Investigation into factors leading to disease-related mortality of young-of-year smallmouth bass *Micropterus dolomieu* in the Susquehanna River Basin: 2011

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INTRODUCTION

Wide-scale, disease-related mortality of young-of-year (YOY) smallmouth bass *Micropterus dolomieu* was first documented in 2005 in the West Branch Susquehanna, Susquehanna, and Juniata rivers. These fish were seen swimming weakly near the surface with noticeable white lesions, sores, and eroded fins and some were dead. Outbreaks occurred in 2005, 2007, 2008, and 2010 and to a lesser extent in 2006 and 2009. While disease has had a large impact on YOY smallmouth bass, it appeared to have little if any effect on other species. Early pathological analysis indicated that the bacterium *Flavobacterium columnare* or columnaris was responsible for the mortality. It was believed that stressful water quality conditions in near-shore habitat required by YOY smallmouth bass were suppressing their immune systems and making them more susceptible to infection. Water quality investigations conducted by the USGS Pennsylvania Water Science Center in 2008 (Chaplin et al. 2009) confirmed the assumption that stressful water quality conditions occurred in this critical near-shore habitat. They determined that water quality, specifically dissolved oxygen (DO), temperature, and pH, were statistically different between near-shore locations and main-channel locations. Water temperature of the Susquehanna and Juniata rivers were also typically higher and varied more than comparable Allegheny or Delaware River locations (Chaplin and Crawford 2012). Both factors are stressful to some fish. This strengthened support of the hypothesis that stressful water quality conditions were contributing to the disease manifestation in YOY smallmouth bass.

Analysis of tissues from smallmouth bass from the Susquehanna River and a number of tributaries has since identified several other issues. Further analysis of YOY smallmouth bass confirmed the presence of columnaris, but also identified several other pathogens. One of these is an unrecognizable pathogen that was found in YOY smallmouth bass collected in 2008 and 2009 and in adult smallmouth bass in 2009 (V.Blazer, USGS, personal communication). Future collections will provide more evidence to identify the unknown pathogen. Analyzed smallmouth bass also were determined to have substantial myxozoan and trematode parasite infections. These infections could be a result of the disease but could also contribute to its presence by causing stress. Collection of adult smallmouth bass from the West Branch Susquehanna River (2009), and Susquehanna River (2008-2009) also provided more information. Adult male smallmouth bass were determined to have very high rates of intersex: a condition in which female egg precursor cells are found in the testes of males. Although rare, it is natural for smallmouth bass to display this condition. However, the proportion of the fish and the severity of the cases are not considered typical. Rock bass, which share many of the same characteristics as smallmouth bass, did not display the condition. Largemouth bass virus (LMBV) was also discovered in smallmouth bass from the Susquehanna River system. Smallmouth bass, like many other species, have been identified as carriers but are not believed to be directly affected by the virus. However, it is unknown if being a carrier of the virus has the ability to add additional stress to the fish's immune system. As more information is gathered it appears less likely that columnaris is the primary pathogen.

Identification of the disease and its causes has become more complex as more evidence is gathered. It appears that that yet unmeasured variables may be acting on the smallmouth bass population. The study will continue to focus on water quality with continuous monitoring of DO, temperature, and pH at locations used in previous years of this study. Drastic differences in hydrologic conditions among the years resulted in different water quality values for the parameters of focus. Development of long-term data records will help to answer essential questions about this disease issue as well as the overall ecology of the Susquehanna River. Continued monitoring of DO, temperature, and pH develop long-term data records, which allow for trend comparisons. The Susquehanna River lacks these records. In addition to conventional water quality parameters, endocrine disrupting compounds (EDC) and emerging contaminants (EC) have major impacts on aquatic environments. EDC and EC come from a number of sources including pharmaceuticals, fertilizers, and household cleaning products. The severity of these impacts is only now starting to be understood. These contaminants can cause various physiological imbalances in fish and other aquatic organisms and alter many aspects of the aquatic ecosystem. These can cause undue stress to fish and other organisms and predispose them to diseases, similar to what has been seen in smallmouth bass.

The fish health issue presented is one of the primary issues facing the Susquehanna River system. The disease observed could simply be an independent occurrence or the result of a larger, ecosystem-wide problem. Future research will help identify the disease and its causes with the goal of eliminating it from the Susquehanna River.

Study elements for 2011

The Susquehanna River smallmouth bass technical committee (tech committee) prioritized four areas to focus studies on for 2011 these included:

1. Continuation of YOY and adult smallmouth bass surveys
2. Installation of large-river, long-term water quality gages
3. Tributary fish health and contaminants
4. Fish pathology (bacteria, parasites, and virus identification)

YOY and adult smallmouth bass surveys

Annually, there are three primary components of the smallmouth bass surveys to address the effects that disease outbreaks are having on the population: early YOY black bass surveys, directed YOY black bass surveys, and adult black bass surveys. Each of these has both specific and secondary purposes for the data that is collected. Early YOY surveys are used to judge the development of the YOY for use in scheduling of traditional, directed YOY black bass surveys but also to collect specimens for disease and parasite analysis to develop a timeline of susceptibility of these fish to bacterial and parasitic infections. The directed YOY black bass surveys are typically held in mid-July of each year and serve as the estimate of reproductive success and the historic baseline for comparison. Since 2005, these surveys have also served to track both incidence and prevalence of disease among YOY smallmouth bass populations within the Commonwealth and to provide specimens for bacterial, viral, and pathological analysis as part of ongoing disease investigations. Adult black bass surveys are conducted during summer and early autumn of each year to estimate the relative abundance of black bass in a given reach of river state-wide. Within the Susquehanna River system, in recent years these surveys have also been used to track the impacts that disease-related mortality of YOY smallmouth bass populations and provide guidance for regulatory action.

Early YOY smallmouth bass collections

Two collections of early YOY smallmouth bass were conducted during 2011. Surveys occurred on June 27 and July 5, 2011 to gauge the development of YOY smallmouth bass and to provide specimens for pathological analysis, respectively. The techniques used for these collections are not uniform because habitats utilized by these fish at this point in their development is hard to systematically electrofish so results are not quantifiable as a relative abundance.

Tables 1 and 2. Length-frequency distribution of young-of-year (YOY) smallmouth bass captured at the Susquehanna River at various locations in Section 01 and 02 on June 27, 2011 (left) and at the Susquehanna River at Section 02 (Liverpool) on July 5, 2011 (right).

Total Length (mm)	Number of Individuals
15-19	1
20-24	2
25-29	12
30-34	5
35-40	0

Total Length (mm)	Number of Individuals
15-19	0
20-24	9
25-29	18
30-34	10
35-40	1

All YOY smallmouth bass collected (n=38) on July 5, 2001 at the Liverpool location were submitted to the U.S. Geological Survey, Leetown Science Center, National Fish Health Research Laboratory for bacterial and pathological analysis. During the analysis, no bacterial or parasite infections were observed (V. Blazer, USGS, Personal Communication).

Directed YOY smallmouth bass surveys

Historically, Division of Fisheries Management Staff has conducted surveys to estimate abundance of YOY smallmouth bass on major rivers in the Commonwealth during mid-July. These data were used to approximate annual reproductive success and in recent years were incorporated into an effort to develop a recruitment index to predict density and characteristics of future adult populations. Following disease outbreaks in the Susquehanna River Basin in 2005, these surveys were also used to identify disease incidence and quantify prevalence throughout the Commonwealth.

Catch rates varied among the different reaches of the Susquehanna River with the lowest catch rate of 0.9 individuals/ 50 meters at the lower Susquehanna River and the highest catch rate of 7.9 individuals/ 50 meters at the upper Susquehanna River (North Branch). Catch rates at the West Branch Susquehanna River, middle Susquehanna River, and lower Susquehanna River were all approximately half the historic median catch rates for those respective reaches (Table 3). The low catch rates at these reaches are not surprising as river discharges during the early portion of the spawning period were not conducive to wide-scale spawning activity. Spawning conditions improved later in the spawning period and some successful spawning did occur in these reaches. The catch rates at the upper Susquehanna River were slightly above the historic median for that reach (Table 3). This reach has historically been the least affected by variable river discharge conditions.

Table 3. Comparison of catch-per-unit-effort (CPUE; individuals/ 50m) of young-of-year smallmouth bass for 2011 and historical medians and means at each reach of the Susquehanna River.

Waterbody	CPUE (ind./ 50 m)	Historic Median	Historic Mean
West Branch Susquehanna River	1.0	2.3	4.5
Susquehanna River (upper)	7.9	5.8	8.2
Susquehanna River (middle)	2.6	5.3	7.6
Susquehanna River (lower)	0.9	2.0	2.8

Disease incidence and prevalence – large rivers

Disease incidence and prevalence were assessed at all large river locations across the Commonwealth as part of the directed, YOY black bass surveys. Similar to previous years, cases of disease incidence were limited in the upper Susquehanna River during 2011 and at locations where diseased fish were found the prevalence was low (Table 4; Figure 1). Prevalence of disease was low to moderate in both the West Branch Susquehanna River and middle Susquehanna River: 22% and 33%, respectively. Prevalence varied among sites within each of the reaches (Figure 2). These values were lower than prevalence values observed in previous years. Disease prevalence in the lower Susquehanna was 36%, similar to what was observed in the middle Susquehanna River; however, this was the highest disease prevalence observed in the lower Susquehanna River during the course of this study.

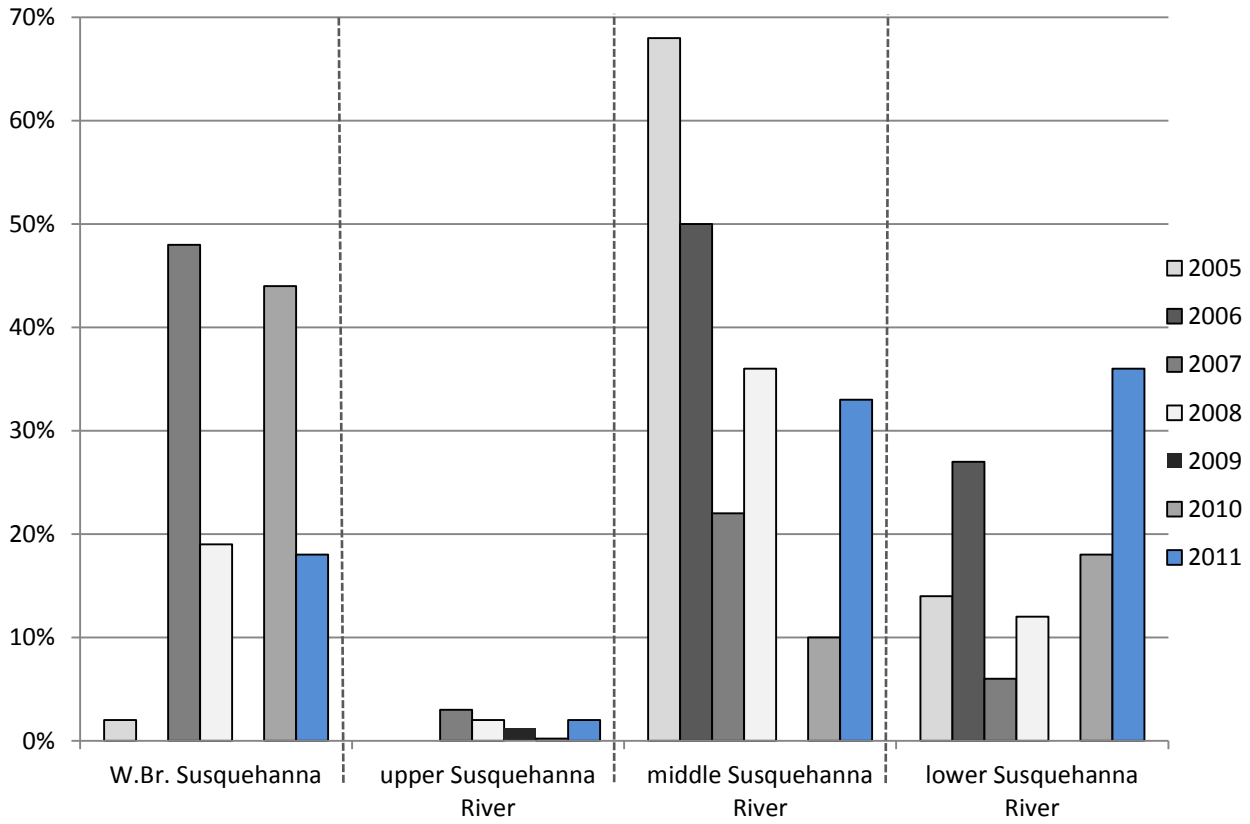

Figure 1: Disease prevalence observed during directed, young-of-year black bass surveys at the different reaches of the Susquehanna River; 2005-2010.

For the first time since disease outbreaks were observed in the Susquehanna River Basin, large-scale incidence of disease was observed outside of the basin during 2011. Directed YOY black bass surveys of the upper Allegheny River, Schuylkill River, and Delaware River each yielded YOY smallmouth bass with bacterial infections above back ground levels (Table 4, Figure 2).

Table 4. Total number of young-of-year smallmouth bass captured, number of captured smallmouth bass displaying symptoms of disease, and prevalence of disease at major river reaches surveyed during 2011 directed surveys.

Waterbody	Number of Fish	Number of Diseased Fish	Prevalence (%)
Delaware River (upper)	76	0	0
Delaware River (middle)	34	0	0
Delaware River (lower)	109	18	17
Lehigh River (middle)	58	0	0
Lehigh River (lower)	195	0	0
Schuylkill River	369	14	4
Allegheny River (upper)	123	34	28
Allegheny River (lower)	16	3	19
Ohio River	18	0	0
Monongahela River	4	0	0
West Branch Susquehanna River	63	14	22
Juniata River	129	5	4
Susquehanna River (upper)	757	15	2
Susquehanna River (middle)	110	36	33
Susquehanna River (lower)	36	13	36

Pathological analysis of moribund YOY smallmouth bass was conducted at two locations at the middle Susquehanna River, one location at the lower Susquehanna River, two locations at the Schuylkill River, one location at the Delaware River, and four locations at the Allegheny River during 2011. Smallmouth bass at the lower Susquehanna River were found to have external infections by columnaris and systemic infections by columnaris, motile *Aeromonas* sp., and *Plesiomonas* sp. bacteria (Appendix A). A largemouth bass from the same location had columnaris infections of the gills and systemic infections of columnaris but was asymptomatic clinically (Appendix A). Smallmouth bass from the Schuylkill River had external protozoan (*Apiosoma* sp.) infections and systemic columnaris and motile *Aeromonas* sp. infections (Appendix A). Sympatric largemouth bass from the Schuylkill River were asymptomatic with the exception of one fish, which had columnaris and monogenean trematodes (*Dactylogyrus* sp.) infections and systemic motile *Aeromonas* infections (Appendix A). Smallmouth bass from the Delaware River had external protozoan (*Apiosoma* sp.) infections and systemic motile *Aeromonas* sp. infections while sympatic largemouth bass had external protozoan (*Apiosoma* sp.) infections, no systemic infections, and were asymptomatic of disease (Appendix A). Smallmouth bass from four locations at the Allegheny River exceeded typical holding times before being delivered to the laboratory for analysis so only limited bacterial analysis could be completed. Systemic infections of motile *Aeromonas* sp. were identified from

these fish (Appendix A). All lots from the large river locations tested positive for Largemouth Bass Virus (LMBV) with the exception of the Allegheny River locations which were not tested (Appendix A).

Directed YOY surveys – tributaries

Eleven tributary streams were sampled during the traditional YOY smallmouth bass index period to determine catch rates of YOY smallmouth bass and determine if diseased fish were present in those waters and , if so, what the proportion of fish displayed symptoms of the disease condition. Catch rates at tributary sites ranged for 0.5

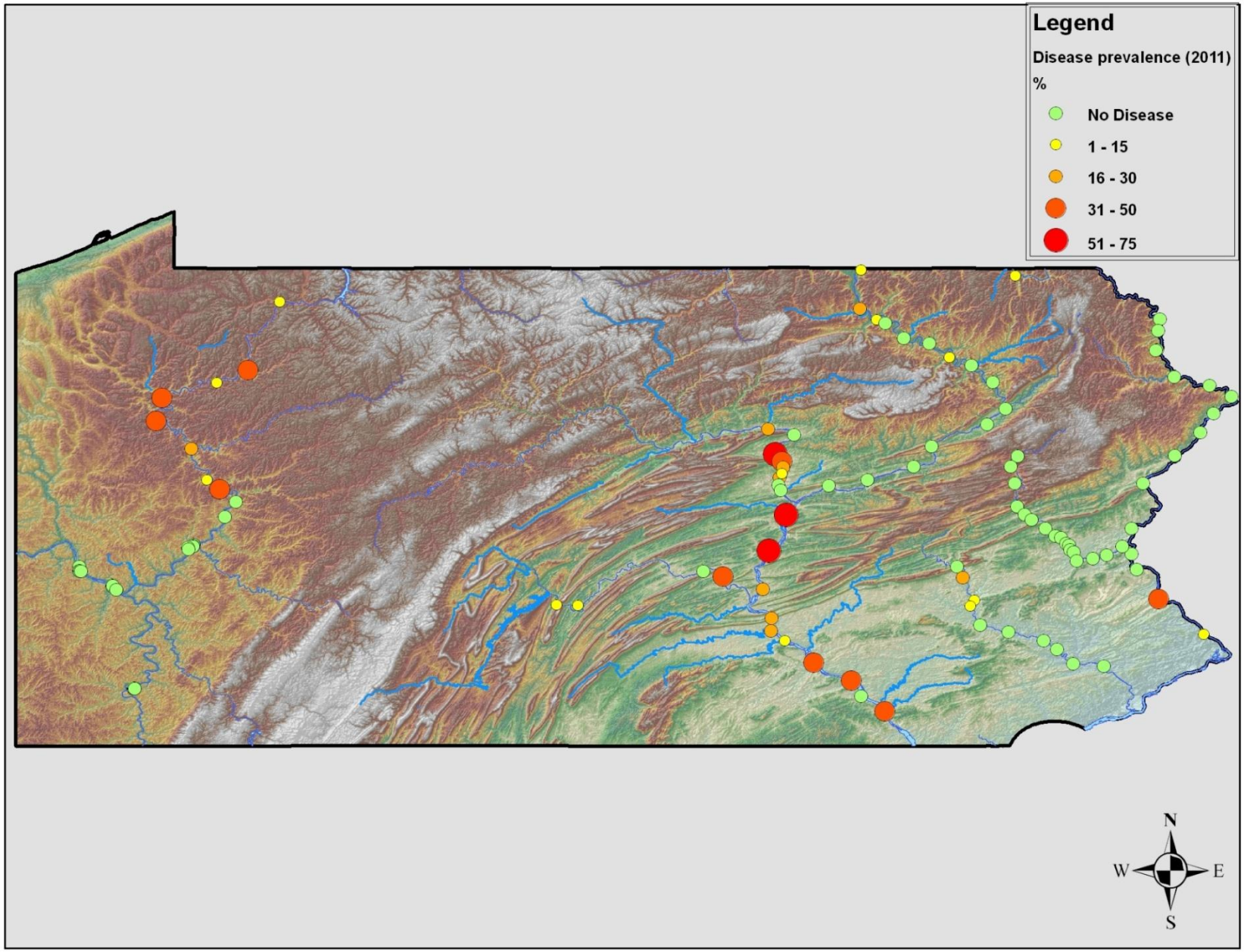

Figure 2: Incidence and prevalence of disease among YOY smallmouth bass collected as part of directed YOY black bass surveys at large river locations during 2011.

individuals/ 50m at the Conestoga River to 11.2 individuals/ 50m at Shermans Creek. Catch rates at Chillisquaque Creek, Conodoguinet Creek, Penns Creek, Swatara Creek, and Wyalusing Creek could not be generated because of differences in sampling gear (Table 5). Due to serviceability issues, backpack electrofishers capable of generating Alternating Current (AC) were not available and pulsed Direct Current (PDC) electrofishers had to be used for these collections. Catch rates in the historic dataset have been established using AC waveforms and there is uncertainty whether YOY smallmouth bass catch rates are comparable using PDC waveforms. In these instances, surveys were used to assess incidence of disease, collect specimens for pathological analysis, and provide qualitative assessment of disease prevalence where it existed.

Table 5. Catch rates (individuals/ 50 m) and disease prevalence (%) of YOY smallmouth bass collected during directed, YOY smallmouth bass surveys at tributary locations during 2011.

Waterbody	Location	Tributary to	Catch rate (ind./ 50 m)	Disease prevalence (%)
Chemung River		Susquehanna River (upper)	9.2	73
Conestoga River	Slackwater	Susquehanna River (lower)	0.5	0
Loyalsock Creek	Loyalsockville	W. Br. Susquehanna River	11.2	12
Pequea Creek		Susquehanna River (lower)	1.3	13
Pine Creek	Hamilton Bottom	W. Br. Susquehanna River	4.7	75
Shermans Creek	Pine Hill Road Br.	Susquehanna River (middle)	9.2	38
Chillisquaque Creek	SR 405 Br.	W. Br. Susquehanna River	N/A	Moderate
Conodoguinet Creek	Mechanicsburg	Susquehanna River (middle)	N/A	Low
Penns Creek	Penns Creek	Susquehanna River (middle)	N/A	Low
Swatara Creek	Vine Rd.	Susquehanna River (middle)	N/A	Low
Wyalusing Creek	SR 706 Br.	Susquehanna River (upper)	N/A	None

Disease incidence and prevalence – tributaries

As previously mentioned, collections were made at 11 tributary locations during the 2011 season (Table 5), seven of which were submitted for pathological analysis. Diseased and asymptomatic YOY smallmouth bass and individuals of other species of fish that were displaying symptoms of clinical disease were submitted for laboratory analysis alive, preserved on ice, or preserved in Z-fix formalin solution based on proximity to the laboratory and laboratory availability for analysis based on backlog of samples. Incidence of disease was

documented at nine of 11 tributaries surveyed during 2011 (Figure 3). Diseased YOY smallmouth bass were documented for the first time at Chillisquaque Creek, Conodoguinet Creek, and Shermans Creek. Of the tributaries where prevalence could be reliably estimated, Pine Creek had the most severe outbreak in 2011 followed closely by the Chemung River (Table 5).

Specimens from Pine Creek and Pequea Creek were submitted to the PFBC, Fish Health Unit for bacterial analysis. Specimens for Loyalsock Creek, Penns Creek, Pine Creek, and Pequea Creek were submitted to USFWS, Northeast Fishery Center for viral analysis. Specimens collected from Pine Creek were suffering from severe external parasite infestations along with systemic bacterial diseases and the presence of Largemouth Bass Virus (LMBV). The severity of the external parasite infestations observed at this site in both years is not consistent with observations from other sites within the Susquehanna drainage where moribund fish with lesions have been collected. The continued occurrence and severity of disease at this location may suggest that there is a reoccurring environmental stressor associated with this particular site (Appendix A). The smallmouth bass specimen from Pequea Creek had no external bacterial or parasite infections but systemic infections of columnaris and motile *Aeromonas* sp. were found. Smallmouth bass (n=1) and asymptomatic largemouth bass (n=3) submitted for viral analysis from this location were negative for LMBV (Appendix A). Specimens from Loyalsock Creek and Penns Creek submitted for viral analysis by USFWS were positive for LMBV; however, no bacterial analyses were conducted on these fish (Appendix A). Symptomatic and asymptomatic YOY smallmouth bass were collected and preserved in Z-fix formalin solution for pathological analysis by USGS, National Fish Health Research Laboratory from Conodoguinet Creek, Swatara Creek, and Wyalusing Creek. Results of pathological analyses are pending.

Follow-up surveys allowed for documentation of disease incidence at two locations during 2010 that would have otherwise gone undetected. Follow-ups were scheduled for locations where no diseased smallmouth bass were found to determine whether temporal difference in outbreaks occurred. High stream flow events; however, precluded revisiting of these sites during a timeframe when YOY smallmouth bass would have been susceptible to capture using backpack electrofishing equipment.

<u>Diseased adult smallmouth bass – lower Susquehanna River</u>

During autumn 2011, initial collections of adult smallmouth bass with severe lesions occurred at the lower Susquehanna River by PFBC, Fisheries Management Area 6 staff. In these instances, an estimated 40 – 50% of the adult smallmouth bass collected had lesions that affected 20 – 40% of their body surface (M. Kaufmann, PFBC, email correspondence). A high flow event shortly after documentation prevented initial collection to provide specimens for pathological analysis. Once river discharge receded, anglers began to report catching affected smallmouth bass in this same stretch of the Susquehanna River. Another attempt to collect specimens after the second report failed to capture affected fish for analysis. Timing of these efforts is poor because smallmouth bass behavior during this time of the year congregates them in waters too deep to be effectively captured using boat electrofishing. To date, no definitive specimens have been provided for pathological analysis. We are uncertain as to whether the condition observed in this isolated stretch of river is in any way linked to what has been observed in YOY smallmouth bass since 2005. It is presumed that, so far, this condition has been restricted to the stretch of the Susquehanna River between York Haven and Safe Harbor dams as there have been no reports of anglers catching any adult smallmouth bass with this condition outside of that area. During 2012, PFBC staff will pay close attention to adult smallmouth bass in this and neighboring areas for evidence of disease outbreaks.

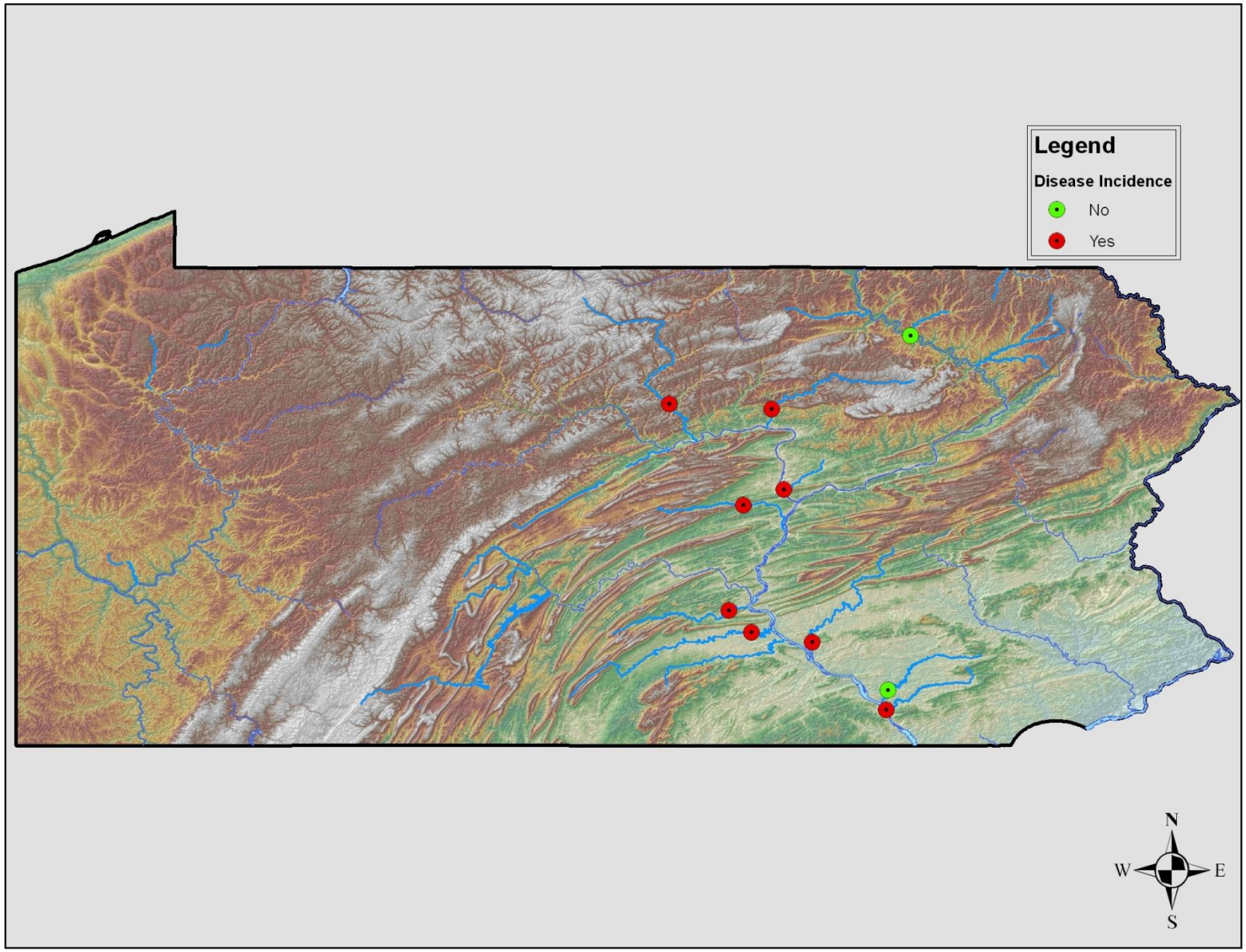

Figure 3. Incidence of disease among YOY smallmouth bass collected as part of directed YOY black bass surveys at tributary locations during 2011.

Water Quality

Various components of water quality were assessed in 2011; both as part of disease investigation as well as in unassociated projects. Beginning in July 2011, three existing USGS gages were retrofitted with water quality sondes as part of an expansion of PADEP's Water Quality Network (WQN). These gaging stations allow for year-round water quality assessment at three large river locations important to this study. Also beginning in 2011, USEPA Region 3 provided water quality sondes on loan agreement to support this project. These sondes were deployed in select tributaries to monitor physical water quality parameters that may be related to disease outbreaks (e.g., dissolved oxygen, pH, temperature). As in previous years, the Susquehanna River Basin Commission (SRBC) sampled nutrient concentrations and generated loading information throughout the basin as part of an unassociated project.

Large River Water Quality

Beginning in 2011, USGS retrofitted three existing gaging stations with instantaneous water quality sondes as part of the PADEP's state-wide water quality network. These gages were located at the Susquehanna River at Danville, Susquehanna River at Harrisburg, and Juniata River at Newport. These long term data were lacking from large river reaches in the Susquehanna River Basin until 2008 when temporary deployments of sondes were made to support this project annually.

Susquehanna River at Danville (USGS 01540500)

In 2010, a sizable disease outbreak was documented by PADEP near Danville and follow-up collections were made by PFBC staff for pathological analysis. This was the most substantial outbreak observed on the upper reach of the mainstem Susquehanna River that had been documented to date and occurred after directed YOY smallmouth bass surveys were conducted by Fisheries Management Area 4. This portion of the Susquehanna River is also the first upstream of the middle Susquehanna River which has been the reach most affected by disease outbreaks. As such, this location is important to document, especially if a progression of large-scale outbreaks begins to extend upstream. Generally, physicochemical water quality parameters were not very stressful at the Danville location in 2011. Minimum daily dissolved oxygen concentrations only fell below 5.0 mg/L briefly during the critical period (Figure 4). Similarly, daily maximum pH never neared the PADEP maximum criterion of 9.0 specific units (Figure 5). Temperature, however, did reach stressful values for during 2011. Daily maximum temperature exceeded 30 degrees Celcius (^{o}C) on nine occasions in mid- to late July, including two occasions where the mean daily temperature also exceeded 30^{o}C (Figure 6). These measurements were preceded by an apparent equipment error so it is uncertain as to the full duration of this high temperature event.

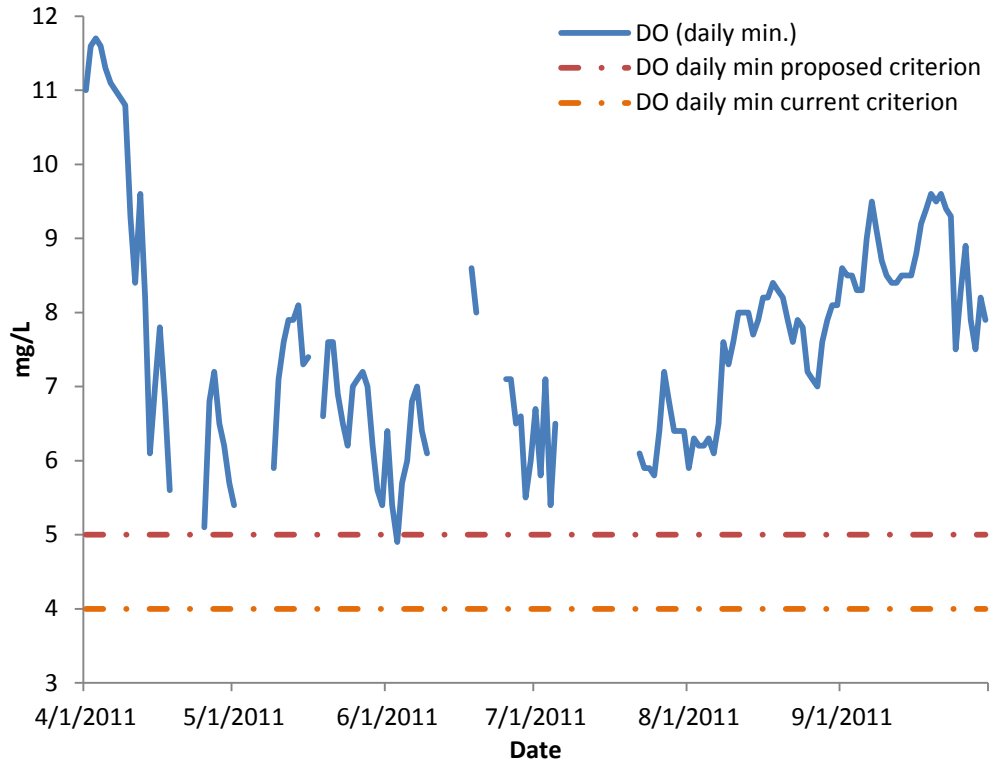

Figure 4. Daily minimum dissolved oxygen concentration (mg/L) at the Susquehanna River at Danville (USGS 01540500): April 1 – September 30, 2011

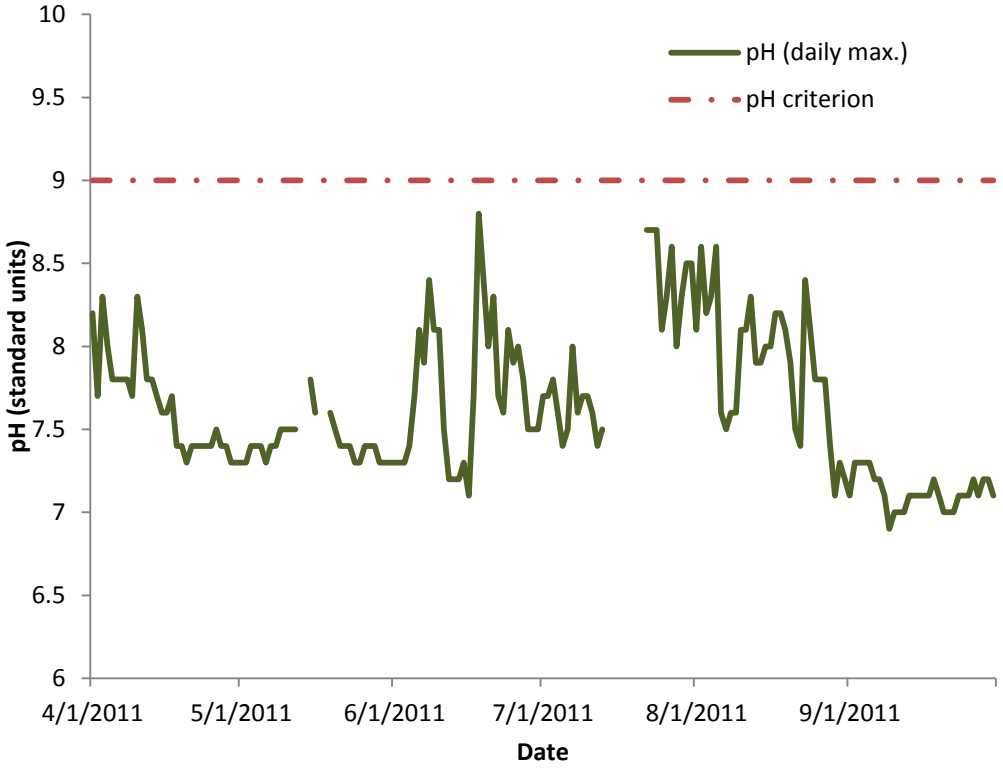

Figure 5. Daily maximum pH (standard units) at the Susquehanna River at Danville (USGS 01540500): April 1 – September 30, 2011.

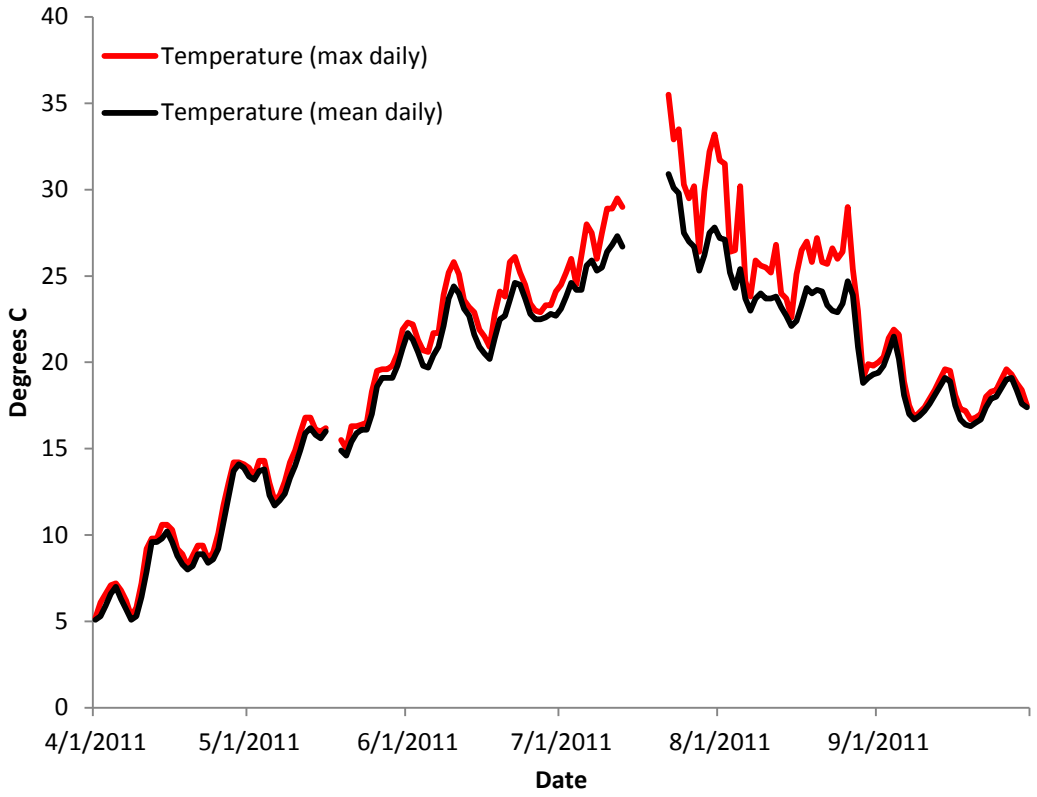

Figure 6. Daily maximum and mean water temperature (degrees Celcius) at the Susquehanna River at Danville (USGS 01540500): April 1 – September 30, 2011.

Susquehanna River at Harrisburg (USGS 01570500)

The middle Susquehanna River, which includes the Harrisburg area, has been the most affected by disease outbreaks among YOY smallmouth bass. As a result, water quality studies focused on this reach of the Susquehanna River beginning in 2008. These studies indicated that there were large spatial and temporal differences in dissolved oxygen, pH, and temperature within this reach (Chaplin et al. 2009; Chaplin et al. 2012). Conditions were also shown to exceed values considered stressful to warm water fish species, including smallmouth bass.

Daily minimum dissolved oxygen concentrations and water temperature reached stressful levels during 2011 at the Susquehanna River at Harrisburg . Minimum daily dissolved oxygen concentrations only fell below 5.0 mg/L on seven occasions during the critical period; two of which fell below the PADEP criterion of 4.0 mg/L (Figure 7). Daily maximum pH never exceeded the PADEP maximum criterion of 9.0 specific units during summer 2011 but did meet that value on one occasion (Figure 8). Water temperatures did reach stressful values during 2011. Daily maximum temperature exceeded 30^{o}C on fourteen occasions in mid- to late July, including six occasions where the mean daily temperature also exceeded 30^{o}C (Figure 9). Further, on July 22nd, the maximum daily temperature reached 35^{o}C; a value believed the maximum for short-term exposure during the summer growth period to avoid potential lethal effects (Wrenn 1980).

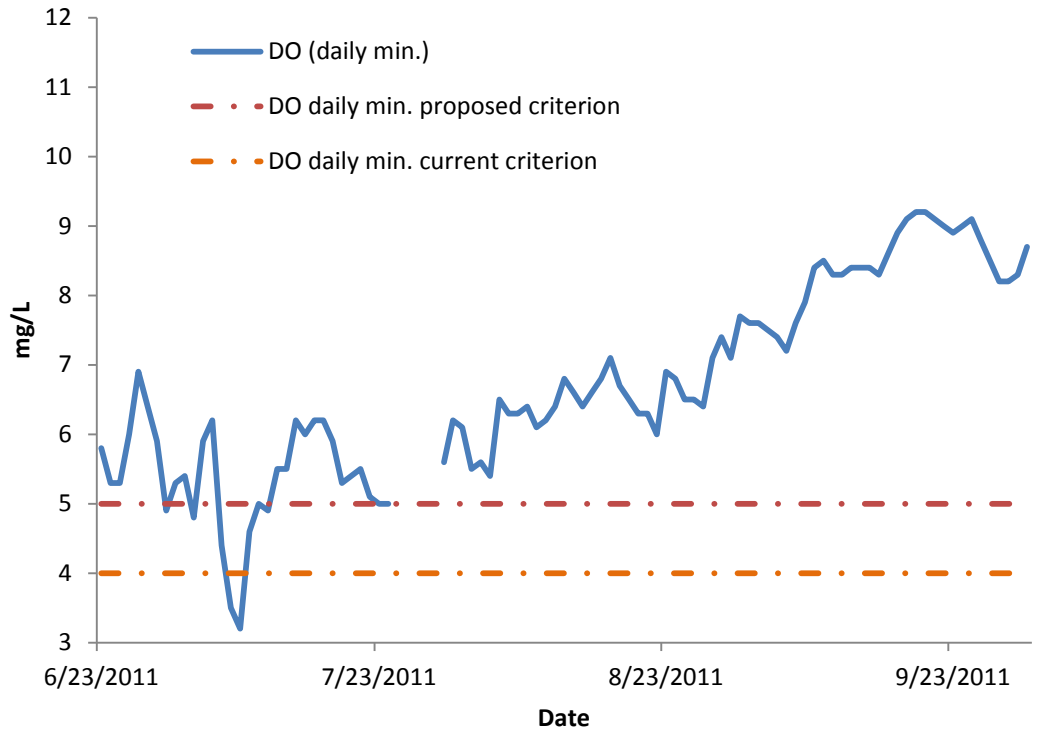

Figure 7. Daily minimum dissolved oxygen concentration (mg/L) at the Susquehanna River at Harrisburg (USGS 01570500): June 23 – September 30, 2011.

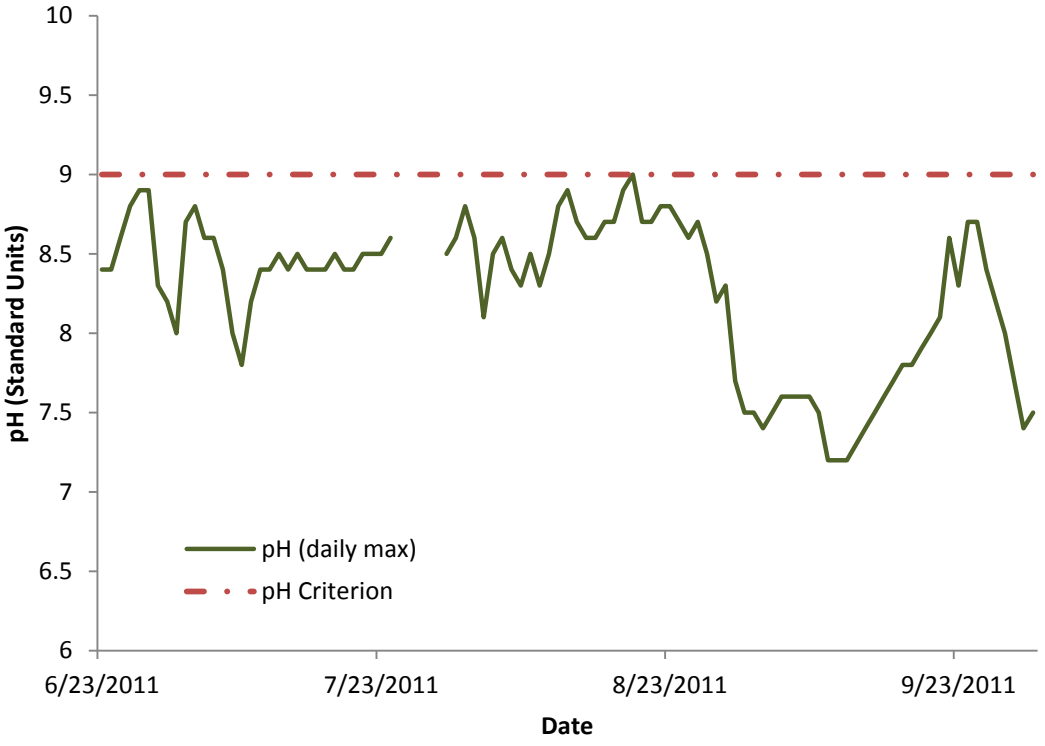

Figure 8. Daily maximum pH (standard units) at the Susquehanna River at Harrisburg (USGS 01570500): June 23 – September 30, 2011.

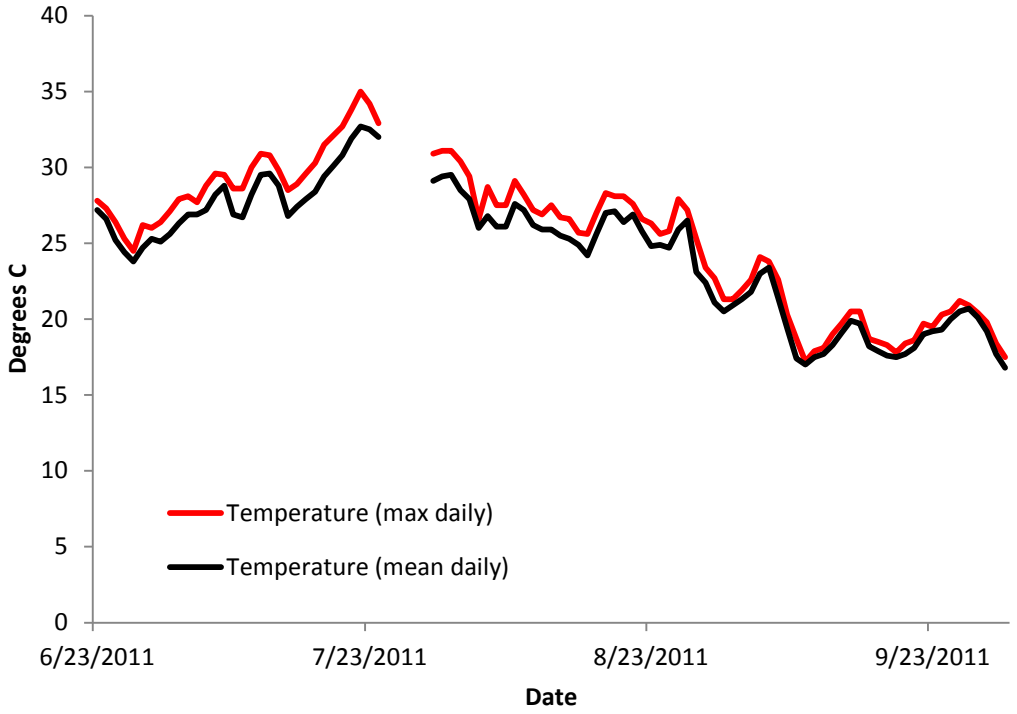

Figure 9. Daily maximum and mean water temperature (degrees Celcius) at the Susquehanna River at Harrisburg (USGS 01570500): June 23 – September 30, 2011.

Tributary water quality

Sondes were placed in select tributaries to collect physicochemical water quality data using equipment obtained on loan agreement from U.S. EPA Region 3 and maintained by PADEP Central Office staff. The tributaries chosen have been locations where previous disease outbreaks have been observed and include Aughwick Creek, Loyalsock Creek, Penns Creek, Pine Creek, and Wyalusing Creek. Loyalsock, Penns, and Pine creeks all had incidence of disease during 2011. Prevalence of disease in Pine Creek was severe (75%, Table 5) while prevalence in both Loyalsock and Penns creeks was low. There was no incidence of disease at Wyalusing Creek at the time of survey. No YOY smallmouth bass survey was conducted at Aughwick Creek during 2011.

Aughwick Creek experienced a brief period of stressful water temperature and longer period of stressful dissolved oxygen concentrations. A maximum water temperature of 32.79 ^{o}C was observed on July 22, 2011. Water temperatures only approached or exceeded 30 ^{o}C during a four-day period. A minimum dissolved oxygen concentration of 4.11 mg/L was recorded on July 24, 2011; the lowest concentration measured among tributary streams monitored. Dissolved oxygen concentrations met or exceeded 5.0 mg/L on 13 days during summer 2011. The 5.0 mg/L concentration threshold is widely accepted as the concentration below which early life-stages of warm water fish become stressed.

Conditions observed during 2011 at Loyalsock Creek included a brief period of stressful water temperature but dissolved oxygen concentrations were not considered stressful. A maximum temperature of 32.91 ^{o}C was observed on July 22, 2011 (Appendix B). For only a four-day period beginning July 21 and ending July 24 did water temperatures approach and exceed 30 ^{o}C; a stressful water temperature for most warm-water fish

species. Despite high water temperature, a minimum dissolved oxygen concentration of 6.18 mg/L on July 23, 2011; a concentration that generally is not considered stressful to warm-water fish species (Appendix B).

Penns Creek experienced a period of stressful high water temperature and low dissolved oxygen concentrations during 2011. A maximum water temperature of 34.94 ^{o}C was observed on 7/22/2011. Unlike Loyalsock Creek, the water temperature approached or exceeded 30 ^{o}C on several occasions during the mid- to late-July period that has been critical in disease outbreaks among YOY smallmouth bass. A minimum dissolved oxygen concentration of 4.67 mg/L was observed on July 23, 2011. Daily minimum dissolved oxygen concentrations also neared and exceeded 5.0 mg/L on July 22 and July 24, 2011, respectively.

Pine Creek experienced a long period of stressful high water temperatures; however, dissolved oxygen concentrations were not as stressful as was observed in 2010. A maximum water temperature of 35.34^{o}C was observed on July 22, 2011. Water temperatures approached or exceeded 30 ^{o}C daily for most of July and early August. The minimum dissolved oxygen concentration observed during 2011 was 5.16 mg/L on July 23, 2011. The minimum dissolved oxygen concentration observed during 2010 was 4.40 mg/L observed on July 9, 2010. There were seven occasions during 2010 when dissolved oxygen concentrations fell below 5.0 mg/L compared to none during 2011.

Wyalusing Creek, like Loyalsock Creek, experienced a brief period of stressful water temperature but dissolved oxygen conditions were not considered stressful. A maximum temperature of 34.56 ^{o}C was observed on July 22, 2011. Water temperatures exceeded 30 ^{o}C on seven days during 2011, six of which were between July 17 and July 24, 2011. The minimum dissolved oxygen concentration of 5.46 mg/L was observed on July 24, 2011. The four-day period of July 22 – July 25, 2011 were the only instances when dissolved oxygen concentrations fell below 6.0 mg/L.

Nutrients

Analysis of nutrient data that could spur algal growth is crucial since respiration-driven dissolved oxygen demands are among the theorized causes of stress leading to wide-scale bacterial infections. Recent reports have indicated that dissolved forms of phosphorus (dissolved phosphorus and orthophosphorus) have increased in recent years (Langland et al 2006, McGonigal 2009, McGonigal 2010). The Susquehanna River Basin Commission (SRBC) currently maintains a nutrient monitoring program within the basin including many of the mainstem Susquehanna River and tributary locations where outbreaks have occurred.

Comparison between orthophosphate concentrations for large river locations during January to August of years when disease incidence were high and years when disease incidence were low to non-existent did not prove to be conclusive. Concentrations were found to be significantly higher when incidence of disease was low to non-existent at the Susquehanna River at Marietta and Juniata River at Newport (chi-squared = 45.0927, df = 1, p = 1.879e-11; chi-squared = 18.2908, df = 1, p = 1.896e-5, respectively) and similar at the Susquehanna River at Danville (chi-square = 3.5946, df = 1, p = 0.5797)(Figure 10). A similar relationship is true for large tributary streams where disease incidence has been observed. A comparison between orthophosphate concentrations in tributaries during years when disease is present and years when disease is low to non-existent yielded no significant differences at most sites (Figure 11; Conoduguinet Creek: chi-squared = 1.7853, df = 1, p = 0.1815; Swatara Creek: chi-squared = 1.2066, df=2, p = 0.547; Shermans Creek: chi-squared = 1.2201, df = 1, p = 0.2693; Penns Creek: chi-squared = 1.2491, df = 1, p = 0.2637) and was found at the Chemung River to be higher during years when disease prevalence is low to non-existent (chi-square = 9.1501, df = 2, p = 0.01031). Dodds (2002)

cautions the use of dissolved inorganic nutrients, which include orthophosphate, in analyses because they are commonly under high demand and turnover rapidly, therefore measured concentration may be low yet supply could be high. Further investigation into inorganic phosphorus paired with quantitative and qualitative analysis of algal communities should provide insight on the role of this parameter in the Susquehanna River system.

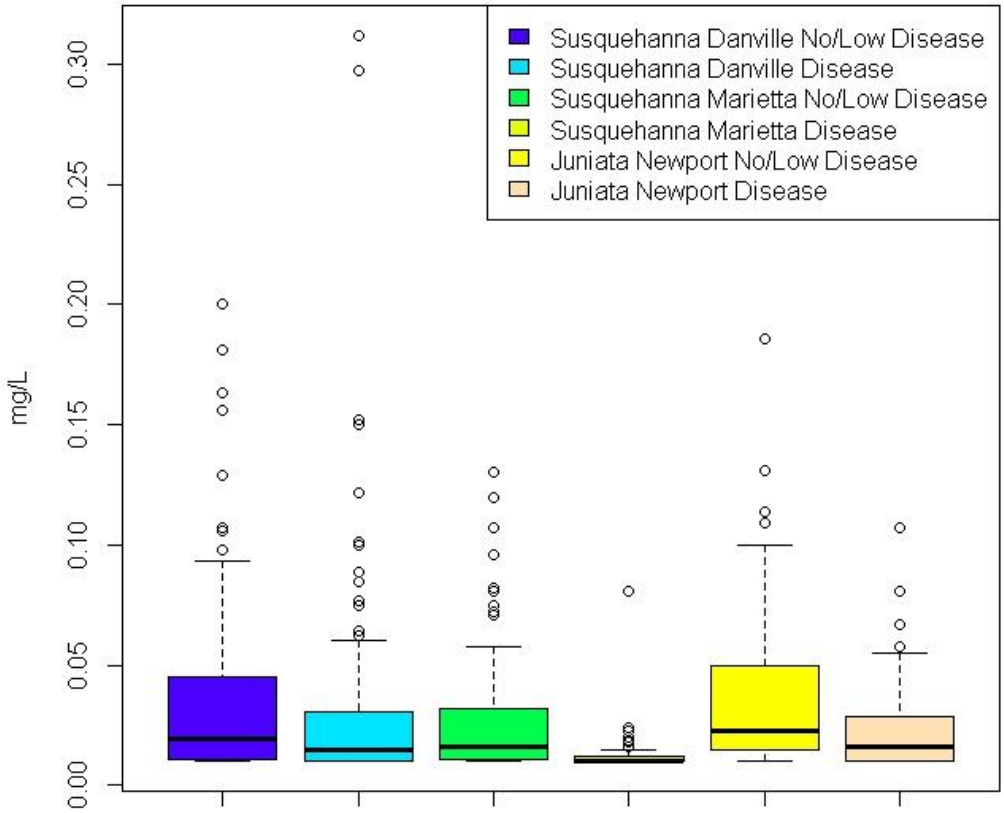

Figure 10. Comparison of orthophosphate concentrations (mg/L) for large river locations during January - August of years when disease incidence were high ("Disease"; 2005, 2007, 2008, 2010, 2011) and years when disease prevalence was low to non-existent ("No/Low Disease"; 2001, 2002, 2003, 2006, 2009). Nutrient data provided by SRBC Nutrient and Sediment Monitoring Program.

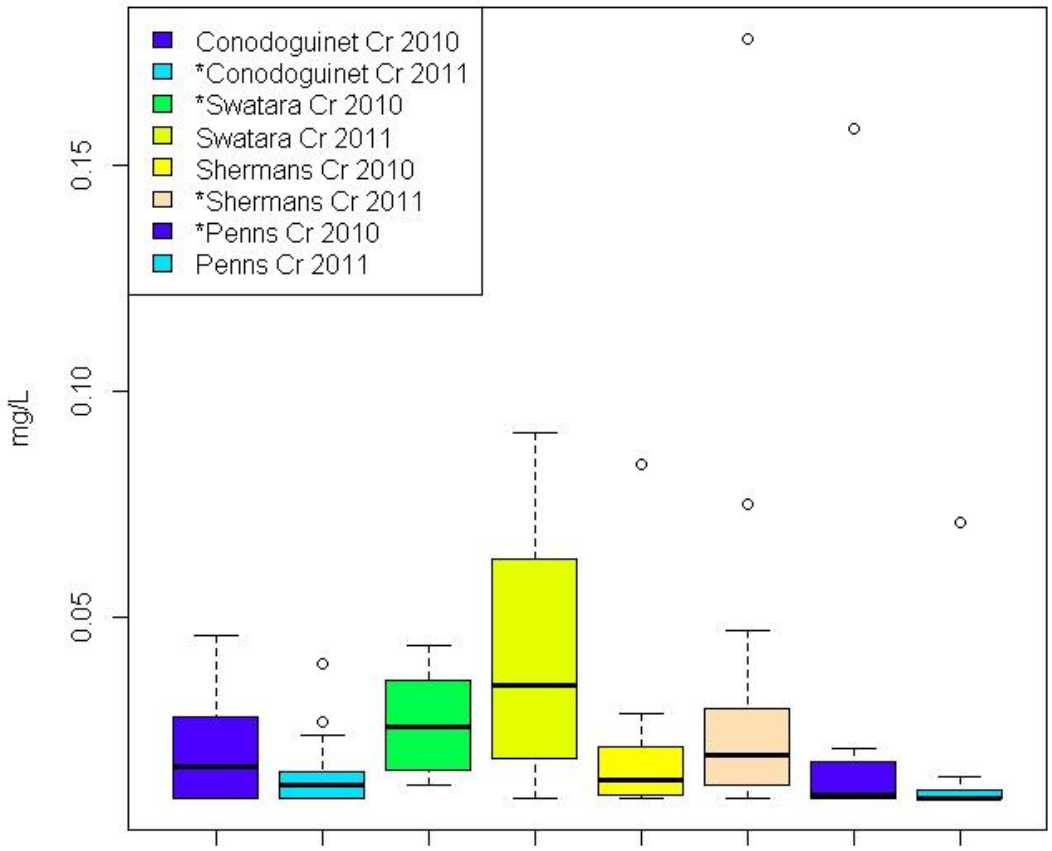

Figure 11. Comparison of orthophosphate concentrations (mg/L) for tributaries where YOY smallmouth bass surveys were conducted to track disease incidence. * indicate tributaries and years when disease prevalence was detected at high frequency. Nutrient data provided by SRBC Nutrient and Sediment Monitoring Program.

Discussion

Disease incidence and prevalence varied spatially during 2011 as they have in past years. Disease prevalence was highest in the lower Susquehanna River among all large river reaches. Disease prevalence in 2011 at the lower Susquehanna River also marked the highest value there since outbreaks began in 2005. The middle Susquehanna River, the reach historically affected the most by disease outbreaks, had moderate prevalence during 2011 compared to previous years. Prevalence in this reach was low across downstream portions but reach-wide prevalence was buoyed by high prevalence at Mahantango and Shady Nook sites. Similarly, the West Branch Susquehanna River also encountered disease prevalence that was moderate compared to previous years. The upper Susquehanna continued to have incidence of disease; however, it was only encountered at a few locations and prevalence in those instances was low to moderate. Overall, patterns of incidence and prevalence remain similar although there is annual variation in locations where incidence occur and prevalence. Hydrologic and meteorologic conditions were optimal to see large-scale outbreaks during 2011; however, YOY smallmouth bass densities below historic median values could have resulted in the low to moderate prevalence values.

In 2011 incidence of disease was also documented in a few tributary streams which have not had symptomatic fish in previous surveys. Past surveys by PFBC staff at Shermans Creek had documented strong YOY smallmouth bass densities during 2009 and 2010 and all fish observed were asymptomatic of clinical disease. The 2011 survey again documented strong YOY smallmouth bass densities but moderate prevalence of disease was affecting the population at the time of the survey. During past years, Shermans Creek has been used as a control stream for comparison with other tributaries in the southern portion of the basin. Chillisquaque Creek, Conodoguinet Creek, and Pequea Creek also had outbreaks of disease during 2011 for the first time since investigations began in tributaries in 2009. The presence of disease in these streams may signify that environmental conditions are changing in these watersheds as well and/ or the pathogen(s) affecting these fish are expanding in range.

Disease incidence was also noted in a number of other tributary systems in 2011 where outbreaks have been documented in the past. The Chemung River, which had fish affected by disease in 2009 had high prevalence of disease in 2011. Loyalsock Creek, Pine Creek, and Penns Creek again were documented to have incidence of disease in 2011 as they did in 2010. The high prevalence and severe condition of the specimens from Pine Creek during 2010 and 2011 may warrant additional attention during future efforts. Swatara Creek, which had high prevalence during PADEP surveys in 2010, had much lower prevalence during 2011 surveys.

Outside of the basin

During 2011, fish from outside of the Susquehanna River basin were observed with lesions similar to those observed in the Susquehanna Basin during previous years. Fish from the upper Allegheny River and Schuylkill rivers had columnaris and motile Aeromonas infections similar to those collected from the Susquehanna River Basin. It is uncertain whether the infections were a result of high water temperatures or characteristic of infections observed in the past in the Susquehanna River Basin. Pathological analysis of tissues will determine whether some of the characteristics that have been observed in fish from the Susquehanna River are present in those from the Allegheny and Schuylkill rivers. The extremely high water temperatures at the time of collection at these sites ($32^{o}C – 35^{o}C$) are typical of temperatures where these bacterial infections occur and could be a result of stress from that alone as opposed to some of the other factors present in previous analysis of Susquehanna River fish. At this point it is impossible to link what was documented in the Allegheny and

Schuylkill rivers during 2011 to what has been affecting Susquehanna River Basin smallmouth bass populations since 2005.

Follow-up collections in these and other waterbodies where disease fish have not been observed to submit fish for viral and pathological analysis is recommended to develop a baseline for those sites and to determine whether disease incidence is spreading to areas outside of the Susquehanna River Basin. A large proportion of the fish that have been submitted for pathological, bacterial and viral analyses state-wide have come as part of this project. Submission of samples from different areas will help to identify the true range of some of the pathogens that have been linked to this disease outbreak. This will also help to better elucidate which of the factors are contributing to the condition. Since most of the fish submitted for analysis have been symptomatic and from areas where disease is known, there is autocorrelation of several of the pathogens to the condition occurring in the Susquehanna River Basin. The presence or absence of some of these pathogens will help to identify what factors are contributing to the condition and those that may be ubiquitous and likely have less or no role in the outbreaks. This will help to streamline research and result in better use of limited funding.

Largemouth Bass Virus

Many of the smallmouth bass submitted during 2011 and during previous years have tested positive for Largemouth Bass Virus (LMBV). This pathogen is not believed to clinically affect smallmouth bass but they have been documented as a carrier of the virus. It is hypothesized that carrying the viral load may cause an additional stress on the fish but this has not been evaluated in smallmouth bass. Most of the samples that have been submitted for viral analysis have come from this investigation and its repeated presence in samples raises suspicion that it may, in some way, be associated with disease outbreaks. Samples from areas where we have not seen outbreaks or from asymptomatic fish are limited. In order to better understand the role that LMBV has on the disease outbreaks that have been observed, more comprehensive collections from across the state must be submitted for analysis. Specimens could be submitted from other large river locations from future collections for viral analysis. Samples will also help to populate the U.S. Fish and Wildlife Service's National Wild Fish Health Database.

Additionally, there is some question as to whether the strain of LMBV that has been documented has not changed or mutated over time and may now be affecting smallmouth bass. Confirmation of LMBV is done molecularly and the region where the mutation may occur that potentially affects its virulence to smallmouth bass may not be recognized in the analysis. The complete viral genome for isolates of LMBV should be sequenced and compared from those isolates from Susquehanna River Basin smallmouth bass to determine if there are variations and those variations result in protein changes that could increase their virulence to smallmouth bass

Parasites

The density of myxozoan and trematode parasites present in the YOY smallmouth bass that have been analyzed has been surprisingly high throughout the course of this investigation. The presence of parasites is another potential stressor that could be contributing to the suppression of the immune systems of the YOY smallmouth bass that could be leading to bacterial infections. Recently, parasites found in YOY smallmouth bass have been identified as *Myxobolus inornatus* (Walsh et al. 2012). This species was first identified from YOY largemouth bass *Micropterus salmoides* from a Montana hatchery during the 1930s. Myxozoans, in general, have complex life cycles that utilize intermediate hosts as part of their development. Information about this stage of their

development may help to identify the factor leading to the relatively abrupt onset of this condition. Also, identifying the intermediate host may also identify a way to manage the outbreaks and limit their spread by controlling or eliminating that species.

An unrecognizable tissue inflammation was observed early in the pathological analyses and as more information is gathered it is believed to be linked to the myxozoan parasites. This inflammation has been considered characteristic of these disease outbreaks in the Susquehanna Basin. The presence of this inflammation and the tissue damage caused could be contributing to the compromised immune system of the fish as well as potentially causing the wound that later is the site of the bacterial infection. Further molecular analysis of the tissues affected by this inflammation would identify whether the cause of the inflammation is an early stage of the parasite or have a bacterial origin. The consistency of this inflammation in pathological samples identifies the importance of this factor in the disease outbreaks and indentifying the source may elucidate the mechanism of the manifestation of the disease.

Like LMBV, samples submitted for pathological analysis to observe parasites have largely come from affected populations of YOY smallmouth bass. There are very few samples from unaffected populations and asymptomatic fish. A subset of samples from outside of the Susquehanna Basin where we have not observed diseased fish as well as asymptomatic fish from areas where we have observed diseased fish would shed some light on the importance of parasite infections in the final bacterial infections. If the parasites are ubiquitous among populations of YOY smallmouth bass, included ones where disease has not been observed, it would weaken the role that parasitic infections are playing in the outbreaks that have been observed.

Summary and Recommendations for 2012

Efforts in 2012 should focus on indentifying the roles that a few factors may be playing in disease outbreaks in order to better direct the investigation's course for the future. Definitively identify the roles that LMBV and parasites are playing in bacterial infections by looking at places where outbreaks have not occurred as well as at fish that are asymptomatic of disease will help to achieve this end. The presence or absence of these factors in areas where there have not been disease incidence will strengthen and weaken their importance in the outbreaks, respectively.

Further, the investigation should continue to assess some measure of algal productivity to better understand how eutrophication is affecting productivity and subsequently respiratory demand for dissolved oxygen. Previous reports (Smith 2011) and study plans have identified this as a crucial task to better understand that relationship but technical committee expertise and financial resources have prevented these efforts from moving forward. As such, this type of study is again recommended for future funding.

Early research into the disease outbreaks identified spatial and temporal differences in physical parameters in the Susquehanna River, of which dissolved oxygen was the most prominent. Continuation of these studies has been prohibitively expensive and they have been discontinued. With the newly installed water quality sondes at mainstem Susquehanna River and Juniata River gaging stations, determining the feasibility of developing a predictive dissolved oxygen model to estimate the YOY smallmouth bass microhabitats from the existing main channel gages is in order. Assessing the strength of the microhabitat – main channel dissolved oxygen concentration relationship as well as collecting more contemporary data to identify potential shifts in this relationship would be required.

Recommended studies for 2012

1. Continued investigations into Largemouth Bass Virus (LMBV) including distribution of the disease and genetic analysis to determine if mutations are occurring increasing its virulence to YOY smallmouth bass.
2. Identification of parasites and the role that they play in bacterial infections by identifying their distribution, determining if they are the cause of unidentifiable inflammations, and identifying the intermediate host(s).
3. Develop a predictive model for determining microhabitat dissolved oxygen concentrations from main channel sondes for the Susquehanna and Juniata rivers.
4. Assess algal communities to determine how nutrients are affecting the Susquehanna River and whether eutrophication from increases in dissolved phosphorus is occurring.

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Appendix A

PENNSYLVANIA FISH & BOAT COMMISSION

Bureau of Fisheries
Fish Production Services

2011 Smallmouth Bass Lesions and Mortality

Prepared By:
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Fish Health Unit Leader

10/28/2011

Beginning in 2005 large numbers of moribund or diseased young of the year (YOY) smallmouth bass with lesions were observed in the Susquehanna River basin. These disease events coincided with poor recruitment leading to weak or non-existent year classes of fish. 2011 marked the first time that substantial numbers of YOY smallmouth bass were observed with lesions outside of the Susquehanna River Basin. Lesions were observed on smallmouth bass in the mainstem of the Allegheny, Schuylkill and Delaware rivers.

Fish were collected from multiple sites within each drainage. In addition to smallmouth bass, other species exhibiting clinical signs of disease as well as sympatric populations of largemouth bass showing no signs of disease were collected and submitted to the PFBC Fish Health Lab and/or the US Fish and Wildlife Northeast Fish Health Center for examination. Bacteriology, parasitology and virology were conducted on all specimens when applicable.

Species Collected:

smallmouth bass (*Micropterus dolomieu*)
largemouth bass (*Micropterus salmoides*)
greenside darter (*Etheostoma blennioides*)
green sunfish (*Lepomis cyanellus*)
white sucker (*Catostomus commersoni*)
satinfin shiner (*Cyprinella analostana*)
spotfin shiner (*Cyprinella spiloptera*)
spottail shiner (*Notropis hudsonius*)
fallfish (*Semotilus corporalis*)

While similar symptoms were observed in other species; of particular concern are the smallmouth bass. Symptoms and diagnosis were similar for smallmouth bass examined from each of the drainages (Tables 1, 2, and 3). Bacteria, and parasites observed in 2011 are endemic to the Commonwealth's waters, thus they do not provide evidence of a new fish pathogen. All bacterial and parasitic pathogens observed in 2011 are known to be opportunistic pathogens, infecting the host when its immune system becomes compromised. Bacterial and external parasite infestations such as the ones observed are usually the by-product of environmental stress such as a sudden increase in water temperature or other water quality issue. Although other species were collected during the disease events, the consistency and dominance of diseased smallmouth bass indicates that the species may be less tolerant of a particular environmental stressor or pathogen. Specific evidence supporting this

assumption is the presence of relatively healthy largemouth bass collected alongside diseased smallmouth bass. Both species are similar in life history and ecological needs and generally can live in the same environmental conditions.

Largemouth bass virus (LMBv) is known to be present in Pennsylvania's smallmouth bass population and was detected at the majority of sites in 2011 (Tables 1, 2, and 3). LMBv is known to cause mortality in adult largemouth bass; however, it has not been linked to fish kills involving juvenile fish or other species. It has been theorized that LMBv may have been an additional stressor, weakening the immune system of the affected fish allowing for the onset of the other conditions. However, the consistency of LMBv detections in the YOY smallmouth bass associated with disease outbreaks is concerning. As a result the PFBC is currently investigating the theory that the LMBv found in fish in its large river systems may be a different strain than that previously investigated in other geographical regions. LMBv samples collected in 2011 are being submitted to the Fish Health Branch of the USGS Leetown Science Center in Kearneysville, West Virginia. Samples will undergo full genome sequencing for comparison to authentic LMBv samples previously collected from other geographical regions. If differences are observed, the next step would be a challenge study to determine if the genomic differences observed alter the virulence of the virus in respect to Pennsylvania's smallmouth bass populations.

Susquehanna Drainage

The severity of the external parasitic infestations and bacterial infections varied between sites within the Susquehanna River drainage in 2011. As in other years, the bacteria belonging to the Motile *aeromonas* group and *Flavobacter columnaris* were cultured internally, the lesions observed on the fish were also characteristic of both Aeromonad septicemia and Columnaris disease (Table 1).

Largemouth Bass Virus was detected at all sites except the Pequea Creek site. However the sample size (one smallmouth bass) may not have been adequate for detection. The three healthy largemouth bass from the Pequea Creek site were also negative from the LMBv (Table 1).

Diseased fish were also collected in 2011 from Pine Creek. In both 2010 and 2011, collected fish were suffering from severe external parasite infestations along with systemic bacterial diseases and the presence of LMBv. The severity of the external parasite infestations observed at this site in both years is not consistent with observations from other sites within the Susquehanna drainage where moribund fish with lesions have been collected. The continued occurrence and severity of disease at this location may suggest that there is a reoccurring environmental stressor associated with this particular site.

Largemouth bass were the only species actually collected from the Lake Raystown site. However, diseased smallmouth bass were observed at the site several weeks prior to the collection of YOY largemouth bass.

Delaware Drainage

Although external parasites were observed on fish collected in the Delaware drainage the severity of the parasitic infestation was relatively mild (Table 2). While the majority of smallmouth bass collected exhibited signs of disease only one largemouth bass was collected with signs of disease. The lesions observed were characteristic of systemic bacterial infections caused by Motile *aeromonas* and *Columnaris*. LMBv was detected in both smallmouth bass and largemouth bass at all sites within the drainage (Table 2). Because 2011 marks the first year symptoms of disease were observed in the

Delaware River basin it will be important to monitor for and collect fish from future disease events within the drainage.

Allegheny River

Diseased smallmouth bass collected at sites located on the Allegheny River exhibited lesions similar to those observed in both the Susquehanna and Delaware drainages. Motile *Aeromonas* was cultured from all specimens collected (Table 3). Unfortunately fish could not be delivered to the PFBC Fish Health Lab until 48 hours after collection. Thus, virology and parasitology analysis were performed. The similarity of symptoms suggests that their most likely is a connection between the diseased smallmouth in the Susquehanna and Delaware drainages. This is the first observation of disease in the Allegheny River smallmouth bass population. It will be important to monitor and collect fish from future outbreaks.

Table 1. Susquehanna River Basin

Site	Date	Species	Number Submitted	Clinical signs of disease	External Parasites	Internal Parasites	Microbiology	Virology
Pequea Creek	7/28/2011	Smallmouth bass	1	Yes	None	None	*Columnaris* *Motile aeromonas*	None
Pequea Creek	7/28/2011	Largemouth bass	3	No	None	None	None	None
Pequea Creek	7/28/2011	White Sucker	1	Yes	None	None	*Columnaris* *Motile aeromonas*	Not Analyzed
Susquehanna R. (Wrightsville)	7/28/2011	Smallmouth bass	12	Yes	*Columnaris* (skin and gills)	Trematodes (white Grub)	*Columnaris* *Motile aeromonas* *Plesiomonas*	LMBv
Susquehanna R. (Wrightsville)	7/28/2011	Largemouth bass	1	No	*Columnaris* (gills)	None	*Columnaris*	LMBv
Susquehanna R. (Wrightsville)	7/28/2011	Greenside Darter	2	Yes	Protozoans (*Apiosoma)* *Columnaris*	None	*Motile Aeromonas*	LMBv
Susquehanna R. (Wrightsville)	7/28/2011	Spottail Shiner	1	Yes	*Columnaris* *(skin and gills)*	None	*Motile Aeromonas*	None
Susquehanna R. (Wrightsville)	7/28/2011	Green Sunfish	1	Yes	*Columnaris* *(skin and gills)*	None	*Motile Aeromonas*	None
Loyalsock Creek*	7/27/2011	Smallmouth bass		Yes	NA	NA	NA	LMBv
Penns Creek*	7/26/2011	Smallmouth bass		Yes	NA	NA	NA	LMBv
Pine Creek	7/20/2011	Smallmouth bass	15	Yes	Fungus, *Columnaris*, Monogenean Trematodes (*Dactylogyrus*), Protozoans (*Trichodina, apiosoma and ambiphrya*)	Larval nematodes, Trematodes (White Grub)	*Columnaris* (Not Cultured) *Motile Aeromonas*	LMBv
Pine Creek	7/20/2011	Fall Fish	1	Yes	*Columnaris* (skin and gills)	None	*Columnaris* *Motile aeromonas*	None
Raystown Lake	8/18/2011	Largemouth bass	15	No	*Protozoans (Trichodina, Trichodonella, Epistylis, apiosoma),* Monogeneans Trematode(*Gyrodactylus*)	Trematodes (White Grub), *Proteocephalus Ambloplitis* (Bass Tapeworm)	*Motile Aeromonas, Serratia,* Miscellaneous non-fermentor group	LMBv

* Examination completed by USFWS

Table 2. Delaware River Basin

Site	Date	Species	Number Submitted	Clinical signs of disease	External Parasites	Internal Parasites	Microbiology	Virology
Schuylkill River (Fritz Island)	8/3/2011	Smallmouth bass	4	Yes	Protozoans (*Apiosoma*)	None	*Columnaris* *Motile Aeromonas*	LMBv
Schuylkill River (Fritz Island)	8/3/2011	Largemouth bass	5	Yes (1 fish)	Columnaris, Monogenean Trematodes (Dactylogyrus)	None	*Motile Aeromonas*	LMBv
Schuylkill River (Five Points)	8/3/2011	Smallmouth bass	10	Yes	Protozoans (*Apiosoma*)	None	*Motile Aeromonas*	LMBv
Schuylkill River (Five Locks)	8/3/2011	Largemouth bass	5	No	None	None	None	LMBv
Delaware River (Point Pleasant)	8/3/2011	Smallmouth bass	4	Yes	Protozoans (*Apiosoma*)	None	*Motile Aeromonas*	LMBv
Delaware River (Point Pleasant)	8/3/2011	Largemouth bass	4	No	Protozoans (*Apiosoma*)	None	None	LMBv
Delaware River (Point Pleasant)	8/3/2011	Spottail Shiner	1	Yes	*Columnaris*	None	NA	Not analyzed

Table 3. Allegheny River

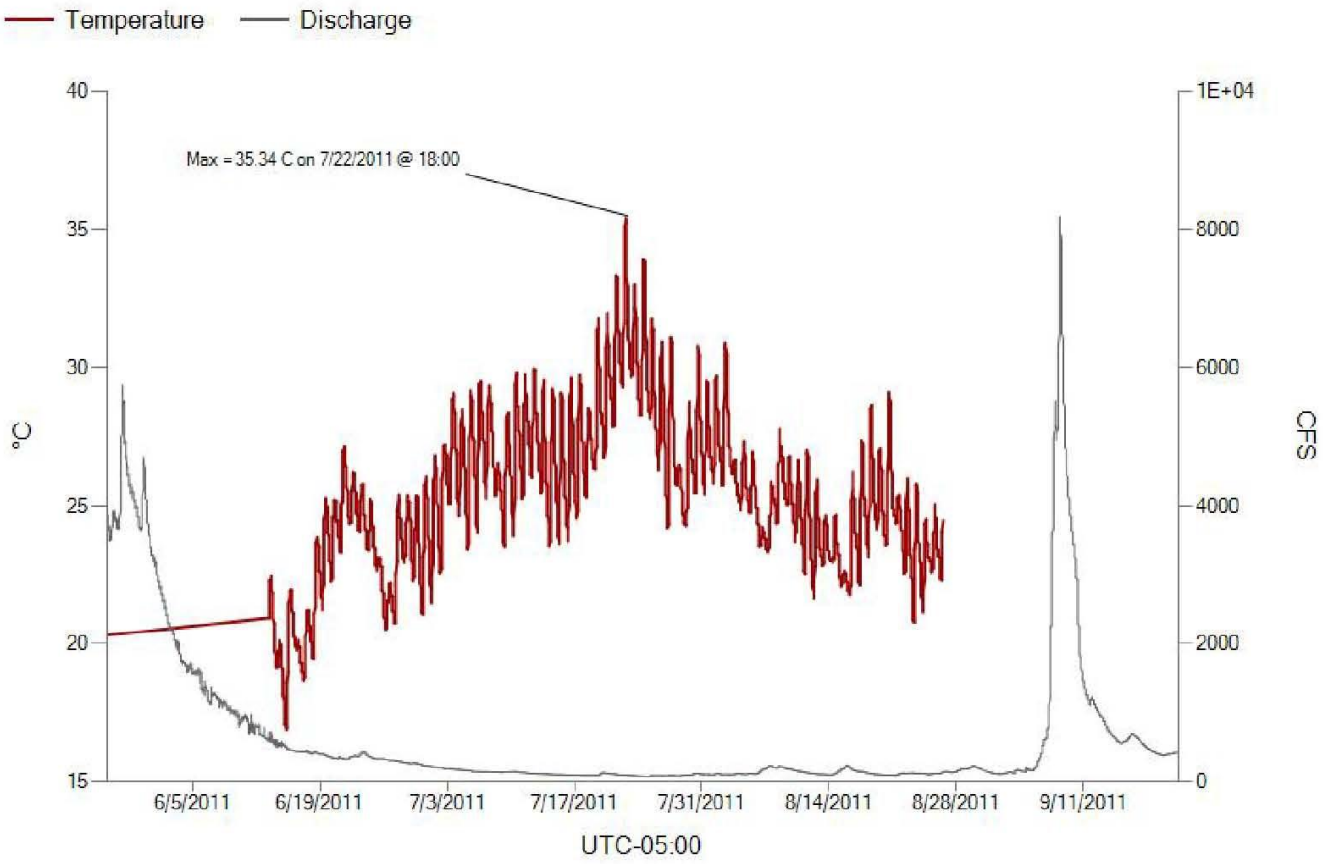
Site	Date	Species	Number Submitted	Clinical signs of disease	External Parasites	Internal Parasites	Microbiology	Virology
Allegheny River (Franklin)	7/27/2011	Smallmouth bass	2	Yes	NA	NA	*Motile Aeromonas*	NA
Allegheny River (Kennerdell)	7/27/2011	Smallmouth bass	5	Yes	NA	NA	*Motile Aeromonas*	NA
Allegheny River (Foxburg)	7/27/2011	Smallmouth bass	2	Yes	NA	NA	*Motile Aeromonas*	NA
Allegheny River (President)	7/27/2011	Smallmouth bass	1	Yes	NA	NA	*Motile Aeromonas*	NA

Appendix B

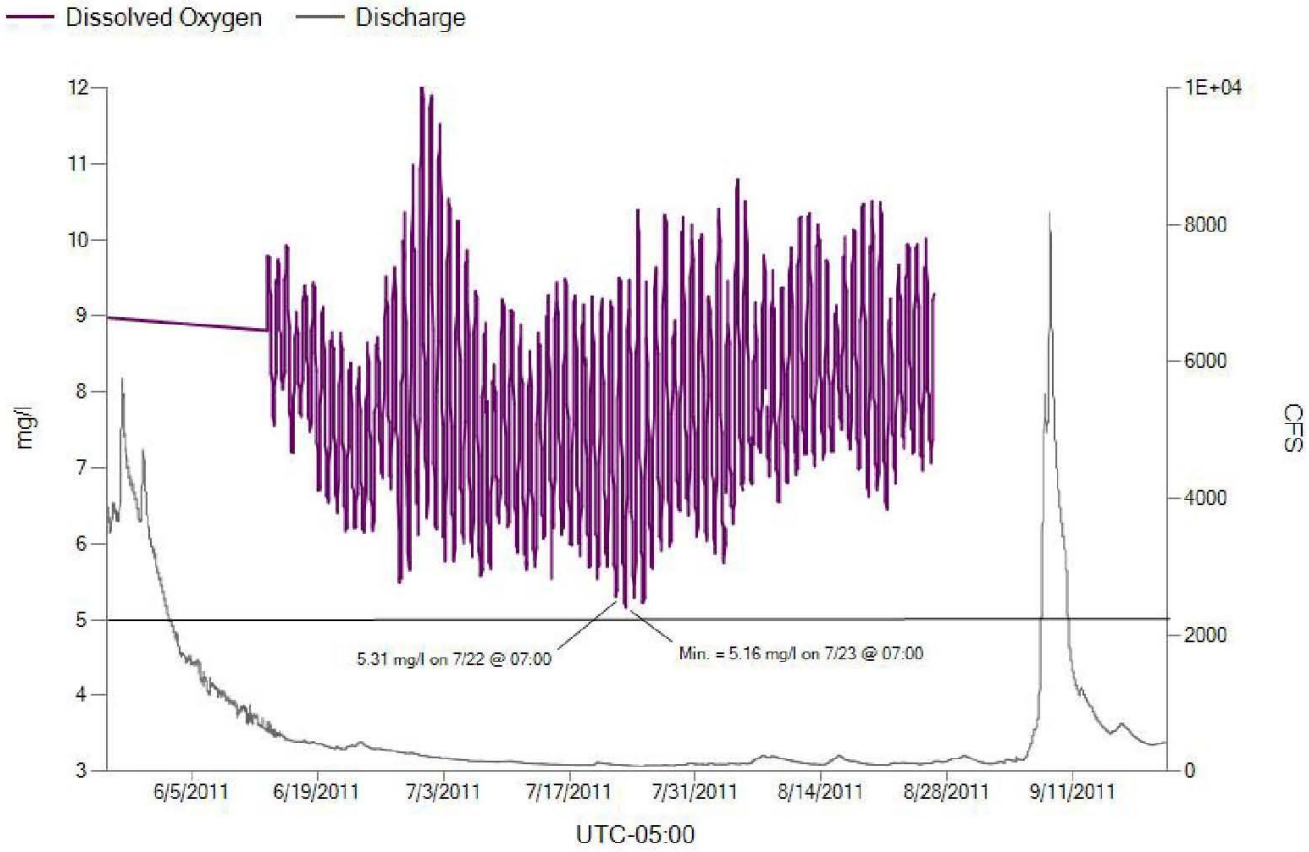
2011 PA DEP Continuous Instream Monitoring (CIM)

Smallmouth Bass - Warm Water Temperature and Dissolved Oxygen Targets

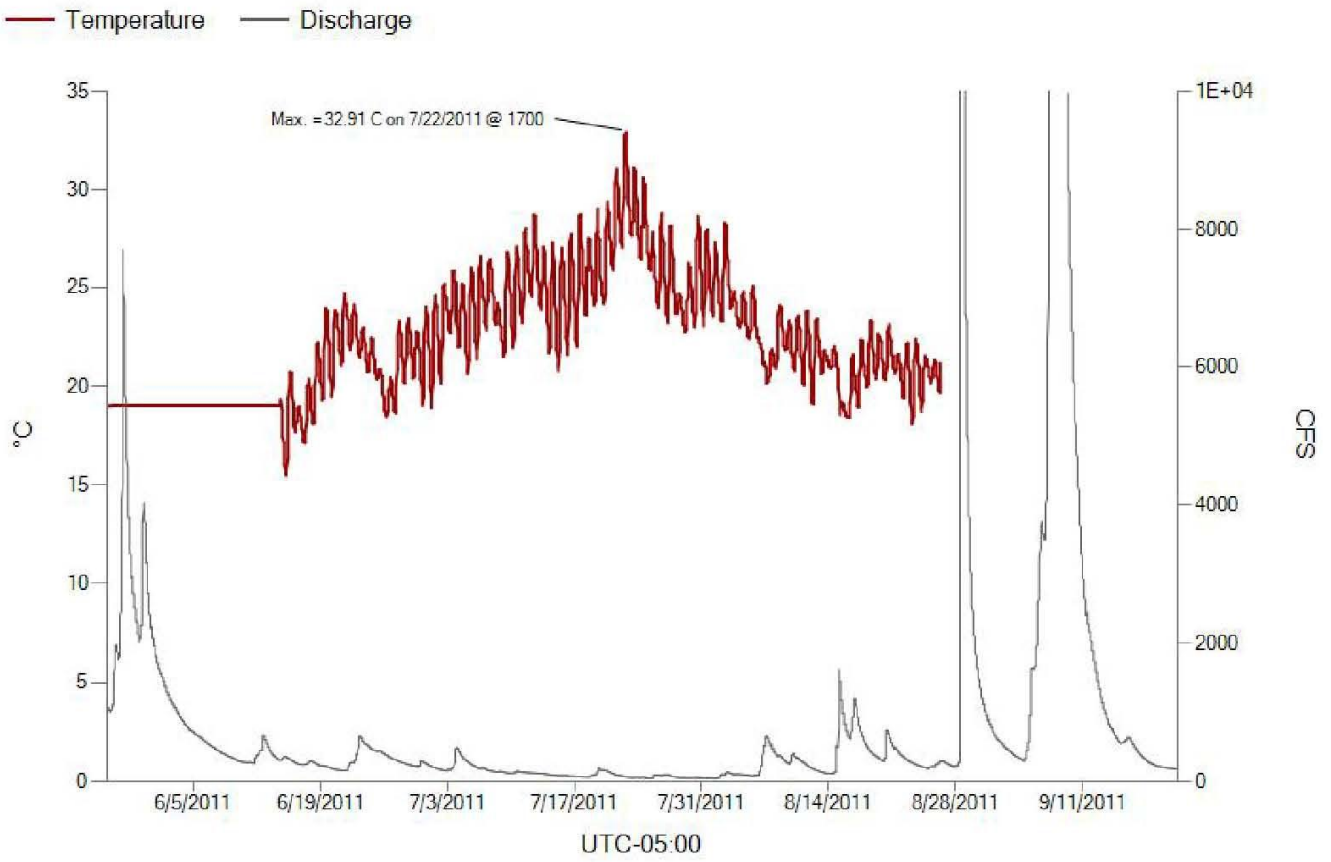
Pine Creek @ Hamilton Bottom



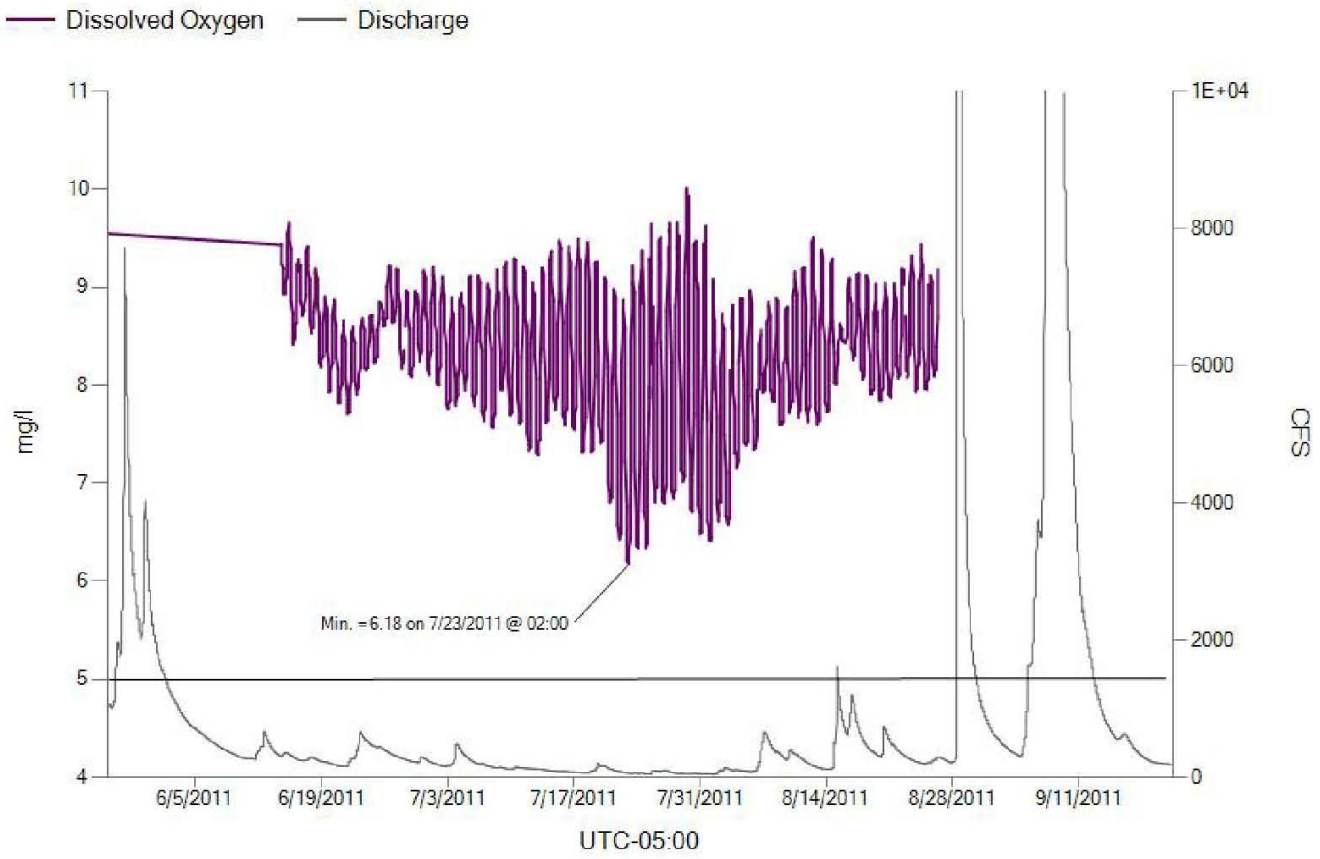
Pine Creek @ Hamilton Bottom



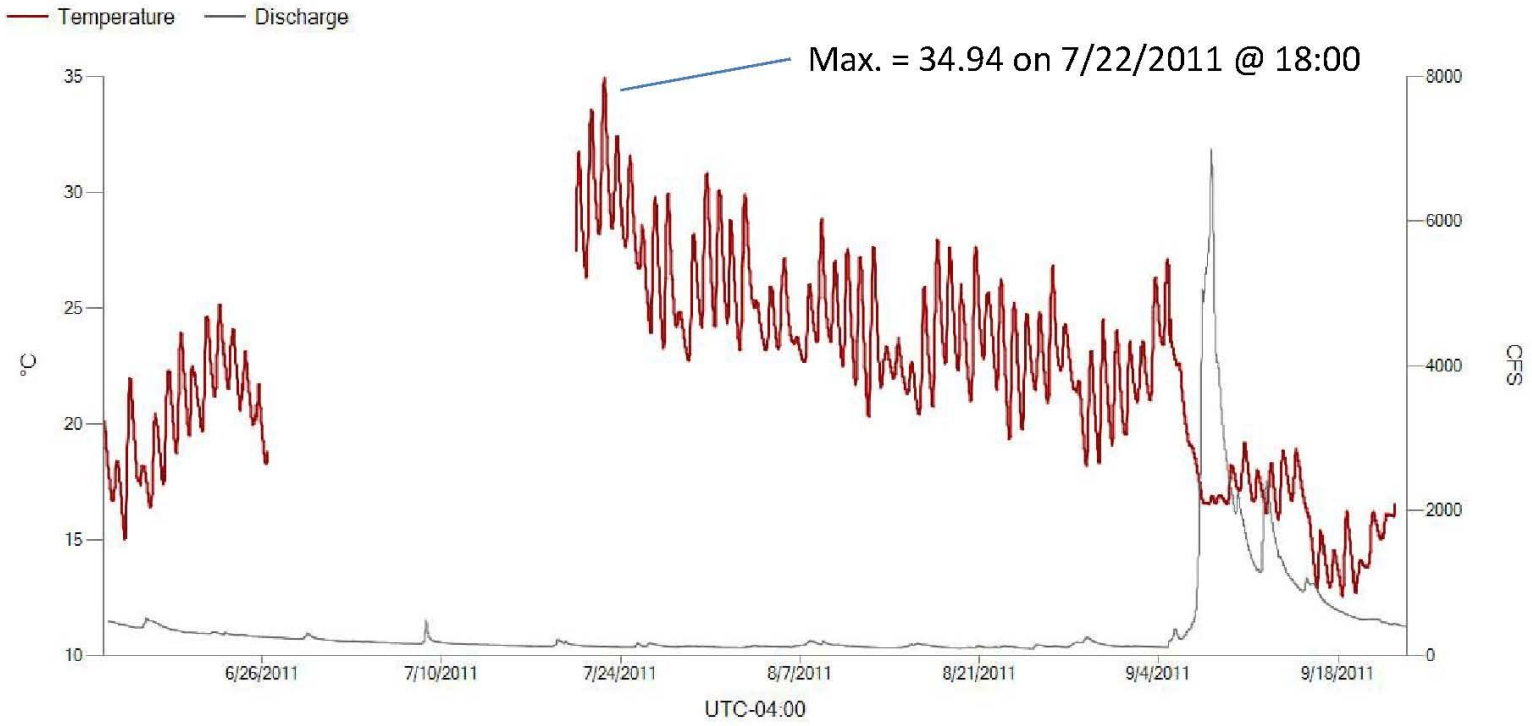
Loyalsock Creek @ Loyalsockville



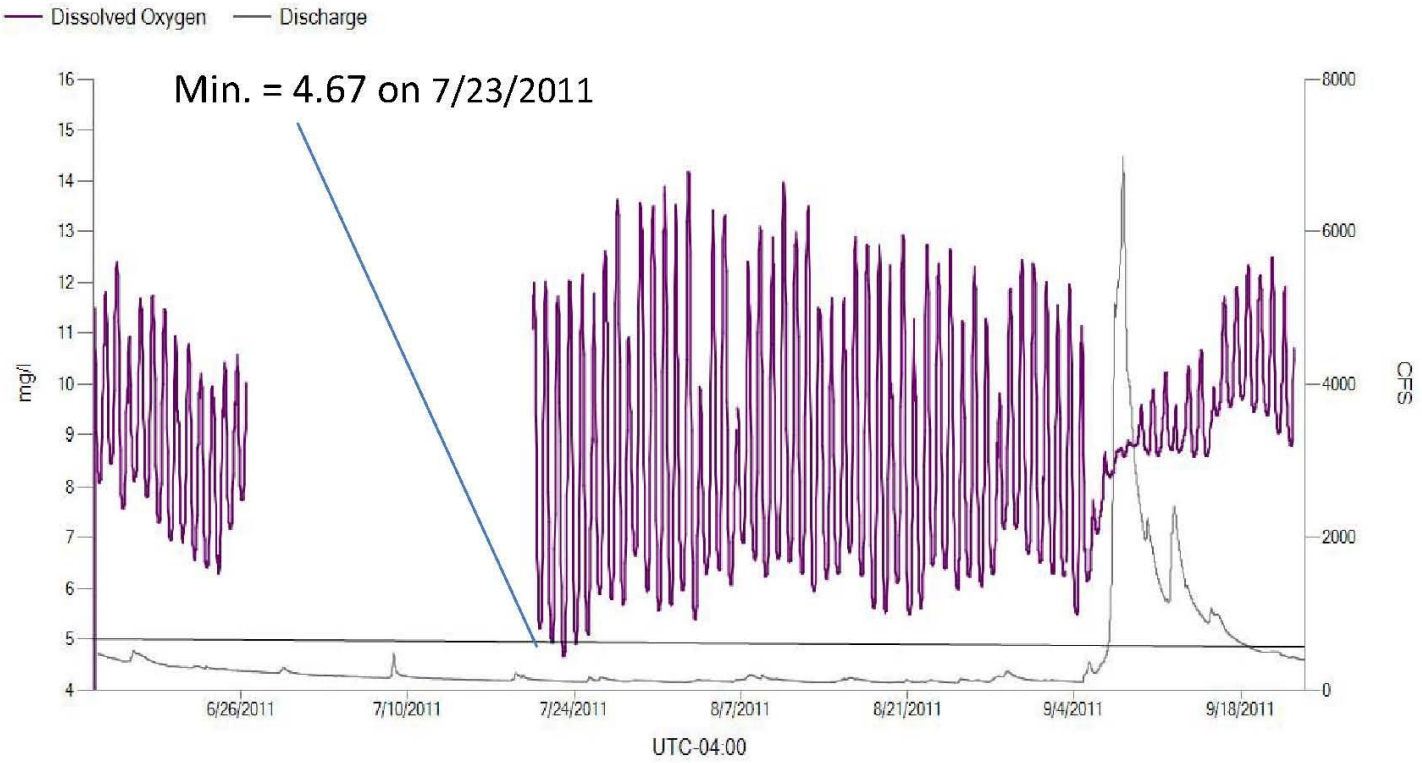
Loyalsock Creek @ Loyalsockville



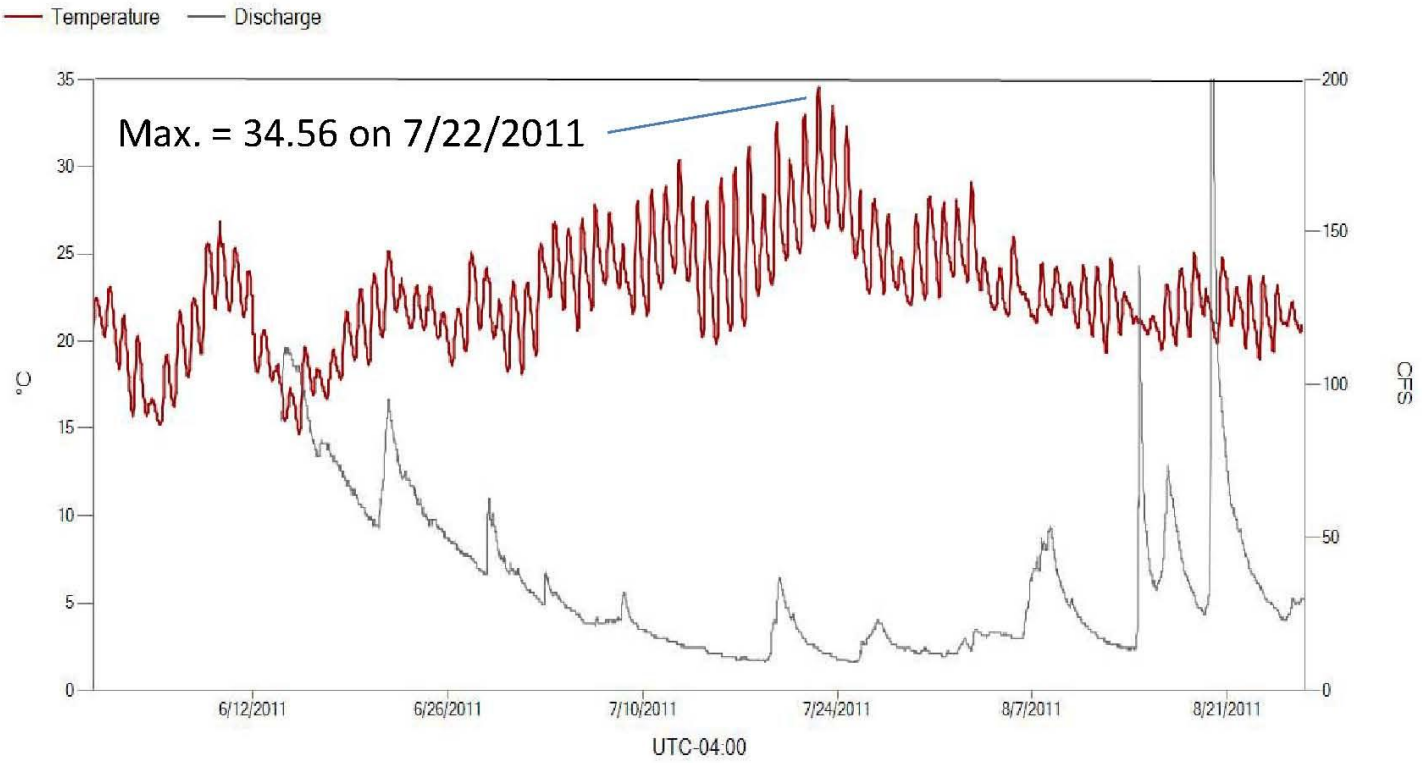
Penns Creek @ Penns Creek



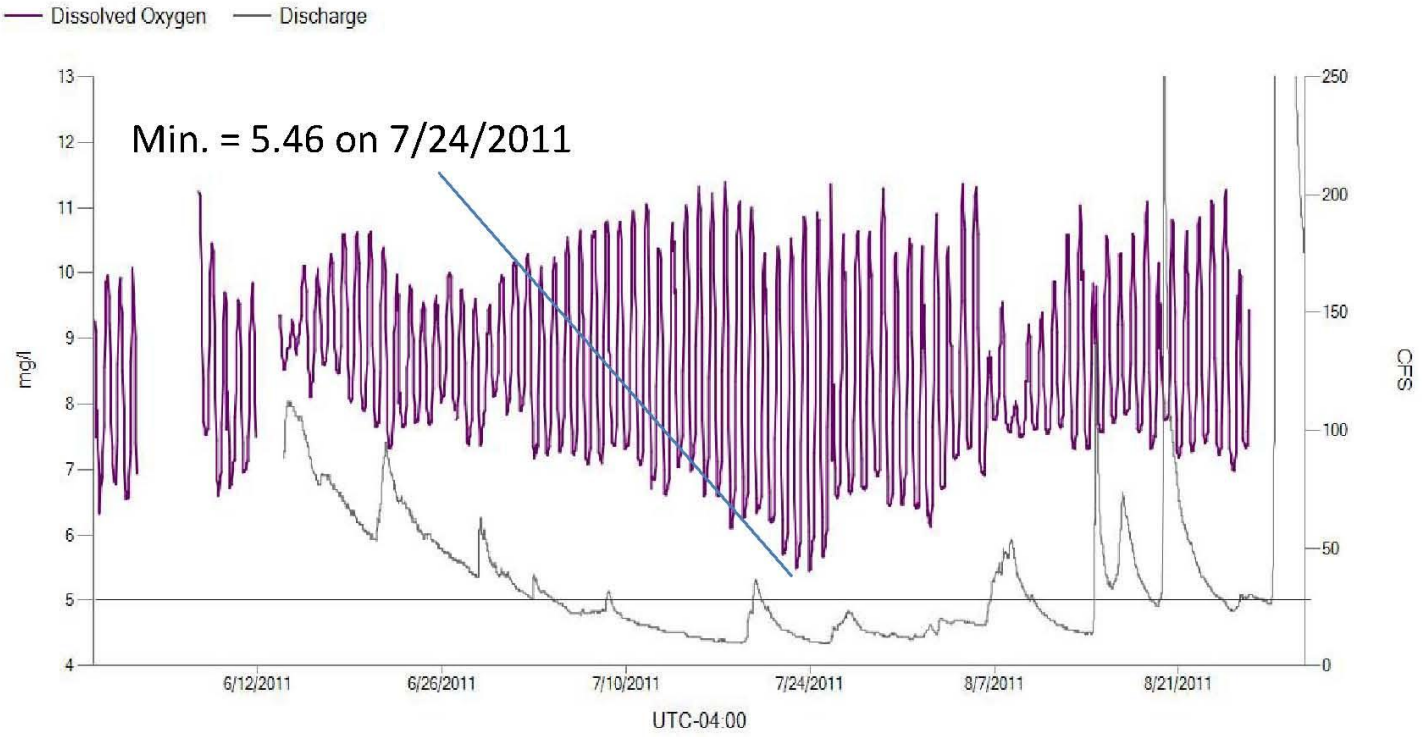
Penns Creek @ Penns Creek



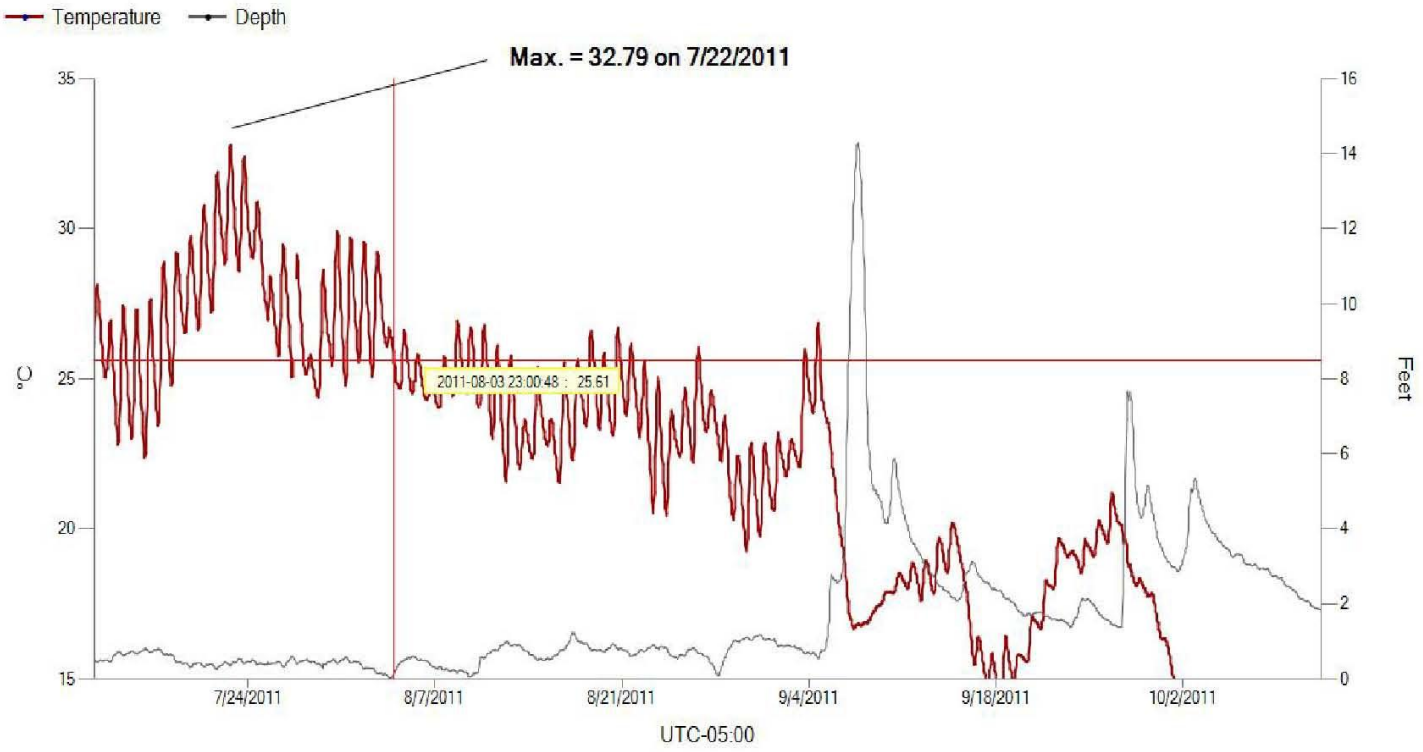
Wyalusing Creek @ Merryall



Wyalusing Creek @ Merryall



Aughwick Creek @ Shirleysburg



Aughwick Creek @ Shirleysburg