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Causal Analysis of Fish Kills in the Shenandoah and Potomac Rivers: A Report from a Workshop held January 16-18, 2007

National Center for Environmental Assessment
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1. INTRODUCTION

Mass mortalities of fish (kills) have occurred each year in the Shenandoah River since 2002 and, to a lesser extent, in the South Branch of the Potomac River. A workshop was convened by Region III of the U.S. Environmental Protection Agency (U.S. EPA), the Virginia Department of Environmental Quality and the West Virginia Department of Environmental Protection at Cacapon State Park, WV, January 16-18, 2007. The workshop addressed two kills that occurred in the spring of 2006. The goals of the workshop were to apply the stressor identification process to those kills using available data, to make a preliminary determination of the causes of the kills and to develop a prioritized list of data needs for the 2007 season. This scope and set of goals were determined during a preliminary workshop in September 2006. Participants in this workshop included representatives of the States of Virginia and West Virginia, the U.S. EPA, U.S. Geological Survey, and stakeholders and technical experts.

The method of analysis was the U.S. EPA's stressor identification process, as implemented in the CADDIS system (http://www.epa.gov/caddis). Stressor identification involves five steps: define the case, list candidate causes, evaluate data from the case, evaluate data from elsewhere and identify probable causes. It is a weight-of-evidence approach in which all relevant and available data are collected, data are analyzed to produce evidence of potentially causal relationships, the evidence is classified into types, each type of evidence is scored, the results are summarized for each candidate cause and, if possible, the probable cause is identified. The CADDIS process serves to organize the available information for the candidate causes and present the evidence for and against each one.

This report presents the results of the workshop, which narrowed the problem by eliminating some candidate causes and identified data needs for future kills in the Shenandoah or upper Potomac. Because essential data from field data sheets and pathology reports were unavailable, the report does not demonstrate ability of CADDIS to determine the causes of fish kills. However, it shows that even a preliminary application of the CADDIS method can be useful.

2. THE CASES

2.1. CASE 1—2006 FISH MORTALITIES IN LOWER NORTH FORK SHENANDOAH RIVER, VA

Case 1 is the fish mortality event that occurred between Woodstock and Passage Creek on the North Fork Shenandoah River in Virginia during the spring of 2006 (Figure 1). Dead and dying fish were found along an approximately 30-mile stretch of the river beginning in early March and continuing through late May of that year. A fish survey performed 2 weeks prior to the initiation of the kill found that the fish were apparently healthy. The species reported to be affected consisted primarily of smallmouth bass (*Micropterus dolomieu*) and redbreast sunfish (*Lepomis auritus*); some dying hogsuckers (*Hypentelium roanokense*) were also found. Adult fish appeared to be the dominant age class affected, though some sub-adult smallmouth bass also died. Biologists observed fin rot and other signs of stress in the dead fish and also in a small number of rock bass (*Ambloplites rupestris*), green sunfish (*Lepomis cyanellus*), bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*). Overall, reproduction of smallmouth bass and recruitment of young stock appeared to be good for the season.

Two distinct clinical phases appeared to occur during this mortality event on the Lower North Fork. First, an acute phase in which large numbers of dead fish appeared began shortly after the first dead fish were reported and lasted for several days around March 20. The dying fish did not show any external lesions. Some fish were noted to be lethargic and swimming at the surface of the water just prior to dying. In a second phase, affected fish had skin lesions, eroded fins and gill hyperplasia (thickening of the gill filaments). Several smallmouth bass that were sampled later that year exhibited circular areas of regenerating scales that may indicate that some fish recovered from the skin lesions. In addition, some smallmouth bass from these die-offs were found on post-mortem examination to be “intersex” (males with eggs in their gonads). It is unknown yet whether this condition is related to the fish deaths. Despite this intersex condition, reproduction of the smallmouth bass in these waters was noted to be good.

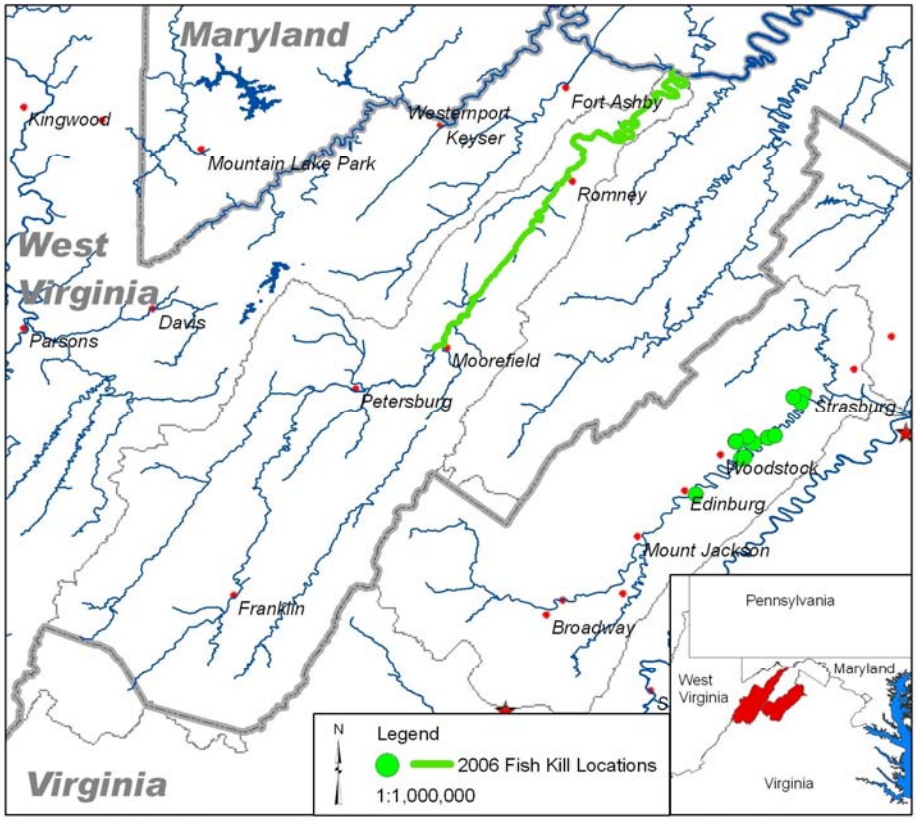

FIGURE 1

Locations of the Reported 2006 Fish Kills in the South Branch of the Potomac River and the North Fork of the Shenandoah River

The reference streams selected for the Virginia Shenandoah fish kill study are Cedar Creek and the Cowpasture and Maury Rivers (Figure 2). Cedar Creek is a large tributary of the North Fork Shenandoah River, and enters the North Fork just northwest of the town of Strasburg. The Cowpasture River serves as an out-of-basin reference. It and the Jackson River meet near Clifton Forge, VA to form the James River. The Maury River is also an out of basin reference stream, and is a tributary of the James River. The collection site on the Cowpasture River is located in the Walton Tract of the George Washington National Forest, at the end of County Route 632 in Bath County. The collection site selected for the Maury River is just downstream from a sewage treatment plant (as are several sites on the North Fork Shenandoah River, and the reference site on Cedar Creek) for the city of Lexington. Fish kills have not been reported for any of these streams since the first kills were observed in the North Fork Shenandoah River in 2004. All of these streams were selected as references because they are similar to the Shenandoah North and South Fork Rivers in that they are limestone buffered and agriculturally influenced. However, these influences are less pronounced than those observed in the North and South Forks of the Shenandoah River.

2.2. CASE 2—2006 FISH MORTALITIES IN THE SOUTH BRANCH POTOMAC RIVER, WV

Case 2 includes fish mortalities that occurred in the South Branch of the Potomac River Watershed in West Virginia during the late spring of 2006. The mortalities extended from Moorefield and continued down stream for approximately 55 miles (Figure 1). Multiple locations within this area were surveyed and found to have a few dead fish at each location. Dead and dying fish were found beginning around May 25th and continued through Memorial Day weekend. No additional dying or dead fish were observed after May 31st of that year. The majority of dead fish were redhorse suckers (*Moxostoma* spp.) along with some northern hogsuckers (*Hypentelium nigricans*) and a few smallmouth bass (*Micropterus dolomieu*). Adult fish appeared to be the dominant age class reported to be affected. Reproduction and recruitment was found to be good for the season despite the kill.

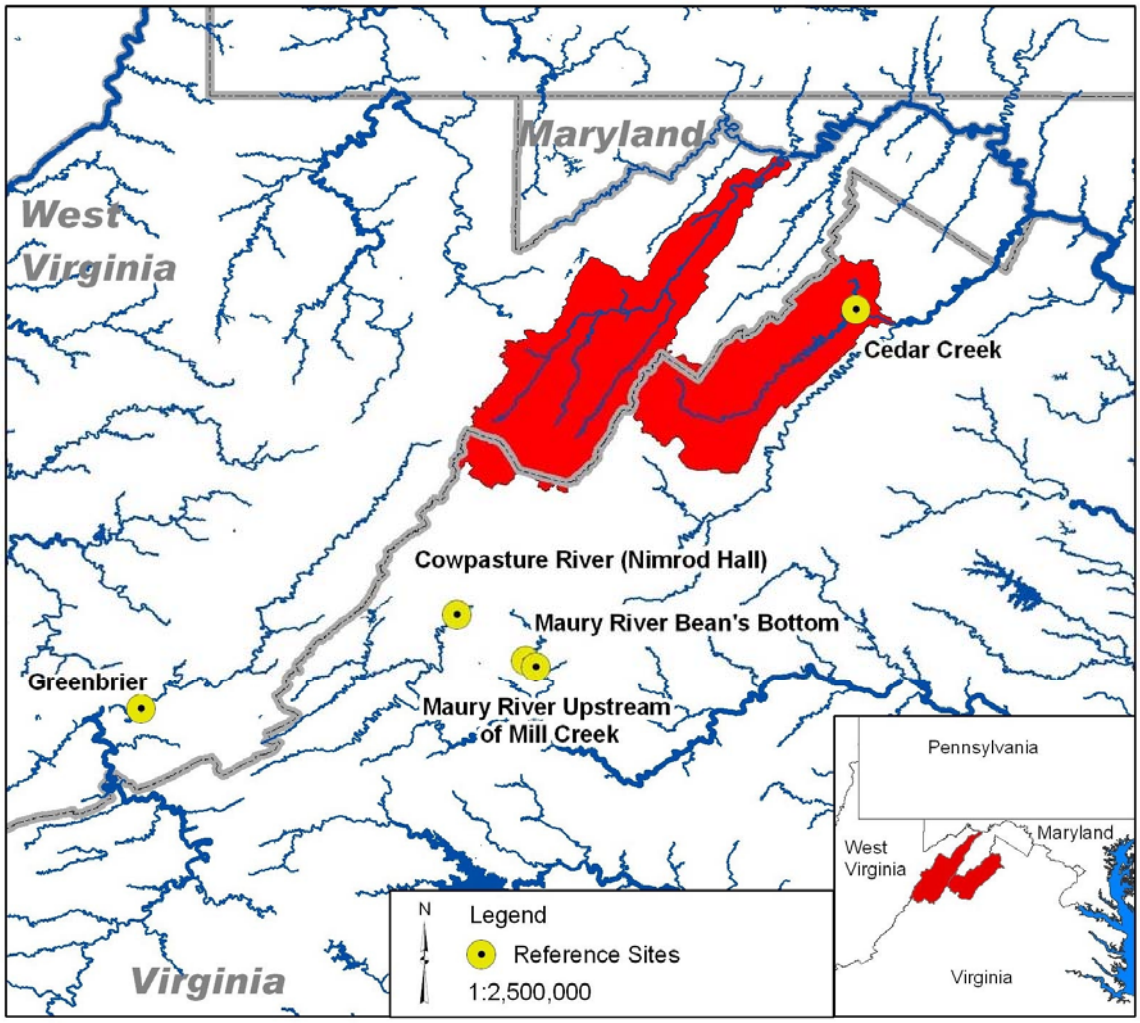

FIGURE 2

Locations of Reference Sites for Comparison with the River Reaches in which the 2006 Fish Kills Occurred. Red areas are the watersheds of the South Branch of the Potomac River and the North Fork of the Shenandoah River. For the South Branch, only the primary reference site on the Greenbrier River is shown.

In this case, it appeared that the affected fish underwent only an acute phase in which mortality was sudden. Fish were reported to be at the water's surface, gasping and behaving abnormally prior to dying. Upon examination, affected fish had gill hyperplasia (thickening of the gill filaments) that diminished the surface area for oxygen exchange.

Reference sites (no reported fish kills in spring 2006), both within basin and out of basin, were set up for comparison of fish health and water chemistry data (Figure 2). Continuous water quality monitors measure pH, dissolved oxygen (DO), conductivity, and temperature at six reference sites; Patterson Creek, North Fork of the South Branch, South Fork of the South Branch, North Branch of the Potomac, Opequon Creek and Cacapon River. With the exception of the North Branch and Opequon Creek, these sites are similarly dominated by agricultural impacts. The North Branch is a mining impacted watershed and Opequon Creek is influenced heavily by municipal wastes. The North Fork and South Fork monitoring sites are both upstream of the 2006 fish kills. Smallmouth bass were collected for intersex and relative health determinations on the Gauley River (at Camp Ceasar in Nicholas County), the Back Fork of the Elk River (above Webster Springs) and the West Fork of the Greenbrier River (above Durbin) in October of 2005. These three sites are considered as clean references due to their wilderness nature.

3. CANDIDATE CAUSE DEFINITIONS

Nine candidate causes have been proposed for the North Fork Shenandoah and South Branch Potomac fish kills. These candidate causes were chosen by participants in a workshop held at Cacapon, WV, in September 2006. At that workshop, which included stakeholders as well and academic, state and federal scientists, the available evidence was presented, causal hypotheses were discussed and conceptual models of the hypotheses were generated. All causal hypotheses that were still advocated by any participant at the end of that workshop were included as candidate causes in the January 2007 workshop.

The nine candidate causes include three different mechanisms for inducing anoxia. A fourth is mortality due to pathogenic or parasitic infections acting through mechanisms other than gill damage. These four candidate causes and the sources and pathways that result in exposure of fish to those candidate causes are illustrated in a single conceptual model (Figure 3). High pH levels, large pH fluctuations and high ammonia (NH_3) concentrations are other possible causes. Their sources and pathways are illustrated in Figure 4. Another candidate cause is unspecified toxic chemicals (Figure 5). The last candidate cause is starvation (Figure 6).

3.1. ANOXIA DUE TO LOW DISSOLVED OXYGEN

This candidate cause includes mortality from anoxia (extremely low blood oxygen concentrations) due to low aqueous DO concentrations. Mortality in fish may occur when DO levels fall below 4.0 mg/L. Low DO may result when nutrient enriched streams produce large amounts of aquatic plant biomass. When the biomass decomposes, bacteria consume available oxygen below levels required by fish for survival. Low DO may also occur when point or non-point source discharges to streams have a high biological or chemical oxygen demand. In addition, at night when photosynthesis stops but respiration continues, algae contribute to oxygen depletion.

3.2. ANOXIA DUE TO GILL INJURY

Injuries to gills may result in insufficient gas exchange and death due to anoxia even if aqueous DO concentrations are high. Injuries may result from mechanical abrasion, parasitic or microbial infection, hyperplasia (thickening of the gill epithelium)

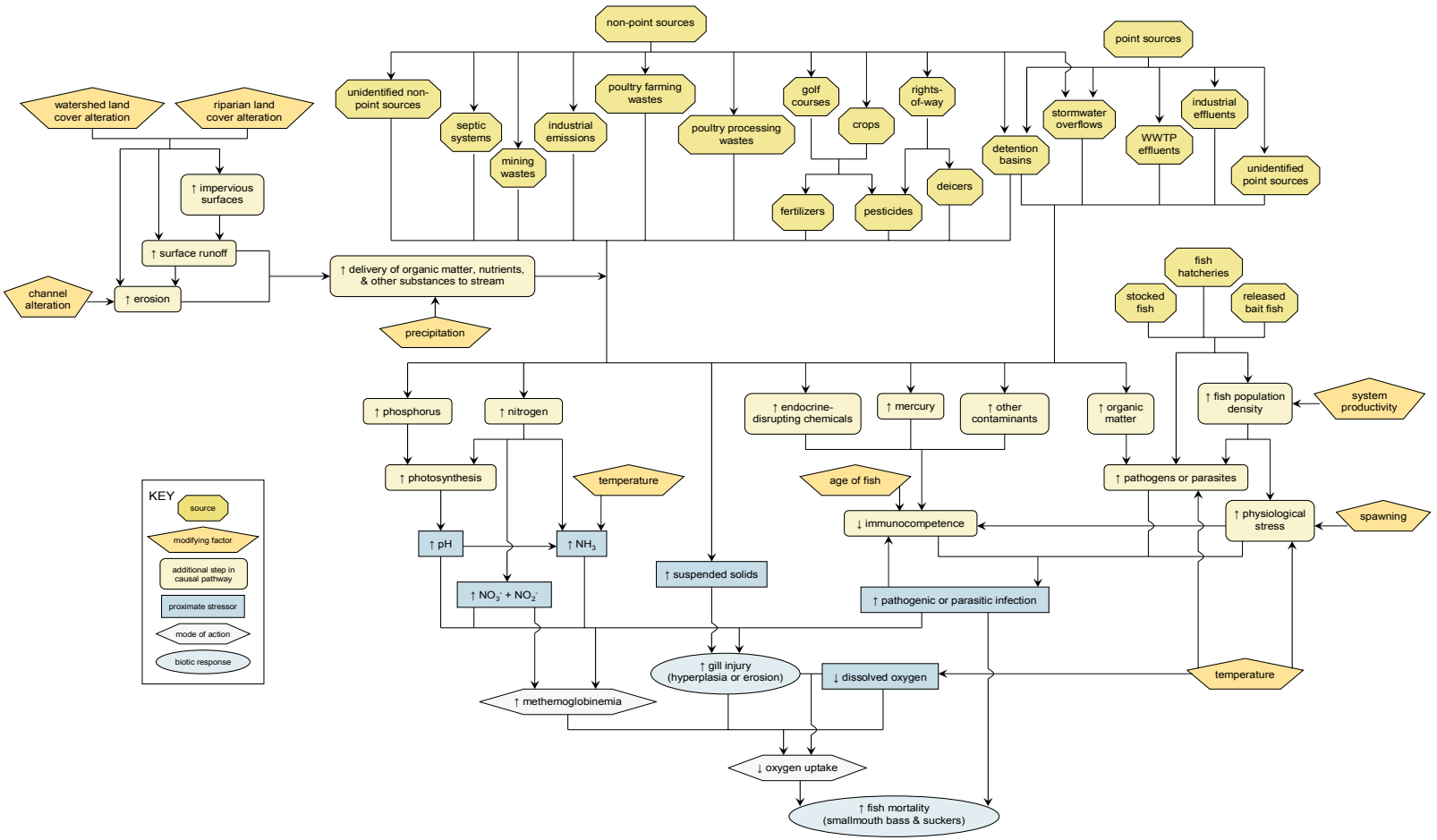

FIGURE 3

Conceptual Model of Candidate Causes 1-4, all of which Result in Fish Mortality due to Low Blood Oxygen Levels

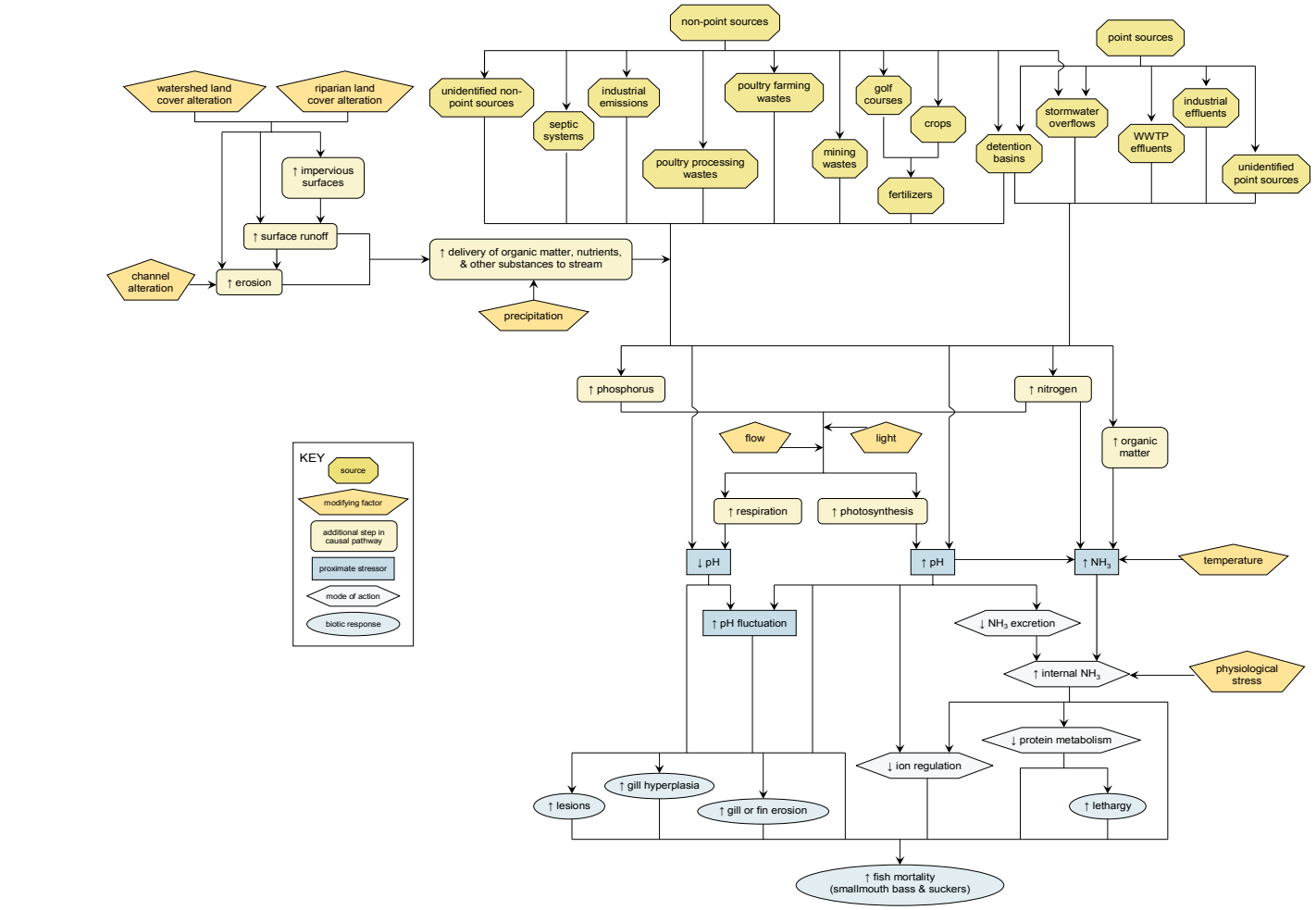

FIGURE 4

Conceptual Model of Fish Mortality due to Candidate Causes 5-7, High pH Fluctuations, High pH Levels and High NH_3 Levels

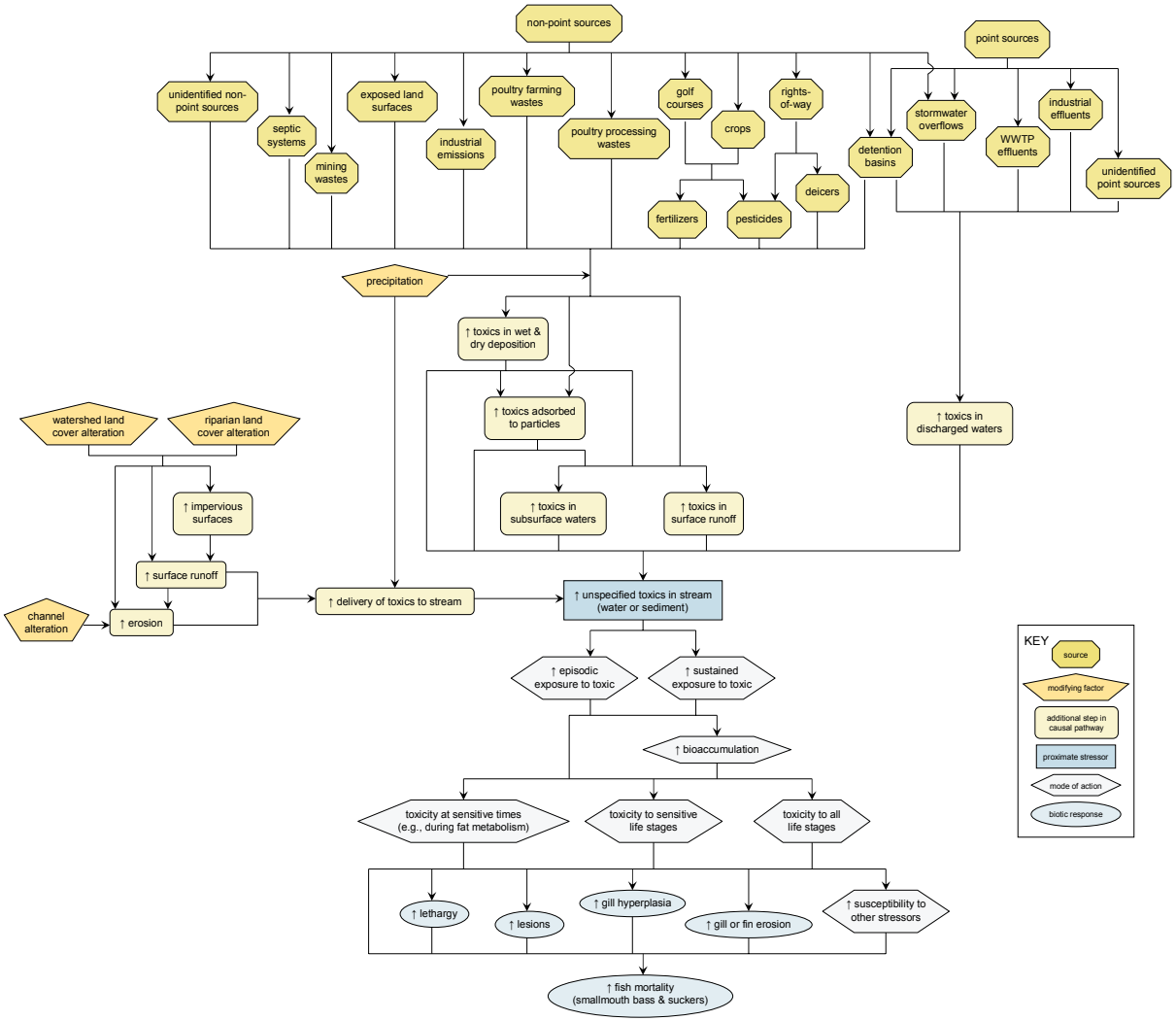

FIGURE 5

Conceptual Model of Candidate Cause 8, Unspecified Toxic Chemicals

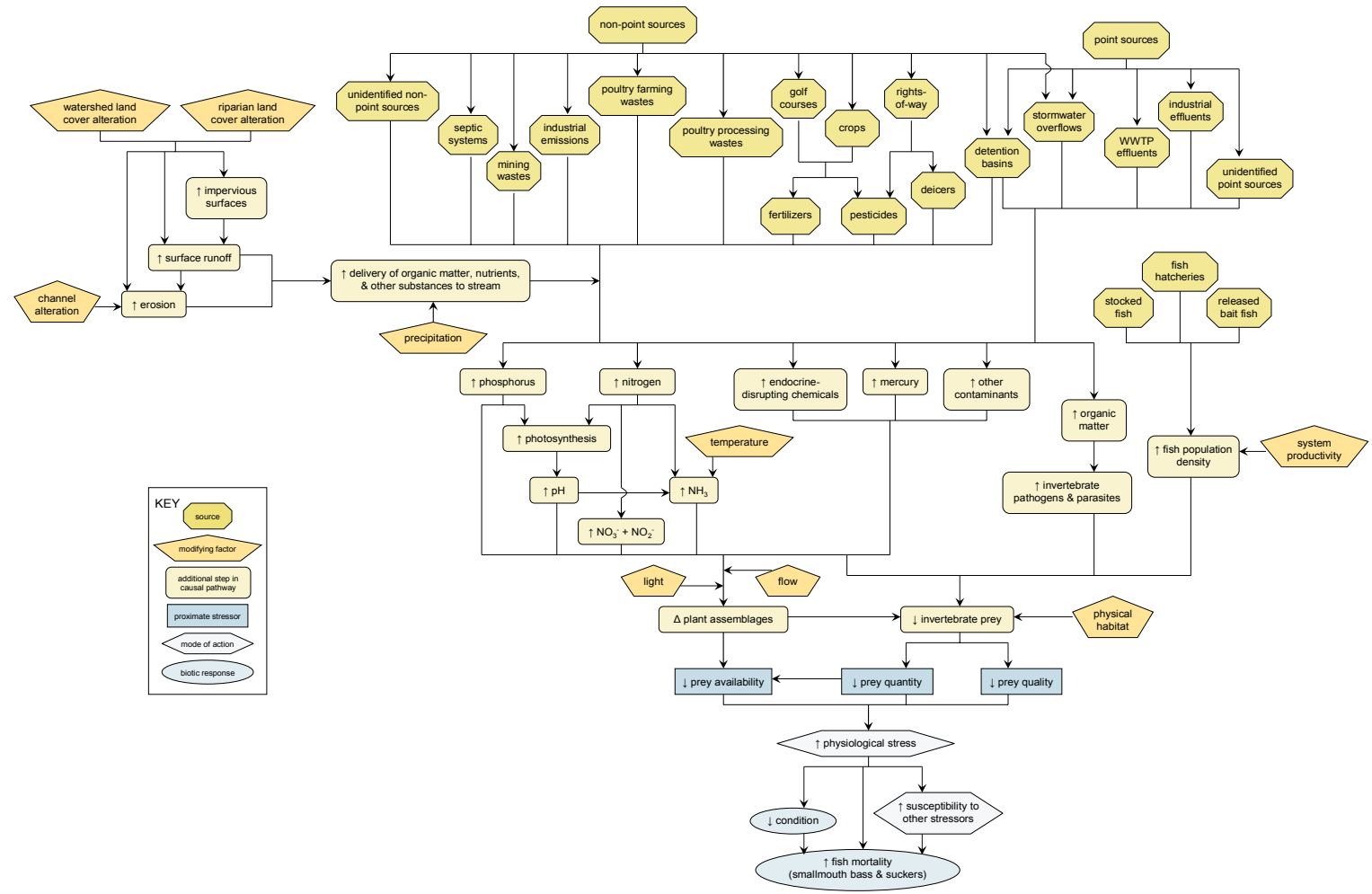

FIGURE 6

A Conceptual Model of Candidate Cause 9, Starvation

and effects of toxicity. Any injuries that substantially reduce the ability of the gill to extract oxygen from the water, through reductions in gill surface area or tissue permeability to oxygen, may result in mortality.

3.3. ANOXIA DUE TO LOW BLOOD OXYGEN AFFINITY

Oxygen is exchanged from the water through the gill and is transported to fish tissues via hemoglobin in red blood cells. Even if aqueous DO levels are high and gas exchange by the gills is unimpaired, death may result from reduced ability of the blood to carry oxygen. The most important cause is methemoglobinemia, a condition that occurs in the presence of high concentrations of nitrites, in which the oxygen carrying ferrous ion (Fe^{2+}) of the heme group of the hemoglobin molecule is oxidized to the ferric state (Fe^{3+}), converting hemoglobin to methemoglobin, a non-oxygen binding form of hemoglobin. High plasma pH may also result in reduced oxygen delivery to the tissues. Severe cases of either condition may result in mortality.

3.4. MORTALITY DUE TO OTHER PATHOGENIC MODES OF ACTION

Mortalities may result from bacterial, viral, parasitic or fungal infections whenever homeostatic functions in an organism are substantially compromised. In addition to impairing gill function (candidate cause 2), pathogens can cause death by tissue damage or wasting, septicemia, increased susceptibility to opportunistic secondary infections, or other mechanisms. Heavy body burdens of parasites increase stress on fish and render them more susceptible to infection and environmental perturbations, and may result in mortalities during energy-intensive activities such as spawning and nest building/ defense. Finally, some pathogens act by releasing toxins to the water.

3.5. MORTALITY DUE TO HIGH pH

High pH can directly kill fish by disrupting blood chemistry. Gill damage may reduce delivery of oxygen to tissues and can reduce the effectiveness of gill ion exchange and excretion. Compromised gill ion exchange can also potentiate the toxicity of NH_3 by reducing the ability of the fish to maintain sufficiently low plasma NH_3 concentrations. Elevated ambient pH may also cause increased plasma pH, denaturing proteins and contributing to tissue oxygen deficiency (Candidate Cause 3).

3.6. MORTALITY DUE TO pH FLUCTUATIONS

Sufficiently high pH can result in mortality, even if the duration at those levels is brief. Rivers frequently exhibit diel fluctuations resulting from photosynthesis. As plants photosynthesize during the day, they sequester dissolved carbon dioxide, resulting in elevated pH. However, at night photosynthesis ceases but plants continue to consume DO and release carbon dioxide, which lowers pH. Typical fluctuations during sunny days under low-flow conditions may cause pH to range from 8.0 to 9.3 or greater; in some instances hydronium ion concentration may vary by as much as a factor of 100. Extremely high concentrations may cause similar damages as observed for sustained high pH, and substantial fluctuation can challenge the fish's ability to maintain homeostasis.

3.7. MORTALITY DUE TO HIGH AMMONIA CONCENTRATIONS

High concentrations of aqueous NH_3 may kill fish, independently of their potential contributions to gill injury and anoxia (Candidate Cause 2). The toxicity of NH_3 is a function of the un-ionized concentration. The fraction of un-ionized NH_3 is determined primarily by the pH and temperature of the water. In general, the higher the pH, the greater the total fraction of un-ionized NH_3. Toxic concentrations of un-ionized NH_3 are temperature- and species-specific, and susceptibility of fish varies with age. Stress during the reproductive season or when coming out of winter or high blood ammonia due to protein metabolism may also lower fish tolerance to NH_3.

3.8. MORTALITY DUE TO UNSPECIFIED TOXIC CHEMICALS

The chemical composition of the rivers, particularly during the periods of the fish kills, is not well known. It is plausible that some episodic increase in exposure to an unknown chemical or combination of chemicals could have caused the kills. Both rivers receive input from point sources including WWTPs, industrial discharges and non-point sources including urban runoff. These sources release agricultural and residential nutrients, pesticides, herbicides and other chemicals. Atmospheric deposition may play a substantial role in toxicant inputs, such as in the case of mercury and acid rain. In sufficient concentrations, toxicants from any of these diverse sources may result in fish mortalities. Observed patterns of mortality will vary depending on the source of the input.

3.9. MORTALITY DUE TO STARVATION

Starvation may occur due to lack of food, inability of the fish to capture or ingest food or inability of the fish to assimilate nutrition from ingested materials. In general, health indices such as length to weight ratios give an acceptable indication of whether starvation is occurring.

4. IDENTIFICATION OF PROBABLE CAUSES

Because the field and laboratory data sheets for fish collected during both kills were not available at the time of the workshop in January 2007, it was not possible to identify the probable cause of either case. The consistency of the available evidence and the information gaps can be identified by examining scoring Tables 1 and 2.

For each candidate cause, those of us attending the workshop analyzed the data that were available data to produce evidence which was evaluated in terms a system of scores applied to each type of evidence. Details of the analysis of evidence and scoring can be found in Appendix A.

The following scores were applied to the evidence.

+++	Convincingly supports
---	Convincingly weakens
++	Strongly supports
--	Strongly weakens
+	Somewhat supports
-	Somewhat weakens
0	Ambiguous
NE	No Evidence
P	Pending evidence
R	Refutes.

TABLE 1

Summary of Evidence Concerning the 2006 Fish Kill in the North Fork of the Shenandoah River

South Branch	Anoxia due to Low DO		Anoxia due to Gill Injury or Hyperplasia		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Evidence that uses data from the case																		
Spatial/ Temporal Co-occurrence	DO was not low during the kill	R	Pathology data are missing.	NE(P)	Pathol-ogy data are missing.	-/ NE(P)	Pathol-ogy data nega-tive or missing.	-/ NE(P)	Kill did not co-occur with highest fluctu-ations	- - -	Kill did not co-occur with high pH	- - -	NH_3 not elevated at time of kill	- - -	Little analy-sis of toxics	NE	Dead fish were not notice-ably starved	---
Evidence of Exposure or Biological Mechanism			Charac-teristic behaviors but multiple causes	0	Charac-teristic behavior but multiple causes	0	Behavior undiag-nostic;	0		NE		NE		NE	No useful tissue analyse s	NE	No stomach content data	NE
Causal Pathway			pH 0; NH_3 0; Temp +; Autointox cycle +; nitrate -; nitrite -; TSS -; conduct -; seasonal stress +; behavioral stress +; flow -; diatoms -	+	Sources possible, nitrite unknown, other mecha-nisms possible	0	Some possible sources of patho-gens	+	Potential sources are present	+	Poten-tial sources are present	+	Poten-tial sources are present	+	Poten-tial sources are present	+	No food data	NE
Laboratory Tests of Site Media															Tests of post-kill water	NE		
Verified Predictions																		

1

TABLE 1 cont.

South Branch	Anoxia due to Low DO		Anoxia due to Gill Injury or Hyperplasia		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Symptoms			Possibly consistent, data are missing.	+(P)	Pathol-ogy data are missing.	NE(P)	Pathol-ogy data are missing.	NE(P)	Pathol-ogy data are missing.	NE(P)	Pathol-ogy data are missing.	NE(P)	Pathol-ogy data are missing.	NE(P)	Pathol-ogy data are missing.	NE(P)	No pathology data	NE
Evidence that uses data from elsewhere																		
Stressor-Response Relationships from other Field Studies									Similar fluctuatio ns non-lethal	-	Compari son to previous year	0	Local ref. sites are not elevated	-				
Stressor-Response Relationships from Laboratory Studies			Some evidence suggestive but more analysis needed	+(P)	Cen-trarchids are resistant	---					pH not at lethal levels during kills	-	NH_3 levels too low	--				
Analogous Cases			2002 kill may be analogous but data anecdotal or unavailable	+							2005 kill may be analogo us	0						
Considerations Based on Multiple Lines of Evidence																		
Consistency	Co-occur-rence alone refutes	NA	Consistent but few and weak lines of evidence	+	Inconsiste ncies	-	Few, weak and inconsist ent data	-	Negative except for sources	-	Lack of co-occur-rence	-	Evidence consist-ently negative, but some ambiguity	---	Evi-dence too meager	0	Only evidence is negative	-

TABLE 1 cont.																		
South Branch	Anoxia due to Low DO		Anoxia due to Gill Injury or Hyperplasia		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Reasonable Explanation of the Evidence					None	0	None	0	Sources are weak evidence	+	Sources are weak evi-dence	+						

1

TABLE 2

Summary of Evidence Concerning the 2006 Fish Kill in the South Branch of the Potomac River

North Fork	Anoxia due to Low DO		Anoxia due to Gill Injury		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Evidence that uses data from the case																		
Spatial/ Temporal Co-occurrence	DO was not low during the kill	R	Pathology data are missing	NE(P)	Brown gills were not observed	-/NE(P)	Pathol-ogy data are missing	-/NE(P)	pH fluctua-tions high before and after kill	- - -	pH was declining at time of kill	- - -	Peak in early March, but not during rest of kill	- - -		NE	Dead fish were not starved	---/R(P)
Evidence of Exposure or Biological Mechanism			Ambiguous behavior	0	Am-biguous behavior	0	Am-biguous behavior, Pathol-ogy data are missing	NE(P)	Pathol-ogy data are missing	NE(P)	Pathol-ogy data are missing	NE(P)	Pathology data are missing	NE(P)	Pathol-ogy data are missing	NE(P)		NE

1

TABLE 2 cont.

North Fork	Anoxia due to Low DO		Anoxia due to Gill Injury		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Causal Pathway			H2O pH +; pH fluctuations +; H2O NH_3 -; H2O temp +; autointox cycle +; nitrate -; nitrite +; TSS -; conduct -; spawning/ prespawning -; seasonal hab changes -; flow +; planktonic diatoms 0; periphytic diatoms -; wash of dead diatoms -	+	Sources of precusors are known but not the pathway	0	Sources of path-ogens were present	+	Potential sources were present	+	Potential sources were present	+	Potential sources were present	+	Poten-tial sources were present	+	Food avail-ability unknow n	NE
Laboratory Tests of Site Media															Tests of water after the kill	NE		
Temporal Sequence																		
Verified Predictions																		
Symptoms			Pathology data are missing	NE(P)	Patholog y data are missing	NE(P)	Pathol-ogy data are missing	NE(P)	Patholog y data are missing	NE(P)	Patholog y data are missing	NE(P)	Pathology data are missing	NE(P)	Pathol-ogy data are missing	NE(P)	Pathol-ogy data are missing	NE(P)

TABLE 2 cont.

North Fork	Anoxia due to Low DO		Anoxia due to Gill Injury		Anoxia due to Low Blood Oxygen Affinity or Methemoglo-binemia		Mortality due to Other Pathogenic Modes		Toxicity from pH Fluctuations		Toxicity from High pH		Toxicity from NH_3 (in the environment)		Unspecified Toxic Substances		Starvation from Low Food Resources	
Type of Evidence	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score	Notes	Score
Evidence that uses data from elsewhere																		
Stressor-Response Relationships from other Field Studies									Similar fluturations non-lethal	-			Similar NH_3 did not cause kills	-	No relevant analy-ses	NE		
Stressor-Response Relationships from Laboratory Studies			Some evidence suggestive but more analysis needed	+(P)	Ambient concentrations are too low	- -		NE	No relevant lab studies found	NE	Field pH non-lethal in lab but other species	-	NH_3 levels too low	--				
Analogous Cases			2002 kill is analogous but data were unavailable	NE	No data from prior kills	NE	Parasites ob-served in 2002 kill	+	Data from prior kills lacking	NE	Data from prior kills lacking	NE	Data from prior kills lacking	NE	Data from prior kills lacking	NE	Data from prior kills lacking	NE
Considerations Based on Multiple Lines of Evidence																		
Consistency	Only co-occur-rence is need-ed	NA	Ambiguous	0	Inconsis-tent and weak	-/0	Positive but weak	+	Few but consis-tent	-	Few but consis-tent	-	Consis-tent and negative	--	Evi-dence too meager	NE	All data weaken, but meager	--
Reasonable Explanation of the Evidence					Nitrite elevated but not enough	-			Sources are not sufficient	-	Sources are not sufficient	-	Sources are not sufficient	-				

1

The workshop participants reached the following conclusions.

- Anoxia as a result of low DO was not a possible cause in either case (Sections A.1.1 and A.2.1).
- Starvation was highly unlikely in both cases because the fish did not appear starved and the condition of surviving fish was similar to that of fish at reference locations (Sections A.1.9 and A.2.9).
- Elevated NH_3 and pH levels and pH fluctuations also appear not to be causes of either kill. Levels do not appear to have been elevated at the time of either kill relative to times when no kills occurred, and they do not appear to have been sufficiently elevated to cause the kills. However, they may have contributed to the susceptibility of fish to other agents.
- The evidence for anoxia due to low blood oxygen affinity or methemoglobinemia was strongly negative for the Potomac because measured nitrite concentrations were low. Evidence was strongly negative for the Shenandoah because the species that were killed are resistant to that candidate cause.
- Data were insufficient to evaluate unspecified pathogens or toxic chemicals as causes. Either one is plausible but both are largely unsupported.
- Anoxia due to gill injury is supported by anecdotal reports of apparently injured gills and by the occurrence of agents that could cause gill injury (although not necessarily at sufficient levels). Hence, the evidence is weak and it is not clear which of the causal pathways would be responsible.

In summary, of the 9 candidate causes, 6 are unsupported or unlikely given the evidence. Anoxia due to gill injury, pathogens and toxins remain as candidate causes. Some additional analysis of data from these two cases and of data from other laboratory and field studies could improve the causal analysis. However, the greatest improvement would come from high quality field and laboratory pathology data. Data gaps identified during the workshop are summarized in the following section and listed in Appendix B.

5. DATA NEEDS

Workshop participants identified areas where additional data would reduce uncertainty and provide additional types of evidence. In general, taking measurements and collecting samples at the same time and place of the observed effect and at least one matched reference site was the most important aspect of sampling. Appendix B gives the outcome of this brainstorming session, which outlines data needs in three lists. The first list is of data needs by general categories of information. The second list provides specific needs for two of the candidate causes. The third list gives the highest priority needs as identified by individual workshop participants.

The data gap receiving the most attention at the workshop was a baseline of fish health; that is, what does a healthy fish in these rivers look like? When we expand this question by considering the number of species involved—smallmouth bass, redbreast sunfish, rockbass, golden redhorse and northern hogsucker—combined with the large numbers of parameters that could be measured, the question gets very complex. However, Appendix B represents a good starting point for data needs that should be re-visited and revised as needs arise and are filled.

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APPENDIX A
EVALUATION OF DATA

This appendix presents the evidence that was available at the workshop for each candidate cause with respect to the North Fork Shenandoah case and then the South Branch Potomac case. For each candidate cause, the evidence is analyzed in terms of the types of evidence for which data were available. Data that were not available but may exist are noted.

A.1. CASE 1—2006 LOWER NORTH FORK SHENANDOAH RIVER, VA

A.1.1. Anoxia due to Low Dissolved Oxygen.

A.1.1.1. Spatial/Temporal Co-occurrence —

Data Used Data from USGS continuous YSI DO monitors are not available for the fish mortality event due to instrument failure.
There are temporal data from a reference site on the South Fork Shenandoah River.
Ad hoc measurements were taken during the kill.

Analysis Co-occurrence inferred from observations of dying fish and concurrent ad hoc DO measurements of 8 mg/L by Steve Reeser.

Discussion The co-occurrence of dying fish and normal DO levels refutes the possibility that low DO caused anoxia. Mortality from low DO is rapid. Therefore, if DO concentrations are high but fish are still dying, low DO cannot be the cause.

Score R, These data are sufficient to refute low dissolved oxygen as the cause of the kill.

A.1.2. Anoxia due to Gill Injury.

A.1.2.1. Spatial/Temporal Co-occurrence —

Data Used Gross and histopathology samples and field data from the kill and from reference sites on the South Fork Shenandoah and Cowpasture Rivers were taken. However, those data were not provided for this analysis.

Analysis None

Discussion The USGS verbally reported gill hyperplasia but data are unavailable.

Score NE (P) No evidence is available from the pathology reports, which are pending.

A.1.2.2. Evidence of Exposure or Biological Mechanism —

Data Used Observations of affected fish reported as lethargy and decreased flight behavior response, but documentation was unavailable.

Analysis None

Discussion Lethargy and decreased flight response are common to many causes of death and, therefore, are diagnostically non-specific.

Score 0 Ambiguous because the evidence also applies to other causes.

A.1.2.3. Causal Pathway —

Data Used Water pH (including pH fluctuations), water temperature, water NH_3 levels, water nitrite/nitrate levels and total suspended solids data.
Pathogen and parasite data may become available from samples sent to the pathology lab.

Analysis Analyses of pH and NH_3 data are presented in Sections A.1.5 through A.1.7. Analyses of pathogen occurrence await the data sheets.

Discussion Gill injury could have been caused by several causal pathways. Evidence for occurrence of a causal pathway consists of the occurrence of an agent in a pathway leading to the proximate cause. Some of the agents in the pathway to this candidate cause are also candidate causes themselves (i.e., pH, NH_3 and pathogens). However, the evidential requirements here are different. Elevated concentrations of an agent that

is a proximate cause must occur at the time of a kill, but agents in a causal pathway may have been elevated in the past. It is necessary only that their effects continue.

pH - Data showed wide pH fluctuations on a daily basis, pH levels rose to 9.5. Although large pH fluctuations did not consistently co-occur with the kill (Section A.1.6), they may have contributed to gill injury and stress to the fish through alkalosis or ammonia autointoxication.

NH_3 - It was not elevated at the time of the kill (Section A.1.7), but it could have contributed to the susceptibility of fish to another proximate cause of gill injury and anoxia. Toxic gill necrosis can result from autointoxication (metabolism of proteins in excess of excretory capacity) which may be triggered by starvation, a sudden drop in temperature, an increase in pH above 9, an increase in aqueous ammonia, or an increase in protein in the diet (Smutna et al., 2002). Of these, only elevated pH was observed (see above).

Nitrate and Nitrite - Data were unavailable, but may be elevated by nitrification of NH_3.

Temperature - Water temperature rose at the time of the kill, which increases biological activity, including oxygen requirements and toxic responses.

Auto-intoxication - The elevated NH_3, pH and temperature suggest that conditions could have existed for an NH_3 auto intoxication cycle to be initiated internally in the fish.

Conductance - Conductance was not elevated (USGS provisional data, Strasburg gage, 2006).

Pathogens - Data were available from analyses of bacterial and protist communities in mucus from fish with and without lesions collected during the mortality event (Gillevet et al., 2006). Parasite levels were potentially increased, but pathology data were unavailable.

General stress - The kill did not correspond to any particular phase in the spawning cycle. Seasonal behavior and habitat changes were noted that year; the fish did not make typical habitat selection and movements apparently because of unusually low water flow.

Abrasion - Concentrations of total suspended solids were not high. Periphytic, but not planktonic, diatoms were quite heavy resulting in observations of “white rocks,” but

there were no high flows at the time of the kill to wash off diatom frustules and cause abrasion.

Score Water pH 0
Water NH_3 0
Water Nitrate & Nitrite NE
Water Temperature +
Auto intoxication cycle +
Conductance -
Stress, Spawning Season -
Stress, Season behavior and habitat changes +
Abrasion, Total Suspended Solids -
Abrasion, Flow -
Abrasion, Planktonic diatoms -
Abrasion, Wash off of dead periphytic diatoms -

A.1.2.4. Stressor-Response from Laboratory Studies —

Data Used Toxicologic benchmarks from laboratory tests of fish for pH, NH_3 and temperature.

pH

pH 9 causes carp mortality (Schaperclause, 1952)

When water pH > blood pH, NH_3 excretion is reduced; above pH 9.5 it is blocked (Schaperclause, 1952; Wood, 2001).

Other effects of high pH at the gills include increased CO_2 excretion leading to alkalosis and reduced uptake of sodium (Na) and chloride (Cl) ions (Wood, 2001).

Reported gill injuries were limited to hyperplasia of the chloride cells, not gross hyperplasia (Wood, 2001).

Temperature

Rapid changes in temperature can increase morbidity and mortality in fish (Stoskopf, 1993).

NH_3

0.2 mg/L caused gill injury in brown trout (U.S. EPA, 1999).

Gill lamellae obtained from parental fish exposed to un-ionized NH_3 concentrations ranging from 0.02 mg NH_3-N/L to 0.05 mg NH_3-N/L for 4 months, and 0.05 mg NH_3-N/L and 0.06 mg NH_3-N/L for 7 and 11 months, showed mild to moderate

fusion, aneurysms and separation of the epithelia from the underlying basement membrane (U.S. EPA, 1999).

"In contrast to acute exposures, a variety of morphologic changes in the gills have been described during chronic sublethal ammonia exposure. Most prominent are an overall swelling of the respiratory lamellae, proliferation of epithelial cells, increased diffusion distance and an increased prevalence of bacterial gill disease… These responses would be expected to decrease the respiratory gas exchange capacity of the fish, and thus its swimming performance and tolerance to hypoxia" (Wood, 2001).

Smallmouth bass are fairly sensitive. The growth effect concentrations ranged from 0.05 mg NH_3-N/L at pH=6.6 to 0.71 mg NH_3-N/L at pH=8.68 (U.S. EPA, 1999, pg 118).

Redhorse sensitivities are unknown

As fish emerge from torpor in the spring, toxic NH_3 concentrations drop from 1.7 to 0.2 mg/L (Schaperclause, 1952).

0.5 mg/L total NH_3 highest observed in spring.

Nitrite and Nitrate

Chronic exposures to nitrite can cause gill hyperplasia (Kroupova et al., 2005).

Analysis Data were not available at the workshop to evaluate the potential for observed levels of stressors to injure gills. Benchmark values should be compared to concentrations measured at the time of the kill and shortly before. Also differences in sensitivity in the laboratory should be compared to apparent differences in response among species during the kill.

Discussion NA.

Score +, (P) Some evidence supports the candidate cause but more is pending

A.1.2.5. Analogous Cases —

Data Used Reports of state biologists.
Water quality data are potentially available from prior kill events.

Analysis Comparison of potentially analogous kills

Discussion Two similar kills of smallmouth bass have occurred in the Shenandoah River previously:

North Fork 2004 spring mortality event
South Fork 2005 spring mortality event

These kills differ from the 2006 kill in that they occurred directly after a major run-off event while the 2006 kill did not. The 2004 and 2005 kills were more widespread. Also, the water was extremely warmed in 2004 and 2005, but the warming was less in 2006. In 2005, the smell of NH_3 from a terrestrial source was quite strong. Also in 2005, white suckers displayed gill hyperplasia, trematodes and parasites and skin lesions. Some water chemistry data and water temperatures are potentially available for those kills. Analysis of these data may provide information relevant to the 2006 kill.

Score +, Reports of gill injury in 2005 weakly support the candidate cause.

A.1.3. Anoxia due to Low Blood Oxygen Affinity—(Methemoglobinemia)

A.1.3.1. Spatial/Temporal Co-occurrence —

Data Used Plasma samples and data from the fish mortality event and reference sites on the South Fork Shenandoah and Cowpasture Rivers are potentially available. Observations of blood by State biologists recounted at the workshop.

Analysis Pending

Discussion Ideally, co-occurrence would be established by signs of methemoglobinemia in fish from the kill but not elsewhere. State biologists observed

neither brown gills nor blood in these fish. High pH can also cause low blood affinity without brown blood. Data from samples sent to the pathology lab were not available.

Score -/NE (P) Field observations were negative but more definitive data are needed from the pathology reports.

A.1.3.2. Evidence of Exposure or Biological Mechanism —

Data Used See A.1.2.2.

Analysis See A.1.2.2.

Discussion See A.1.2.2, behavioral evidence is consistent with many causes.

Score 0 Ambiguous

A.1.3.3. Causal Pathway —

Data Used Knowledge of sources and processes in the watershed.

Analysis None.

Discussion Nitrite accumulates in water when NH_3 concentrations are elevated and the second stage of nitrification is inhibited. Sources of NH_3 are present in the watershed, but nitrification rates or processes controlling the rates are unknown. Nitrite concentrations were not available at the workshop.

Score 0 Ambiguous because sources are known but the conversion processes are not.

A.1.3.4. Stressor-Response from Laboratory Studies —

Data Used Literature reviews.

Centrarchids (includes sunfish and black bass) are refractory to methemoglobinemia (Kahn, 2005).

Methemoglobinemia symptoms occur at 0.10 to 0.50 mg/L in sensitive species (channel catfish and trout) and LC50 values range from 0.60 to 200 mg/L (Animal Disease Diagnostic Laboratory, 1998).

Analysis Logic and comparison to ambient concentrations.

Discussion If methemoglobinemia was the cause of the kill, Centrarchids would have been among the last rather than the first to die.

Score - - This evidence greatly weakens methemoglobinemia as a candidate cause.

A.1.4. Mortality due to Other Pathogenic Modes of Action.

A.1.4.1. Spatial/Temporal Co-occurrence —

Data Used Gross and histopathology samples and field data from the mortality event and from reference sites on the South Fork Shenandoah and Cowpasture River were taken, but the data were not available for this analysis.

Data were available from analyses of bacterial and protest communities in livers from fish with and without lesions collected during the mortality event (Gillevet et al., 2006).

Analysis Pending for pathology data.

Authors' reported results for Gillevet et al. (2006).

Discussion The USGS verbally reported occurrences of parasites at the September preliminary workshop, but data are unavailable. Gillevet et al. (2006) reported no differences in bacteria and protists from livers.

Score -/NE(P) Results from Gillevet et al. were negative but not definitive. The pathology reports are necessary to confirm or refute this candidate cause.

A.1.4.2. Evidence of Exposure or Biological Mechanism —

Data Used Observational data on affected fish demonstrating lethargy and decreased flight response.

Analysis Minimal analysis due to non-specific observational nature of data.

Discussion Observational data in this case are diagnostically non-specific.

Score 0 Ambiguous, because the behavios have multiple causes.

A.1.4.3. Complete Exposure Pathway —

Data Used Knowledge of State biologists.

Analysis None.

Discussion Sources of pathogens may include stocked fish, released bait fish or effluents from hatcheries. Trout but not smallmouth bass are stocked in the Shenandoah. Bait minnows inevitably are released. Hatcheries occur at and below the kill site.

Score + Somewhat supports because some steps are present.

A.1.4.4. Analogous Cases —

Data Used Reports of State biologists.

Analysis None.

Discussion In the 2005 Shenandoah River fish kill event (Section A.1.2.5), white suckers displayed trematodes and other parasites and skin lesions.

Score + Reports of parasites in 2005 somewhat support the candidate cause.

A.1.5. Mortality due to High pH.

A.1.5.1. Spatial/Temporal Co-occurrence —

Data Used Continuously monitored pH data from 2005-2006 on the North Fork of the Shenandoah at Woodstock and Strasburg sites collected by the USGS, Ken Hyer (kenhyer@usgs.gov).

Analysis Daily maxima were plotted with respect to time and the period of the kill.

Plot of pH over time with respect to the interval of the kill.

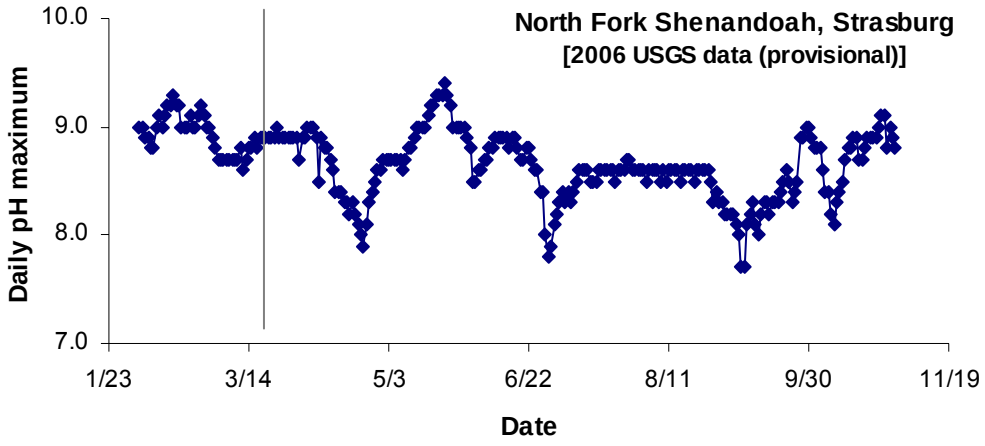

FIGURE A-1

The daily maximum pH values in the North Fork Shenandoah River at Strasburg. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

Discussion pH values were high (above 9) beginning as early as February, however, there was no kill at this time (Figure A-1). The kill did not begin until mid March, when pH values were actually dropping. The highest pH of the recorded period came in middle to late May, which was still during the timeframe of the kill, but by this point, fish had been dying for 2 months, so this elevation alone wouldn't have been the cause of the kill. All this evidence demonstrates a lack of temporal alignment of peaks in pH and the onset of the fish kill in the North Fork.

Score --- The effect both does not occur when the candidate cause occurs and does occur when the candidate cause does not occur.

A.1.5.2. Complete Exposure Pathway —

Data Used Observations of State biologists.

Analysis None.

Discussion High pH in the Shenandoah results from the karst geology of the valley and, during the day, from high plant production.

Score + Somewhat supports because sources are present.

A.1.5.3. Stressor-Response Relationship in the Lab —

Data Used Scott et al. (2005) addresses effects of pH as high as 9.5 on behavior of rainbow trout and perch.

An article by Serafy and Harrell (1993) addressing sublethal effects of high pH on bluegill, striped bass and killifish.

Analysis Maximum pH values during the kill were compared with those causing effects in these studies.

Discussion The pH maximum during the study by Scott et al. (2005) was as high as 9.5, somewhat higher than observed on the North Fork in 2006 during the kill. Since fish exposed to that pH were shown to have reduced ability to excrete ammonia (as measured in the fish's blood), but exhibited no lethality or change in swimming behavior, the fish in this case should not have been dying due to similarly high pH levels alone. Note that this experiment was carried out on different species.

The fish in the Serafy and Harrell (1993) experiment were subjected to pH increases of about 1 unit over the course of less than 1 hour, with the highest replicate reaching a

final pH of about 9.5. Because the exposures are so short in duration, the comparability with our case is severely reduced. Also, while these results may be useful for assessing fish stress response, they are not very useful for assessing lethality since none of the fish died.

Score - The fish exposed to pH levels similar to those found in our case showed reduced ammonia excretion capabilities, but did not die.

A.1.5.3. Stressor-Response Relationship in the Field —

Data Used Continuous pH data from 2005-2006 on the North Fork of the Shenandoah at Woodstock and Strasburg sites collected by the USGS, Ken Hyer (kenhyer@usgs.gov).

Analysis Maximum pH values from 2005 of 8.9 at Strasburg and 8.3 at Woodstock were selected from this period and compared to maximum values during the case.

Discussion We compared the pH maximum during the analogous kill period from 2005 to the maximum during the kill of 2006. Because there were reports of lesions from the North Fork in 2005 but very few kills, we might consider this a non-kill year. However, the status of the 2005 scenario as a kill is confounded by the fact that there was a large kill on the North Fork in 2004, and Virginia biologists hypothesize that the lack of a large-scale kill in 2005 may simply have been a result of overall lower numbers of fish and thus lower numbers available to die.

Score 0 The ambiguity of 2005 as a kill/non-kill year prevents us from drawing any conclusions from the comparison of maximum pH levels.

A.1.6. Mortality due to pH Fluctuations.

A.1.6.1. Spatial/Temporal Co-occurrence —

Data Used Continuous pH data from 2005-2006 on the North Fork of the Shenandoah at Woodstock and Strasburg sites collected by the USGS, Ken Hyer (kenhyer@usgs.gov).

Analysis Calculated pH shifts over 24 hour windows were plotted against the date and with respect to the kill interval.

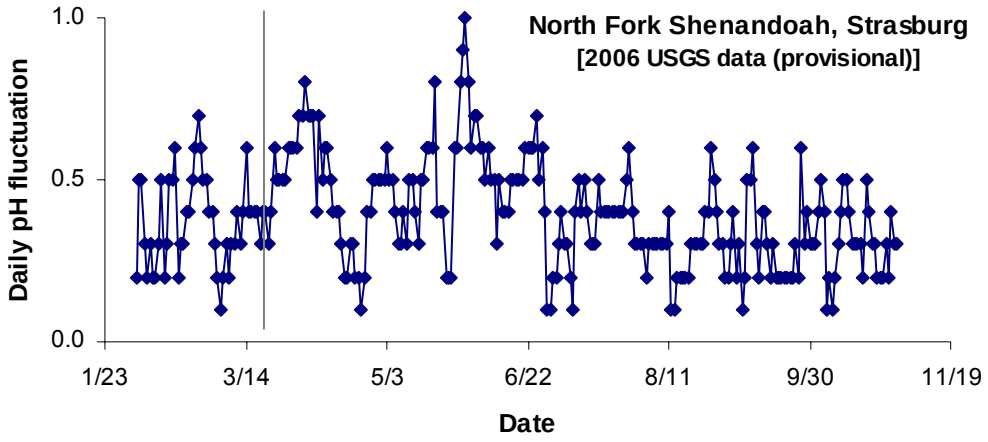

FIGURE A-2

The Daily Difference Between Maximum and Minimum pH Values in the North Fork Shenandoah River at Strasburg. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

Discussion pH fluctuations were high (0.6-0.8 units) before, during and after the kill, with the highest fluctuations coming at the end of the kill (Figure A-2). pH fluctuations dropped to a low of only 0.1-0.2 units in April/May and the kill did not cease. Also, since data begins only in February and the kill started in March, there are few preceding data to establish a trend. Though there may be some interaction/combination of temperature and pH effects on the fish, when considering pH fluctuations alone, there is not significant evidence of temporal co-occurrence to support pH fluctuations as a candidate cause.

Score --- The effect both does not occur when the candidate cause occurs and does occur when the candidate cause does not occur.

A.1.6.2. Complete Exposure Pathway —

Data Used Knowledge of State biologists.

Analysis None.

Discussion The source of high pH fluctuations is the high primary production during the day, which raises pH. High production is in turn due to high nutrient levels.

Score + Somewhat supports because some steps are present.

A.1.6.3. Stressor-Response Relationship in the Field —

Data Used Serafy and Harrell (1993) addressed sublethal effects of pH fluctuations on natural fish communities including pumpkinseed, eel, and killifish.

Analysis We compared fish behavior in our case with that observed in the field experiment under similar pH conditions.

Discussion The fish communities in this experiment were monitored during a natural shift in pH through the course of a day while pH was monitored. The observers noted shifts in community density, biomass and species richness. None of these changed drastically through the course of the pH shift. pH was measured as varying from ~8.5 up to a maximum of ~9.8 and then down to a minimum of ~7.6. The pH regime in this experiment is probably the most relevant external scenario to ours because of the magnitude of pH shift and the high average pH around which the pH was centered. On the other hand, the other environmental/ecological factors in this setup decreased comparability between the experimental scenario and ours on the North Fork. The study was conducted in a tidal freshwater environment of the Chesapeake Bay with different fish species. We question whether, if this pH regime is natural and common for

that area, then are the native fish more accustomed and better adapted to these conditions? Also, the observers noted whether or not fish fled the study site, which was a hydrilla grass bed, and interpreted this as a potential reaction to undesirable pH levels. But if pH remained the same at distances further from the grass bed, we shouldn't expect fleeing the grass bed to be of any benefit, so perhaps fleeing is an inappropriate endpoint of assessment. Also, parameters other than pH that weren't measured may have influenced the fishes' behavior.

Score The experiment somewhat weakens this candidate cause by showing that pHs fluctuations of over 2 units centered around a pH of ~8.5, similar to the fluctuations measured in our case, are not lethal to fish. However, the key differences of conditions in each scenario reduce their comparability.

A.1.6.4. Stressor-Response Relationship in the Field —

Data Used Continuous pH data from January-October 2006 on the Rappahannock River at gage #1668000 collected by the USGS, Ken Hyer (kenhyer@usgs.gov).

Analysis Calculated daily pH shift plotted against time.

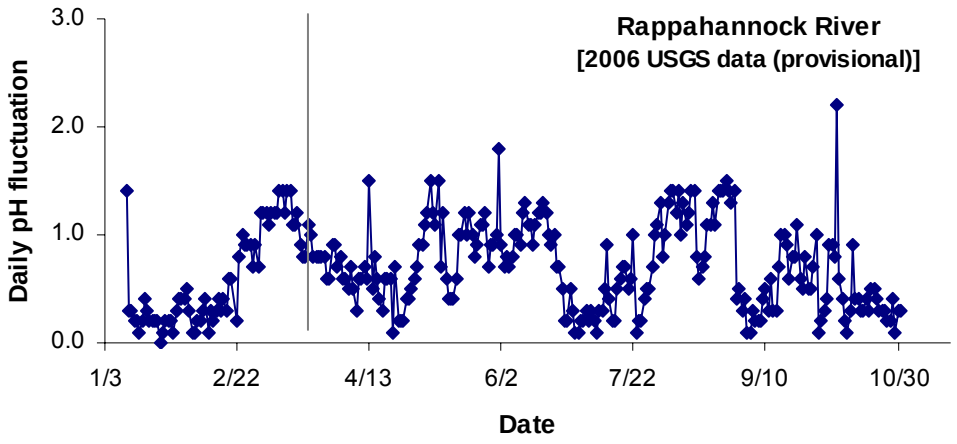

FIGURE A-3

The Daily Difference Between Maximum and Minimum pH Values in the Rappahannock River. The grey band covers the period of the kill in the Shenandoah River and the vertical line denotes the peak of the acute portion of that kill.

Discussion The Rappahannock River is being used here as an out-of-basin reference stream, though it should be noted that there is no solid evidence of similarities in geology, ecology, and water quality between this reference stream and the North Fork. That being said, these data show pH fluctuations of even greater magnitude (1.5-2 units, Figure A-3) than those seen on the North Fork (Figure A-2), and there were no reported kills there.

Score - A difference in response relative to exposure to the candidate cause was observed at non-spatially linked sites, but the difference is not in the expected direction. The Rappahannock shows greater pH fluctuation than the South Branch but with no increase in fish kill occurrence (in fact, no observed fish kills). This was not given a stronger negative score because of the weak similarities in conditions between the kill site and this reference site.

A.1.7. Mortality due to High Ammonia Concentrations.

A.1.7.1. Spatial/Temporal Co-occurrence —

Data Used Ammonia samples taken at an average of 2.5 days per week for sites at north and south-run extremities and 5 days per week for sites in between north and south sample runs at random days of the week from March-July 2006 on the North Fork of the Shenandoah at Woodstock, Strasburg and Mount Jackson sites as well as South Fork sites, Cedar Creek, Cowpasture River and Maury River at Bean's Bottom and upstream of Mill Creek sites. This was a continuation of a similar weekly sampling regime in 2005 except that the 2005 project included only North Fork Shenandoah sites from Timberville downstream to Strasburg. Sampling has continued since July 2006 at weekly intervals. pH readings were taken with these samples to allow for calculation of un-ionized ammonia. Data was collected by the Valley Regional Office of the Virginia DEQ and is in the possession of Robert (Ted) Turner, biologist for the DEQ.

Analysis We calculated un-ionized ammonia concentrations from the total ammonia measurements using temperature and pH measurements taken at the time of sampling. Then we plotted both un-ionized and total ammonia concentrations against time (Figures A-4 and A-5).

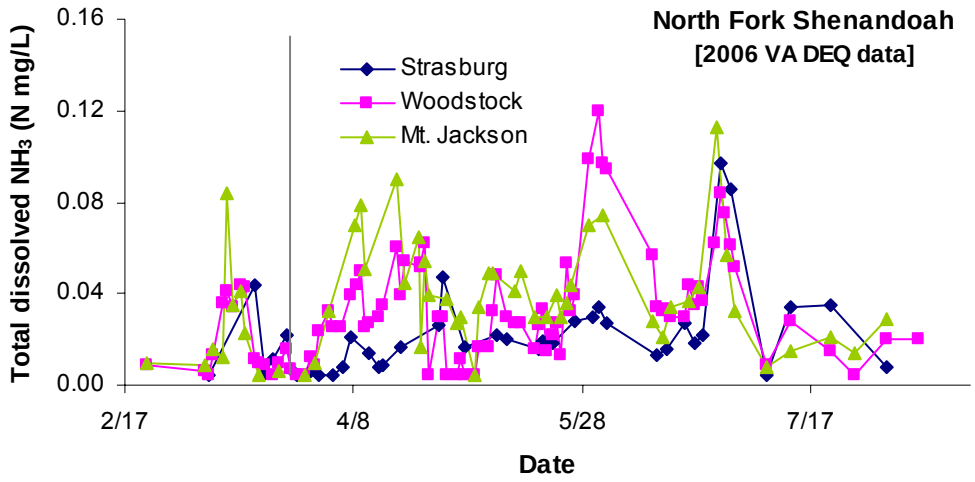

FIGURE A-4

Total Dissolved Ammonia Values in the North Fork Shenandoah River at Three Locations. The Strasburg and Woodstock locations are within the kill zone, but the Mt. Jackson location is an upstream reference site. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

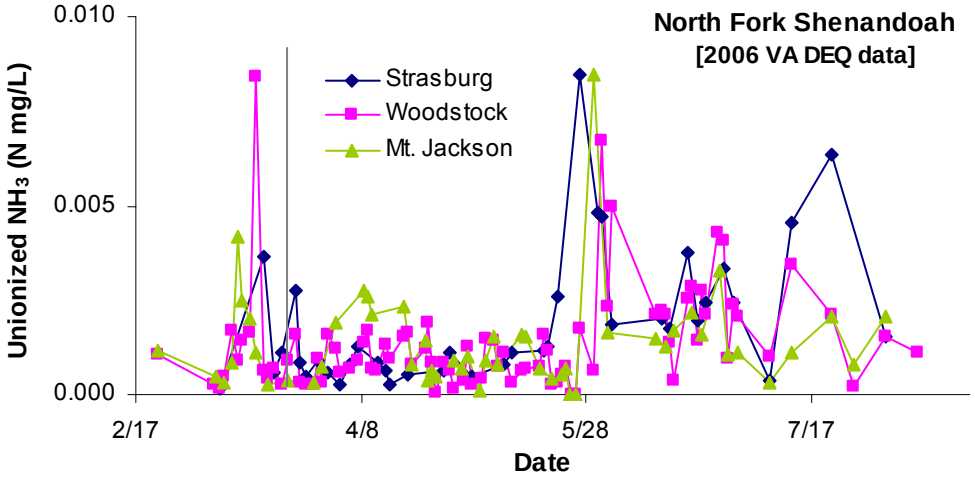

FIGURE A-5

Un-ionized Ammonia Values in the North Fork Shenandoah River at Three Locations. The Strasburg and Woodstock locations are within the kill zone, but the Mt. Jackson location is an upstream reference site. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

Discussion Because un-ionized ammonia we calculated its concentrations. The amount of un-ionized ammonia increases with pH. Also, since pH fluctuates through the day, if ammonia is sampled in the morning and pH is recorded then, we expect pH to rise by the afternoon, with a concurrent rise in un-ionized ammonia concentration. Therefore, we may not be recording the day's maximum un-ionized ammonia value if we record in the morning.

Score --- The effect both does not occur when the candidate cause occurs, and does occur when the candidate cause does not occur.

A.1.7.2. Complete Exposure Pathway —

Data Used Knowledge of State biologists.

Analysis None.

Discussion Sources of ammonia include sewage treatment plants, poultry wastes, and nitrogenous fertilizers.

Score + Somewhat supports because some steps are present.

A.1.7.3. Stressor-Response Relationship in the Field —

Data Used Measurements of ammonia at similar sites both in the same basin and out-of-basin in Virginia from March-October 2006.
Rivers/sites sampled include the North Fork of the Shenandoah at the Mt. Jackson gauging station (upstream of the zone with reported kills), Cedar Creek, the Cowpasture River, the Maury River at Bean's Bottom and the Maury River upstream of Mill Creek.

Analysis Using total ammonia measurements, pH and temperature data, we calculated un-ionized ammonia concentrations. We then plotted those values against time (Figures A-4 and A-5). The Mt. Jackson data is plotted on the same graph presented above alongside the data from sites within the kill zone. In addition, the other reference

streams are plotted in the same manner (Figures A-6 and A-7). The first pair of plots shows total ammonia and the second pair, un-ionized ammonia.

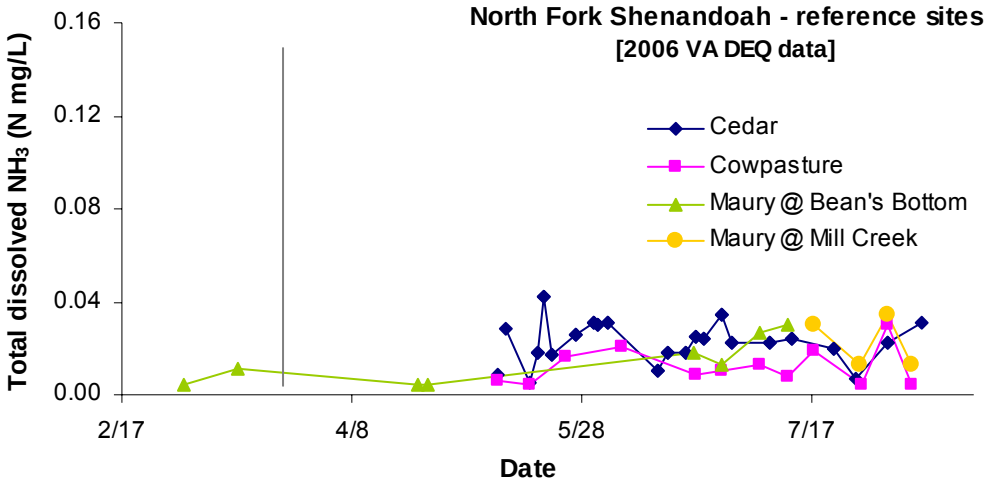

FIGURE A-6

Total Ammonia Values in Four Reference Streams for the North Fork Shenandoah River. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

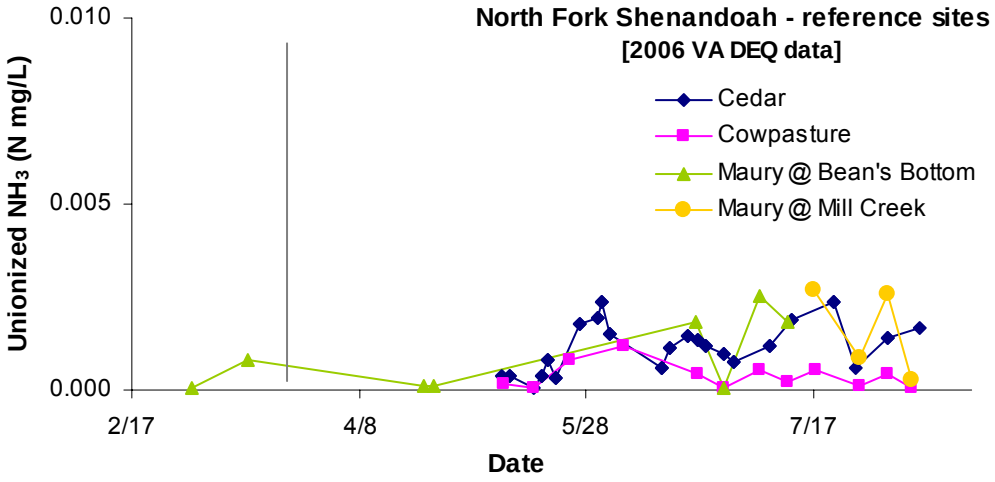

FIGURE A-7

The Daily Un-ionized Ammonia Values in Four Reference Streams for the North Fork Shenandoah River. The grey band covers the period of the kill and the vertical line denotes the peak of the acute portion of the kill.

Discussion While the data recorded at the site of the kills does show a peak in the concentration of ammonia in mid March, around the time of the first observed kills, we also see a corresponding increase upstream of where kills were reported at the Mt. Jackson gauging station. If ammonia alone were responsible for killing the fish, we would expect to see kills everywhere we see these elevated ammonia levels, yet we do not. Furthermore, ammonia levels drop again after the spike, yet fish continued to die. If ammonia concentration falls to levels similar to those in the reference streams with no observed kills, we would expect a cessation in the fish kill on the North Fork. As a final caveat, we have not considered time of day of sampling here, and since pH fluctuates throughout the day, it is possible that the variation we observe in these plots is partially due to choice of sampling time.

Score - We observed a qualitative difference in response relative to exposure to the candidate cause, at reference sites, but the difference was not in the expected direction. The reference streams here are non-spatially linked sites and show an increase in ammonia concentration similar to that in the North Fork but with no increase in fish kill occurrence (in fact, no observed fish kills).

A.1.7.4. Stressor-Response Relationship in the Field

Data Used Monitored natural ammonia levels on the Rappahannock are frequently as high as 0.6-0.9 mg/L. There have not been any reported kills there. This anecdotal evidence comes from studies by Steve McIninch, Virginia Commonwealth University.

Analysis None, data are pending.

Discussion This evidence was available only as a verbal communication.

Score - (P) We saw a large difference in response relative to exposure to the candidate cause at a reference site, but the difference is not in the expected direction. The reference stream shows higher ammonia concentrations than the South Branch but no occurrence of fish kills.

A.1.7.4. Stressor-Response Relationships in the Field —

Data Used Ammonia measurements covering the analogous kill period of 2005 recorded on the North Fork at Strasburg and Woodstock.

Analysis We calculated maximum un-ionized ammonia values of 7.3 μg/L at Strasburg and 3.8 μg/L at Woodstock from this period.

Discussion The un-ionized maximum ammonia level during the analogous kill period from 2005 is compared here to the max during the kill of 2006 under consideration. Because there were reports of lesions from the North Fork in 2005 but few deaths, we might consider this a non-kill year. However, the status of the 2005 scenario as a kill is confounded by the fact that there was a large kill on the North Fork in 2004, and Virginia biologists hypothesize that the lack of a large-scale kill in 2005 may simply have been a result of overall lower numbers of fish and thus lower numbers available to die. So, while ammonia levels were lower in 2005, we cannot draw any conclusions since we don't know whether to consider this a kill.

Score 0 The ambiguity of 2005 as a kill/non-kill year prevents us from drawing any conclusions from the comparison of maximum un-ionized ammonia levels.

A.1.7.5. Stressor-Response Relationship from the Lab —

Data Used Articles including Milne et al. (2000) and Constable et al. (2003) and the U.S. EPA (1999) criteria document.
Exposure data come from ammonia levels reported above from the Strasburg and Woodstock sites on the North Fork.

Analysis We compared results reported in literature to ammonia levels reported above from the Strasburg and Woodstock sites on the North Fork, above.

Discussion Reported ammonia thresholds for lethal or even sublethal toxic effects were at least 10 times higher than what we've seen in the North Fork. For example, Milne et al. (2000) reported needing ~400 μg/L un-ionized ammonia to show significant lethality over a 24-hour exposure, ~750 μg/L over a 6-hour exposure, and ~850 μg/L for a 1-hour exposure in rainbow trout. In longer term pulsed exposure experiments of up to 6 weeks in duration, ~200 μg/L of un-ionized ammonia was needed to cause sub-lethal effects assessed as "very severe gill damage." Milne also noted the trend that increased exposure frequency is more significant than increased total concentration of ammonia in terms of effects on fish. Constable et al. (2003) reported an LC_{50} of ~1.3 mg/L of un-ionized ammonia for acute exposure in fathead minnows. TheU.S. EPA (1999) criteria document reports values of EC_{20} of 4.79 mg/L of total ammonia at pH 8 for white sucker, 1.35 and 2.8 mg/L ammonia for bluegill and a geometric mean of 4.56 mg/L total ammonia at pH 8 for smallmouth bass. The document also reports an LC_{50} of 50 mg/L of total ammonia during an acute exposure test in fathead minnow. Highest values in the North Fork were about 1-5 μg/L of un-ionized ammonia during the kill, reaching a maximum for the sampling period of ~8 μg/L of un-ionized ammonia but only at the end of the kill. It is definitely worth noting that none of these lab tests exposed the fish to elevated or pulsed levels of ammonia for as long a period as our the fish in this case may have been exposed. Indeed, it would be impractical to reproduce those conditions. However, we're also comparing the peak concentrations of ammonia observed on the North Fork with reported lethal and sub-lethal levels from the literature. So even if these observed levels approach those shown to cause effects in fish in the literature, we know that ammonia levels fall far below this in between peaks and so the average ammonia concentration the fish are exposed to as a sustained exposure is considerably lower and definitely comparable with observed levels in reference streams without kills.

Score -- If ammonia alone were responsible for the kills, we would expect the levels recorded in the North Fork to be more similar to those shown to have killed fish in lab tests, and that's not the case.

A.1.8. Candidate Cause: Unspecified Toxics.

A.1.8.1. Evidence from the Case: Spatial-Temporal Co-occurrence —

Data Used Sediment, metals/organics and water chemistry data from two sites on the North Fork of the Shenandoah. Virginia Probabilistic Monitoring Program (ProbMON) VADEQ daily and continuous water column monitoring measures nutrients and NH_4 but no metals or organics.
Data from Friends of the Shenandoah.

Analysis NA.

Discussion Barium (Ba) was detected in the water column in 2006. No other data were available for 2006 on toxic metals, organics, pesticides, pharmaceuticals.
Table A-1 presents the results of analyses of various media collected in the North Fork. The utility of the data is limited, because they are not temporally and spatially relevant to the fish kill events. The table should, however, be reviewed to determine similarities and differences between the two watersheds to help design future studies by identifying contaminants that should be monitored.

Score NE No Evidence.

A.1.8.2. Evidence from the Case: Evidence of Exposure or Biological Mechanisms —

Data Used Arsenic (As) was observed in fish tissue data in 2005 (the year before); 2006 fish tissue data not yet available.
USGS Virginia laboratory (Paul McCormick) is investigating periphyton at 14 sites on the NF Shenandoah. Data are pending.

1

Table A-1

Chemicals Detected in Various Media at Virginia Department of Environmental Quality (North Fork Shenandoah) and USGS (South Branch Potomac) Sample Stations

	North Fork Shenandoah (river miles)			South Branch Potomac												
	0.57	54.75	93.53	Cabins	South Fork near Moore-field	Mainstem near Moorefield	Near Spring-field	Near Upper Tract	At Peters-burg Gap	Pilgrim's Pride Slaughter-house Discharge	At Sycamore	Franklin	Spring Run Hatchery effluent near Masonville	Small Mouth Bass Plasma	Peters-burg STP	Moore-field STP
Anhydroerythromycin													WW		WW	WW
Ciprofloxacin															WW	
Erythromycin															WW	WW
Lincomycin															WW	WW
Ofloxacin										WW			WW		WW	WW
Sulfamethoxazole															WW	WW
Trimethoprim															WW	WW
Tylosin															WW	WW
Pentachloroanisole (PCA)	FT			PS		PS	PS	PS	PS		PS					
4-tert-octylphenol														PL		
Diethoxynonylphenol						WC^{est}	WC^{est}									
Polybrominated diphenyl ether congeners (PBDEs)	FT	FT	FT	PS,		PS	PS,	PS,	PS,		PS			PL		
Arsenic	FT		FT	WC	WC			WC		WC	WC					
Caffeine						WC^{est}	WC^{est}									
Chromium	FT		FT													
Mercury	FT		FT													
1,3-dimethylnaphthalene	FT															
1,6-dimethylnaphthalene	FT															
1-methylnapthalene	FT					$PS^{est.}$	PS					PS				
2,6-dimethylnaphthalene	FT					$PS^{est.}$	PS					PS				

Table A-1 cont.

	North Fork Shenandoah (river miles)			South Branch Potomac												
	0.57	54.75	93.53	Cabins	South Fork near Moore-field	Mainstem near Moorefield	Near Spring-field	Near Upper Tract	At Peters-burg Gap	Pilgrim's Pride Slaughter -house Discharge	At Sycamore	Franklin	Spring Run Hatchery effluent near Masonville	Small Mouth Bass Plasma	Peters-burg STP	Moore-field STP
2-methyl naphthalene	FT					PS$^{est.}$	PS					PS		PL		
Acenaphthene	FT															
Fluoranthene	FT															
Naphthalene	FT						WC^{est},PS$_{est}$					PS$^{est.}$		PL		
PAH	FT															
Phenanthrene	FT					PS	PS					PS				
Pyrene	FT					PS	PS					PS				
PCB	FT	FT	FT													
MethTriclosan	FT	FT														
Tonalide (AHTN)						PS	PS					PS				
Methyl salicylate						WC^{est}	WC^{est}		WC^{est}							
Chlordane	FT	FT	FT	PS		PS	PS	PS	PS		PS					
Chlorpyrifos				PS					PS		PS					
Nonachlor				PS		PS	PS	PS	PS		PS					
DDT and metabolites	FT	FT	FT													
Fipronil																
gamma-HCH				PS		PS	PS	PS	PS							
Hexachlorobenzene	FT			PS		PS	PS	PS	PS		PS					
Trifluralin						PS	PS		PS		PS					
1,4-Dichloro benzene						WC^{est}	WC^{est}		WC^{est}							
diethyl phthalate														PL		
diethylhexyl phthalate														PL		
Acetophenone						PS$^{est.}$	PS,					PS		PL		
d-limonene														PL		
Isophorone						PS	PS,WC^{est}					PS				
Tributyl phosphate									WC^{est}							

est = Concentration estimated; FT = Fish Tissue; PL = Smallmouth bass plasma; PS = Passive sampler ; WC = Water Column; WW = Waste water (effluent sample)

Discussion Although there was some evidence of As exposure in 2005, this was one year before the 2006 fish kill. Ba was not observed in fish tissue. Intersex fish were observed on the NF in 2005 which suggests that some chemical contaminant was available at biologically active concentrations. No other data were available that would constitute evidence. Neither pathology data from dead fish of the 2006 kill nor intersex data were yet available.

Score NE, The available data provide no evidence of exposure or biological mechanism.

A.1.8.3. Evidence from the Case: Complete Causal Pathway —

Data Used Locations of potential sources constitute evidence of potential causal pathways.

National Pollution Discharge Elimination System (NPDES) discharges provide evidence of sources of waste water (Figure A-8).

Agricultural sources were described in Bill van Wart's presentation at the September 2006 CADDIS workshop, derived from the U.S. Census of Agriculture.

Industrial chemicals used in the watershed are indicated by the Toxics Release Inventory.

Analysis NA.

Discussion Sources are summarized in Table A-2.

National Pollution Discharge Elimination System (NPDES) discharges constitute evidence of potentially toxic chemicals. Although discharge permits do not allow know toxic discharges, unknown discharge constituents or unreported exceedances of permitted levels may result in toxic exposures.

Virginia did not have a comprehensive background document for the North Fork Shenandoah River; however, Turner referred to the Bill Van Wart presentation. The Shenandoah Basin, both North and South Forks, has a high concentration of livestock-based agriculture, both poultry and cattle (van Wart presentation).

TABLE A-2 Potential Sources of Toxic Substances in North Fork Shenandoah	
Source	Potential Toxic Stressors
Poultry farms, poultry processing plants	Pesticides, antibiotics, cleaners
Agriculture	Pesticides, metals
Highways	PAHs, metals
POTWS	Pesticides, pharmaceuticals, PAHs, metals
Industrial	Unregulated substances, accidental discharges
Legacy industrial	PCB, other chlorinated organics, metals, PAHs, etc.
Accidental/illegal dumping	Any
Golf courses	Pesticides

The fish kills in the North Fork do not correspond to the location of POTWs or but other discharges cannot be eliminated (Figure A-8).

Chicken processing houses may ship wastes via pretreatment plants to POTW. Constituents of those wastes were unknown to workshop participants and should be investigated.

Toxic Release Inventory (TRI) data suggest other possible water pollutants. See Tables A-3, A-4 and A-5 for 2005 releases.

Score + Evidence somewhat supports because the identified sources constitute at least one step in each causal pathway (Figure 5).

1

TABLE A-3

2005 TRI-eFDR Reported Releases to Streams, POTW, or Other for Facilities Within the Two Watersheds

County	Facility Name	Compound(s)	Quantity (pounds)	Disposal Method
Shenandoah	GLOBAL STONE CHEMSTONE CORP	LEAD COMPOUNDS	4	Other onsite
				(Air/Stormwater also)
	PERRY JUDD'S INC. STRASBURG DIV	CERTAIN GLYCOL ETHERS	200	STRASBURG WATER TREATMENT PLANT (also Air emissions)
		ETHYLENE GLYCOL	6,100	STRASBURG WATER TREATMENT PLANT (Also Air emissions)
	GEORGE'S CHICKEN LLC	NITRATE COMPOUNDS	123,828	Stoney Creek
				(also Offsite)
Pendleton	GREER INDUSTRIES INC. DBA GREER LIME CO	LEAD COMPOUNDS	2,167	other onsite
				(Air / Stormwater also)
		MERCURY COMPOUNDS	16	other onsite
				(Air / Stormwater also)
Hardy	PILGRIM'S PRIDE CORP MOORFIELD PERPARED FOOD PLANT	AMMONIA	171	South Fork of the South Branch of Potomac
				(Air / Offsite also)
		NITRATE COMPOUNDS	93,583	South Fork of the South Branch of Potomac
				(Offsite also)
	PILGRIM'S PRIDE CORP MOOREFIELD FRESH FACILITY	NITRATE COMPOUNDS	127,000	South Fork of the South Branch of Potomac
				(Offsite also)

2

1

TABLE A-4

2005 TRI-eFDR Reported Releases for Dominion Mount Storm Power Station, Grant County

Compound	Quantity (pounds)	Disposal
Ammonia	1,200	Stony River
	36,000	onsite landfill
		(also air emissions and offiste)
Arsenic Compounds	150	Stony River
		(also air, landfill, offsite)
Barium Compounds	630,000	onsite landfill
		(also air, offsite)
Beryllium Compounds	9,200	onsite landfill
		(air, offsite also)
Chromium Compounds (except chromite ore mined in the Transvaal Region)	110,000	onsite landfill
		(also air, offsite)
Cobalt Compounds	40,000	onsite landfill
		(also air, offsite)
Copper Compounds	11	Stony River
	130,000	onsite landfill
		(also air, offsite)
Lead Compounds	1	Stony River
	61,662	onsite landfill
		(air, offsite)
Manganese Compounds	1,200	Stony River
	160,000	onsite landfill
		(air, offsite)
Mercury Compounds	5	Stony River
	1,358	onsite landfill
		(air, offsite)
Nickel Compounds	1	Stony River
	115,000	onsite landfill
		(air, offsite)
Selenium Compounds	170	Stony River
	170,000	onsite landfill
		(air, offsite)
Vanadium Compounds	240,000	onsite landfill (offsite)
Zinc Compounds	160	Stony River
	140,000	onsite landfill (air, offsite)

1

TABLE A-5 Other Compounds Found in 2005 TRI-eFDR for the Two Watersheds
These compounds were disposed of either in Air, Offsite, or reported on Form A and thus have no disposal information:
1,2,4-Trimethylbenzene
Antimony Compounds
Benzene
Chlorine
Copper
Diisocyanates
Dioxin and Dioxin-like Compounds
Ethylbenzene
Hydrochloric Acid
Hydrogen Fluoride
Lead
Methyl Isobutyl Ketone
Mercury
Methanol
Methyl Tert-Butyl Ether
Mixture
n-Butyl Alcohol
n-Hexane
Naphthalene
Ozone
Polycyclic Aromatic Compounds
Styrene
Sulfuric Acid
Toluene
Xylene (Mixed Isomers)

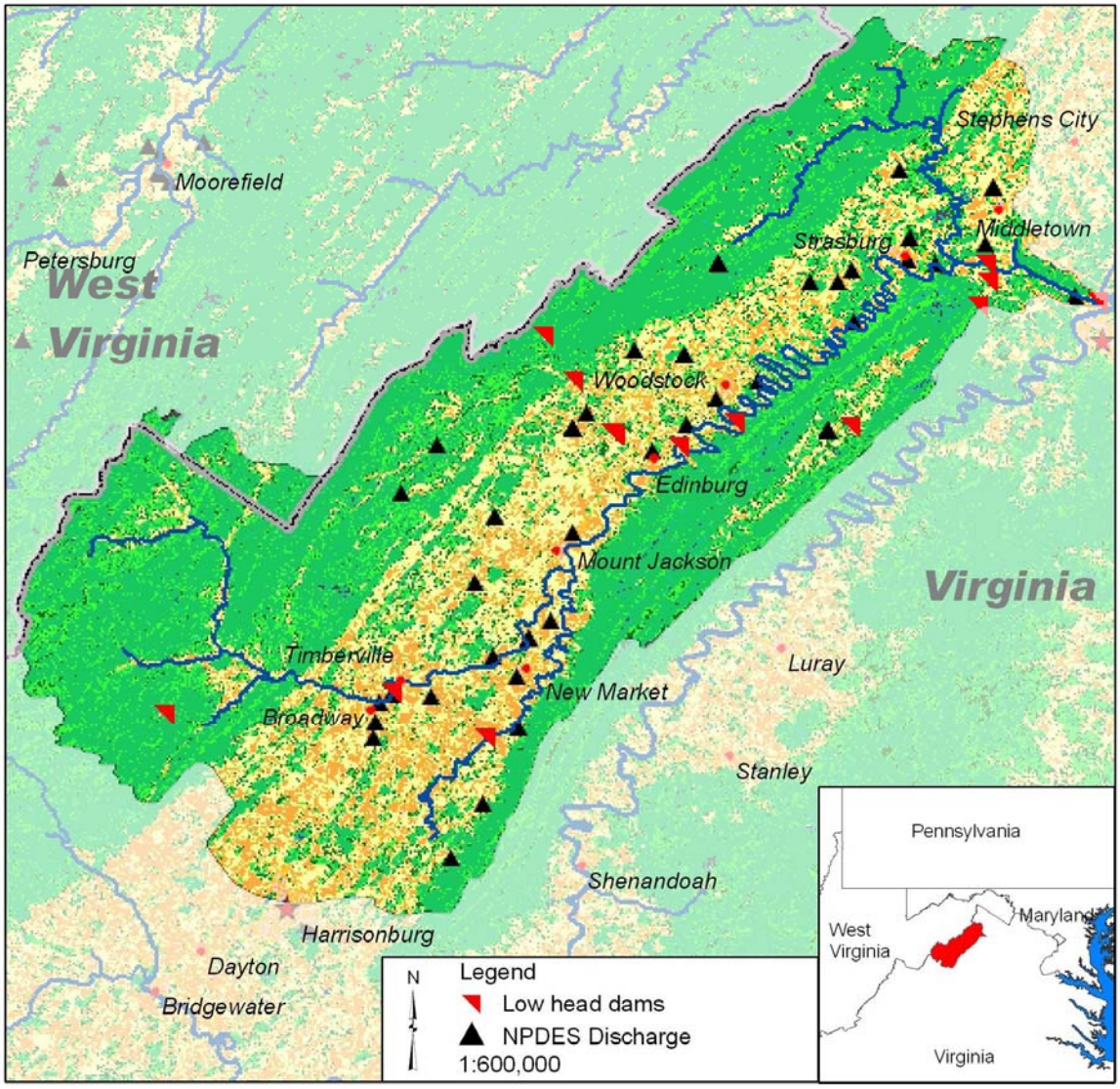

FIGURE A-8

Locations of All Permitted Waste-Water Discharges on the North Fork Shenandoah River

A.1.8.4. Evidence from the Case: Tests of Media in Laboratory —

Data Used U.S. EPA toxicity tests of South Branch media (Amy Bergdale).

Analysis None.

Discussion Toxicity tests were performed on media from the North Fork Shenandoah and the Cowpasture River, a reference site. Both *Ceriodaphnia* and fathead minnows were tested. Results were equivocal, with some observed toxicity but toxicity was similar in the reference river as in the North Fork of the Shenandoah. The samples for the toxicity tests were taken after the fish kills.

Score NE Toxicity tests were on post-kill waters.

A.1.8.6. Evidence from other Studies: Stressor-Response from Laboratory Studies —

Data Used Although data are available concerning the toxicity of many potential contaminants, ambient concentrations are not available for comparison.

Analysis NA.

Discussion NA.

Score NE, No evidence.

A.1.8.7. Evidence from Other Studies: Analogous Cases —

Data Used None

Analysis NA.

Discussion Prior kills in the Shenandoah could be analyzed as analogous cases, but data for chemical exposures from those kills are lacking.

Score NE.

A.1.9. Candidate Cause: Starvation.

A.1.9.1. Evidence from the Case: Spatial/Temporal Co-occurrence —

Data Used Length, weight and age observations of smallmouth bass before, during and after kill.

Analysis NA.

Discussion Very low weight relative to length is considered the measure of starvation, not just a symptom. Observations on weights of dead smallmouth bass showed no indication of low weight or weight loss compared to before the kill, after the kill, or in other rivers. Killed fish did not appear different from healthy fish in terms of body weight.

Score - - - Evidence strongly suggests that the candidate cause did not occur.

A.1.9.2. Evidence from the Case: Evidence of Exposure or Biological Mechanisms —

NE, No evidence exists on stomach contents of fish in the 2006 fish kills.

A.1.9.3. Evidence from the Case: Complete Causal Pathway —

NE, No evidence exists on food availability in the North Fork of the Shenandoah.

A.1.9.4. Evidence from Other Studies: Analogous Cases —

Data Used Unpublished 2005 letter report to Don Kain of the Virginia DEQ, Valley Regional Office, from Dr. Stephen Smith of Virginia Polytechnic Institute and State University (Virginia Tech).

Analysis NA.

Discussion In the 2005 South Fork Shenandoah fish kills, dead long-eared sunfish, smallmouth bass and suckers all had food in their stomachs, appeared normal in body weight and had normal body fat.

Score Negative, -. Candidate cause did not occur in an analogous kill.

A.2. CASE 2—2006 SOUTH BRANCH POTOMAC RIVER, WV

A.2.1. Anoxia due to Low Dissolved Oxygen.

A.2.1.1. Spatial/Temporal Co-occurrence —

Data Used USGS continuous YSI D.O. monitor spatial and temporal data from the fish mortality event; temporal data from a reference control site on the South Fork Shenandoah River. (Data on team room) Ad hoc measurements during the kill.

Analysis Co-occurrence inferred from observations of dying fish and concurrent ad hoc DO measurements of 8 mg/L by Jim Hendrick.

Discussion The co-occurrence of dying fish and normal dissolved oxygen levels refutes the possibility that low dissolved oxygen caused anoxia. Mortality from low dissolved oxygen is rapid. Therefore, if DO concentrations are high but fish are still dying, low DO cannot be the cause.

Score R, The evidence is sufficient to refute low dissolved oxygen concentrations as the cause.

A.2.2. Anoxia due to Gill Injury (Gill Hyperplasia).

A.2.2.1. Spatial/Temporal Co-occurrence —

Data Used Gross and histopathology samples and data from the mortality event and from reference sites on the Greenbrier River were taken. However, those data were not available for this analysis.

Analysis Pending pathology data.

Discussion None.

Score NE (P), No Evidence at this time.

A.2.2.2. Evidence of Exposure or Biological Mechanism —

Data Used Observational data from Jim Hendrick (WV DNR) on affected fish demonstrating clinical signs of gasping, abnormal surfacing, lethargy and decreased flight behavior response.

Analysis Minimal analysis due to non-specific observational nature of the data.

Discussion Some fish displayed gasping behavior but most were simply lethargic.

Score + Data are positive but weak and inconsistent.

A.2.2.3. Causal Pathway —

Data Used Data was obtained on water pH (including pH fluctuations by USGS), changes in water temperature, water ammonia levels, water nitrite / nitrate levels, total suspended solids (WV Dept of Agriculture), possible parasite data (unavailable pathology reports).

Analysis Pending.

Discussion Gill injury could have been caused by several causal pathways. Evidence for occurrence of a causal pathway consists of the occurrence of an agent in a pathway leading to the proximate cause. Some of the agents in the pathway to this candidate cause are also candidate causes themselves (i.e., pH, NH_3, and pathogens). However, the evidential requirements here are different. Elevated concentrations of an agent that is a proximate cause must occur at the time of a kill, but agents in a causal pathway may have been elevated in the past. It is necessary only that their effects persist.

pH – Data showed wide pH fluctuations on a daily basis, pH levels went to mid 9's. Fluctuations were high immediately before the kill but were even higher at the beginning of April (Section A.2.6). pH fluctuations may have contributed to gill injury and stress to the fish through alkalosis or ammonia autointoxication.

NH_3 – Ammonia was not elevated at the time of or immediately before the kill. Toxic gill necrosis can result from autointoxication (metabolism of proteins in excess of excretory capacity) which may be triggered by starvation, a sudden drop in temperature, an increase in pH above 9, an increase in aqueous ammonia, or an increase in protein in the diet (Smutna et al., 2002). Of these, only elevated pH was observed (see above).

Nitrate and Nitrite – Data were unavailable, but may be elevated by nitrification of ammonia in the watershed.

Temperature – Water temperature rose, which increases biological activity, including oxygen requirements and most toxic responses.

Auto-intoxication – The elevated pH and temperature but not ammonia suggest that conditions provide ambiguous evidence for an ammonia auto intoxication cycle to be initiated internally in the fish.

Conductance – Conductance was not elevated.

Pathogens – Data were also available from analyses of bacterial and protist communities in mucus from fish with and without lesions collected during the mortality event (Gillevet et al., 2006). Parasite levels were potentially increased, also adding further physiological stressor, but pathology data were unavailable.

Stress – The kill did not co-occur with spawning and seasonal behavior was normal.

Flow – A high flow event occurred prior to the kill, which could carry contaminants into the river or resuspend material in the river potentially injuring the gills.

Abrasion – Concentrations of total suspended solids were not high. Neither periphytic nor planktonic diatoms appeared to be heavy.

Score	
	Water pH 0
	Water Ammonia 0
	Water Nitrate & Nitrite 0
	Water Temperature +

Auto intoxication cycle 0
Conductance -
Stress, Behavioral -
Abrasion, Flow event +
Abrasion, Total Suspended Solids -
Abrasion, Planktonic diatoms -
Abrasion, Wash off of dead diatoms -

A.2.2.4. Stressor-Response from Laboratory Studies —

Data Used Toxicological benchmarks from laboratory tests of fish were obtained for pH, NH_3, and temperature.

pH

9 causes carp mortality (Schaperclause, 1952)
When water pH > blood pH, NH_3 excretion is reduced; above pH 9.5 it is blocked (Schaperclause, 1952; Wood, 2001).
Other effects of high pH at the gills include increased CO_2 excretion leading to alkalosis and reduced Na^+ and Cl^- uptake (Wood, 2001).
Reported gill injuries were limited to hyperplasia of the chloride cells, not gross hyperplasia (Wood, 2001).

Temperature

Rapid changes in temperature can increase morbidity and mortality in fish (Stoskopf, 1993).

NH_3

0.2 mg/L caused gill injury in Brown trout (U.S. EPA, 1999).

Gill lamellae obtained from parental fish exposed to un-ionized ammonia concentrations ranging from 0.02 mg NH_3-N/L to 0.05 mg NH_3-N/L for four months, and 0.05 mg NH_3-N/L and 0.06 mg NH_3-N/L for seven and eleven months, showed mild to moderate fusion, aneurysms, and separation of the epithelia from the underlying basement membrane (U.S. EPA, 1999).

"In contrast to acute exposures, a variety of morphologic changes in the gills have been described during chronic sublethal ammonia exposure. Most prominent are an overall swelling of the respiratory lamellae, proliferation of epithelial cells, increased diffusion distance, and an increased prevalence of bacterial gill disease … These

responses would be expected to decrease the respiratory gas exchange capacity of the fish, and thus its swimming performance and tolerance to hypoxia" (Wood, 2001).

Smallmouth bass are fairly sensitive. The growth effect concentrations ranged from 0.05 mg NH_3-N/L at pH=6.6 to 0.71 mg NH_3-N/L at pH=8.68 (U.S. EPA, 1999, pg. 118).

Red horse sensitivities are unknown.

As fish emerge from torpor in the spring, toxic ammonia concentrations drop from 1.7 to 0.2 mg/L (Schaperclause, 1952).

0.5 mg/L total ammonia highest observed in spring.

Nitrite and Nitrate

Chronic exposures to nitrite can cause gill hyperplasia (Kroupova et al., 2005).

Analysis Comparison of aqueous concentrations of stressors to concentrations causing effects in laboratory studies.

Discussion Data were not sufficiently available at the workshop to confidently evaluate the potential for observed levels of stressors, singly or in combination, to injure gills. Although the type of injury is unknown and the laboratory studies are not clearly comparable to the field, the available evidence suggests that chronic exposures to some contaminants may be sufficient. Also differences in sensitivity in the laboratory should be compared to apparent differences in response among species during the kill.

Score +(P) Some evidence supports the candidate cause but more is Pending.

A.2.2.5. Analogous Cases —

Data Used NA.

Analysis NA.

Discussion A kill in the South Branch in 2002 primarily affecting red horse, but also including smallmouth bass, and redbreast sunfish occurred in the same time frame as the 2006 kill. Also like the 2006 kill, it also occurred after a spring run-off event. Flow, pH & temp data maybe available for the 2002 kill.

Score NE(P) The 2002 kill may be analogous but data were not available to evaluate the candidate cause.

A.2.3. Anoxia due to Low Blood Oxygen Affinity (Methemoglobinemia).

A.2.3.1. Spatial/Temporal Co-occurrence —

Data Used Plasma samples archived and observational data were taken from the fish mortality event and reference sites on the Greenbrier River, but those data were unavailable.

Observations by State biologists were recounted at the workshop.

Analysis None.

Discussion Neither brown gills nor brown blood were not observed in these fish by State biologists. High pH can also cause low blood affinity without brown blood. Pathology reports could provide more definitive data.

Score -/NE (P) Field observations were negative but more definitive data from the pathology reports are pending.

A.2.3.2. Evidence of Exposure or Biological Mechanism —

Data Used Observational data from Jim Hendrick (WV DNR) on affected fish demonstrating clinical signs of gasping, abnormal surfacing, lethargy and decreased flight behavior response.

Analysis Minimal analysis due to non-specific observational nature of data.

Discussion Some fish displayed gasping behavior but most were simply lethargic.

Score + Data somewhat support methemoglobinemia but are inconsistent.

A.2.3.3. Causal Pathway —

Data Used Knowledge of sources and processes in the watershed.

Analysis None.

Discussion Nitrite accumulates in water when ammonia concentrations are elevated and the second stage of nitrification is inhibited. Sources of ammonia are present in the watershed, but nitrification rates or processes controlling the rates are unknown.

Score 0, The evidence is ambiguous because sources are known but not transformation processes.

A.2.3.4. Stressor-Response from Laboratory Studies —

Data Used Literature reviews

Methemoglobinemia symptoms occur at 0.10 to 0.50 mg/L in sensitive species (channel catfish and trout) and LC_{50} values range from 0.60 to 200 mg/L (Animal Disease Diagnostic Laboratory, 1998).

Centrarchids (includes sunfish and black bass) are refractory to methemoglobinemia (Kahn, 2005).

Analysis Logic and comparison to ambient concentrations.

Discussion The maximum reported nitrite concentrations in the South Branch during the period of the kill was 0.0041 mg/L. That is less than half the concentrations reported to cause methemoglobinemia in laboratory studies and less the a tenth of concentrations that cause lethal methemoglobinemia in sensitive species.

This kill involved catastomids rather than centrarchids so the relative sensitivities may be consistent.

Score - - This evidence significantly weakens methemoglobinemia as a candidate cause. Reports of blood color in pathology reports would be definitive.

A.2.4. Mortality due to Other Pathogenic Modes of Action.

A.2.4.1. Spatial/Temporal Co-occurrence —

Data Used Gross and histopathology samples and data from mortality event and from reference sites on the Greenbrier River. However, those data were not available for this analysis.

Analysis None, pending pathology data.

Discussion None.

Score NE (P) No Evidence, but data are pending.

A.2.4.2. Evidence of Exposure or Biological Mechanism —

Data Used Observational data on affected fish demonstrating lethargy and decreased flight behavior response. Pathology data were unavailable.

Analysis Minimal analysis due to non-specific observational nature of data.

Discussion Observational data is in this case is diagnostically non-specific.

Score 0/NE(P) Ambiguous behavior, other data are pending.

A.2.4.3. Complete Exposure Pathway —

Data Used Knowledge of State biologists.

Analysis None.

Discussion Sources of pathogens may include stocked fish, released bait fish or effluents from hatcheries. Trout but not smallmouth bass are stocked in the South Branch. Bait minnows inevitably are released. Hatcheries occur at and below the kill site. Spring Run Hatchery is located upstream of Petersburg on South Fork of Mill Run of South Branch.

Score + Some steps are present.

A.2.5. Mortality due to High pH.

A.2.5.1. Spatial/Temporal Co-occurrence Evidence (Specifically Temporal Co-occurrence) —

Data Used Continuously monitored pH data from January-July 2006 on the South Branch of the Potomac at Springfield (mile 6) collected by the USGS.

Analysis Continuously monitored pH data plotted vs. date and with respect to the kill interval (Figure A-9).

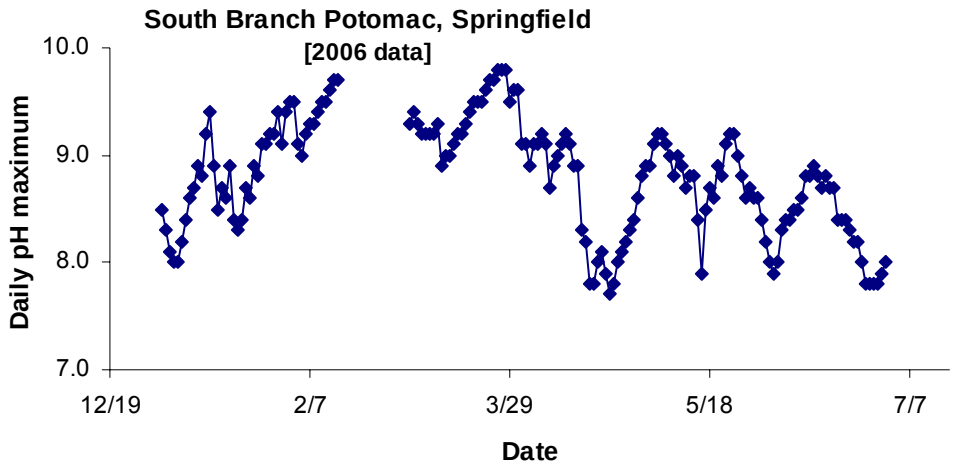

FIGURE A-9

A Plot of the Daily Maximum pH Balues in the South Branch Potomac River at Springfield. The shaded band covers the period of the kill.

Discussion pH values were high (above 9) beginning as early as January, however there was no kill at this time (Figure A-9). The kill did not occur until late May when pH values were actually dropping below the March highs. All of this demonstrates a lack of temporal alignment of peaks in pH and the onset of the fish kill in the South Branch.

Score --- The effect both does not occur when the candidate cause occurs, and does occur when the candidate cause does not occur.

A.2.5.2. Stressor-Response Relationship in the Lab —

Data Used Scott, Lucas, and Wilson in Aquatic Toxicology, 2005 addressed effects of pH as high as 9.5 on behavior of rainbow trout and perch.

Analysis Maximum pH values during the case of interest were compared with those used during this study.

Discussion The pH maximum during the reported experiment was as high as 9.5, similar to what we've observed on the South Branch in 2006 during the kill. Since fish exposed to that pH were shown to have reduced ability to excrete ammonia (as measured in the fishes' blood), but exhibited no lethality or change in swimming behavior, we have to conclude that our fish should not have been dying due to comparatively lower pH levels alone. Note that this experiment was carried out on different species.

Score - Little effect gradient is observed relative to exposure to the candidate cause, in a controlled lab environment. The fish exposed to pH levels similar to those found in our case of interest did showed reduced ammonia excretion capabilities, but did not die.

A.2.5.3. Stressor-Response Relationship in the Lab —

Data Used An article by Serafy and Harrell (1993) addressing sublethal effects of high pH on bluegill, striped bass, and killifish.

Analysis Comparison of fish behavior in our case of interest with that observed in the experiment under similar pH conditions.

Discussion The fish in this experiment were subjected to pH increases of about 1 unit over the course of less than one hour, with the highest replicate reaching a final pH of about 9.5. Because the exposures are so short in duration, the comparability with our case is severely reduced. Also, while these results may be useful for assessing fish stress response, they are not very useful for assessing lethality since none of the fish died.

Score - The experiment shows that pHs as high as 9.5, similar to the peaks measured in our case of interest, are not lethal to fish. However, the key differences of conditions in each scenario reduce the comparability of each situation to the other.

A.2.6. Mortality due to pH Fluctuations.

A.2.6.1. Spatial/Temporal Co-occurrence (Specifically Temporal Co-occurrence) —

Data Used Continuously monitored pH data from January-July 2006 on the South Branch of the Potomac at Springfield (mile 6) collected by the USGS.

Analysis Calculated pH shifts over 24 hour windows plotted against the date (Figure A-10).

Discussion pH fluctuations were high (0.2-1.2 units) before the kill, dropping somewhat after the kill, and with the highest fluctuations coming around early April. Though there may be some interaction/combination of temperature and pH effects on the fish, when considering pH fluctuations alone, there is not significant temporal co-occurrence evidence to support pH fluctuations as a candidate cause.

Score --- The effect both does not occur when the candidate cause occurs, and does occur when the candidate cause does not occur.

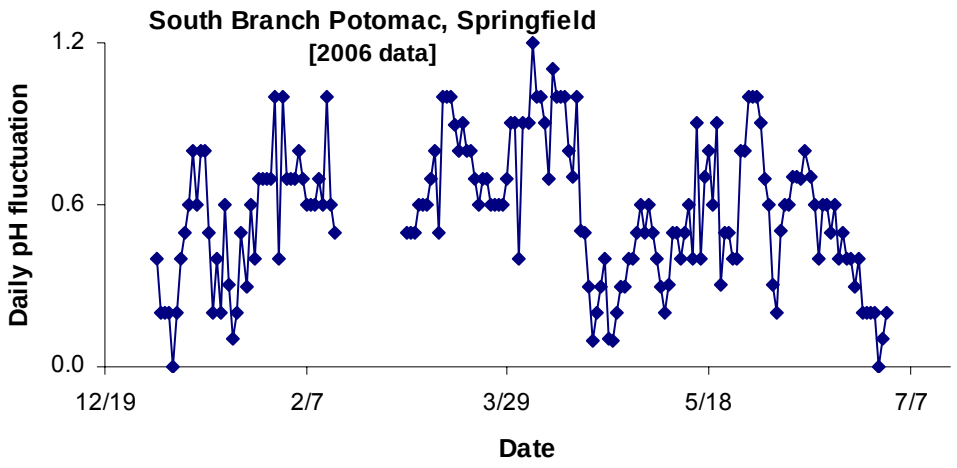

FIGURE A-10

A plot of the differences between daily maximum and minimum pH values in the South Branch Potomac River at Springfield. The shaded band covers the period of the kill.

A.2.6.2. Stressor-Response Relationship in the Field —

Data Used An article by Serafy and Harrell (1993) addressing sublethal effects of pH fluctuations on natural fish communities including pumpkinseed, eel, and killifish.

Analysis Comparison of fish behavior in our case of interest with that observed in the experiment under similar pH conditions.

Discussion The fish communities in this experiment were monitored during a natural shift in pH through the course of a day while pH was monitored. The observers noted shifts in community density, biomass and species richness. None of these changed drastically through the course of the pH shift. pH was measured as varying from ~8.5 up to a maximum of ~9.8 and then down to a minimum of ~7.6. The pH regime in this experiment is probably the most relevant external scenario to ours because of the magnitude of pH shift and the high average pH around which the pH was centered. On the other hand, the other environmental/ecological factors in this setup decreased comparability between the experimental scenario and ours on the South Branch. The study was conducted in a tidal freshwater environment of the Chesapeake Bay with

different fish species. We must question whether, if this pH regime is natural and common for that area, then are the native fish more accustomed and better adapted to these conditions? Also, the observers noted whether or not fish fled the study site, which was a hydrilla grass bed, and interpreted this as a potential reaction to undesirable pH levels. But if pH remained the same at farther distances from the grass bed, we shouldn't expect fleeing the grass bed to be of any benefit, so perhaps fleeing is an inappropriate endpoint of assessment. Also, there could have been parameters other than pH that weren't measured that influenced the fishes' decision to stay or leave.

Score - The experiment shows that pHs fluctuations of over 2 units centered around a pH of ~8.5, similar to the fluctuations measured in our case of interest, are not lethal to fish. However, the key differences of conditions in each scenario reduce the comparability of each situation to the other.

A.2.6.3. Stressor-Response Relationship in the Field —

Data Used Continuously monitored pH readings from USGS gage # 1668000 on the Rappahannock River from January 2006 to October 2006.

Analysis Calculated daily pH shift plotted against time (Figure A-11).

Discussion The Rappahannock River is being used here as an out-of-basin reference stream, though it should be noted that there is no solid evidence of similarities in geology, ecology, and water quality between this reference stream and the South Branch. That being said, this data shows pH fluctuations of even greater magnitude (1.5-2 units) than those seen on the South Branch and there were no reported kills there.

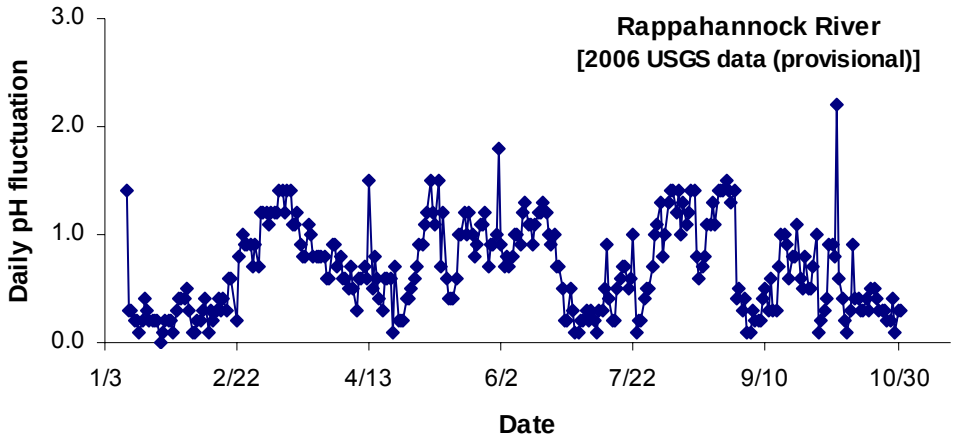

FIGURE A-11

A Plot of the Differences Between Daily Maximum and Minimum pH Values in the Rappahannock River, a Reference Stream. The shaded band covers the period of the kill in the South Branch.

Score - A differential in response relative to exposure to the candidate cause was observed at non-spatially linked sites, but the difference is not in the expected direction. The Rappahannock is a non-spatially linked site and shows greater pH fluctuation than the South Branch, but with no increase in fish kill occurrence (in fact, no occurrence of fish kills). This was not given a stronger negative score because of the weak similarities in conditions at each of the two sites, and therefore the low suitability of the Rappahannock as a reference site.

A.2.7. Mortality due to High Ammonia Concentrations.

A.2.7.1. Spatial/Temporal Co-occurrence (Specifically Temporal Co-occurrence) —

Data Used Ammonia sampled 5 times monthly by the West Virginia Department of Agriculture at random times of the month from October 2005-October 2006 at 11 sites on the South Branch of the Potomac River including: SB11 Petersburg, SB13 Potomac Valley View, SB15 below Moorefield, SB16 Old Fields Bridge, SB17 McNeil, SB18

Harrisons, SB19 Stony Run, SB20 Romney Bridge, SB22 Blues Beach Bridge, SB24 Blue Ford North, and SB26 at the mouth (Figure A-12).

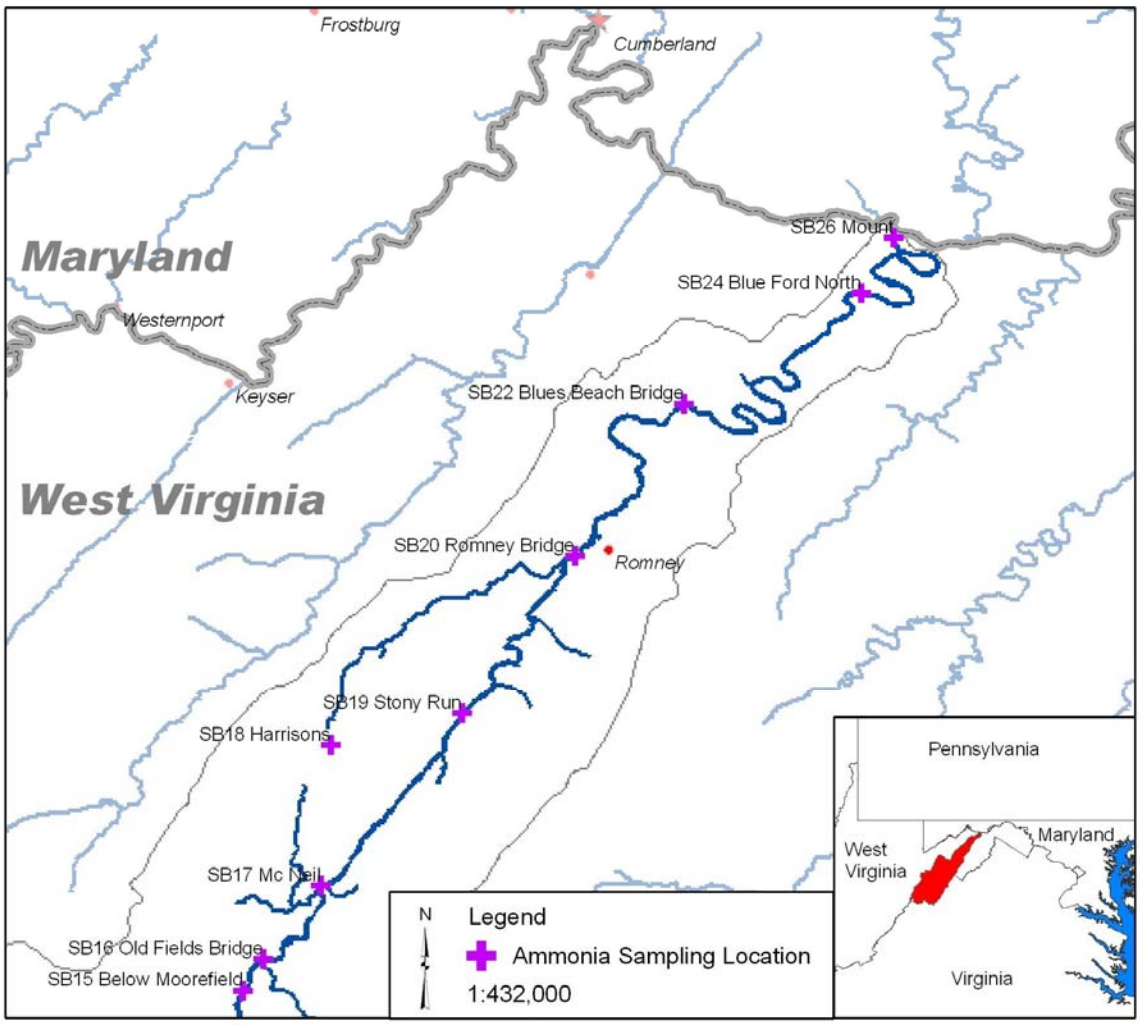

FIGURE A-12

Locations of Ammonia Sampling Stations in the South Branch of the Potomac River

Analysis Unionized ammonia concentrations were calculated from the total ammonia measurements using temperature and pH measurements taken at the time of sampling. Both unionized and total ammonia concentrations were plotted against time (Figure A-13 through A-23).

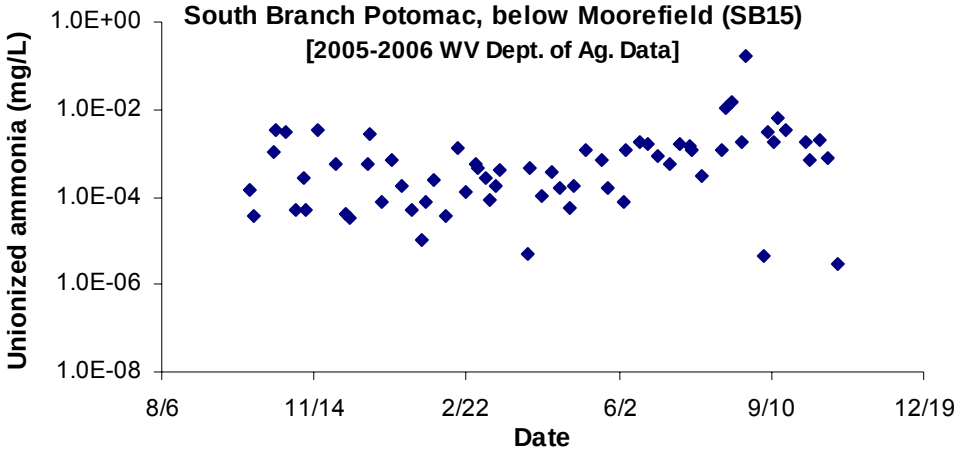

FIGURE A-13

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River Below Moorefield. The shaded band covers the period of the kill.

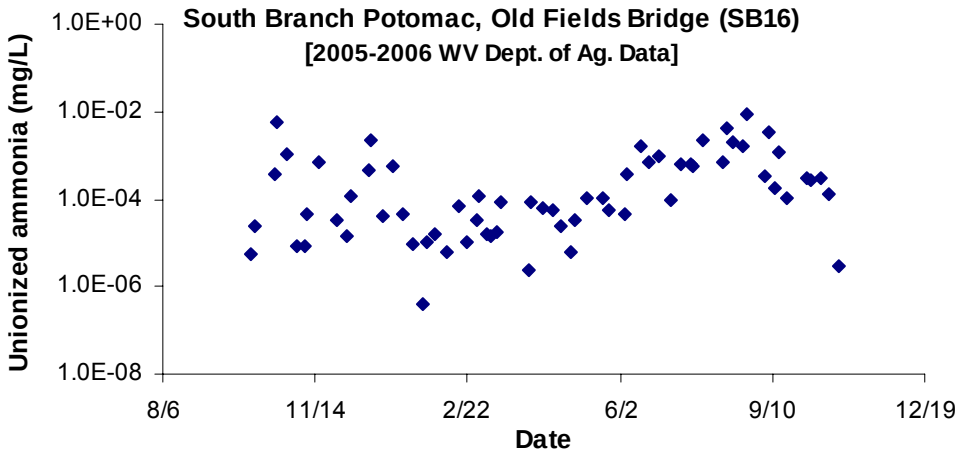

FIGURE A-14

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Old Fields Bridge. The shaded band covers the period of the kill.

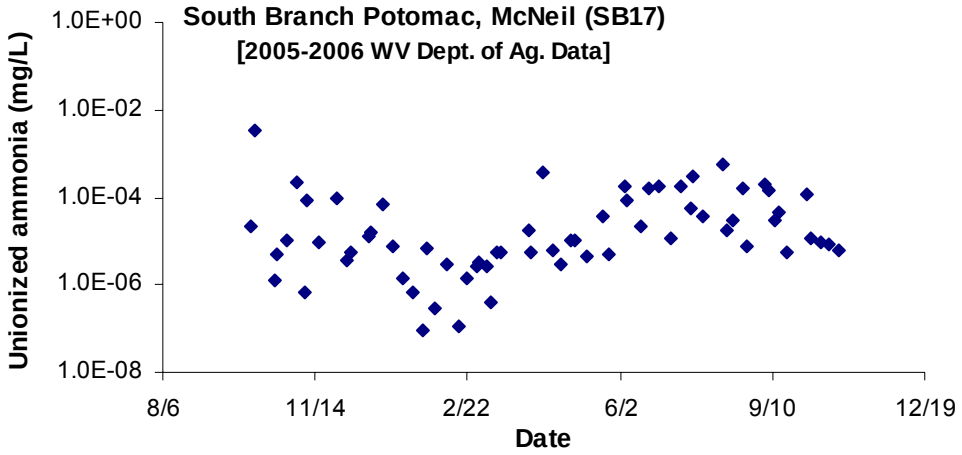

FIGURE A-15

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at McNeill. The shaded band covers the period of the kill.

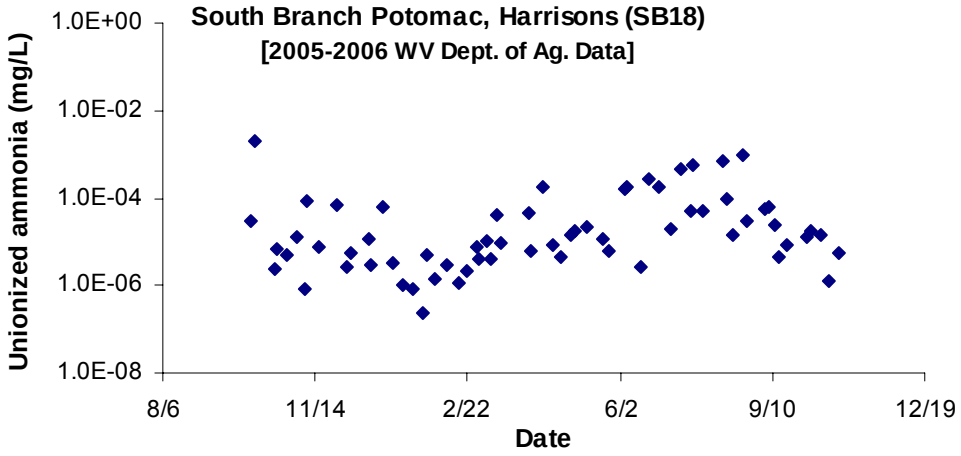

FIGURE A-16

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Harrisons. The shaded band covers the period of the kill.

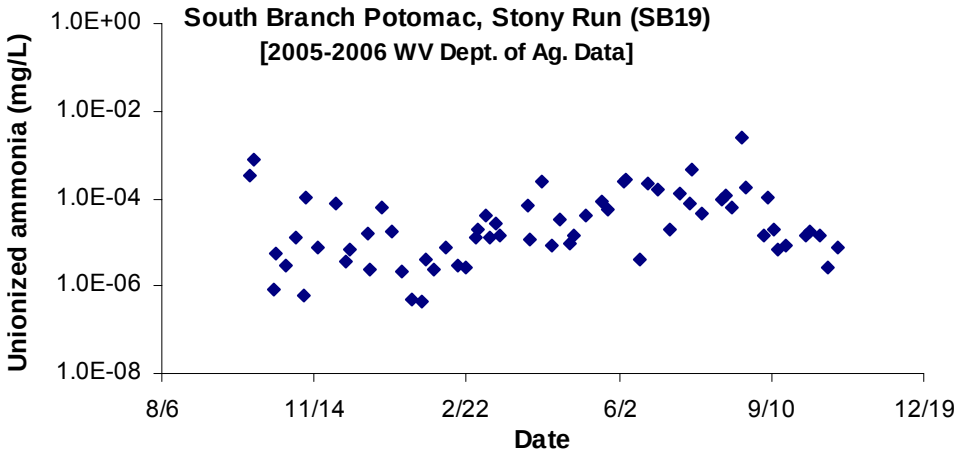

FIGURE A-17

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Stony Run. The shaded band covers the period of the kill.

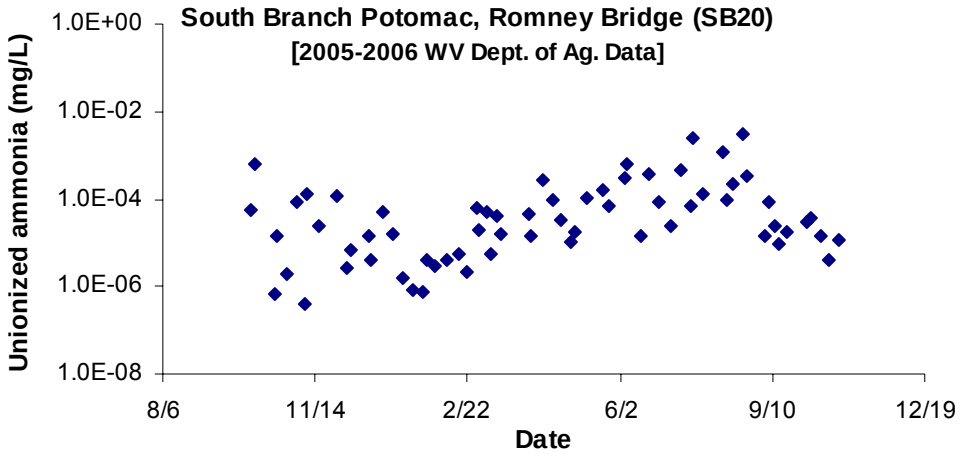

FIGURE A-18

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Romney Bridge. The shaded band covers the period of the kill.

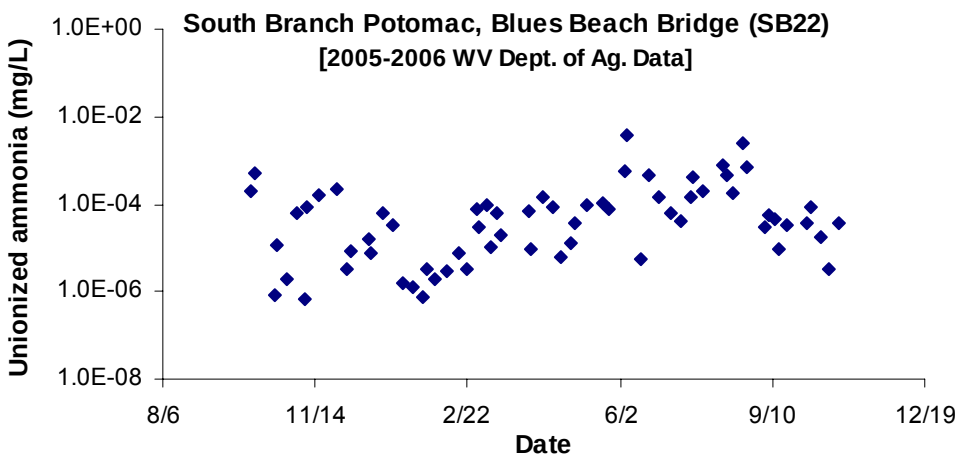

FIGURE A-19

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Blues Beach Bridge. The shaded band covers the period of the kill.

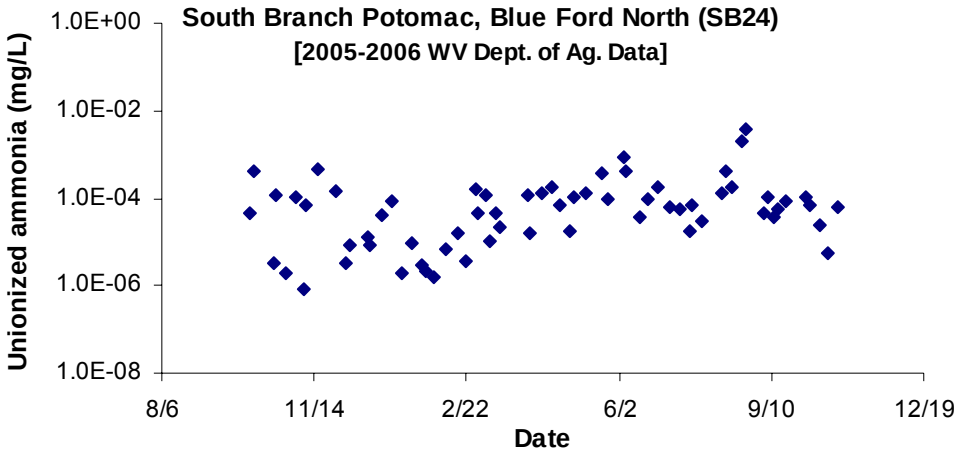

FIGURE A-20

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Blue Ford North. The shaded band covers the period of the kill.

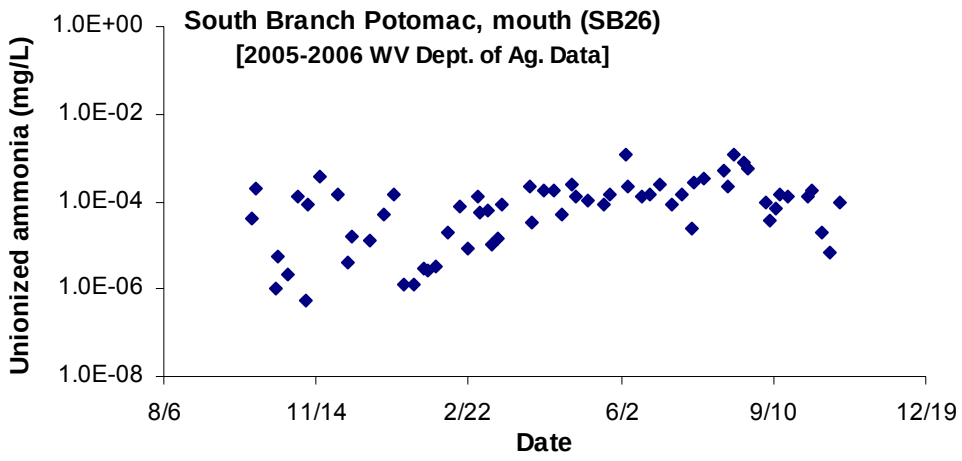

FIGURE A-21

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at its Mouth. The shaded band covers the period of the kill.

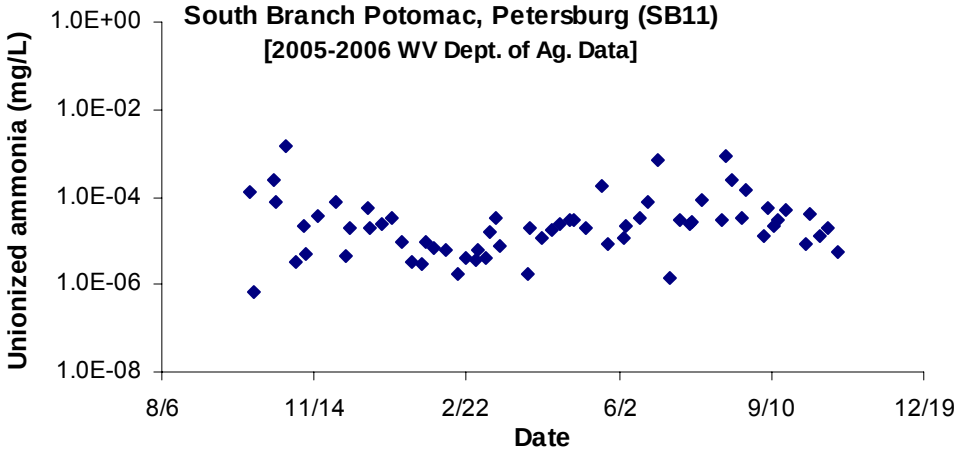

FIGURE A-22

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Petersburg. The shaded band covers the period of the kill.

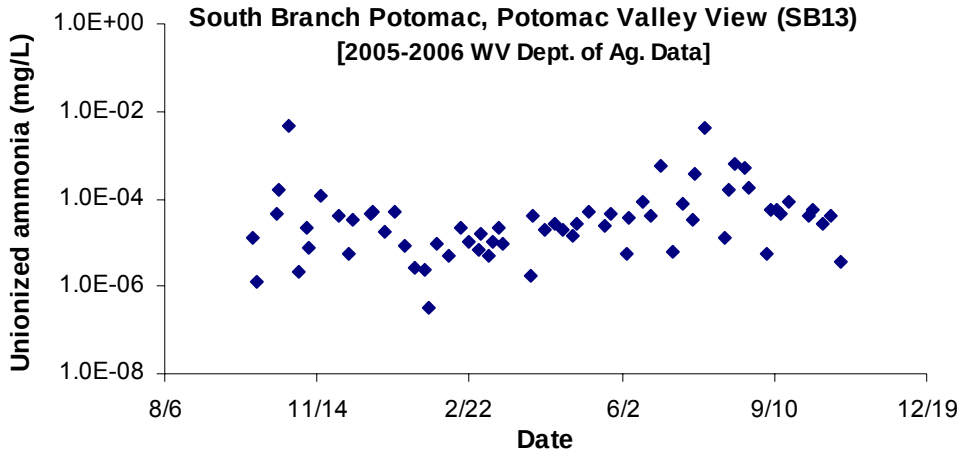

FIGURE A-23

A Plot of Unionized Ammonia Concentrations in the South Branch Potomac River at Potomac Valley View. The shaded band covers the period of the kill.

Discussion Unionized ammonia values were calculated since this is the most toxic form. The amount of total ammonia present as unionized ammonia increases with pH. Also, since pH fluctuates through the day, if NH_3 is sampled in the morning and pH is recorded then, we should expect pH to rise by the afternoon, with a concurrent rise in unionized NH_3 concentration. Therefore, we may not be recording maximum unionized NH_3 values. However, macrophytes, periphyton and phytoplankton take up ammonia during the day, and that process should also be considered. Hence, modeling of maximum unionized ammonia concentrations would require modeling rates of uptake as well as pH increases, which is not possible with available data and is beyond the scope of this workshop. Still, data taken at all sites within the kill region show the same trend of common unionized ammonia levels below 1μg/L with the highest levels often between 1-10 μg/L (Figure A-24). The highest level recorded in the kill zone was 170 μg/L of unionized ammonia observed at site SB 15 just below Moorefield, but this measurement was taken in August 2006. All sites in the kill zone had values below 5 μg/L during the kill. If unionized ammonia alone were responsible for the kill, we would expect to see the peak of ammonia concentrations corresponding temporally with the kill, which they do not.

Score --- The effect both does not occur when the candidate cause occurs, and does occur when the candidate cause does not occur.

A.2.7.2. Stressor-Response Relationship in the Field —

Data Used Frequent measurements of ammonia at sites upstream of the kill on the South Branch from October 2005 - October 2006. Rivers/sites sampled include the SB 11, at Petersburg, and SB 13 at Potomac Valley View.

Analysis Unionized ammonia levels are presented, calculated using pH and temperature data. These values were plotted against time.

Discussion While the data do show slightly lower levels of ammonia upstream of the kill zone (common levels between 0.005 μg/L and 0.5 μg/L) than those observed at Moorefield and below (common levels of 0.05 μg/L to 5 μg/L), if we look at the date of the kill specifically, we see that unionized ammonia levels upstream of the kill are within 1 order of magnitude of the levels observed at some sites within the kill zone. Also, when upstream sites' ammonia levels peak in July/August, they approach 5 μg/L, which is higher than some sites' levels within the kill zone during the kill, yet we see no kills upstream of Moorefield.

Score - Little effect gradient is observed relative to exposure to the candidate cause. The upstream reference sites here show similar ammonia concentrations to those downstream in the kill zone of the South Branch, but with no increase in fish kill occurrence (in fact, no occurrence of fish kills).

A.2.7.3. Stressor-Response Relationship in the Field —

Data Used Monitored natural ammonia levels on the Rappahannock are frequently as high as 0.6-0.9 mg/L. There have not been any reported kills there according to studies by Steve McIninch of Virginia Commonwealth University.

Analysis None – data are actually pending.

Discussion This evidence is currently only present as a verbal communication.

Score - A large difference in response was observed relative to exposure to the candidate cause, at non-spatially linked sites, but the difference is not in the expected direction. The reference stream shows higher ammonia concentrations than the South Branch, but no occurrence of fish kills.

A.2.7.4. Stressor-Response Relationship in the Field —

Data Used Mean ammonia measurements based on data from July 2004-September 2005, taken 5 times per month in 7 streams within West Virginia's Potomac watershed and neighboring watersheds (mean = 0.089 mg N/L, max = 9.46 mg N/L)—streams that did not experience kills (all WVDA sample sites) by the WV Department of Agriculture.

Analysis Mean ammonia values of 80-90 μg/L were selected from this period.

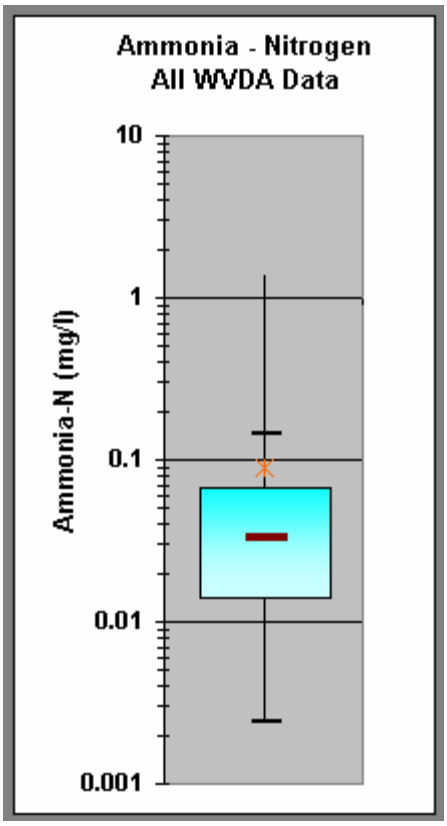

FIGURE A-24

A box and whisker plot of all concentrations of total ammonia nitrogen (Figures A-13 – A-23). The horizontal bars are the median and 95% confidence limits. The x is the mean and the box bounds the 25th-75th percentiles.

Discussion The mean level of total ammonia during the sampling year of July 2004 to September 2005 was ~80-90 μg/L of ammonia.

The average total ammonia level during the analogous kill period from 2005 is compared here to the max during the kill of 2006 under consideration. Although this is an average of many sites spread throughout this part of the state, if we consider this entire agricultural region as a representative reference, then we see that fish in these streams are tolerating higher levels of ammonia. To account for these measurements being total ammonia instead of unionized ammonia, we can estimate that under the worst conditions of pHs around 9 and temperatures around 20°C, these could represent unionized ammonia levels as high as 40-45 μg/L. On average though, if we look back at North Fork Shenandoah data, we see that unionized ammonia levels usually represent about $1/10^{th}$ the amount of total ammonia levels. Using this guideline, we might estimate that average unionized ammonia in all WVDA sampled streams is on the order of 8-9 μg/L, which is still higher than values seen in the zone of the South Branch kill during the 2006 kill. Since there was no kill during 2005, but ammonia values were still comparable if not higher than in 2006, this reference does not support ammonia alone being responsible for the 2006 kill.

Score - While the suitability of the entire WVDA sample set as a comparable reference is not proven, fish in these waters are clearly subject to ammonia levels at least as high as those seen during the 2006 kill in the South Branch with no resulting kill.

A.2.7.5. Stressor-Response Relationship from the Lab —

Data Used Journal articles include Milne et al. (2000) in *Environmental Toxicology and Chemistry*, Constable et al. (2003) in *Human and Ecological Risk Assessment* addressing Canadian fish, amphibian, and invertebrate species' exposure to ammonia and finally the U.S. EPA (1999) criteria document's section on ammonia exposures for various species. Our exposure data come from ammonia levels measured in the South Branch during 2006.

Analysis Literature results were compared to ammonia levels reported above from the South Branch.

Discussion In general, all reported NH_3 thresholds for lethal or even sublethal toxic effects were at least 10 times higher than what we've seen in the South Branch. For example, Milne et al. reported needing ~400 μg/L unionized ammonia to show significant lethality over a 24 hour exposure, ~750 μg/L over a 6 hour exposure, and ~850 μg/L for a 1 hour exposure in rainbow trout. In longer term pulsed exposure experiments of up to 6 weeks in duration, ~200 μg/L of unionized ammonia were needed to cause sub-lethal effects assessed as "very sever gill damage." Milne also noted the trend that increased exposure frequency is more significant than increased total concentration of ammonia in terms of effects on fish. Constable et al. reported an LC_{50} of ~1.3 mg/L of unionized ammonia for acute exposure in fathead minnows. The U.S. EPA (1999) criteria document reports EC_{20}s of 4.79 mg/L of total ammonia at pH 8 for white sucker, 1.35 and 2.8 mg/L ammonia for bluegill, and a geometric mean of 4.56 mg/L total ammonia at pH 8 for smallmouth bass. The document also reports an LC_{50} of 50 mg/L of total ammonia during an acute exposure test in fathead minnow. The highest values in the South Branch were ~2 μg/L or less of unionized ammonia during the kill, reaching a maximum for the sampling period of ~170 μg/L of unionized NH_3, but only after the kill had stopped. It is definitely worth noting that none of these lab tests exposed the fish to elevated or pulsed levels of ammonia for as long a period as our fish may have been. Indeed, it would be very impractical to reproduce those conditions. However, we're also comparing the peak concentrations of ammonia observed on the South Branch with reported lethal and sub-lethal levels from the literature. So even if these observed levels approach those shown to cause effects in fish in the literature, we should remember that ammonia levels fall far below this in between peaks and so the average ammonia concentration the fish are exposed to as a sustained exposure are considerably lower and definitely comparable with observed levels in reference streams without kills.

Score If ammonia alone were responsible for the kills, we would expect the levels recorded in the South Branch to be more similar to those shown to have killed fish in lab tests.

A.2.8. Candidate Cause: Unspecified Toxics.

A.2.8.1. Spatial-Temporal Co-occurrence —

Data Used WVDEP Water chemistry July/August 2006
USGS water column parameters
WV Long term site (6 years) metal, nutrients, etc…
6 sites continuous pH/CON/DO January 2007.

Analysis NA.

Discussion Barium and arsenic were detected in the water column in 2006. No other hits were observed in 2006 for toxic metals. Organics, pesticides, and pharmaceuticals were not analyzed.

Barium in the water column but not fish tissue samples. Wirts is reviewing other datasets to determine extent of the Barium problem—between 18 and 64 μg/L were detected—detected at all 24/40 sites (July-August 2006 sampling index period).

Table A-1 presents the results of analyses of various media collected in the North Fork and South Branch. The utility of the data is limited, because they are not temporally and spatially relevant to the fish kill events. The table should, however, be reviewed to determine similarities and differences between the two watersheds to help design future studies by identifying contaminants that should be monitored.

Score No Evidence, NE. Too few toxics were analyzed in the data sets.

A.2.8.2. Evidence of Exposure or Biological Mechanisms —

Data Used
Wells fish tissue (2005)
Fish tissue data
USGS Blood plasma
See Table A-1.

Analysis NA.

Discussion Data from 2005 or earlier. West Virginia provided fish tissue data (2005) verbally from Martha Wells via Pat Campbell along with fatty acid profiles (indicator of how well an immune system is functioning). Sample size = 12 fish; whole fish tissue analyses. Fewer lipids is taken to be an indicator of stress. The monitoring data (Table 1) provide clues to be investigated with respect to future kills

Score NE, The available data do not constitute evidence that can be interpreted in terms of exposure to a lethal contaminant.

A.2.8.3. Complete Causal Pathway —

Data Used Locations of potential sources constitute evidence of potential causal pathways.

National Pollution Discharge Elimination System (NPDES) discharges provide evidence of sources of waste water (Figure A-25).

Industrial chemicals used in the watershed are indicated by the Toxics Release Inventory.

Discussion Sources are summarized in Table A-6.

National Pollution Discharge Elimination System (NPDES) discharges constitute evidence of potentially toxic chemicals. Although discharge permits do not allow know toxic discharges, unknown discharge constituents or unreported exceedances of permitted levels may result in toxic exposures.

The Toxics Release Inventory data suggest other possible water pollutants. See Tables A-3, A-4 and A-5 for 2005 releases.

The USGS document on the background of the South Branch Potomac River (USGS SPMD/POCIS) (landuse, population, industry, geology, etc.) provided the information on land use as an indicator of potential sources:

Population data for the Communities of Morefield (2500) and Petersburg (2200)
Romney (1940); related these sites to the fish kill locations
12 river miles between Petersburg and Morefield

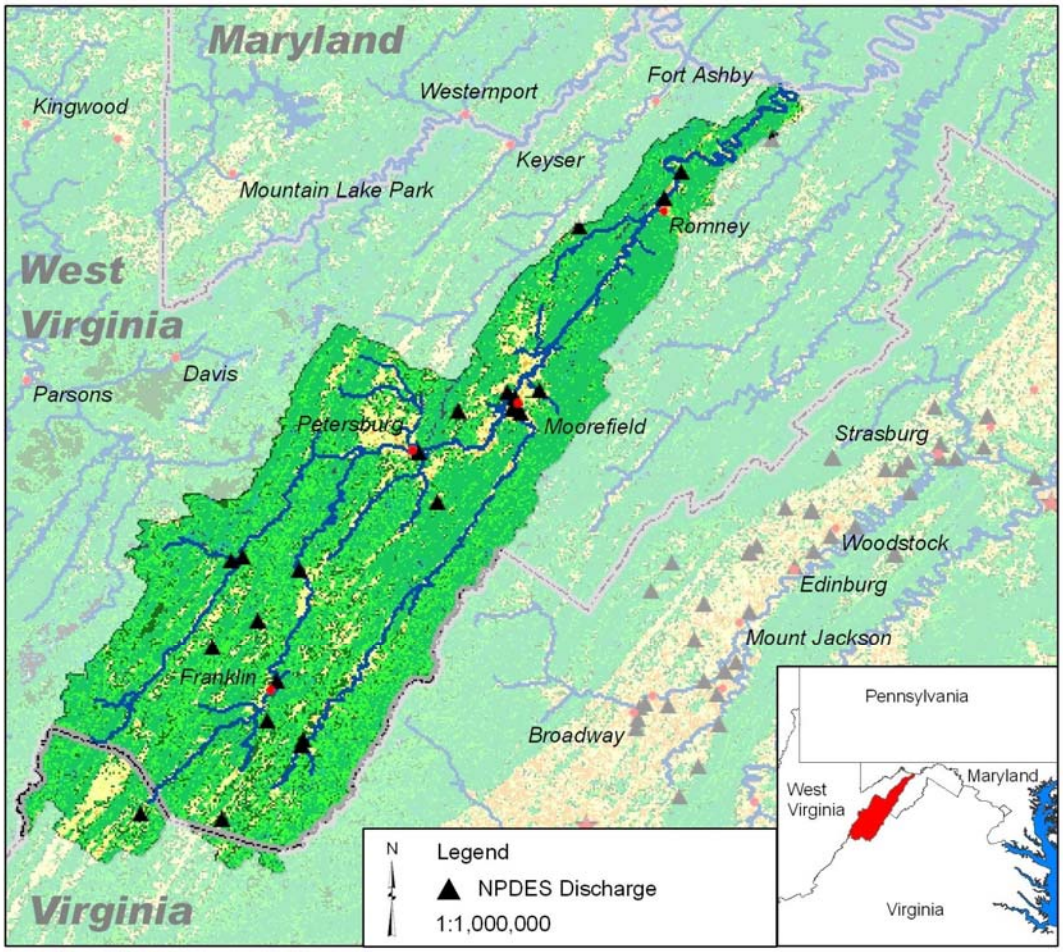

FIGURE A-25

Locations of All Permitted Waste-water Discharges on the South Branch Potomac River

TABLE A-6

Potential Sources of Toxic Substances in South Branch Potomac

Source	Potential Toxic stressors
Poultry farms, poultry processing plants	Pesticides, antibiotics, cleaners, BOD
Agriculture	Pesticides, metals
Highways	PAHs, metals
POTWS	Pesticides, pharmaceuticals, PAHs, metals
Accidental/illegal dumping	Any
Golf courses	Pesticides

Poultry processing plant located in the town of Morefield at mouth of South Fork of South Branch; NPDES outfall within 1 mile of Morefield POTW outfall
Spring Run Hatchery located upstream of Petersburg on South Fork of Mill Run of South Branch

Livestock numbers (cattle and chickens) have declined in the counties around the South Branch PR. Chicken processing houses may ship their wastes via pretreatment plants to POTW.

Score +, At least some steps of the causal pathway are present in the watershed.

A.2.8.4. Tests of Media in Laboratory

Data Used USEPA toxicity tests of South Branch media (Amy B.).

Analysis NA.

Discussion Toxicity tests were performed on media from the South Branch Potomac, and the Cowpasture River as an undisturbed reference site. Fathead minnows were tested. Results were equivocal, with some observed toxicity, but toxicity was similar in the reference river as in the SB Potomac. The samples for the toxicity tests were taken after the fish kills had occurred.

Score NE, No evidence, because the toxicity tests were on post-kill media.

A.2.8.5. Stressor-Response from Other Field Studies

Data Used NA.

Analysis NA.

Discussion Although similar fish kills have been previously observed in the other rivers of the region, pathology data from those kills is not available, and no causes have been identified for the other kills.

Score NE No relevant evidence.

A.2.8.6. Stressor-Response from Laboratory Studies —

NE, No evidence, because aqueous concentrations at the time of the kill are not available for comparison to laboratory toxicity studies.

A.2.8.7. Analogous Cases —

Data Used None.

Analysis NA.

Discussion Prior kills in the South Branch could be analyzed as analogous cases, but data for chemical exposures from those kills are lacking.

Score NE. No data from analogous kills were identified.

A.2.9. Starvation.

A.2.9.1. Spatial-Temporal Co-occurrence —

Data Used Observations of killed suckers by professional fish biologists.

Analysis NA.

Discussion Very low weight relative to length is considered the measure of starvation, not just a symptom. Professional observations of dead suckers indicated that the killed fish were not different from healthy fish in terms of overall size and weight.

Score - - - Convincingly weakens the case, because the candidate cause does not occur.

A.2.9.2. Evidence of Exposure or Biological Mechanisms —

N E. No evidence exists on food availability or stomach contents of fish in the 2006 fish kills.

A.2.9.3. Complete Causal Pathway —

Data Used WVDEP WAP database on benthic macroinvertebrates in South Branch.

Analysis NA.

Discussion Benthic macroinvertebrate data on the SB Potomac indicated a normal to very good benthic community (WVSCI Scores 62-84) with normal abundance.

Score - Somewhat weakens, because there is at least one missing step.

APPENDIX B
DATA NEEDS

This appendix presents the results of discussions of data needs during the workshop.

B.1. GENERAL CATEGORIES

B.1.1. Monitoring Data.

- Chemical Monitoring
  - Continuous Chemical Sampling
    - In the Potomac, Shenandoah and reference rivers, and at locations of kill events.
    - Parameters should include pH, temp, conductivity, and TSS.
  - Grab samples for flow events
    - Particularly need NH_3 levels in high flow, pulse events
  - Investigate spatial variability for pH, temperature, and conductivity
- Biological Monitoring
  - Fish Health & Condition Data
    - Field samples collection protocols
      - Whole fish
      - Blood
      - Select tissues
      - Field data collection recording sheet to accompany
    - Archive protocols
      - Frozen
      - Formalin
      - Sample release protocol
        - Only release the minimum needed to do assay
      - Data base to maintain log of samples and information about the sample such as date collected, GPS coordinates, from mortality event
    - Spatial Sampling protocol
      - Including standardized reference site
      - Sample sizes for base line and kill events.
    - Health Data
      - Behavior of organisms (location in stream)
        - Home range (nests, etc.)
      - Gross pathology
      - Histopathology
        - formalin-splice piece of organ
        - healthy + sick/dead fish
      - Parasitology
      - NH_3 levels in fish (blood)

- Fish population statistics
  - General population structures
    - Periphyton
    - Snails
  - Site selection
    - Fish kills
    - Also reference data
  - Spatial & temporal variability
- Fish community assessment

B.1.2. Literature Reviews.

- Susceptibility
  - Mechanisms
    - Over wintering stress
    - Spawning stress (driven by temperature and photoperiod)
      - Smallmouth Bass
        - (temp related, wide window, work 2 months)
      - Suckers
        - Long spawning seasons (earlier)

B.1.3. Toxin Source Information.

- Point and non-point sources
  - Toxins- priority list
  - Seasonality, pulse, etc…
    - High volume vs. continuous
- Agricultural practices & timing
- TRI data
- Water and sediment profiles

B.1.4. Laboratory Toxicity Tests (including TIE manipulations).

- On samples collected during storm events
- In different seasons

B.1.5. Manipulation of Exposure.

- Field experiments are not feasible

B.1.6. Verified Predictions.

- Periphyton: Algal changes drive changes in pH, these are light + flow limited
- Autointoxication – Consider time lags: can be 2 weeks after change in pH for auto intoxication cycle to reach build up of ammonia for to clinical effect to be seen

B.1.7. Simulation Models.

- Potentially do simulation model using Aquatox

B.2. CANDIDATE CAUSE-SPECIFIC DATA NEEDS

B.2.1. Unspecified Toxic Substances.

- Basic work-up: flows, seasonal variations
- Know chemical compounds list- comparisons
- Sediment sampling
- During spawning can sample spawning beds, include sampling finer particles down in beds (habitats, nests)
- Storm event sampling
  - Include seasonal sampling
- Potential sources of contaminants – "Hit List"- Upfront analysis- narrow down
  - Water column
  - Tissues, whole body analysis
    - bio-accumulated chemicals
    - non-accumulated chemicals
  - Sediment
  - Passive samplers
  - Characteristic responses in fish
- Land use – spatial relation to affected fish
  - chemical
  - agricultural

- TRI plus MSDS-
  - pKa's degradation
  - general property of chemicals
  - total tons discharged
- Sewage discharge (average)
- Poultry waste amount

B.2.2. Hypoxia.

- Blood ammonia
- Ammonia in Water – at run off event
  - Increases not observed in continuous monitoring.
  - Fish can undergo the auto intoxication cycle which can delay clinical effects up to 3 weeks.
  - Criteria for reference site – temporal
    - Fish population, geology, size, water quality, other aquatic based species, invasive species
  - pH
  - Temperature
    - Both air and water
    - Cheap and continuous
  - TSS / Turbidity Probes
    - Conductivity

- Weather
    - Storm Flow Events
- Periphyton

B.3. ROUND ROBIN-KEY PARAMETER/NEED

Each participant was asked to identify their highest priority data need. Data needs have been roughly grouped by theme. Data needs receiving multiple votes are noted in parentheses.

- **Total fish diagnostics (5 votes).**
    - Baseline of what's normal, blood and tissue
    - Samples from sites with and without kill for comparison to baseline
    - Build archive of above
    - Blood ammonia levels
    - VHS diagnostic on frozen fish and new fresh samples
    - Archive of sediment, water, fish tissue etc. samples.
- **Baseline of fish community conditions and structure (2 votes)**
- **Broader geographic fish kill assessment (3 votes):**
    - When a fish kill occurs, look more broadly within the Potomac River Watershed, tribs and mainstems).
    - Spatial extent assessment of the fish kills
- **Spatial Concerns**
    - Consider combining the VA and WVA efforts—remove state boundaries
    - Centralized database of info for both WV and VA research
- **Toxins**
    - Toxic chemical hit list screen
    - Inventory of unspecified toxics list based on TRI and historic state/USGS studies plus known sources.
    - Metal concentrations in gills
- **Temporal Concerns**
    - Continuous monitoring data: site with kills and reference sites
    - Concurrent collection of all needed info to interpret fish kill
    - Time windows for sampling
    - Appropriate time data collection related to fish kills
    - Rapid-response kits.
- **Algae**
    - Algae: Planktonic sampling for ID after storm events
        - Community structure
        - Spatial distribution
        - Role in pH issue
        - (Paul McCormick research on North Fork—periphyton research at 17 sites)

- Toxic algal blooms—the possibility need investigated.

➢ **Virus**

- Species jump between poultry and fish—possible?

➢ **Other Laboratory Work**

- Conduct laboratory exposures of pH/NH_3 using fish of concern