newport than the Allegheny River at Kittaning when considering the Spring 2010 samples, though the intersex prevalence was high in small mouth bass collected at both sites in 2010 (Juniata 67% and Allegheny 75%).

Of the 5 hormones analyzed in the discrete samples, estrone and *cis*-androsterone were the only hormones that were detected above the reporting limit (Table 8, reproduced from Blazer et al. 2014). Estrone and *cis*-androsterone were not detected in grab samples collected from the Ohio Basin. Estrone was highest in the grab samples collected from the Schuylkill River at Fall Bridge, but concentrations also exceeded reporting limits at samples collected from Brodhead Creek (downstream), Conodoguinet Creek near Hogestown, Susquehanna River (downstream of Danville WWTP), and Juniata River at Newport. *Cis*-androsterone was only detected in the grab sample collected from Yellow Breeches Creek at New Cumberland.

Estrone concentrations were significantly correlated with testicular oocyte prevalence (rho=.6530, p=0.0238), severity (rho=0.7609, p=0.0055), and the percentage of male SMB with measurable plasma vitellogenin (rho=0.7914, p=0.0033).

Estrone was the most commonly found hormone in the bed sediment with concentrations above the reporting limit at all basin sites except for the Swatara upstream site and Conodoguinet site (Table 9). Estrone was also found in the sediment at comparable levels in the out-of-basin sites including Schuylkill, Ohio, Allegheny (up and down), Monongahela (up and down), and Ohio. Estrone tended to be higher downstream of WWTPs on the Susquehanna River (Danville WWTP), and Swatara Creek. Estrone was also higher downstream of the Quittapahilla confluence. The only hormone that was found in the basin but not in the controls was progesterone, which was found at the Swatara mid and down locations, though concentrations were below the reporting limit. The androgen *cis*-androstene was only detected at the Ohio location.

In 2010, all estrogenic and androgenic compounds tested for by the passive samplers were higher at the Juniata River site at Newport than the Allegheny River site at Kittaning (Table 10). Four out of the six pesticides tested by the passive samplers in 2010 tended to be higher at the Allegheny site including atrazine, acetochlor, metolachlor, and simazine. Prometon and desethylatrazine were below the reporting limits or detection limits at both sites.

Table 4 Reproductive endpoints of male smallmouth bass collected from three river drainages in Pennsylvania in 2007–2010

Site	Sample size	Intersex severity[a]	Mean vitellogenin[b] (μg/mL)
Delaware drainage			
Schuylkill	10	0.6±0.9 ab	166.7±11.9 (9)
Susquehanna drainage			
Susquehanna up	14	1.4±0.3 a	4.3±1.2 (3)
Susquehanna down	8	1.8±0.4 a	113.6±61.8 (5)
Swatara up	4	0.6±0.4 ab	0
Swatara mid	6	0.9±0.7 ab	8.0±3.8 (3)
Swatara down	6	2.0±1.2 a	0
Juniata Summer	7	2.6±0.5 a	155.4±27.9 (7)
Spring 2010	9	2.0±0.5 A	431.7±823.5 (6)
Ohio drainage			
Ohio	9	0.1±0.2 b	66.0±0.0 (1)
Monongahela up	10	0.1±0.2 b	37.0±0.0 (1)
Monongahela down	7	0.1±0.2 b	13.0±0.0 (1)
Allegheny up	6	0.2±0.3 b	31.0±0.0 (1)
Spring 2010	12	0.3±0.5 B	338.9±373.5 (9)
Allegheny down	10	0.1±0.3 b	36.5±26.2 (2)

[a] Mean±standard deviation. Values followed by the same lowercase letters collected during summers of 2007–2008 or uppercase letters collected in Spring 2010 were not significantly different

[b] Mean±standard deviation of only those individuals with measurable vitellogenin. Number in parentheses indicates sample size of positive males

Table 4. reproduced from Blazer et al. 2014, p. 6478

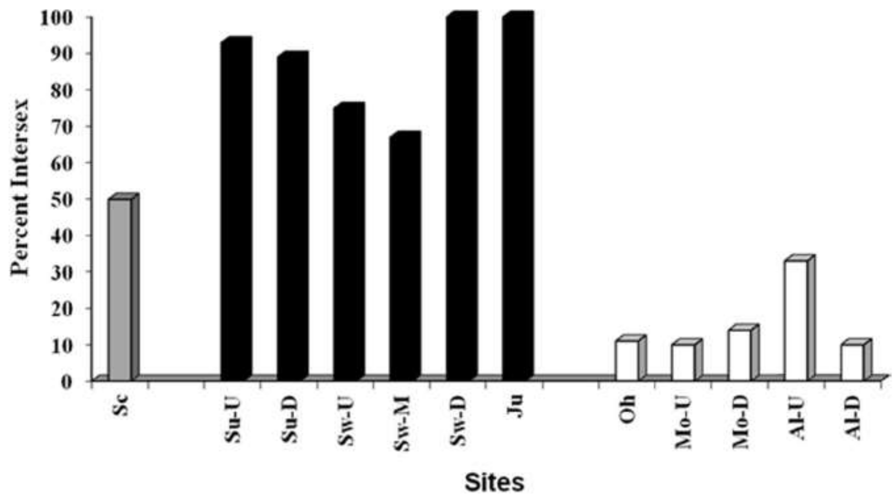

Fig. 2 Percentage of male smallmouth bass with testicular oocytes collected in the three major river drainages in Pennsylvania in 2007–2008. One site, Schuylkill River (*Sc*) in the Delaware drainage (*gray bar*); six sites, upstream (*Su-U*) and downstream (*Su-D*) on the Susquehanna River, upstream (*Sw-U*), middle (*Sw-M*), and downstream (*SW-D*) Swatara Creek and Juniatia River (*Ju*) in the Susquehanna drainage (*black bars*); Ohio River (*Oh*), upstream (*Mo-U*) and downstream (*Mo-D*) Monongahela River, upstream (*AL-U*) and downstream (*Al-D*) Allegheny in the Ohio drainage (*white bars*)

Figure 2. reproduced from Blazer et al. 2014, p.6478

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Table 8 Hormones (ng/L) measured in discrete water samples collected at fish health sites

Site	17-β Estradiol	Estriol	Estrone	*cis*-Androsterone	4-Androstene-3,17-dione
MDL[a]	0.05	0.05	0.05	0.05	0.05
Schuylkill	BD	BD	**2.72**	BD	BD
Brodhead up	BD	BD	*0.32*	BD	*0.40*
Brodhead down	BD	BD	**1.71**	BD	BD
Conodoguinet	BD	BD	**1.20**	BD	BD
Susquehanna up	BD	BD	*0.77*	BD	BD
Susquehanna down	BD	BD	**1.06**	BD	*0.65*
Swatara up	BD	BD	BD	BD	BD
Swatara mid	BD	BD	*0.77*	BD	BD
Swatara down	BD	BD	*0.68*	BD	BD
Juniata	*0.39*	BD	**1.31**	BD	BD
Yellow Breeches	BD	*0.23*	*0.73*	**1.53**	*0.39*
Ohio	BD	BD	BD	BD	BD
Monongahela up	BD	BD	BD	BD	BD
Monongahela down	BD	BD	BD	BD	BD
Allegheny up	BD	BD	BD	BD	BD
Allegheny down	BD	BD	BD	BD	BD

[a] The reporting level (RL) is set at least two times higher than the method detection limit (MDL). Values at or above the RL are presented in bold; those in italics are below the RL but above the MDL and are estimated (Foreman et al. 2012). Below detection (BD) denotes below the MDL

Table 8. reproduced from Blazer et al. 2014, p. 6484.

Table 9. Hormones and plant sterols (µg/kg) measured in sediment samples collected at fish health sites

Site	**17-β estradiol**	**17-α Estradiol**	**Estrone**	**Progesterone**	**cis-Androstene**	**β-Sitosterol**	**β-Stigmastanol**
MDL[a]	0.05	0.05	0.05	0.25	0.05	*363*	*367*
Schuylkill	*0.102*	BD	**1.336**	BD	BD	*632*	*218*
Brodhead up	BD	BD	BD	BD	BD	*445*	BD
Brodhead down	BD	BD	BD	BD	BD	*441*	*70*
Conodoguinet	*0.452*	BD	BD	BD	BD	*3,072*	*833*
Susquehanna up	BD	BD	**0.444**	BD	BD	*1,058*	*265*
Susquehanna down	BD	BD	**1.336**	BD	BD	*2,184*	*53*
Swatara up	BD	BD	BD	BD	BD	*1,917*	*384*
Swatara mid	*0.569*	BD	**2.857**	*3.504*	BD	*6,625*	*1,424*
Swatara down	*0.246*	*0.061*	**1.319**	*1.015*	BD	*713*	*367*
Juniata	*0.414*	BD	**1.437**	BD	BD	*593*	BD
Yellow Breeches	BD	BD	**0.386**	BD	BD	*876*	*280*
Ohio	**0.49**	BD	**2.861**	BD	**0.78**	*889*	BD

Monongahela up	**0.222**	BD	**1.851**	BD	BD	*4,615*	*739*
Monongahela down	**0.181**	**0.082**	**0.5**	BD	BD	*2,667*	*299*
Allegheny up	BD	BD	**0.478**	BD	BD	*196*	BD
Allegheny down	BD	BD	**0.289**	BD	BD	*177*	BD

[a] The reporting level (RL) is set at least two times higher than the method detection limit (MDL). Values at or above the RL are presented in bold; those in italics are below the RL but above the MDL and are estimated, except for β-sitosterol and β-stigmastanolwhich are all estimated due to variable method performance (Foreman et al. 2012). Below detection (BD) denotes below the MDL.

Table 9. reproduced from Blazer et al. 2014, p. 6484.

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Table 10 Hormones and pesticides detected in extracts of passive samplers deployed in the Juniata and Allegheny Rivers spring 2010

Chemical compounds[a]	Juniata River	Allegheny River
Estrogenic Compounds (ng/POCIS)		
17-α-Estradiol	**0.42**	BD
17-β-Estradiol	**1.12**	*0.18*
Estrone	**3.70**	**1.73**
Total EEQ[b]	2.94	0.78
Androgenic Compounds (ng/POCIS)		
cis-Androsterone	**0.70**	BD
4-Androstene-3,17-dione	**2.77**	**1.65**
Testosterone	**0.48**	BD
Pesticides estimated (ng/L) from POCIS extracts		
Desethylatrazine	*2.60*	*2.00*
Atrazine	**5.70**	**15.00**
Acetochlor	BD	**0.88**
Metolachlor	**1.70**	**4.90**
Prometon	*0.36*	BD
Simazine	BD	**3.80**

POCIS polar organic chemical integrative samplers, *EEQ* estrogen equivalent quotient, *BD* below detection

[a] Values at or above the RL are presented in bold; those in italics are below the RL but above the method detection limit (MDL)

[b] EEQ is the estrogen equivalent quotient as measured using the bioluminescent yeast estrogen screen (BLYES)

Table 10. reproduced from Blazer, et al., 2014, p. 6485.

Conclusion:

The research conducted by Blazer et al. (2014) gives support to the hypothesis that the prevalence of testicular oocytes and severity of intersex in male smallmouth bass may play a role in the decline of YOY SMB in the lower Susquehanna basin because these reproductive benchmarks were significantly higher at the majority of the basin sites, though mean vitellogenin was similar between the Schuylkill site and the Susquehanna sites. In addition, correlation analysis demonstrated that higher levels of estrone in the water column may be associated with reproductive benchmarks in SMB but the evidence that estrone was detected at comparable levels in the sediment between basin and out of basin controls weakens the case that estrone concentration in the sediment is the cause for the decline in young of the year. The passive sampler results in 2010 gives further support that estrogenic and androgenic compounds, including 17-α-estradiol, 17-β-estradiol, estrone, total EEQ, cis-androstene-3,17-dione, and testosterone, may play a role as these compounds were higher at the Juniata site than the Allegheny site, though passive samplers were just deployed at these two locations. The 2010 passive sampler pesticide results at the two sites somewhat weakens the case that the six pesticides tested for play a role because they were either below reporting limits (desethylatrazine, and prometon) or were higher at the site on the Allegheny River versus the site on the Juniata River (atrazine, acetochlor, metolachlor, and simazine).

Literature Cited:

Blazer, V., Iwanowicz, D., Walsh, H., Sperry, A., Iwanowicz, L., & Alvarez, D. 2014. Reproductive health indicators of fishes from Pennsylvania watersheds: association with chemicals of emerging concern. *Environmental Monitoring and Assessment,* 186(10), 6471-6491.

Title: Total Estrogenicity (WS #49)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: EDCs increase YOY susceptibility to disease

Introduction:

EDCs could negatively affect YOY survival and/or susceptibility to disease. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

Data:

PA DEP collected grab samples to be tested for total estrogenicity at 29 sites within the Susquehanna River basin. Samples were analyzed by USGS:

Stream Name
Buffalo Creek
Cocolamus Creek
Crosskill Creek
Earlakill Creek
Elizabeth Run
Frankstown Branch Juniata River
Honey Creek
Hungry Run
Jacks Creek
Juniata River below Huntington STP
Juniata River below Lewistown STP
Kishacoquillas Creek
Little Juniata River
Little Kishacoquillas Creek
Little Swatara Creek 1
Little Swatara Creek 2
Lost Creek
Manada Creek
Quittapahilla Creek
Raystown Branch Juniata River

Reeds Creek
Schuylkill River
Spring Creek
Susquehanna River @ Danville
Susquehanna River @ Lewisburg
Swatara Creek 1
Swatara Creek 2
Swatara Creek 3
Tuscarora Creek

Analysis and Results:

Total estrogenicity was detected at only five sites:

Crosskill Creek	0.28	ng/L
Elizabeth Run	0.24	ng/L
Kishacoquillas Creek	0.48	ng/L
Little Swatara Creek 1	0.36	ng/L
Little Swatara Creek 2	0.88	ng/L

These arevery low amounts. There is a chance that the detection limit is not low enough to detect contaminants at the other sites.

Conclusion:

The evidence weakens the argument for total estrogenicity as the cause of low recruitment of YOY SMB. Few consistent patterns are noticeable in the data, but hormones remain a parameter of concern.

Candidate Cause 14: Pathogens and Parasites

Worksheets: 27, 29, 30, and 31

Title: YOY Smallmouth Bass and abiotic factors (WS #27)

Agency: Pennsylvania Fish and Boat Commission

Stressor-Response Relationships

Candidate Cause: Pathogens and parasites kill YOY directly or they are more effective because YOY have become more susceptible due to candidate causes 2-13

Introduction:

One of the hypotheses is that abrupt or stressful physicochemical water quality changes at certain timeframes increases susceptibility of YOY SMB to disease. There is a complex interplay of factors that regulate disease at the individual and population levels. The presence of a pathogen is necessary but typically not sufficient to cause disease alone (Snieszko 1974). Chaplin et al. (2010) and Chaplin and Crawford (2012) documented stressful water quality conditions in nearshore, microhabitats during critical times during their development. Seifert et al. (1974) found dissolved oxygen concentrations below 4.4 mg/L resulted in a 20% reduction in survival of SMB embryos and larvae in laboratory experiments. In addition to acute effects, sub-lethal effects such as earlier hatch time and reduced growth of larval Smallmouth Bass were documented at dissolved oxygen concentrations of 4.4 mg/L (Seifert et al. 1974) while behavioral responses such as agitation and rising to the surface were documented in larval Smallmouth Bass at dissolved oxygen concentrations up to 4 mg/L (Spoor 1984).

Comparisons of the Susquehanna River with similar locations at the Delaware and Allegheny rivers during 2008 - 2010 found median daily maximum temperatures to be 2.0 ^{o}C, 2.7 ^{o}C, and 1.6^{o}C higher and 3.1 ^{o}C, 1.2 ^{o}C, and 3.4^{o}C higher, respectively (Chaplin et al. 2009; Chaplin and Crawford 2012). Further, a comparison of median daily temperatures from continuous monitoring data at the Susquehanna River at Harrisburg, Pennsylvania for the period critical to YOY SMB development were significantly higher (two-sided Wilcoxon signed-rank test, $p < 0.05$) during 2008-2010 than during the same period in 1974 to 1979, the only other timeframe with a continuous temperature record (Chaplin et al. 2009; Chaplin and Crawford 2012). Water temperatures that YOY SMB were exposed to did not approach lethal temperatures of 37^{o}C suggested by Wrenn (1980) and higher temperatures during this life stage are beneficial for rapid growth, with peak consumption rates at 29^{o}C (Shuter and Post 1990; Hewett and Johnson 1992; Zwiefel et al.1999). Additionally, juvenile black bass (*Micropterus* spp.) are considered to have a higher thermal tolerance than adults (Recsetar et al. 2012). Brewer (2011) found that in spring-fed Missouri streams, age-0

SMB selected warmer available habitats regardless of the amount of groundwater contribution. At finer spatial scales, temperature is one of the primary factors with age-0 SMB habitat selections (Brewer 2011). While habitat selection based on higher temperature may benefit YOY SMB metabolically, it also potentially increases the risk of exposure to pathogens. Grant et al. (2003) found that temperatures above 30°C increased the viral replication rate of LMBV and that Largemouth Bass infected with LMBV had higher rates of mortality and higher viral loads at 30°C than at 25°C in laboratory experiments. Similarly, some common bacterial pathogens such as *Flavobacterium columnare* (20 – 25°C, Starliper and Schill 2010) and *Aeromonas hydrophila* (25-35°C, Cipriano and Austin 2011) share optimal temperature ranges to that of YOY SMB.

The intent of this analysis is to compare water quality conditions immediately prior to the YOY SMB surveys to determine if any factors consistently occur that may be contributing to the outbreaks. These would include elevated temperatures, elevated or variable pH, or low dissolved oxygen conditions.

Data:

Back-pack electrofishing CPUE (fish/ 50 m) data for YOY SMB from the Pennsylvania Fish and Boat Commission's (PFBC) state-wide monitoring network was included in the analysis. Site-specific catch and prevalence data were aggregated by river reach and by year. Timing is based on directed surveys that are tied to fish size to allow for comparable catchability among years. Surveys are point-in-time surveys and reflect conditions at the time of capture and cannot be used to infer about other conditions outside of the survey window (i.e., before or after the survey). It is assumed that environmental conditions prior to the identified survey cumulatively affect fish within the sample. Follow-up surveys noted are qualitative as fish catchability changes as they grow; results are not comparable to directed surveys but can be used to comment on presence or absence of fish with signs of disease. For Juniata River figures, "lower" and "upper" values correspond to YOY Smallmouth Bass survey results from the lower Juniata River and upper Juniata River, respectively.

Physicochemical water quality data is from U.S. Geological Survey (USGS) and/or PA DEP sondes from different locations in the subject reaches. Where possible, multiple data points per location were plotted to allow for spatial differences in values to be accounted for by reviewers. The data period used was truncated to May 1 to August 14 of each year to simplify graphics while accounting for conditions that were most influential to YOY SMB. The data period for 2012 was extended to August 23 to account for the later onset of disease during that year.

Analysis and Results:

Continuous, instream monitoring data from subject reaches were overlain with results from PFBC directed YOY SMB surveys for subject reaches. When possible, qualitative surveys results were also overlain on the physicochemical water quality data to allow for further analysis of factors that could have contributed to difference in disease occurrence.

There is evidence of stressful water quality conditions occurring prior to the onset of disease at some reaches during some years but not occurring consistently. Further, there are also incidences where stressful water quality conditions occur where no disease is noted. There are also instances when physicochemical conditions would not be deemed particularly stressful during the time period leading into surveys; however, diseased fish were found. Throughout the different reaches and years of surveys there is no obvious pattern of physicochemical water quality conditions occurring that may be prompting disease manifestation.

Conclusion:

There is no evidence to support or refute the role of physicochemical parameters in the onset of disease. There are no consistent patterns in physicochemical data that precede disease onset among reaches and years. Other unmeasured factors may be present that influence the impact of these parameters.

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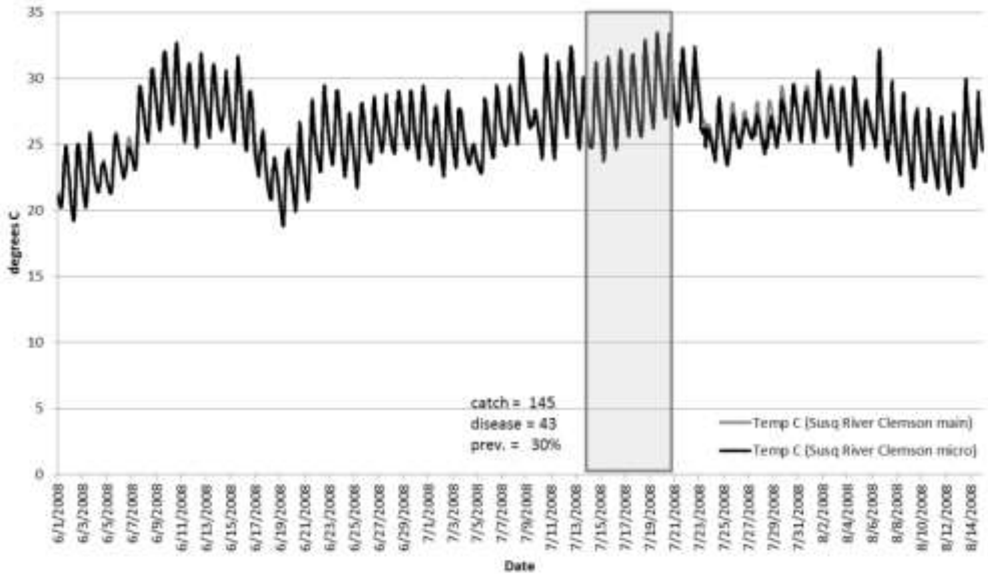
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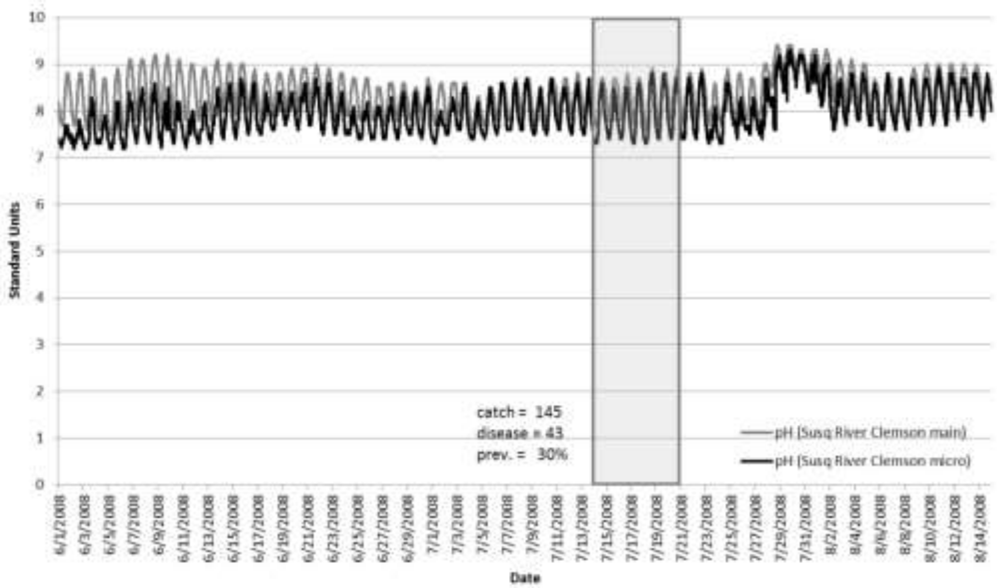
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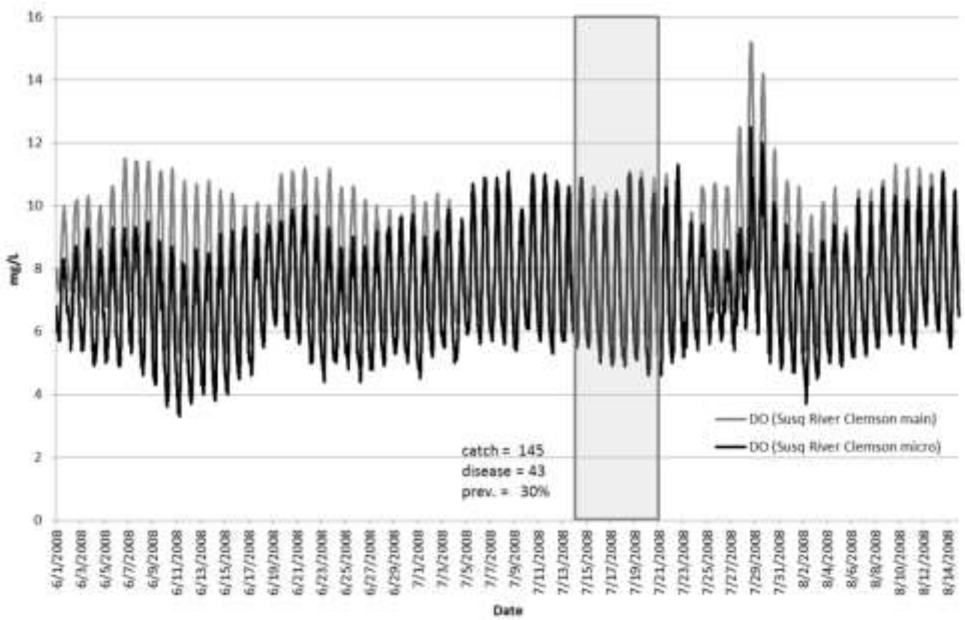
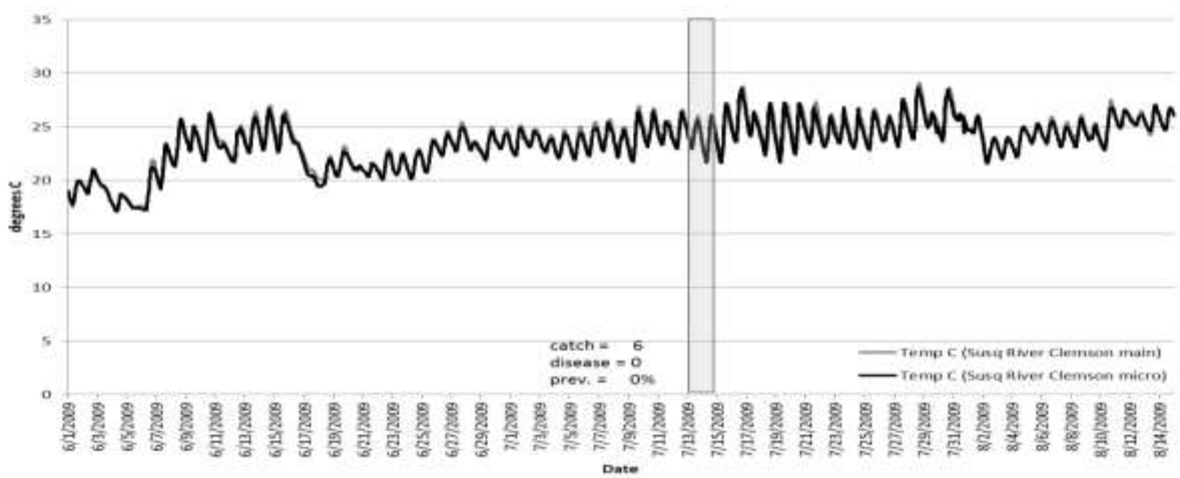
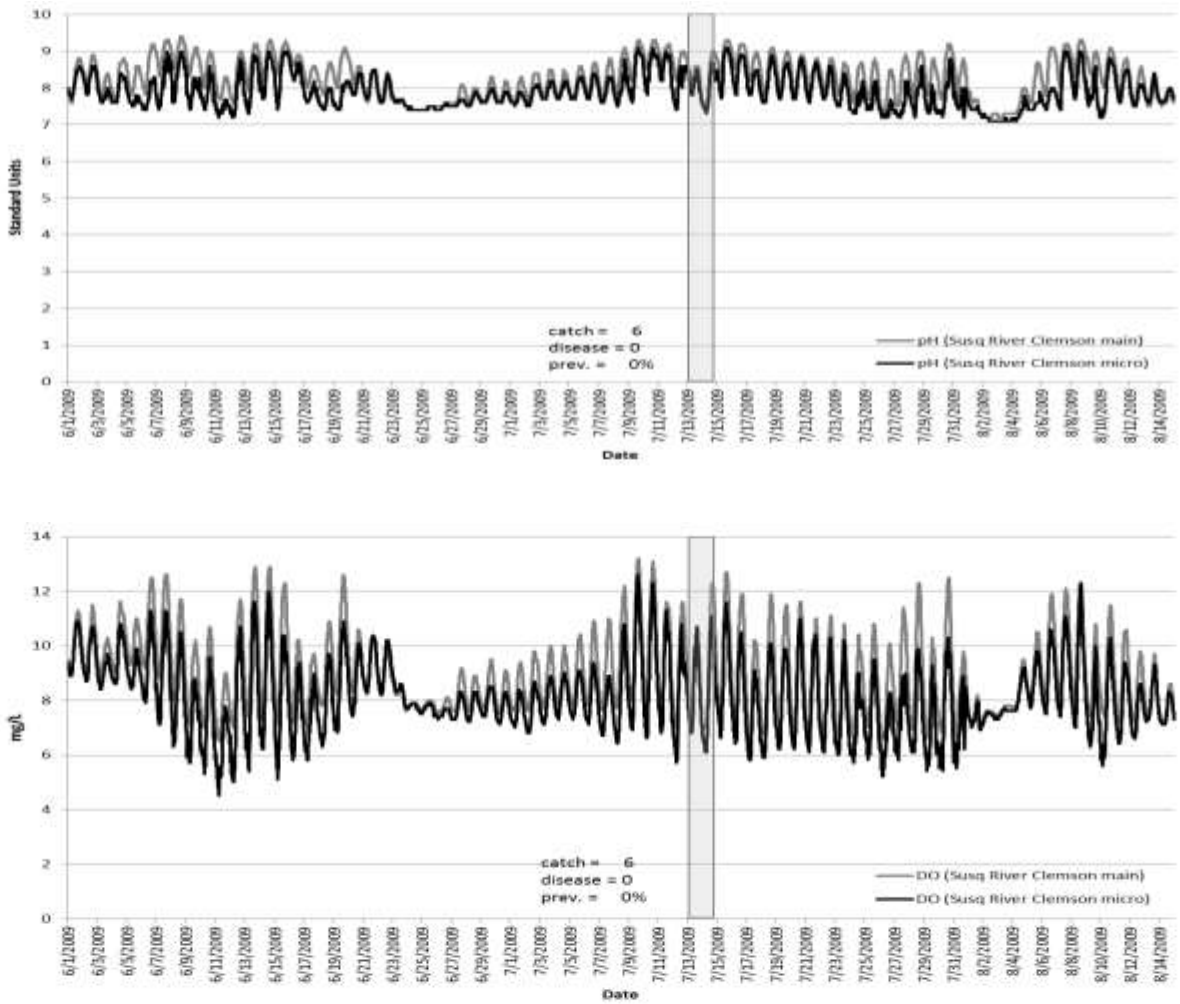
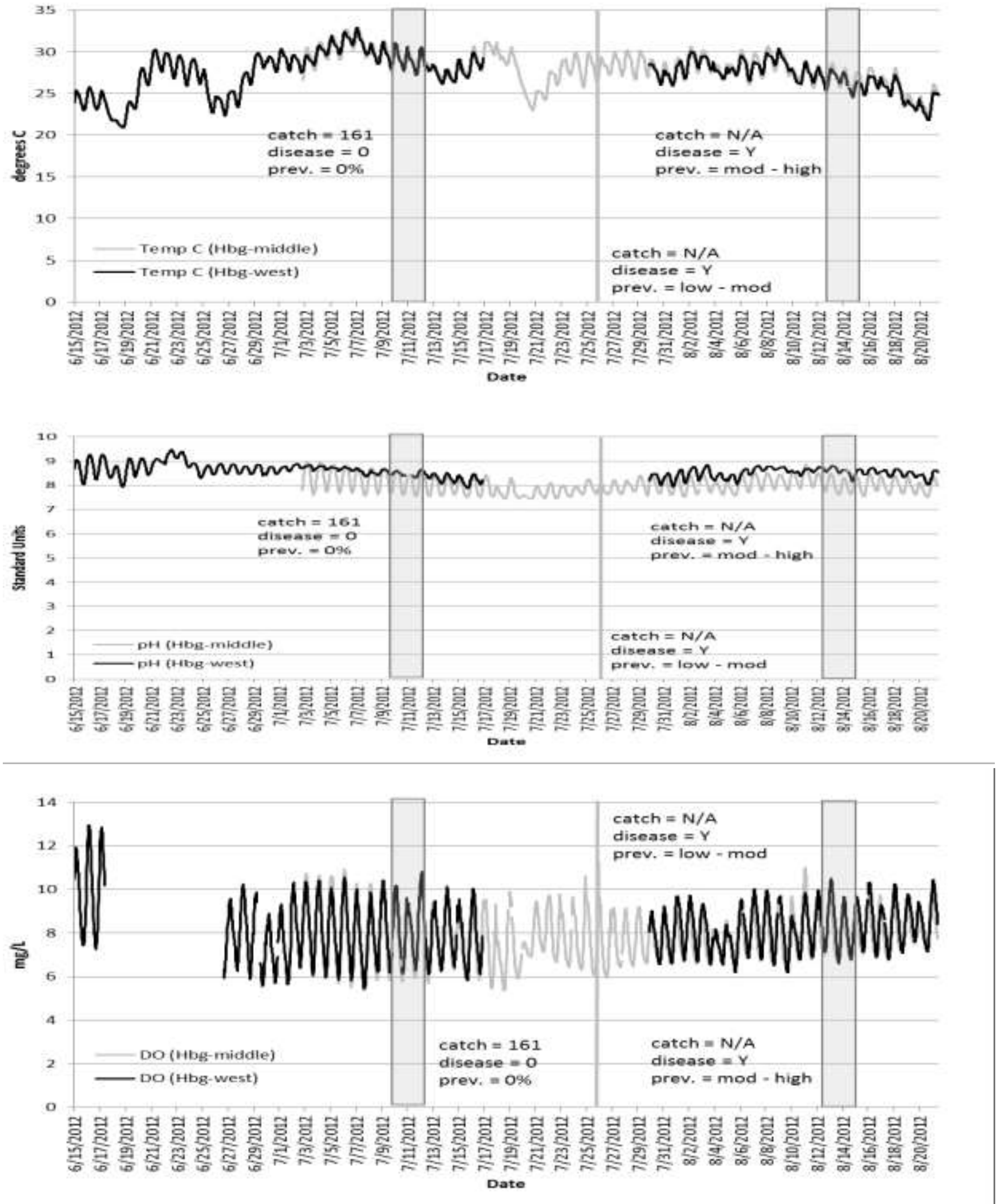


Comparison of temperature (top), pH (middle), and dissolved oxygen concentration (bottom) between main channel (gray) and microhabitat (black) locations at the Susquehanna River at Clemsons Island location during spring-summer 2008. Shaded area indicates survey window during that year with catch values for that survey reach superimposed on the graphic.

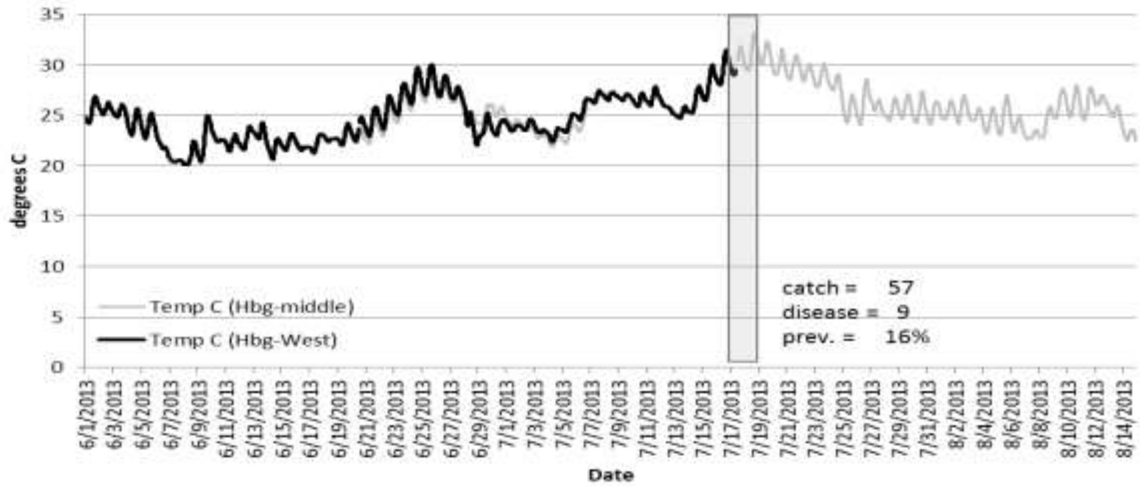


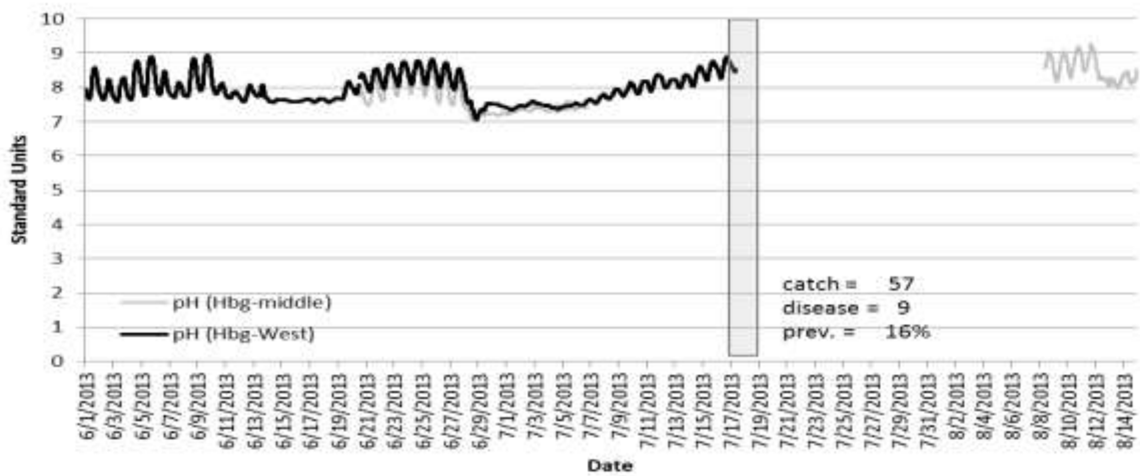


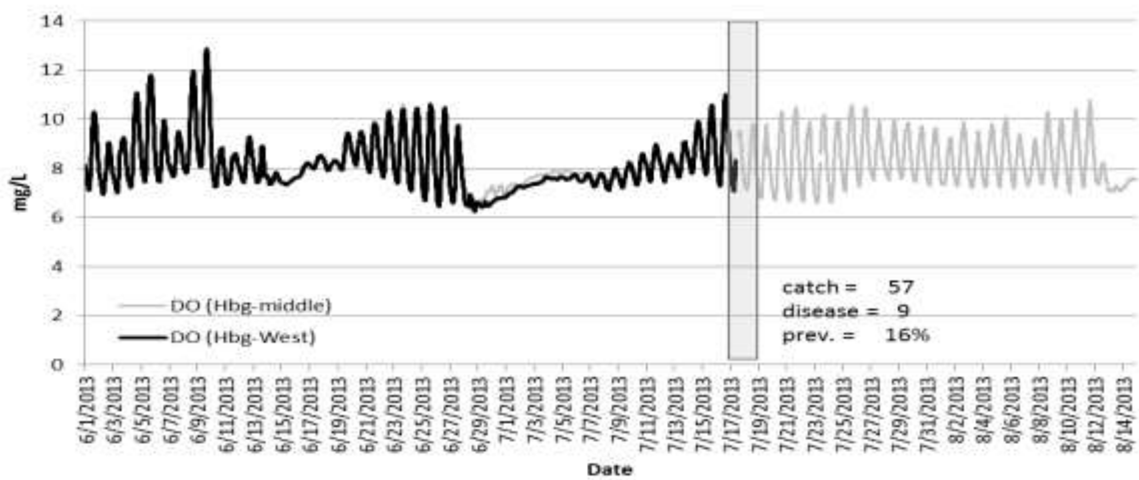
Comparison of temperature (top), pH (middle), and dissolved oxygen concentration (bottom) between main channel (gray) and microhabitat (black) locations at the Susquehanna River at Clemsons Island location during spring-summer 2009. Shaded area indicates survey window during that year with catch values for that survey reach superimposed on the graphic.



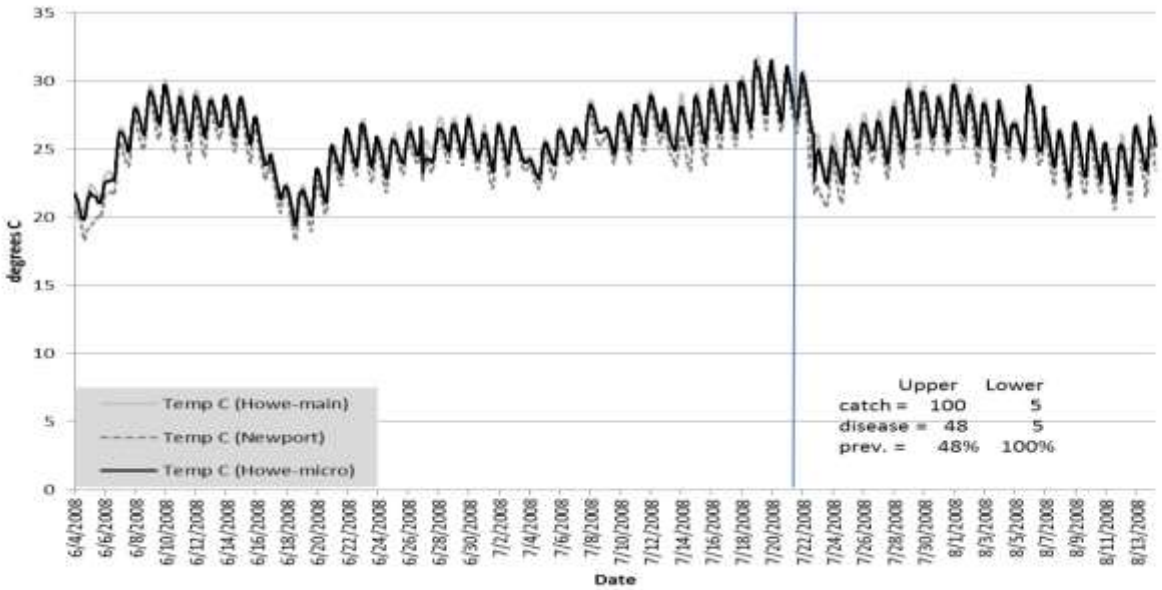
Temperature (top), pH (middle), and dissolved oxygen concentration (bottom) at the Susquehanna River at Harrisburg West channel (black line) and middle channel (gray line) locations during spring-summer 2012. Shaded areas indicate survey windows during that year with catch values for that survey reach superimposed on the graphic. Catch values for follow-up surveys could not be reported accurately because catch biased to favor diseased fish.

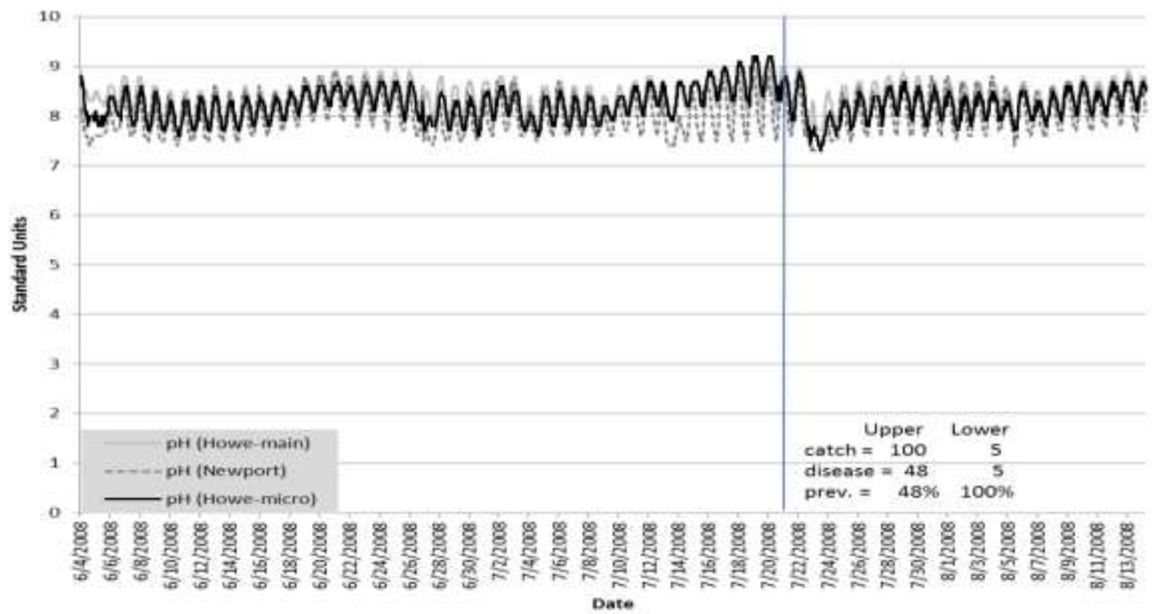


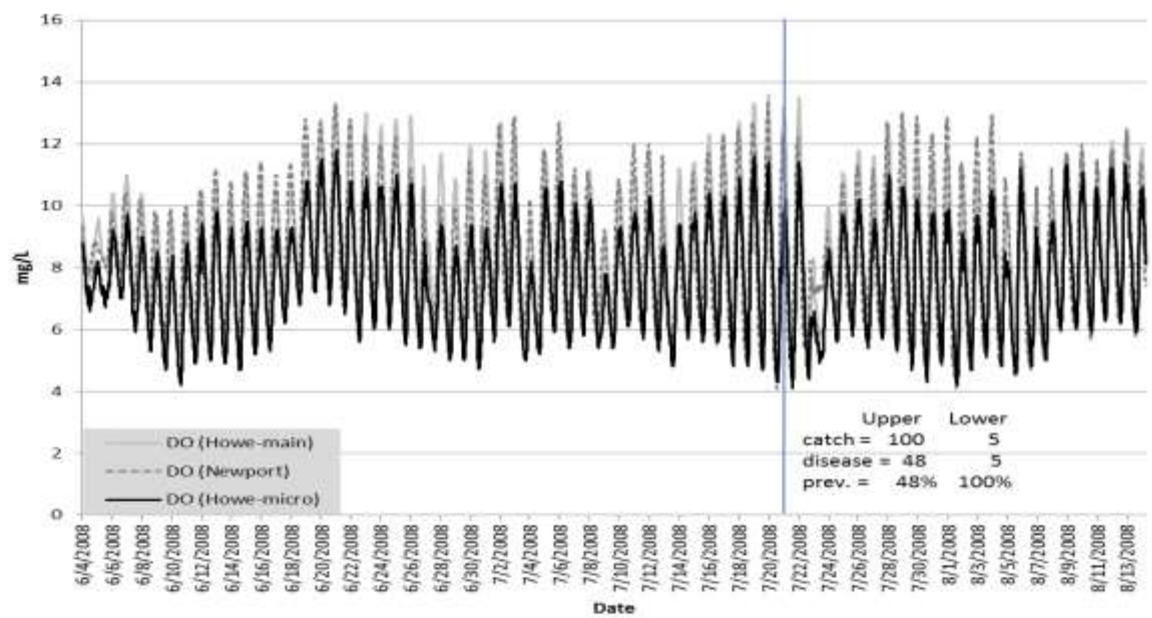




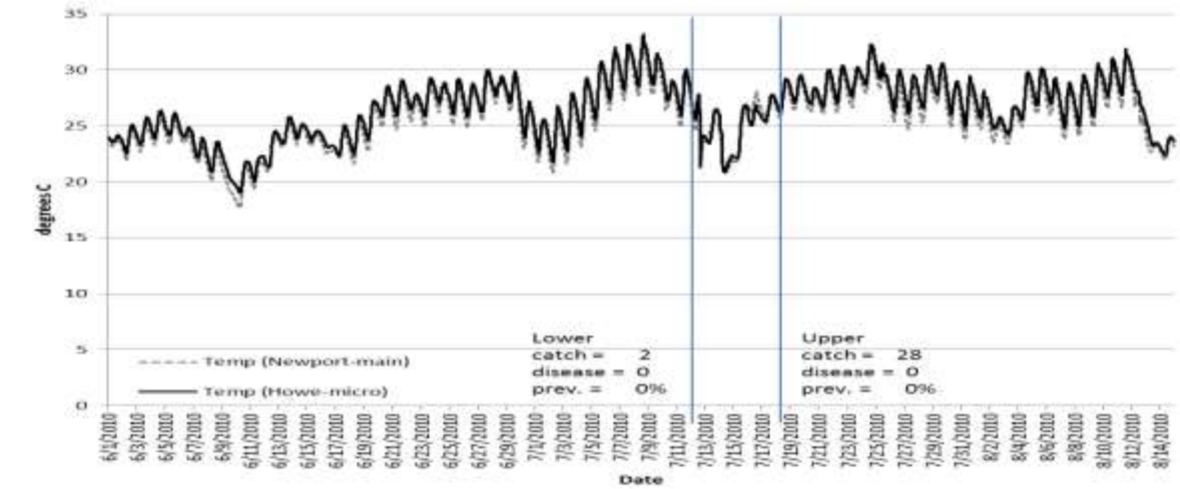
Temperature (top), pH (middle), and dissolved oxygen concentration (bottom) at the Susquehanna River at Harrisburg West channel (black line) and middle channel (gray line) locations during spring-summer 2013. Shaded area indicates survey window during that year with catch values for that survey reach superimposed on the graphic.







Comparison of temperature (top), pH (middle), and dissolved oxygen concentration (bottom) between main channel (gray), microhabitat (black), and alternate (dashed gray) locations at the Juniata River at Howe Twsp Park location during spring-summer 2008. Blue line indicates survey window during that year with catch values for that survey reach superimposed on the graphic.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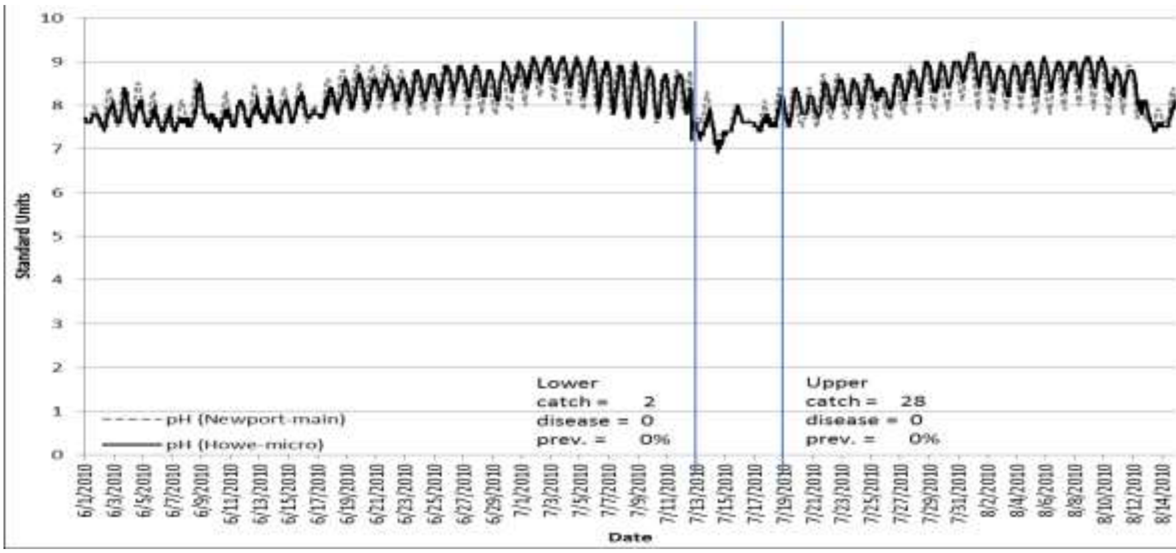
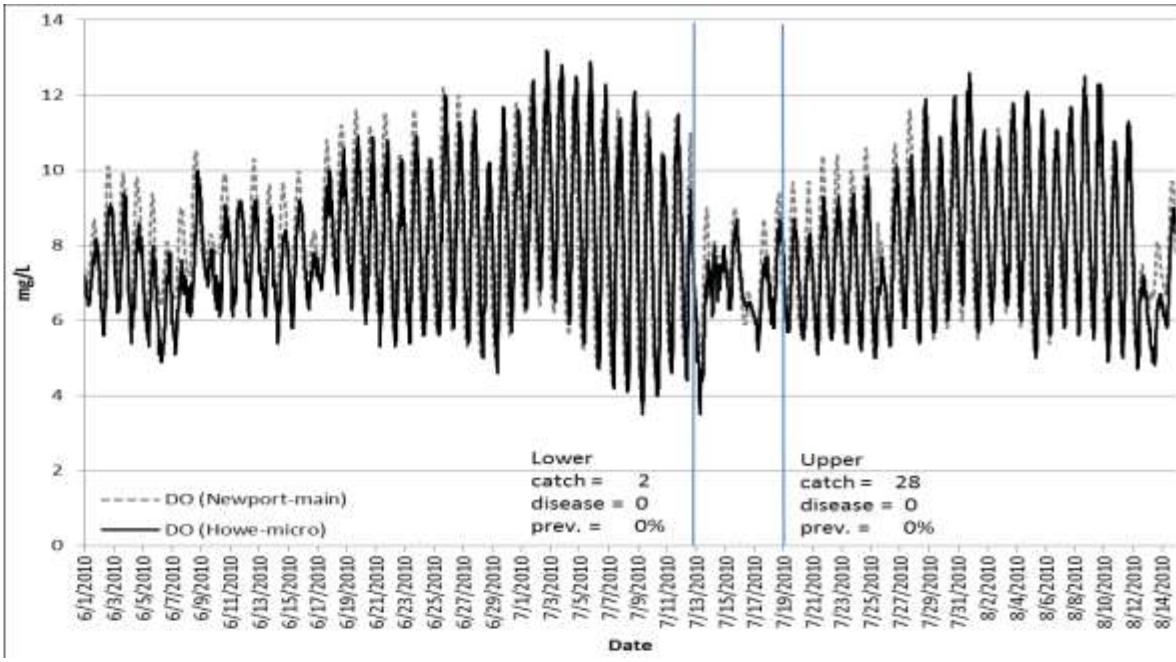
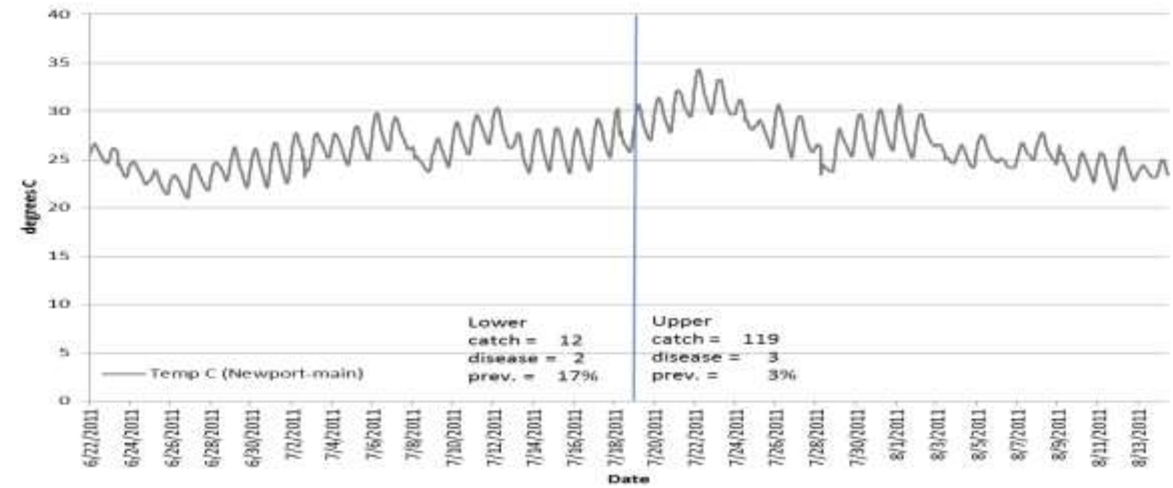
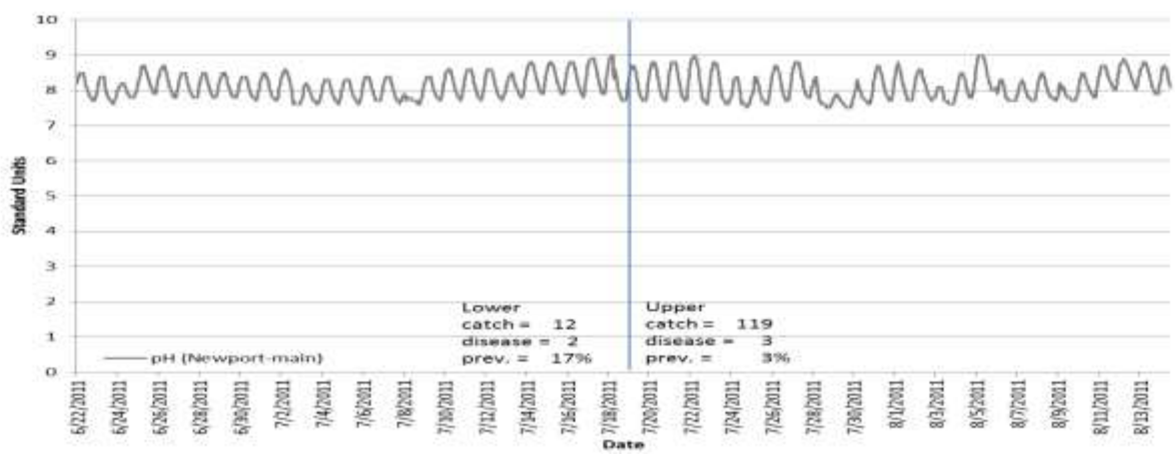
disease = 0
prev. = 0%
Temp (Newport-main)
Temp (Howe-micro)
Date

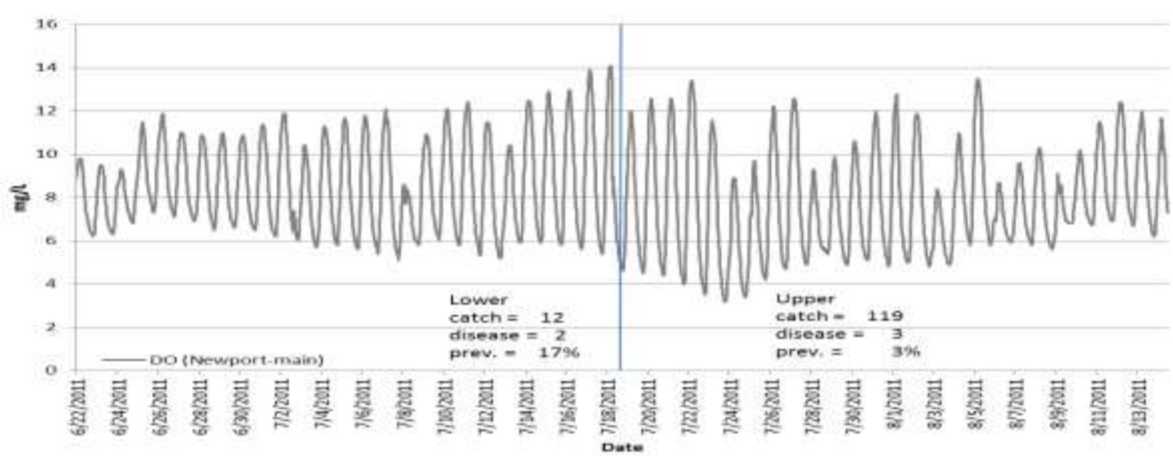

Standard Units
Lower
catch = 2
disease = 0
prev. = 0%
Upper
catch = 28
disease = 0
prev. = 0%
pH (Newport-main)
pH (Howe-micro)
Date


mg/L
Lower
catch = 2
disease = 0
prev. = 0%
Upper
catch = 28
disease = 0
prev. = 0%
DO (Newport-main)
DO (Howe-micro)
Date

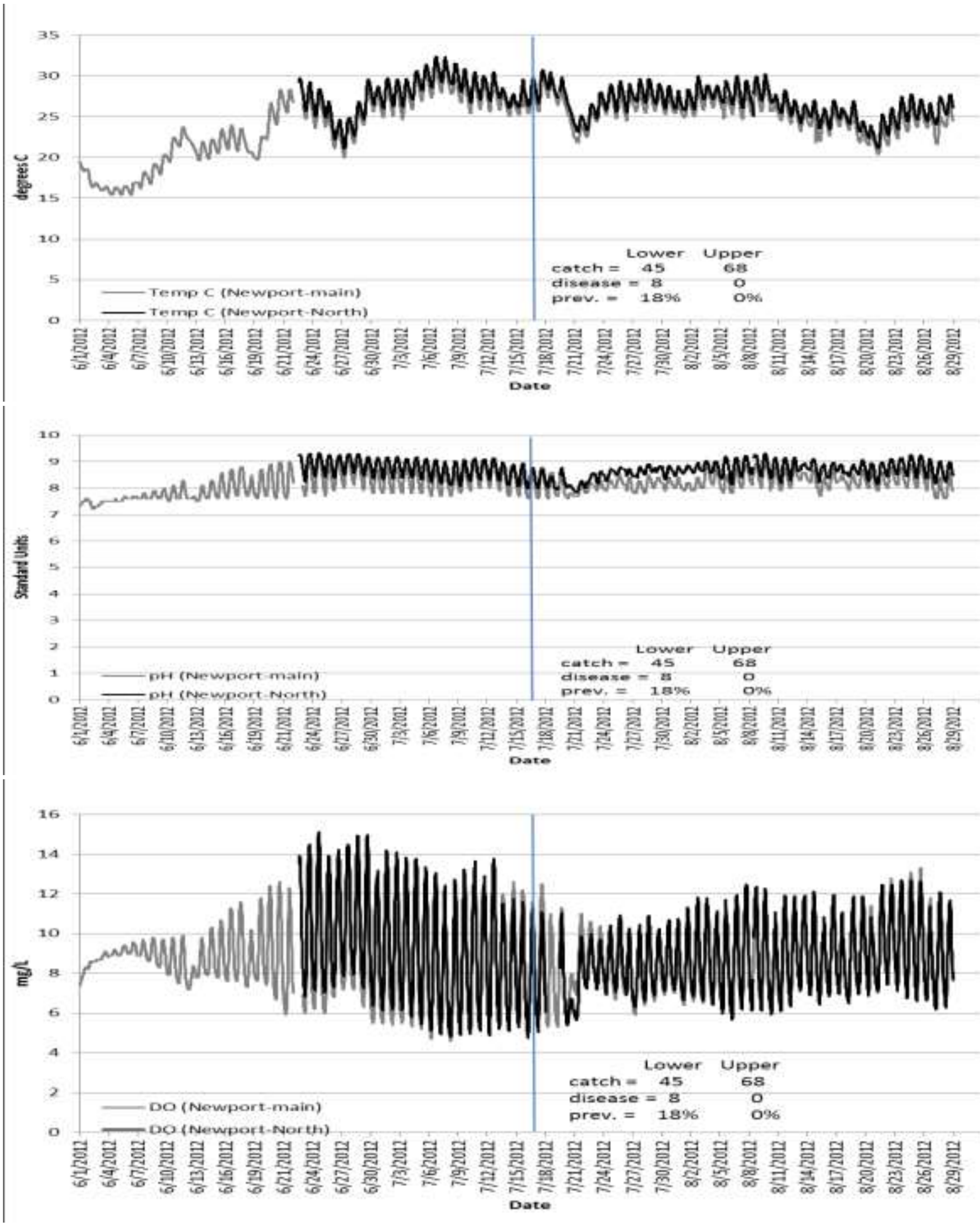
Comparison of temperature (top), pH (middle), and dissolved oxygen concentration (bottom) between microhabitat (black) and alternate (dashed gray) locations at the Juniata River at Howe Twsp Park location during spring-summer 2010. Blue line indicates survey window during that year with catch values for that survey reach superimposed on the graphic.



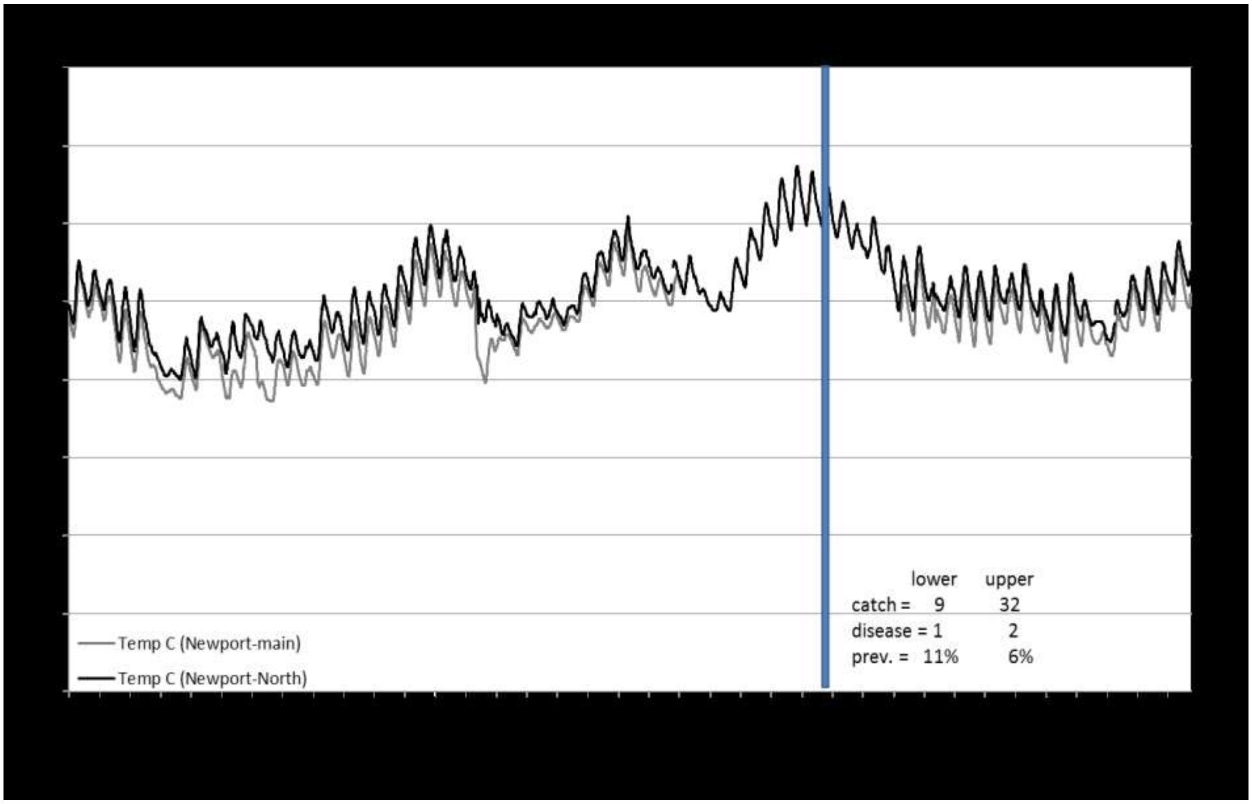


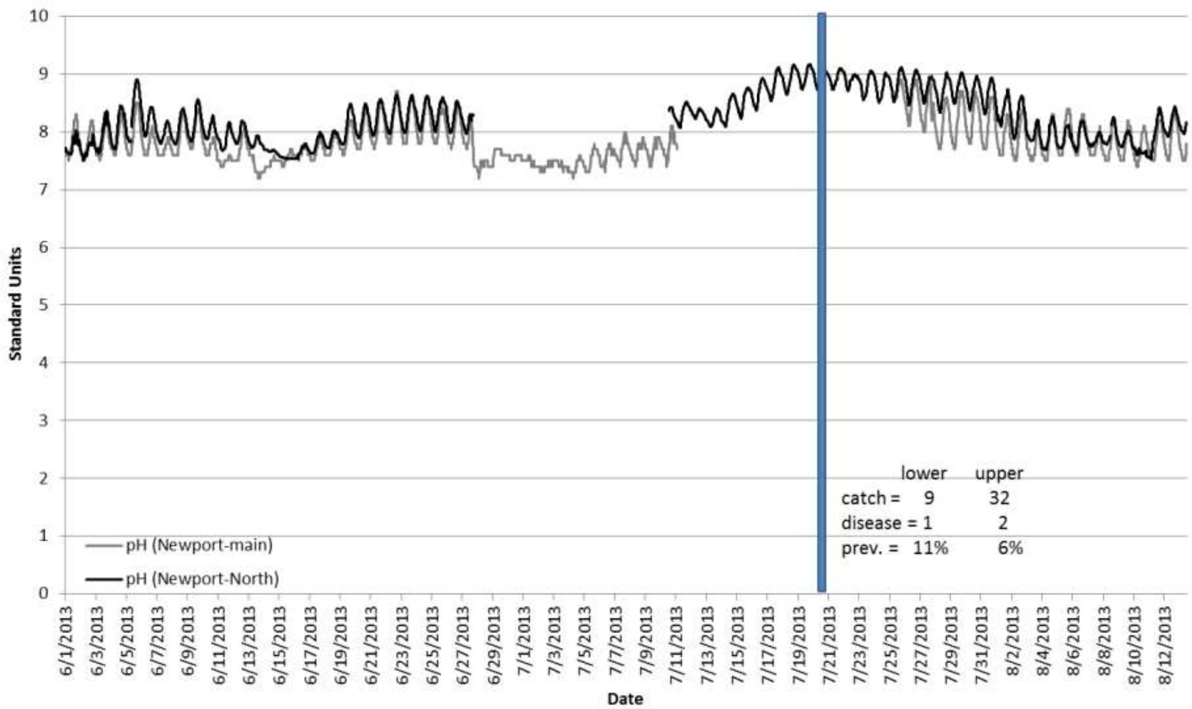


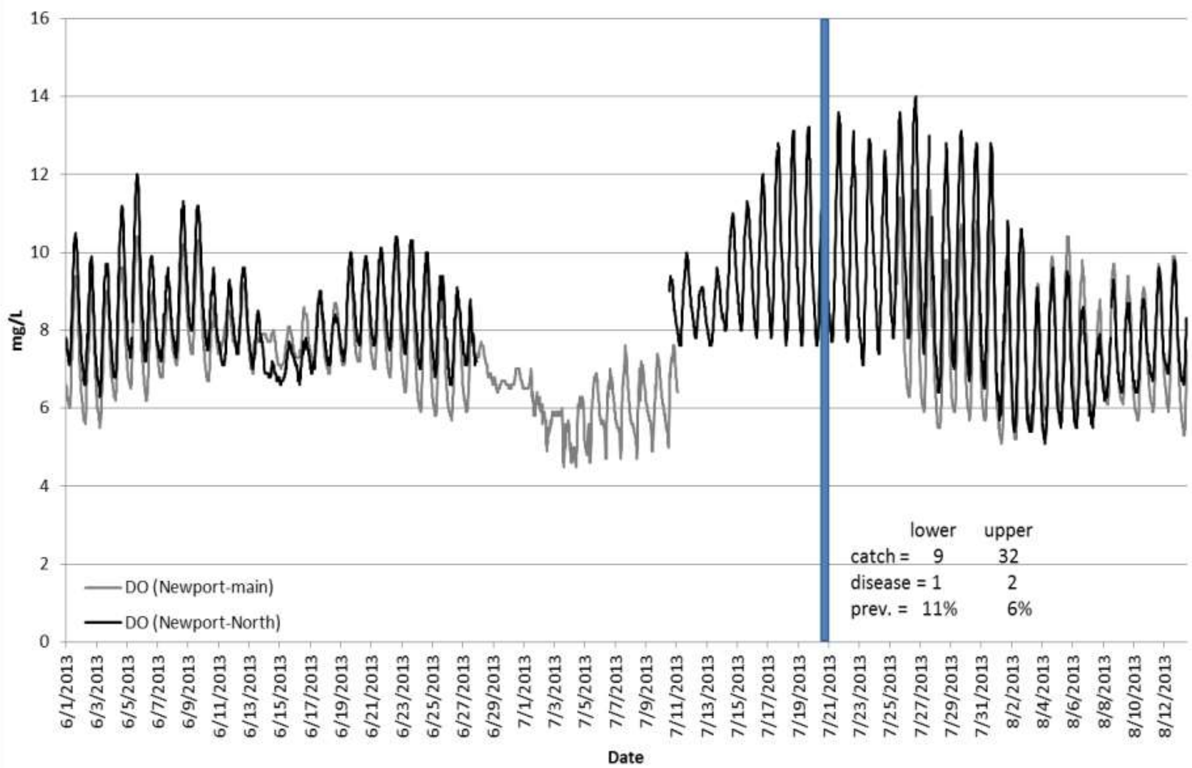
Temperature (top), pH (middle), and dissolved oxygen concentration (bottom) at the Juniata River at Newport (USGS 01567000) location during spring-summer 2011. Blue line indicates survey window during that year with catch values for that survey reach superimposed on the graphic.



Temperature (top), pH (middle), and dissolved oxygen concentration (bottom) at the Juniata River at Newport (USGS 01567000; gray line) and Newport North channel (black line) locations during spring-summer 2012. Blue line indicates survey window during that year with catch values for that survey reach superimposed on the graphic.




Standard Units
lower upper
catch = 9 32
disease = 1 2
prev. = 11% 6%
pH (Newport-main)
pH (Newport-North)
Date


mg/L
lower upper
catch = 9 32
disease = 1 2
prev. = 11% 6%
DO (Newport-main)
DO (Newport-North)
Date

Temperature (top), pH (middle), and dissolved oxygen concentration (bottom) at the Juniata River at Newport (USGS 01567000; gray line) and Newport North channel (black line) locations during spring-summer 2013. Blue line indicates survey window during that year with catch values for that survey reach superimposed on the graphic.

Title: Impacts of Pathogens (LMBV) or Parasites on Small Mouth Bass Populations (WS #29)

Agency: Pennsylvania Fish and Boat Commission

Stressor-Response Relationship

Candidate Cause: New pathogens (LMBV) or parasites or intermediate hosts may have moved into the area ($14b_2$)

Introduction:

Factors including water quality (Chaplin et al. 2009; Chaplin and Crawford 2012; Blazer et al. 2014) and parasite pathogens (Walsh et al. 2012; Smith et al. 2014) have been associated with secondary bacterial infections that are characteristic of outbreaks among YOY SMB. Largemouth Bass Virus (LMBV) has been consistently isolated from moribund SMB specimens submitted for viral analysis. Clinically diseased fish and apparently healthy from the same location have yielded positive results for LMBV during past surveys. Despite testing positive for LMBV via cell culture, fish did not exhibit the typical clinical sign of LMBV infection described by Plumb et al. (1996). Grizzle et al. (2003), demonstrated that fish without clinical signs of disease constitute a major source of LMBV dissemination. The repeated detection of LMBV in juvenile SMB specimens from diseased populations and the coincident onset of disease and discovery of LMBV in the Susquehanna River Basin in 2005 (U.S. Fish and Wildlife Service, National Wild Fish Health Database) necessitates a closer investigation into the potential role of LMBV in causing these annual mortality episodes in juvenile SMB.

Studies investigating LMBV have primarily focused on adult fish as they relate to large-scale fish kills in trophy-size adult Largemouth Bass *Micropterus salmoides*; however, mortality has been induced in laboratory studies involving juvenile Largemouth Bass and suggest susceptibility is higher at young age and that such mortalities can be unnoticed in the field (Plumb and Zilberg 1999b, Grant et al. 2003, Inendino et al. 2005). In clinical studies, dead and moribund juvenile Largemouth Bass infected by intraperitoneal (IP) injections of LMBV had virus concentrations that averaged $10^{8.5}$ TCID50/g (range $10^{6.6}$ - $10^{9.5}$), and $10^{7.7}$ TCID 50/g (range $10^{6.5}$ – $10^{9.5}$) (Plumb and Zilberg 1999b). Titer loads in a small sample of moribund YOY SMB collected from the Susquehanna River Basin have been found between $10^{4.0}$ and $10^{7.0}$ (C. Hillard, Penn State University, unpublished data), which is considered high in non-experimentally infected fish. Despite species differences, similarities would suggest that there would be a similar LD_{50} for LMBV in both species. Certainly, the observed viral loads in the moribund Susquehanna River SMB are similar to those observed to those associated with morbidity and mortality in Largemouth Bass in clinical trials, suggesting that LMBV could factor in the observed mortality in the Susquehanna Basin. Unfortunately, the pathogenicity of LMBV to SMB has not been ascertained and it median lethal dose (LD_{50}) never calculated.

Pathology of infected SMB has not been consistent with that documented in previous studies in the Largemouth Bass (Plumb et al. 1996, Plumb and Zilberg 1999b, Zilberg et al. 2000), suggesting differences in pathogenicity between the two closely related fish species along with the potential effects of stressors that may be prevalent at the time of infection (Groocock et al. 2008). For example, Inendino et al. (2005) found that density of fish and water quality influenced the survival and condition of juvenile Largemouth Bass infected with LMBV. Similarly, stressful water quality conditions (Chaplin et al. 2009, Chaplin and Crawford 2012, Smith et al. 2015) and the presence of opportunistic bacteria (Chaplin et al. 2009, Chaplin and Crawford 2012, Starliper et al. 2013, Smith et al. 2015) and parasite pathogens (Walsh et al. 2012, Smith et al. 2014, Smith et al. 2015) suggest that co-occurrence of these conditions in the Susquehanna River Basin may collectively exacerbate the virulence of LMBV to juvenile SMB. One key factor of interest is water temperature. YOY Smallmouth Bass condition is generally positively related to water temperature. Higher temperatures during this life stage are beneficial for rapid growth, with peak consumption rates at 29^{o}C (Shuter and Post 1990; Hewett and Johnson 1992; Zwiefel et al.1999). Additionally, juvenile black bass are considered to have a higher thermal tolerance than adults (Recsetar et al. 2012). However, Grant et al. (2003) found that temperatures above 30^{o}C increased the viral replication rate of LMBV and that Largemouth Bass infected with LMBV had higher rates of mortality and higher viral loads at 30^{o}C than at 25^{o}C in laboratory experiments. Similar to viral replication rates, many opportunistic bacteria share temperature optima with YOY SMB and may compound or utilize stress from other pathogens such as LMBV. Similarly, some common bacterial pathogens such as *Flavobacterium columnare* (20 – 25^{o}C, Starliper and Schill 2010*)* and *Aeromonas hydrophila* (25-35^{o}C, Cipriano and Austin 2011*)* share optimal temperature ranges to that of YOY SMB. It is plausible that despite the benefit of high water temperature to YOY SMB condition, the increased temperature could increase viral replication rate and expose fish to greater concentrations of opportunistic bacteria and could potentially induce stress and subsequently, disease.

Data:

Observational data for clinical signs of disease of SMB was conducted by Pennsylvania Fish and Boat Commission (PFBC) Division of Fisheries Management and Division of Fish Production Services, Fish Health Unit. Viral analysis was conducted by U.S. Fish and Wildlife Service, Northeast Fishery Center as part of the National Wild Fish Health Survey. Additional data on titer levels for some populations of SMB was provided by Penn State University, Animal Health Diagnostic Laboratory.

Results:

LMBV has widespread distribution within Pennsylvania (Figure 1) since at least 2005. Generally, LMBV has been isolated from YOY SMB and Largemouth Bass from populations where fish with clinical signs of disease have been found (Table 1). This includes both apparently healthy individuals and those with clinical signs of disease, with a few exceptions. Repeated collections over the years at some locations have demonstrated that once established in a system, LMBV is not always present at detectable concentrations (Table 1, Figure 1).

The role that LMBV is having in secondary infections is unknown. On-going laboratory investigations should help to clarify potential of LMBV pathogenicity to YOY SMB.

Conclusion:

The role of LMBV remains unclear and needs further resolution. Evidence of bass with high concentration of LMBV as well as high consistency of detection among population where disease is occurring suggest some role; however, whether it is opportunistic or primary pathogen remains unclear. It is most likely an opportunistic pathogen but it may contribute additional stress to the fish.

Table 1. Waterbody, location, date, species, sample size, disease characterization of Largemouth Bass Virus (LMBV) samples submitted as part of on-going investigations of YOY Smallmouth Bass disease investigations. Cell culture analysis and PCR confirmation was conducted by U.S. Fish and Wildlife Service, Northeast Fishery Center. Titer values were estimated by Penn State University, Animal Health Diagnotics Laboratory.

Waterbody	Site	Date	Species	Number	Clinical Disease	LMBV	Titer	Comment
Pine Creek		8/10/2010	Smallmouth Bass	17	Yes	Yes	NA	
Susquehanna River	Danville	7/13/2010	Smallmouth Bass	21	Yes	Yes	NA	
Pequea Creek		7/28/2011	Smallmouth Bass	1	Yes	No	NA	
Pequea Creek		7/28/2011	Largemouth Bass	3	No	No	NA	
Susquehanna River	Wrightsville	7/28/2011	Smallmouth Bass	12	Yes	Yes	NA	
Susquehanna River	Wrightsville	7/28/2011	Largemouth Bass	1	No	Yes	NA	
Loyalsock Creek		7/27/2011	Smallmouth Bass		Yes	Yes	NA	
Penns Creek		7/26/2011	Smallmouth Bass		Yes	Yes	NA	
Pine Creek		7/20/2011	Smallmouth Bass	15	Yes	Yes	NA	fish condition was very bad at time of capture
Raystown Lake		8/18/2011	Largemouth Bass	15	No	Yes	NA	No signs of clinical disease listed in table; however, picture from the field show fish with lesions
Schuylkill River	Cross Keys	8/3/2011	Smallmouth Bass	4	Yes	Yes	NA	
Schuylkill River	Cross Keys	8/3/2011	Largemouth Bass	5	Yes	Yes	NA	1 fish with lesions
Schuylkill River	Five Locks	8/3/2011	Smallmouth Bass	10	Yes	Yes	NA	
Schuylkill River	Five Locks	8/3/2011	Largemouth Bass	5	No	Yes	NA	
Delaware River	Point Pleasant	8/3/2011	Smallmouth Bass	4	Yes	Yes	NA	
Delaware River	Point Pleasant	8/3/2011	Largemouth Bass	4	No	Yes	NA	
Susquehanna River		7/2/2012	Smallmouth Bass		No	No	NA	
Delaware River	Milford	7/16/2012	Smallmouth Bass		No	No	NA	
Delaware River	Milford	7/16/2012	Largemouth Bass		No	No	NA	
Delaware River	Matamoras	7/16/2012	Smallmouth Bass		No	No	NA	
Delaware River	Delaware Water Gap	7/16/2012	Smallmouth Bass		No	No	NA	
Lehigh River	Catasauqua	7/17/2012	Smallmouth Bass		No	No	NA	
Schuylkill River	Port Clinton	7/18/2012	Smallmouth Bass		No	Yes	NA	
Ohio River	Montgomery	7/17/2012	Smallmouth Bass		Yes	Yes	1×10^4	cell culture negative for fish without signs of disease
Monongahela River		7/18/2012	Smallmouth Bass		No	No	NA	
Monongahela River		7/18/2012	Spotted Bass		No	No	NA	

Table 1. (*cont.*) Waterbody, location, date, species, sample size, disease characterization of Largemouth Bass Virus (LMBV) samples submitted as part of on-going investigations of YOY Smallmouth Bass disease investigations. Cell culture analysis and PCR confirmation was conducted by U.S. Fish and Wildlife Service, Northeast Fishery Center. Titer values were estimated by Penn State University, Animal Health Diagnotics Laboratory.

Waterbody	Site	Date	Species	Number	Clinical Disease	LMBV	Titer	Comment
Susquehanna River	Danville	7/19/2012	Smallmouth Bass	3	No	No	NA	
Susquehanna River	Danville	7/19/2012	Largemouth Bass	1	No	No	NA	
Juniata River		7/19/2012	Smallmouth Bass		Yes	No	1×10^7/ 1×10^5	higher titer value in clinically diseased fish
Shermans Creek		7/23/2012	Smallmouth Bass		Yes	Yes	NA	both clinically diseased and apparently healthy fish
Susquehanna River	Rockville	7/23/2012	Smallmouth Bass		Yes	Yes	NA	both clinically diseased and apparently health fish
Bald Eagle Creek		7/24/2012	Smallmouth Bass		No	No	NA	
Allegheny River		7/25/2012	Smallmouth Bass		Yes	Yes	NA	
Juniata River	Mifflin	7/17/2013	Smallmouth Bass	1	Yes	Yes	NA	
Susquehanna River	Rockville	7/18/2013	Smallmouth Bass	3	Yes	Yes	NA	
Susquehanna River	Rockville	7/18/2013	Smallmouth Bass	1	No	No	NA	
Susquehanna River	Rockville	7/18/2013	Smallmouth Bass	1	No	No	NA	
Pine Creek		7/18/2014	Smallmouth Bass	20	Yes	Yes	NA	

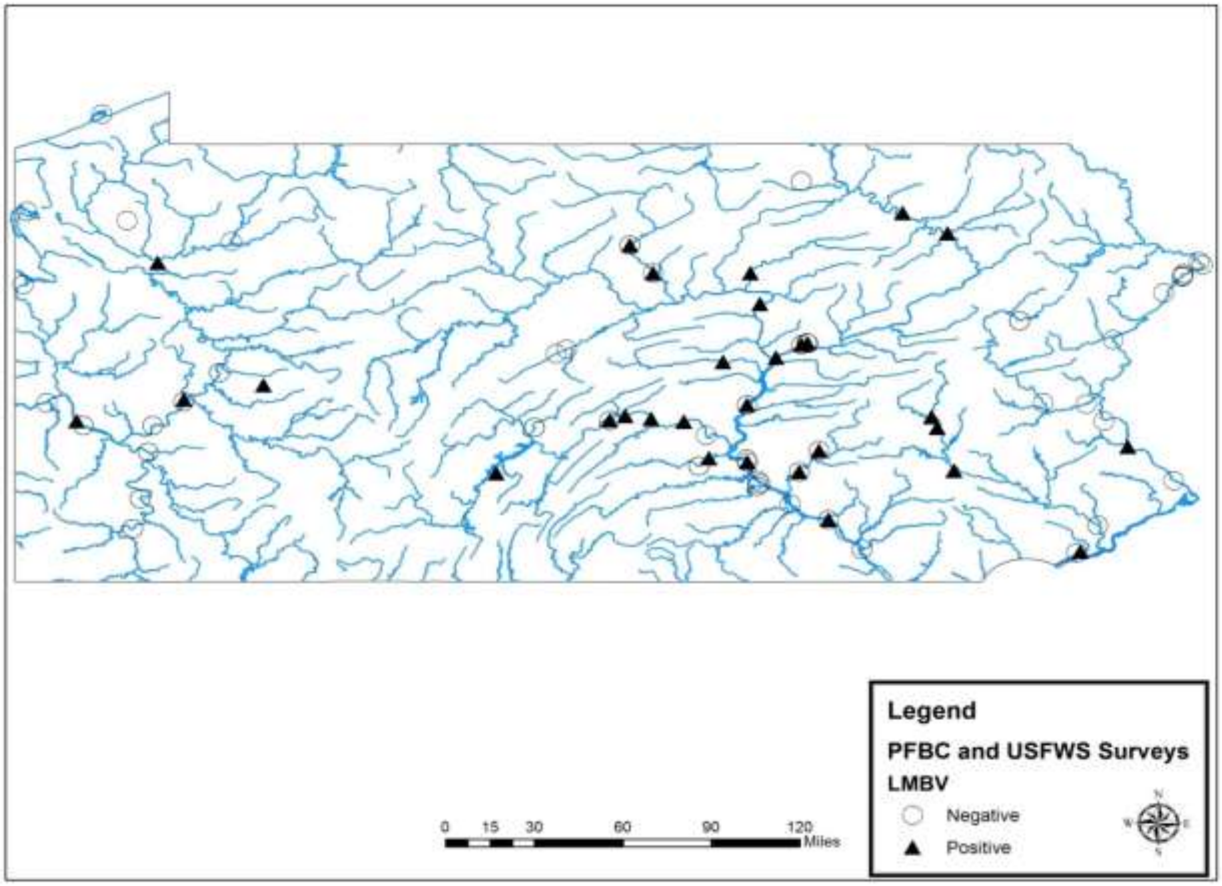


Figure 1: Distribution of samples analyzed for the presence of Largemouth Bass Virus (LMBV) within Pennsylvania. Data from U.S. Fish and Wildlife Service, National Wild Fish Health Database (2001 – 2013).

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Title: Impacts of Pathogens and Parasites on YOY Smallmouth Bass (WS#30)

Agency: Pennsylvania Fish and Boat Commission

Stressor-Response Relationship

Candidate Cause: New pathogens or parasites (*Myxobolus* spp.) or intermediate hosts may have moved into the area.

Introduction:

One candidate cause that is currently being investigated is the presence of the myxozoan parasite *Myxobolus inornatus* (Walsh et al. 2012) within muscle and connective tissue of young-of-year (YOY) SMB *Micropterus dolomieu*. Myxozoan parasite infections can be responsible for important economic losses in fisheries and aquaculture industries (Sitjà-Bobadilla 2008). *Myxobolus cerebralis*, the parasite responsible for whirling disease in salmonids, has caused negative economic impact on recreational trout fisheries in Colorado (Nehring and Walker 1996) and other states where tourism was an important part of local and state economies (Mahoney and Hudson undated; Koel et al. 2006). Proliferate gill disease (PGD), caused by the myxozoan parasite *Aurantiactinomyxon ictaluri*, is the third most common disease diagnosis among commercially farmed Channel Catfish *Ictalurus punctatus* (Hanson et al. 2008). More recently, *Sphaerospora motemarini*-related mortality in juvenile Grey Snapper *Lutjnaus griseus* was predicted to have impacts in commercial and recreational fisheries for that species in the Gulf of Mexico (Holzer et al. 2013). Internal and external signs of parasite infections, both trematodes and myxozoans, are commonly observed in the diseased YOY SMB.

The role that the myxozoan parasites play in the YOY SMB mortalities remains unclear. Parasites may compromise YOY in a number of ways: high parasite loads may contribute to general stress, as well as immune suppression, resulting in increased susceptibility to opportunistic bacteria; sites of parasite entry or exit may cause wounds allowing for bacterial entry; or heavy parasite loads may increase sensitivity to water quality related stressors. *M. inornatus* was first reported from Largemouth Bass fingerlings in a Montana hatchery in 1937 (Fish 1939). To our knowledge, the only other reports since then were from adult SMB in Lake Erie with identification based on spore morphology (Dechitar and Nepszy 1988; Muzzall and Whelan 2011). Since then, the isolate from YOY SMB has been redescribed, genetically sequenced, and primer sets were developed (Walsh et al. 2012).

Data:

Histological data for fish collected during 2013 and 2014 from U.S. Geological Survey, Leetown Science Center, National Fish Health Research Laboratory that was funded by Pennsylvania Sea Grant. Disease incidence, prevalence, and distributional data is from Pennsylvania Fish and Boat Commission directed young-of-year black bass surveys.

Analysis and Results:

Very few YOY SMB were collected in 2013 with clinical signs of disease. *M. inornatus* (or a similar-appearing myxozoan) was observed in fish at sites in the Susquehanna drainage and one fish from the Allegheny River. Of these, only fish from the Susquehanna River at Harrisburg had clinical disease. Myxozoan cysts were not observed at sites in the Delaware drainage (Smith et al. 2014).

At sites within the Susquehanna drainage fewer myxozoan parasite cysts and associated inflammation were observed in 2013 than were observed at sites with previously available data. This may be a result of changes in sample acquisition (i.e., random sample of fish rather than biased toward fish with clinical signs of disease) or lower disease incidence and prevalence during 2013. Detailed results for 2014 are pending; however, collections from 2014 documented presence of *M. inornatus* from fish at the West Branch Susquehanna River and Susquehanna River (Mahantango Access) where no fish were caught during 2013. A single fish with a single myxozoan cyst was found at the Lehigh River during 2014 (Table 1). Further analyses are necessary to determine if time of collection, annual differences water temperature, stream flow, or other water quality factors may influence the prevalence and severity of the infection (V. Blazer, U.S. Geological Survey, unpublished data).

Based on sampling in 2013 and previous years, *M. inornatus* appears to be widespread in the Susquehanna River basin (Figure 1) and present at a lower incidence and severity in bass from the Allegheny and Lehigh rivers. Clinical disease was only observed at one mainstem site in the Susquehanna Basin in 2013 and at the two sites surveyed during 2014; hence evaluating the potential role of the parasite in the disease syndrome of YOY was not possible. Despite the lack of visible lesions, there were signs of disease microscopically at the other sites including the presence of myxozoan cysts and inflammation in the muscle and connective tissue associated with myxozoan infections. It is uncertain if fish with myxozoan infections that did not have clinical disease would have developed clinical disease had they not been captured (Smith et al. 2014).

An in situ hybridization technique (Antonio et al. 1998) utilizing the specific sequences identified in the species description (Walsh et al. 2012) was successfully developed by Heather Walsh, USGS National Fish Health Research Laboratory. The method identifies mature spores and immature stages within the cysts observed on young-of-year SMB. Routine handling of tissues for histopathology which includes fixation in neutral buffered formalin, sometimes for prolonged periods (weeks to months) prior to processing and decalcification of tissues in acid-based solutions were found to interfere with successful results. In 2013, some fish were fixed with the PaxGene system in order to develop the technique. While this is good for select samples, it is too expensive and time-consuming for routine monitoring. Hence, in 2014 we experimented with routine fixation but decalcification with an EDTA solution. This appeared to be successful and the plan it to utilize this methodology in all further collections in order to 1) determine if areas of inflammation that appear to be early lesions of the SMB contain parasite DNA rather than bacterial or other factors and 2) determine if the myxozoans observed in various tissues and in SMB from various watershed are all the same species. Labeled sequences for *Flavobacterium columnare* together with the labeled sequences for *M. inornatus* will be utilized to better understand the role of each of these in the young-of-year disease progression (V. Blazer, unpublished data).

Conclusion:

The role of myxozoan parasites remains unclear and need further resolution. Evidence of bass with high prevalence of parasitic cysts as well as high consistency of detection among populations where disease is occurring suggest some role; however, whether it is opportunity or primary pathogen remains unclear. Regardless of its status, it may contribute additional stress to the fish.

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Table 1. The catch, number and percentage of fish with clinical signs of disease, and the presence of myxozoan cysts in young-of-year SMB at various locations within Pennsylvania during 2013 and 2014. Clinical signs of disease are visual assessments in the field while myxozoan determination is by histological analysis by U.S. Geological Survey, Leetown Science Center, National Fish Health Laboratory.

Waterbody	Location	Drainage	Catch	Diseased # (%)	Presence of Myxozoan
Delaware River	Matamoras	Delaware	0	NF[1]	No
Lehigh River	Northampton	Delaware	15	0	No
Lehigh River	Northampton[2]	Delaware	21	0	Yes[3]
Schuylkill River	Port Clinton	Delaware	25	0	No
Elk Creek		Lake Erie	21	0	No
Allegheny River	Buckaloons	Ohio	32	0	Yes[3]
Allegheny River	Buckaloons[2]	Ohio	9	0	No
Juniata River	Thompsontown	Susquehanna	5	0	Yes
Susquehanna River	Danville	Susquehanna	9	0	No
Susquehanna River	Harrisburg	Susquehanna	28	4 (14.3)	Yes
Susquehanna River	Laceyville	Susquehanna	23	0	Yes
Susquehanna River	Mahantango	Susquehanna	0	NF	NF
Susquehanna River	Mahantango[2]	Susquehanna	18	9 (50.0)	Yes
West Branch Susquehanna River	Watsontown	Susquehanna	0	NF	NF

West Branch Susquehanna River	Watsontown[2]	Susquehanna	11	5 (45.4)	Yes

1 - no fish in initial survey; however, follow-up survey yielded enough fish for analysis

2 - site revisited during 2014 to capture adequate sample

3 - record indicates a single cyst found in a single fish

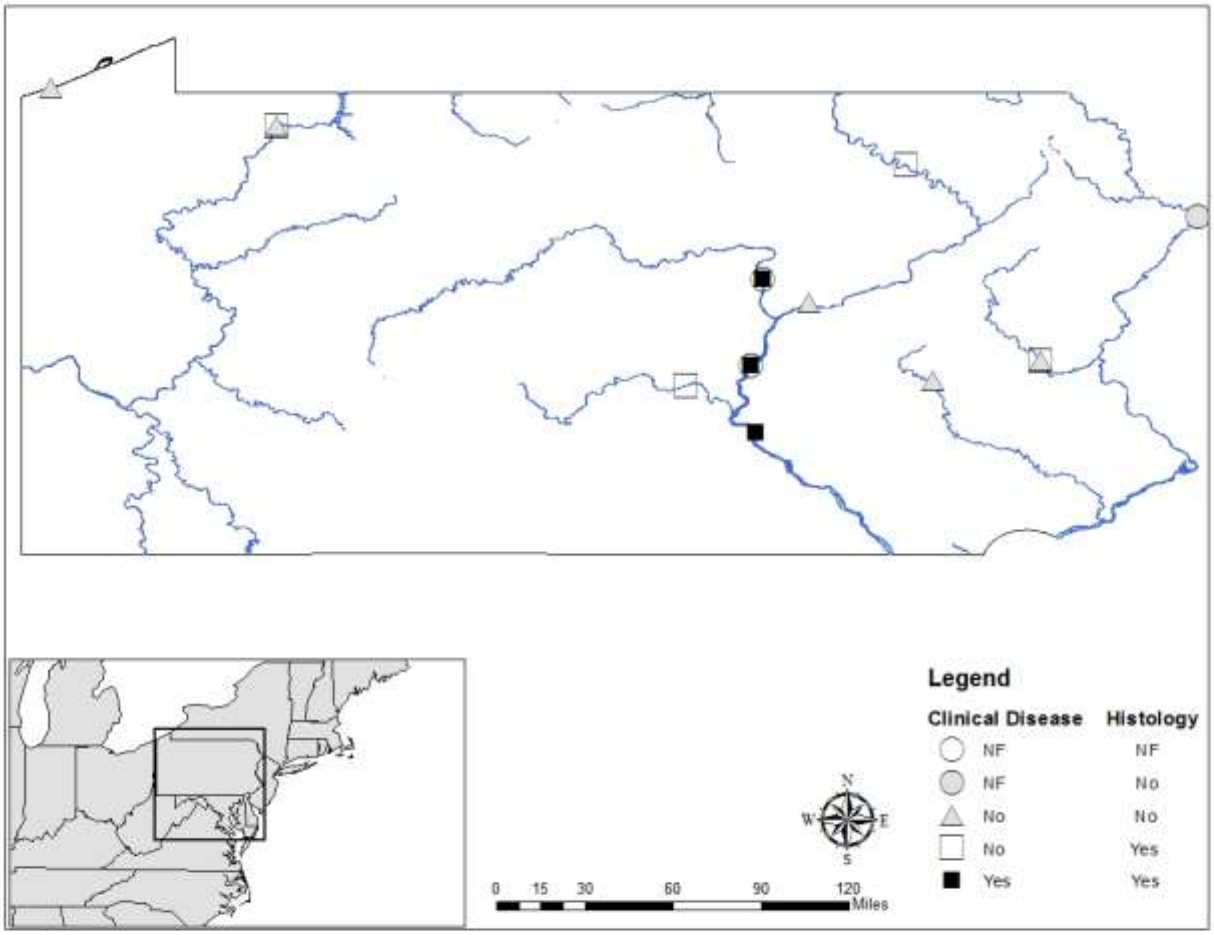


Figure 1. Distribution of *Myxobolus inornatus* in tissues of young-of-year (YOY) SMB *Micropterus dolomieu* during 2013 and 2014. Circles indicate locations where no fish (NF) were captured during initial surveys. Triangles indicate locations where YOY SMB were found but did not display clinical symptoms (No) of disease and did not have myxozoan cysts present during histological analysis. Squares indicate locations where YOY SMB had myxozoan cysts present (Yes) during histological analysis but either had no clinical signs of disease (hollow) or had clinical signs of disease (black filled). Initial surveys at the Delaware River at Matamoras yielded no YOY SMB; however, a follow-up collection produced

enough fish for histological analysis. Additional collections were made at four locations during 2014 where either no fish were found or where additional fish were needed for analysis.

Title: Disease Prevalence in YOY Smallmouth Bass (WS #31)

Agency: Pennsylvania Fish and Boat Commission

Stressor-response associations from the case

Candidate Cause: Disease

Introduction:

If diseases were causing the lack of recruitment into the adult SMB population, we would expect a negative correlation between the prevalence of disease and year 1 catch per unit effort (CPUE).

Data:

Source: Pennsylvania Fish and Boat Commission

Disease prevalence

Disease prevalence is the proportion of YOY SMB captured during backpack electrofishing surveys at each site displaying clinical signs of disease compared to the overall catch at that give site. Prevalence is reported at reach-wide aggregates in this instance (total catch with signs of disease in the reach/ total catch within the reach * 100). Prevalence values are index values reported during initial, directed surveys to maintain consistency among years. Directed surveys focus on a period of time when fish are 45 mm – 75mm TL to maintain similarities in catchability. Catchability of fish outside of that size range decreases and may affect results; primarily, biasing catch towards compromised fish (i.e., sick fish easier to catch, inflating prevalence values)

Age-1 CPUE

Age-1 catch per unit effort (CPUE; fish/h) was calculated reach-wide from boat electrofishing surveys for adult SMB at large river reaches state-wide. Age indices were used to calculate the component of catch across each reach that was attributable to Age-1 fish within a given year. Cumulative, reach-wide effort (electrofishing time (in hours) was then applied to the reach-wide catch data to determine CPUE.

Analysis and Results

The association between disease prevalence and the next year's Age-1 CPUE was calculated using Spearman's rank correlation.

Spearman's rank correlation (r) = -0.90. A scatterplot of the relationship is shown in Figure 1.

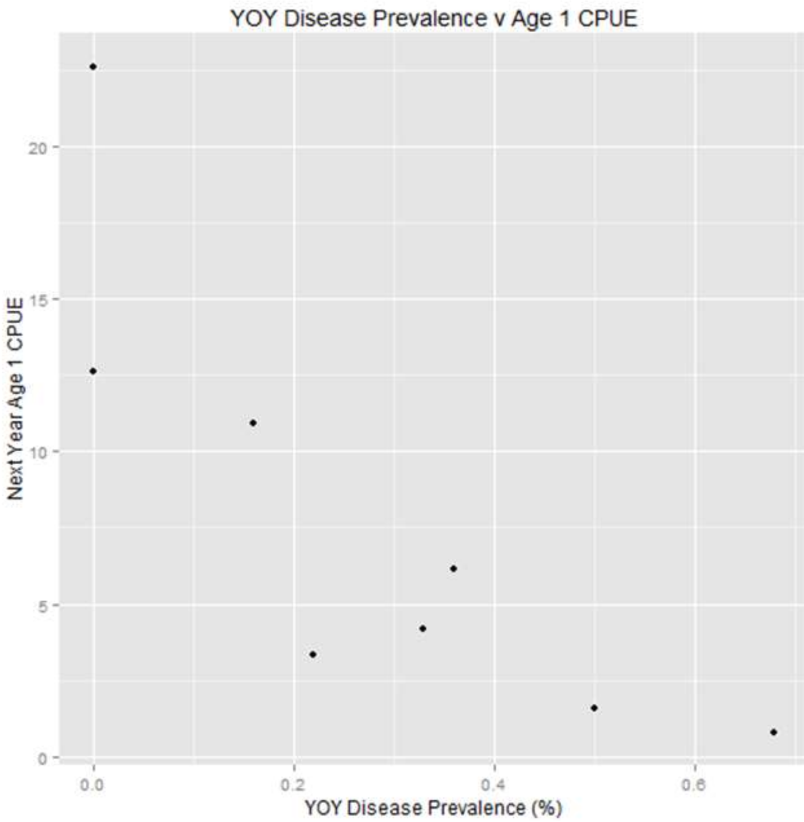


Figure 1. A scatterplot of disease prevalence of young of the year smallmouth bass (YOY) vs. Age 1 smallmouth bass catch per unit effort in the following year.

Conclusion:

Disease was strongly and negatively associated with Age-CPUE. This supports the hypothesis that disease is playing a role in recruitment of SMB to adult age classes.

Worksheets not used as Evidence in the CADDIS Decision Process

Worksheets: 41, 43, and 54

Title: Chemical Compounds in the Susquehanna River Basin (WS # 41)

Agency: Pennsylvania Department of Environmental Protection

Spatial Co-occurrence using Data from the Case

Candidate Cause: Toxic chemicals either kill YOY directly, or increase YOY susceptibility to disease; EDCs increase YOY susceptibility to disease

Introduction:

Toxic chemicals and/or EDCs could negatively affect YOY survival and/or susceptibility to disease. Water concentrations of these compounds could come into direct contact with YOY fish. If this is the case, higher concentrations of these chemicals would be expected at areas where YOY die-offs are seen and lower concentrations where no problems are seen.

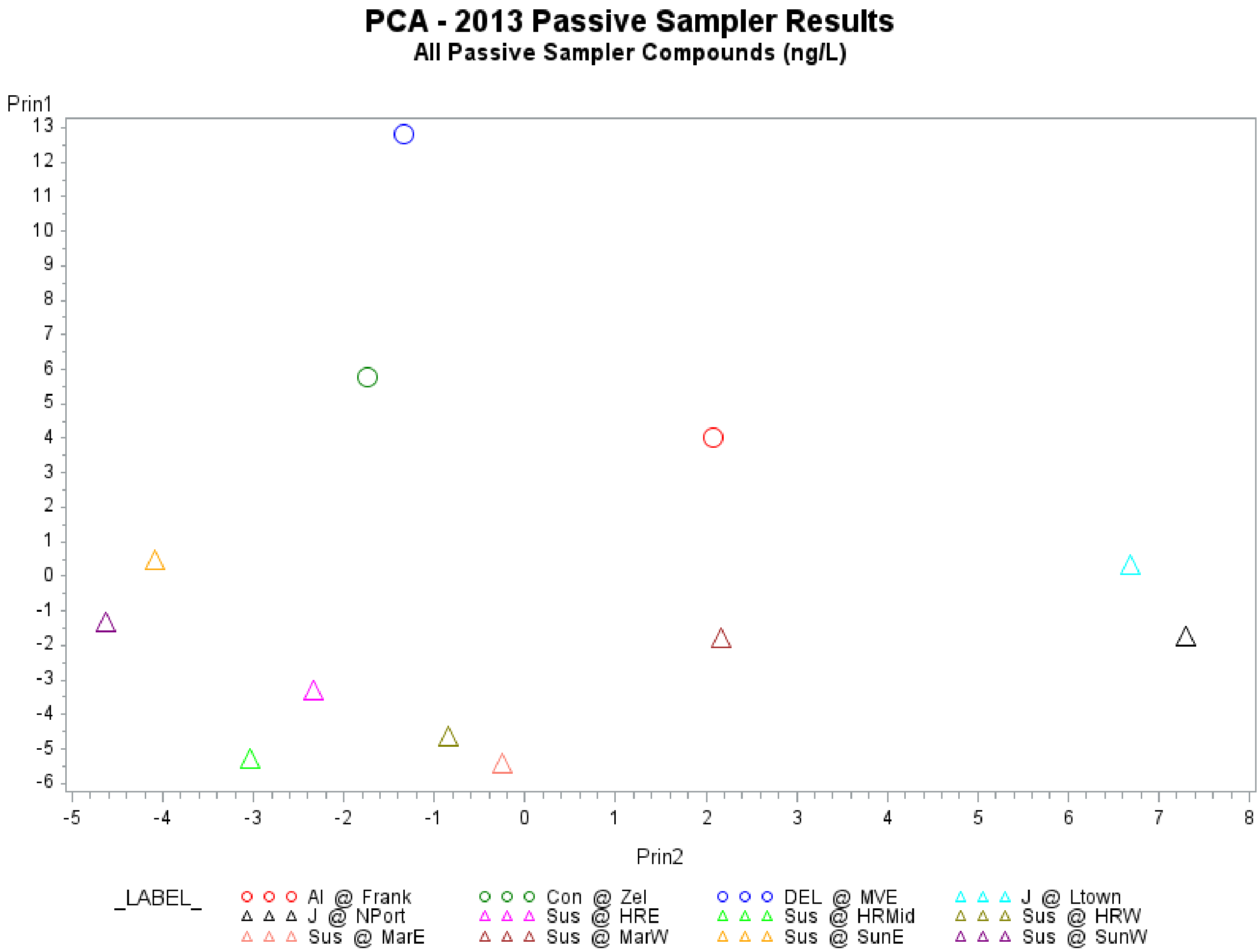
Data:

PA DEP collected passive water samples from 12 sample sites within and out of the Susquehanna basin in 2013 to be tested for a variety of different compounds (organochlorine & currently used pesticides, PCBs, PBDEs, hormones, wastewater indicators, and PAHs – pharmaceutical data is currently pending):

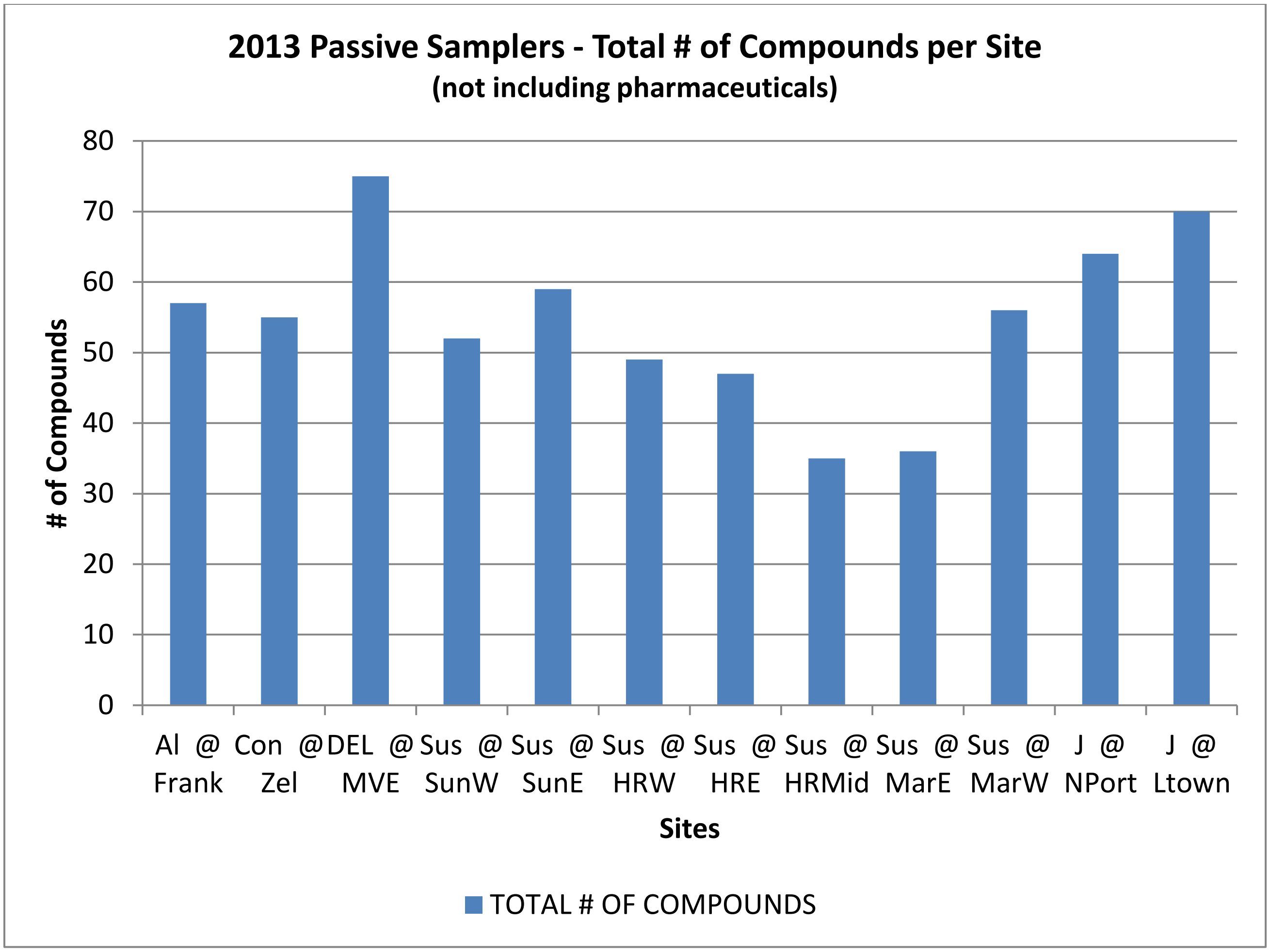
Waterbody	Location Name
Susquehanna River	Marietta West
Susquehanna River	Marietta-Falmouth East
Susquehanna River	Rockville West
Susquehanna River	Rockville Middle
Susquehanna River	Rockville East
Juniata River	Lewistown Narrows
Juniata River	Newport South
Susquehanna River	Sunbury West
Susquehanna River	Sunbury East
Connequenessing Creek	Zelienople
Allegheny River	Franklin
Delaware River	Morrisville East

Analysis and Results:

Juniata R @ Lewistown and Newport are clustered together towards the end of axis 2; Susquehanna R @ Marietta East and West are also clustered together. The remaining Susquehanna sites are clustered together and the control sites are scattered and somewhat separated from the Susquehanna basin sites. Juniata sites are definitely similar, and tend to have a large number of compounds (as can be seen below), while the Susquehanna sites are more similar to each other. The controls, however, also tend to have high numbers of compounds and are not true “reference” sites.



PCA - 2013 Passive Sampler Results
All Passive Sampler Compounds (ng/L)
Prin1
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
Prin2
LABEL
Al @ Frank
Con @ Zel
DEL @ MVE
J @ Ltown
J @ NPort
Sus @ HRE
Sus @ HRMid
Sus @ HRW
Sus @ MarE
Sus @ MarW
Sus @ SunE
Sus @ SunW



2013 Passive Samplers - Total # of Compounds per Site
(not including pharmaceuticals)
# of Compounds
80
70
60
50
40
30
20
10
0
Al @ Frank
Con @ Zel
DEL @ MVE
Sus @ SunW
Sus @ SunE
Sus @ HRW
Sus @ HRE
Sus @ HRMid
Sus @ MarE
Sus @ MarW
J @ NPort
J @ Ltown
Sites
TOTAL # OF COMPOUNDS

Title: Stroud Study – Temperature Cycling and Maximum Temperature Effects on SMB (WS #54)

Agency: Pennsylvania Department of Environmental Protection

Candidate Cause: High Water Temperatures (and changing water temperatures)

Introduction:

DEP contracted Stroud Water Research Center to perform a study on the effect of rate of temperature change (abruptly changing temperatures) and various thermal maxima on fish and macroinvertebrates. The fish that were studied were walleye, white sucker, golden shiner and smallmouth bass; however, this worksheet will focus on smallmouth bass. The smallmouth bass were generally more tolerant than the other fish species to both temperature maxima and rate of temperature change.

Study Design:

The study included 2 experiments and was conducted in 2010 and 2011. It should be noted that Stroud conducted a rate of temperature change study for PPL in which the results were reported in 2009. The PPL study was designed to evaluate sub-lethal effects of temperature changes on biological organisms and therefore included lower temperatures (82°F or cooler) than what see typically see in the Susquehanna in mid-summer in order to avoid mortality. DEP then decided to take the PPL study further and look at higher temperatures which we often see as ambient temperatures in big rivers and also frequently where thermal discharges occur.

In DEP Experiment 1, four fish species were exposed to two constant (82° or 89°F) or one of three fluctuating temperature regimes (2°, 4°, or 8°F/h) for 30 days under mid-summer (75°-89°F, mean 82°F) conditions. The total degree hours were equalized among regimes by adjusting the time spent at the maximum and minimum temperatures. Fish were acclimated to 82°F over three weeks prior to initiating the experiment. Sub-lethal response was assessed by measuring the primary, secondary, and tertiary stress response (cortisol, glucose, or triglyceride, respectively) during the first day of temperature fluctuation and again after 4 weeks of temperature cycling; by periodically measuring growth and survival; and by histopathology analysis of a number of tissues at the conclusion of temperature cycling.

DEP Experiment 2 was aimed at determining the effect of the upper temperature reached during diel fluctuations on the stress response. Fluctuations went from 75°F to a maximum temperature of 82°F (August ambient), 87°F, 89°F, 91°F, or 93°F, where the rate of change was 4°F/hour and time at maximum temperature was 10 hours across treatments. All fish were acclimated to 75°F over two weeks prior to initiating the experiment. The same sub-lethal stress responses were monitored as for Experiment 1.

Results:

Experiment 1:

- SMB did not show a primary stress
- SMB exhibited significantly lower growth in the 89°F treatment (control)
- Some SMB showed some interesting characters but appeared to be unrelated to temperature cycling: curved spines; focal branchitis and lamellar fusion of the gills; intersex gonads; flukes without an associated inflammation reaction in the river and spleen
- Antagonistic behavior among SMB during acclimation period led to low initial survival of SMB but there was 100% survival once they were isolated other than a few that died shortly after being moved due to injuries sustained earlier

Table 2.7-4 Experiment 1 summary table of acute (day 1) and chronic (day 30) biochemical, growth and survival responses to constant temperature of diel temperature cycling between 75 and 89°F at the rate indicated. Symbols indicate statistically significant treatment induced response (*) or no response (~). NF indicates no sample was taken because of treatment induced mortality.

	Treatment	Sublethal	Lethal	Acute			Chronic				
	°F	effect	effect	Cort.	Gluc.	Trig.	Cort.	Gluc.	Trig.	Growth	Survival
Golden shiner	82	N	N	~	~	~	*	~	~	~	~
	2°F/hr	N	N	~	~	~	*	*	~	~	~
	4°F/hr	N	N	~	~	~	~	~	~	~	~
	8°F/hr	N	N	*	~	~	~	*	~	~	~
	89	Y	Y	*	~	~	NF	NF	NF	*	*
Smallmouth bass	82	N	N	~	~	~	~	~	~	~	~
	2°F/hr	N	N	*	~	~	~	~	~	~	~
	4°F/hr	N	N	~	~	~	~	~	~	~	~
	8°F/hr	N	N	*	~	~	~	~	~	~	~
	89	Y	N	~	~	~	~	~	~	*	~
Walleye	82	N	N	*	~	~	*	*	*	~	~
	2°F/hr	N	N	*	~	~	*	*	*	~	~
	4°F/hr	Y	N	~	~	~	~	*	*	*	~
	8°F/hr	Y	N	*	~	~	~	*	*	~	~
	89	Y	N	~	*	*	~	*	*	*	~
White sucker	82	N	N	~	~	~	~	~	~	~	~
	2°F/hr	N	N	~	~	~	~	~	~	~	~
	4°F/hr	N	N	~	~	~	~	~	~	~	~
	8°F/hr	Y	Y	*	~	~	~	~	~	~	*
	89	Y	Y	~	~	~	NF	NF	NF	~	*

Experiment 2:

- Cycling to 91°F stressed or killed SMB
- Temperature cycling appeared to drive the increase in cortisol at the peak temperatures but returned to baseline levels during the ramp down and at the lower 'soak temperature' (decreasing temperatures offered relief)
- Cortisol levels in SMB in the 91°F and 93°F treatments were significantly elevated at the peak temperature after 30 days of temperature cycling. They were also elevated in the 91°F treatment at the bottom of the diel cycle

- Metabolism did not appear to be affected by treatments (glucose and triglycerides)

- 5 SMB developed curved spines in experiment thought to be unrelated to temperature

- 2 SMB found to be moribund during quarantine showed severe infestations of gills, kidney necrosis hemorage, flukes in the gastrointestinal tract and liver, suggested that fish arrived from Schuylkill river in a heavily infested state

- Almost all SMB showed signs of lesions, inflammation or parasite infestation and some had extreme liver vacuolation. Tissue damage or parasite infestation appeared to increase sensitivity of other species (walleye and golden shiner) to extreme temperature changes but did not appear to alter the sensitivity of the SMB (or white sucker)

- Withdrawing blood from SMB for bioassays created more stress than expected

- Among SMB not sampled for blood, there was significant mortality among fish reaching 93°F

- All [normal spine] SMB that died, did so within 48 hours of reaching the peak temperature for the first time

Table 2.7-5 Experiment 2 summary table of acute (day 1) and chronic (day 30) biochemical, growth and survival responses to diel temperature cycling from 75°F to the maximum temperature indicated. Symbols indicate statistically significant treatment induced response (*) or no response (~). The ~/* indicates a significant response in only fish sampled for blood. NF indicates no sample was taken because of treatment induced mortality.

	Treatment °F	Sublethal effect	Lethal effect	Acute Cort.	Gluc.	Trig.	Chronic Cort.	Gluc.	Trig.	Growth	Survival
Golden shiner	82	N	N	*	~	~	*	~	~	~	~
	87	N	N	~	~	~	~	~	~	~	~
	89	Y	Y	*	~	~	~	~	~	*	*
	91	Y	Y	~	~	~	~	~	~	*	*
	93	Y	Y	*	~	~	NF	NF	NF	*	*
Smallmouth bass	82	N	N	~	~	~	~	~	~	~	~
	87	N	N	~	~	~	~	~	~	~	~
	89	N	N	~	~	~	~	~	~	~	~
	91	Y	N	~	~	~	~	~	~	~	~/*
	93	Y	Y	*	~	~	~	~	~	*	*
Walleye	82	N	N	NF	NF	NF	~	~	~	~	~
	87	Y	N	NF	NF	NF	~	*	*	~	~
	89	Y	Y	NF	NF	NF	NF	NF	NF	NF	*
	91	Y	Y	NF	NF	NF	NF	NF	NF	NF	*
	93	Y	Y	NF	NF	NF	NF	NF	NF	NF	*
White sucker	82	N	N	~	~	~	*	~	~	~	~
	87	Y	Y	~	~	~	~	~	~	*	*
	89	Y	Y	*	~	~	NF	NF	NF	NF	*
	91	Y	Y	*	~	~	NF	NF	NF	NF	*
	93	Y	Y	NF	NF	NF	NF	NF	NF	NF	*