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DEPARTMENT OF ENVIRONMENTAL
PROTECTION

Causal Analysis of the Smallmouth Bass decline in the Susquehanna and Juniata Rivers

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The following individuals were unable to attend the workshops; however, each gave valuable presentations remotely during a workshop.

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Executive Summary

Beginning in 2005, large die offs of young-of-year (YOY) Smallmouth Bass (SMB) and associated bacterial infections were observed throughout the Susquehanna Basin which resulted in reduced abundance of adult SMB at the middle Susquehanna River (Sunbury to York Haven) and lower Juniata River (Port Royal to the confluence with Susquehanna River). Fish mortality due to disease, predation, angling injuries, and senescence is common. However, prior to 2005, no substantial mortality events were noted among YOY SMB at the Susquehanna River. Additionally, the population size and age structure of adult SMB during pre-2005 surveys conducted by the Pennsylvania Fish and Boat Commission (PFBC) were substantially different than post-2005 surveys, suggesting a change in population characteristics resulting from reduced YOY recruitment.

In 2014, the Pennsylvania Department of Environmental Protection (PADEP) requested assistance from the United States Environmental Protection Agency (EPA) in identifying the causes of the SMB declines on the Susquehanna River. EPA's stressor identification process, as described on the Causal Analysis/Diagnosis Decision Information System website (CADDIS (www.epa.gov/caddis)) identifies the cause(s) of an environmental problem by determining which of a set of alternative candidate causes is best supported by the body of evidence. The CADDIS process was chosen because it provides transparency and reduces bias without restricting the types of evidence used. It provides a framework for engaging experts and different sources of data together in order to identify one or more likely causes of an environmental problem. This process will assist PADEP in developing appropriate programmatic strategies that will result in an improved fishery. However, it is important to note that the CADDIS process and subsequent report are not the Susquehanna River water quality assessment process for determining impairments under section 303(d) of the Federal Clean Water Act.

In cooperation with the PFBC, PADEP and EPA scheduled three workshops that included representatives from various State, Interstate, Federal, and academic organizations including, the Susquehanna River Basin Commission (SRBC), United States Geological Survey (USGS), United States Fish and Wildlife Service (USFWS), and Susquehanna River Heartland Coalition for Environmental Studies (SRHCES). These workshops were the framework used to make a preliminary determination for the cause(s) of the case, detailed below.

The case was defined as a decrease in abundance of SMB as a result of poor recruitment into the adult SMB population. The temporal frame of the effect was established as 2005 to the present, since 2005 was the initial year that the decline was recorded. However, some longer term trend data were reviewed in order to elucidate the relationship between several candidate causes and the effect. The geographic range or study area was identified as the Susquehanna River from Sunbury to York Haven and the Juniata River from Port Royal to the mouth. These were the reaches identified to have a decrease in SMB abundance. Comparison sites were selected to represent streams with stable and sufficiently robust SMB populations without documented instances of SMB population decline. Some examples include the Allegheny River at Franklin, Delaware River at Morrisville, upper Juniata River, upper Susquehanna River, lower West Branch Susquehanna River (Lewisburg), Pine Creek (Lycoming Co.), and Loyalsock Creek. Although there have been confirmed instances of disease at comparison sites, SMB populations have not declined in abundance or experienced changes in length and age structure.

After the case is defined, the CADDIS process identifies “candidate causes” and “causal pathways.” Candidate causes are in-stream stressors which may be directly responsible for the observed biological effects. For example, high ammonia concentrations can be toxic to fish and cause overt mortality. Other candidate causes, such as low dissolved oxygen or high temperature can cause direct mortality, or stress leading to disease. Causal pathways describe the interim steps by which human activities, sources and in-stream ecological processes result in a candidate cause. For example, increased nutrient loads are part of a multiple-step causal pathway that can produce stressors that harm fish. Increased nutrient loading (nitrogen and phosphorus) associated with land use and stormwater runoff can increase algal growth. The algae photosynthesize and respire, increasing the range of daily swings in pH and dissolved oxygen levels. Very high pH and low dissolved oxygen levels harm fish. In the CADDIS process, nutrient loadings are considered as part of the causal pathway leading to the candidate causes of low dissolved oxygen and high pH levels.

A total of 14 potential candidate causes were initially identified for the decline in SMB recruitment during the first CADDIS workshop. Two mechanisms by which candidate causes could decrease SMB recruitment were considered throughout the process: direct mortality and increased susceptibility to disease (e.g., high water temperature causing direct mortality vs high water temperature causing increased disease). As the process continued and familiarity with the data improved, several of the candidate causes were subdivided to address multiple mechanisms that were not originally considered. Over 50 worksheets (data and associated analyses) consisting of almost 400 pages of information were used in this evaluation (Appendix B). Each worksheet was evaluated and scored using data available at the time of the third workshop. Each candidate cause was classified as whether it was **Likely**, **Unlikely**, or **Uncertain** that it was contributing to the reduction in SMB recruitment. As more data are collected, conclusions made in this report are understood to be dynamic.

List of candidate causes:

1. High Flows
2. Intraspecific Competition (Competition within the SMB species)
3. Interspecific Competition (Competition with other species, possibly invasive species)
4. YOY Food quality
5. Egg Quality
6. YOY Habitat Degradation
7. High Temperature
8. High pH
9. Low Dissolved Oxygen
10. High Ammonia
11. Algal and Bacterial Toxins
12. Toxic Chemicals: Pesticides/ Polychlorinated Biphenyls (PCBs)/Metals
13. Toxic Chemicals: Herbicides/Endocrine Disrupting Compounds (EDCs)
14. Pathogens and Parasites

The CADDIS process narrowed the scope of concerns that may be affecting SMB populations in the Susquehanna and Juniata Rivers. Based on all available evidence, eight candidate causes were not supported by the data analysis and were considered Unlikely for directly causing the decline of YOY SMB recruitment. The evidence for eight additional candidate causes was judged to be Uncertain. The CADDIS process identified two candidate causes as Likely for the decline in recruitment of YOY SMB into the adult population. However, it is noted that the causal pathways are very important to understanding the modifying effects of other factors not listed as candidate causes. These pathways are analyzed and discussed in the report.

Candidate cause classifications:

Candidate Cause #	Candidate Cause Name

	Likely Causes
13	EDCs and herbicides[1]
14	Pathogens & Parasites with other stressors[2]
	Unlikely Causes
1	High Flows
2	Intraspecific Competition
4	YOY Food Quality: fatty acids
7	Temperature – direct mortality
8	pH
9	Dissolved Oxygen – direct mortality
10	Ammonia (NH_3)
12	Toxic Chemicals: pesticides/PCBs/metals
	Uncertain Causes
3	Interspecific Competition
4	YOY Food Quality: thiaminase
5	Egg Quality
6	YOY Habitat
7	Temperature – increased disease
9	Dissolved Oxygen – increased disease
11	Algal & Bacterial Toxins
14	Pathogens & Parasites alone[2]

[1] The evidence available for herbicides was limited and future monitoring is planned to obtain more data.

[2] The workshop participants concluded that pathogens and parasites were likely interacting with other candidate causes in order to produce the disease. It is uncertain whether they would be capable of doing so alone.

It is critical to note that this report only serves to provide information on the current state of data collection and conclusions related to the SMB decline. There are many data that have yet to be analyzed and interpreted which may affect any final conclusions. Consequently, many sub-lethal, complex interaction hypotheses were not fully developed and analyzed in the CADDIS process. Research on this topic is on-going and will continue for the foreseeable future.

Despite these challenges, this report represents a large amount of work from many dedicated professionals across multiple agencies and organizations. It is the compilation of the current understanding as it relates to the SMB population decline in the Susquehanna and Juniata Rivers and will serve as the foundation for continued research. This report also serves to provide greater transparency on what work has been completed thus far.

One of the most important next steps is to identify factors contributing to immunosuppression, and increased pathogen and parasite abundance. The possible increase of intermediate hosts and other changes within the biological community are critical factors that will be investigated. Potential interactions among physicochemical parameters, nutrients, emerging contaminants and their relationship to SMB YOY need to be clarified. The results of the CADDIS process will lead to more focused action with the goal of increasing recruitment to restore abundance of SMB.

Introduction

Smallmouth Bass *Micropterus dolomieu* (SMB) were introduced to the Susquehanna River watershed from the Potomac River watershed in 1869 (Milner 1874, cited in Bielo 1963) or 1870 (Bean 1892) and were well established within three years (Bean 1892, Meehan 1893). Throughout the Susquehanna River and its larger tributaries SMB angling has been a popular recreational activity, with 65% of angler catch comprised of Smallmouth Bass in a survey of the lower Juniata and middle Susquehanna rivers in 2007 (Smucker et al. 2010). Estimates of economic contribution related to sport fishing on these reaches exceeded $3.35 million in 2007, making the abundance of this species important economically (Martin 2010). Growing concern over the health and abundance of SMB has spurred an unprecedented amount of research and public interest.

The Susquehanna River originates from Otsego Lake in Cooperstown, New York and flows south through Pennsylvania and Maryland to the Chesapeake Bay. The Susquehanna River drains approximately 71,000 km^2 and is the largest source of fresh water to the Chesapeake Bay (Brown et al. 2005). Most of the Chesapeake Bay and tidal tributaries have been declared impaired by EPA and on December 29, 2010, EPA established the Chesapeake Bay Total Maximum Daily Load (TMDL) for nitrogen, phosphorus, and sediment that includes the Susquehanna River. Consequently, the Susquehanna River has received a large amount of attention concerning nutrient and sediment transport.

This report addresses numerous issues surrounding SMB in the Susquehanna River watershed, and begins to refine the scope of future work. It is critical to note that this report only serves to provide information on the current state of data collection and conclusions related to the SMB decline and disease. There are many data that have yet to be analyzed and interpreted that may affect any final conclusions. Research on this topic is on-going and will continue for the foreseeable future.

Population Decline

Reproductive success of adult SMB and recruitment of young-of-year (YOY) to the adult population vary annually throughout their range due to various environmental factors (Blazer et al. 2007, 2010, Funk and Fleener 1974, Lukas and Orth 1995, Smith et al. 2005, Wrenn 1980). Natural causes such as disease, predation, and senescence are components of total mortality, as well as mortality attributable to angling. Prior to 2005, no substantial disease-related YOY SMB mortality events were documented in the Susquehanna River, but beginning in 2005, dead and dying YOY SMB were observed particularly in the middle Susquehanna (between Sunbury and York Haven, Pennsylvania). Due to poor year classes ostensibly attributable to disease/mortality first documented in 2005 and subsequent low recruitment to the adult population, Catch per unit effort (CPUE) for both adult and YOY SMB have decreased during the post-2005 era (Figures 4 & 5). In addition, the size structure of the adult SMB population documented during pre-2005 surveys was noticeably different than during post-2005 surveys (Figure 1). Specifically, the proportional stock density (PSD) of SMB, a measure of balance in freshwater fish populations which compares the proportions of larger, quality length fish (280 mm TL) to stock length fish (180 mm TL; Anderson and Weithman 1978), increased in the post-2005 time period (Figure 2). Pre-2005 PSD values for the middle Susquehanna River were considered optimal at approximately 30 – 40% (Weiss-Glanz and Stanley 1984); post-2005 PSD values for the middle Susquehanna River increased to 35% - 65% (Smith et al. 2015). The post-2005 PSD suggested that there were a limited number of smaller or younger fish in the population and that rates of reproduction, growth, and mortality were no longer reflective of a healthy and stable population.

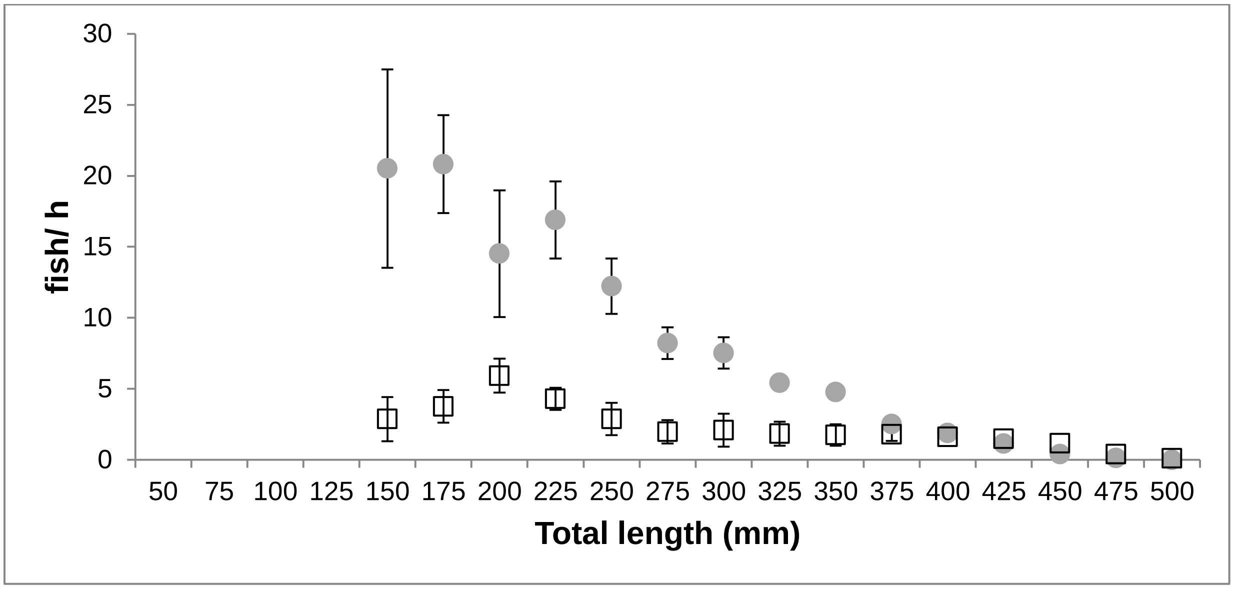

Figure 1. Comparison of length-frequency distributions of adult (age-1 and older) Smallmouth Bass caught during nighttime boat electrofishing surveys at the middle Susquehanna River pre-2005 (i.e., from 1990-2004 filled circles) and post-2005 (i.e., 2005-2014, open squares). Each marker indicated mean catch per unit effort (CPUE: fish/h) for the time period with error bars indicating one standard deviation.

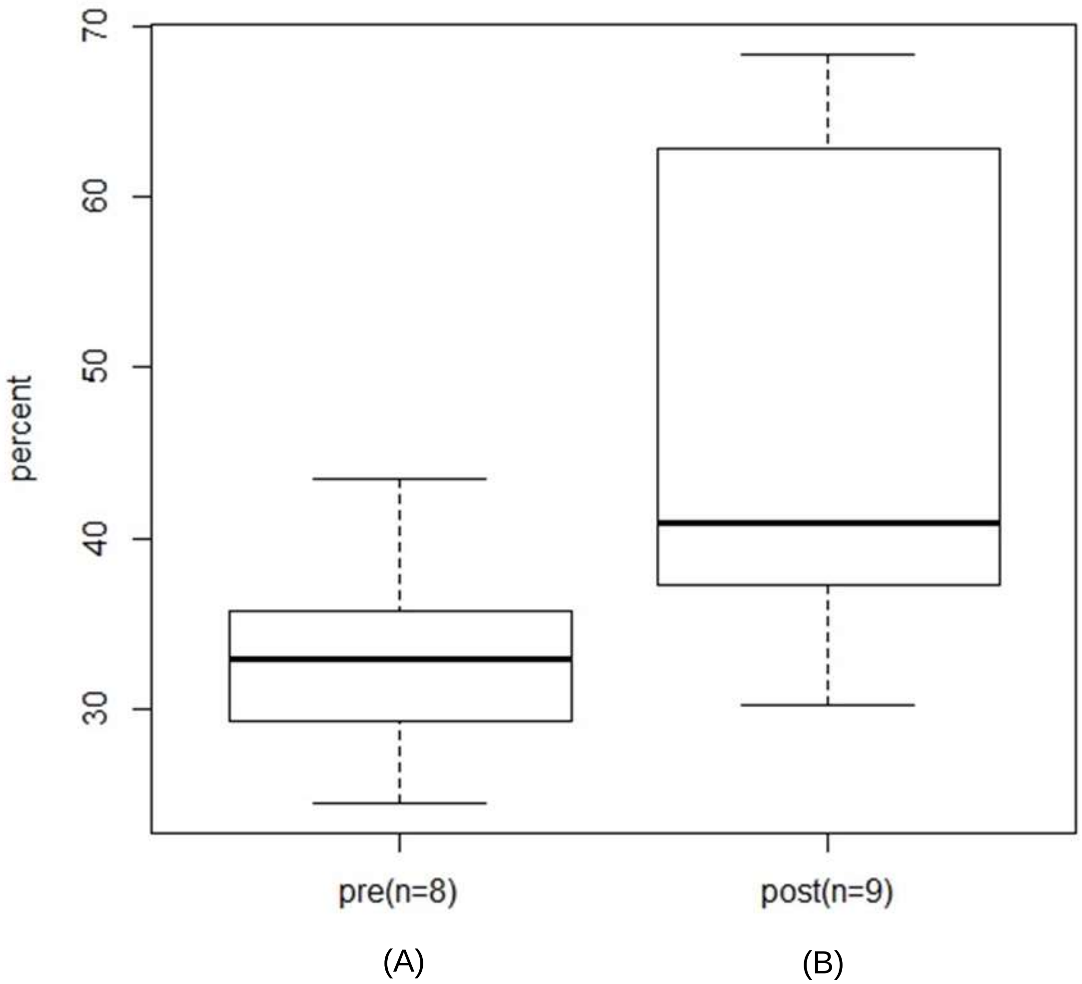

Figure 2. Comparison of proportional stock density (PSD: percent) of Smallmouth Bass prior to (A, 1995-2004) and following (B, 2005-2014) disease outbreaks caught during nighttime boat electrofishing surveys (n = surveyed years) at the middle Susquehanna River (between Sunbury and York Haven, Pennsylvania). Bold line indicates mean PSD value for test period, narrow lines indicate upper and lower quartiles and whiskers denote minima and maxima for the test periods.

Additional Concerns

Disease outbreaks among YOY SMB have occurred annually since 2005 with varying degrees of severity at the West Branch Susquehanna River, Susquehanna River and Juniata River. Fish were typically observed swimming weakly near the surface with noticeable white lesions, sores, eroded fins, and some were found dead. The outbreaks appeared to be limited to SMB; observations of disease of this severity were not (and have not subsequently) reported for other species. Six public meetings have been held to discuss this issue since 2006 and more restrictive fishing regulations have been enacted to reduce fishing mortality.

In September 2007, the Susquehanna River Technical Committee (Committee); composed of representatives from the PFBC, PADEP, USGS, USEPA, and SRBC was formed and met for the first time. The Committee’s first order of business was to identify existing data, determine what data were needed, and develop recommendations for future action. Pathological analysis indicated that secondary bacterial infections by opportunistic bacteria (Chaplin et al. 2009, Chaplin and Crawford 2012, Starliper et al. 2013, 2014, C. Yamashita, Pennsylvania Fish and Boat Commission, unpublished data) were potentially responsible for observed lesions and mortality.

In 2007, a large scale filamentous algal bloom was observed during late summer. In response, PFBC began taking around-the-clock discrete dissolved oxygen (DO) measurements in select habitats on the Susquehanna River and noted low DO concentrations during the early morning period (PFBC, unpublished data). As a result, initial data needs that were identified included a better characterization of temperature and DO concentrations in critical reaches of the Susquehanna River and a determination of whether stressful conditions existed in near-shore YOY nursery areas. Water quality investigations conducted by the USGS Pennsylvania Water Science Center in 2008 documented stressful water quality conditions in critical near-shore habitat (Chaplin et al. 2009). They determined that DO concentrations (DO), were statistically different between near-shore locations and main-channel locations. Water temperature of the Susquehanna and Juniata Rivers were also typically higher and more varied when compared to Allegheny River or Delaware River locations (Chaplin and Crawford 2012).

Further analysis of diseased YOY SMB has identified other pathogens including myxozoan and trematode parasite infections. These parasite infections may have resulted from external stressors but could also have directly contributed as a stressor leading to bacterial infection and mortality. Largemouth Bass Virus (LMBV) has also been detected in SMB from all major basins of Pennsylvania, but to a greater prevalence in the Susquehanna Basin. Smallmouth Bass, like many other species, are known carriers of LMBV but are not believed to be directly affected by the virus. It is unknown if carriers of the virus experience additional stress that may weaken the immune system (G. Smith, Pennsylvania Fish and Boat Commission, personal communication).

In addition to conventional water quality parameters, recent studies have documented effects believed to be caused by endocrine disrupting compounds (EDC) and emerging contaminants (EC) in the Susquehanna River Basin (Reif et al. 2012, Blazer et al. 2014). These compounds are attributable to a number of sources including pharmaceuticals, fertilizers, and household cleaning products and can have major effects on aquatic environments (Hotchkiss et al. 2008, Diament-Kandarakis et al. 2009). The severity of these impacts is only now starting to be understood. EDCs and ECs may cause various physiological imbalances in fish and other aquatic organisms and have the potential to alter aquatic ecosystems. These chemicals can cause stress or immunosuppression in organisms that predispose them to diseases; similar to what has been observed in SMB (Milston et al. 2003, Liney et al. 2005, Vandenberg et al. 2012).

Analysis of adult SMB from the West Branch Susquehanna River (2009), Susquehanna River (2008-2010), and Juniata River (2010) documented high rates of intersex; a condition in which female egg precursor cells are found in the testes of males (Blazer et al. 2014). Although the natural occurrence of intersex in fishes is unknown, observations of this condition in SMB are widespread throughout the United States (Hinck et al. 2009). However, the proportion of SMB affected and the severity of the cases documented in the Susquehanna River Basin are greater than other basins.

In addition to intersex among adult SMB, other issues have been noted over the course of the investigation. During autumn 2011 and winter 2012, numerous adult SMB with lesions were observed in the lower Susquehanna River between York Haven and Safe Harbor dams; however, this appears to be an isolated incident. Also, since 2012, there has been increased interest in melanistic areas observed on the body surface of adult SMB from the Susquehanna River and a number of other locations in Pennsylvania. Angler reports of a high incidence of adult SMB with large, dark markings throughout the mainstem Susquehanna River began in March 2012. Initial pathological analysis by USGS and USFWS diagnosed the condition as melanosis; an accumulation of the pigment melanin in the dermis and epidermis of the fish. Although this condition has been documented in *Micropterus* spp. throughout their range in North America, potential causes and effects on individuals and populations remain unclear. Within Pennsylvania, the condition has been reported from numerous locations, but most frequently from the Susquehanna and Allegheny Rivers. The annual recurrence of the condition has led to questions about pathology, causes or contributing factors and whether the condition recedes on individual fish. Only a small number of fish have been analyzed histologically; however, the pathology observed has been consistent with melanosis or hyperpigmentation (V. Blazer, U.S. Geological Survey, unpublished data).

Sporadic observations of melanosis are not recent; informal investigations in Pennsylvania and New York since the mid-1980s yielded similar results to those of USGS and USFWS for Susquehanna River SMB. The spatial distribution of adult SMB with melanistic characteristics in Pennsylvania rivers is currently unknown. The majority of recent reports originated from anglers and have been largely restricted to the Susquehanna River, presumably as a result of public awareness of on-going SMB issues. However, reports from other portions of Pennsylvania have been received (G. Smith, Pennsylvania Fish and Boat Commission, written correspondence). Due to the need for more data, melanosis is not a topic covered under this report. However, ongoing research is being conducted by several agencies to determine cause and potential linkage to other SMB issues throughout Pennsylvania.

With the vast amount of research conducted since 2005 and multiple biological effects being analyzed, there was a significant need to consolidate resources and data. Agencies and academic institutions also needed to narrow down possible reasons for SMB declines observed on the Susquehanna River and its tributaries. Hence, PADEP requested assistance from the EPA to begin the stressor identification process. This method is described on EPA Causal Analysis/Diagnosis Decision Information System (CADDIS, www.epa.gov/caddis). In cooperation with the PFBC, three workshops were scheduled that included representatives from various state, interstate/federal agencies and academic institutions. These workshops sought to apply the CADDIS process, using available data, to make a preliminary determination of the causes of poor SMB recruitment at the Susquehanna River. This process will assist PADEP in developing appropriate programmatic strategies that will result in an improved fishery. However, it is important to note that the CADDIS process and subsequent report are not the Susquehanna River water quality assessment process for determining impairments under section 303(d) of the Federal Clean Water Act.

Methods: The CADDIS Process

The stressor identification process as described on the CADDIS website (hereafter called the CADDIS process) identifies the cause(s) of a biological problem in the environment by determining which among a set of alternate candidate causes is best supported by the body of evidence. The CADDIS process was chosen because it provides transparency and reduces inferential errors without restricting the types of evidence used. This process also has a clearly defined framework (Figure 3) making it ideal for multi-organizational cooperation and public understanding. Additional information on CADDIS can be accessed at: http://www.epa.gov/caddis/index.html. The CADDIS process began in October 2014 and was punctuated by three workshops (described below).

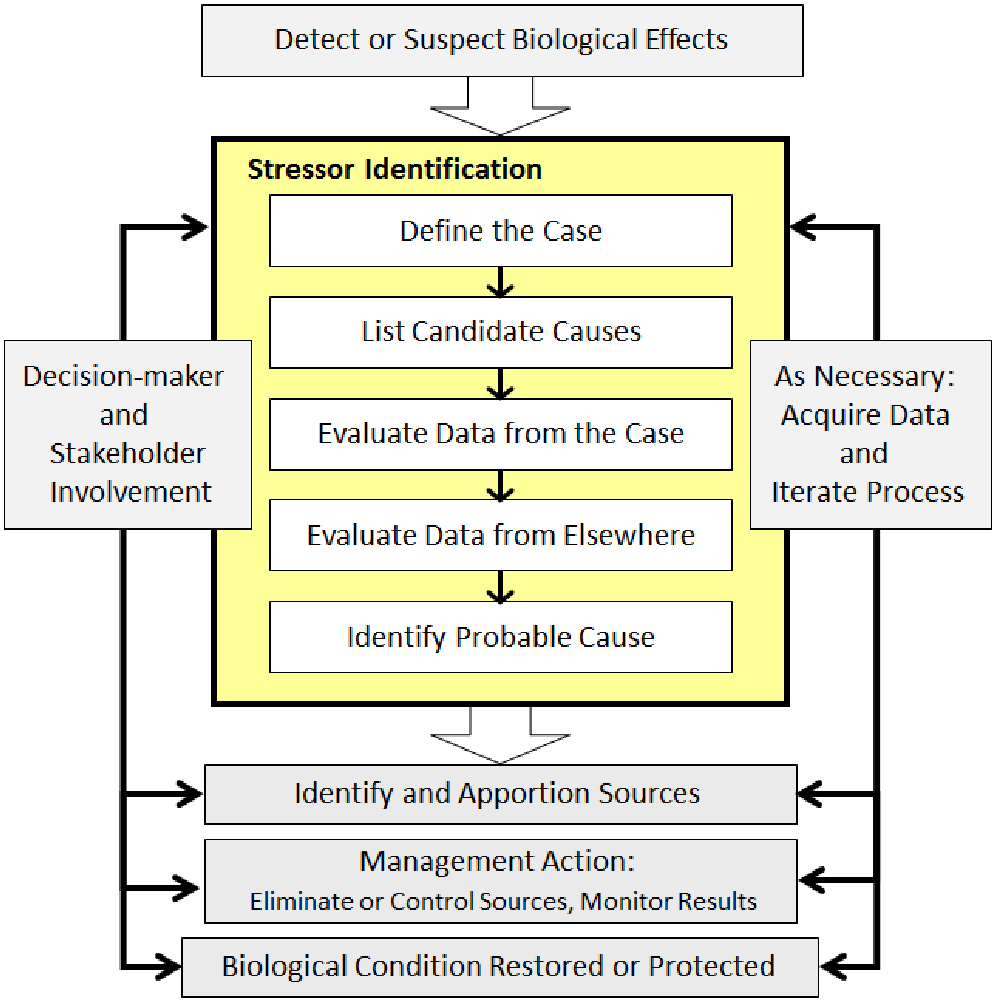

Figure 3. The framework for USEPA's stressor identification progress

CADDIS Workshop 1

The first CADDIS workshop was held in early October 2014 at Bald Eagle State Park, in central Pennsylvania. The main focus of the workshop was to provide background on the concerns for the SMB in the Susquehanna River watershed, define the case for analysis, and identify potential candidate causes.

Presentations pertaining to the history of SMB in the Susquehanna River watershed and the research and monitoring efforts pertaining to the SMB decline were given by four agencies and one academic organization: PFBC, PADEP, USEPA, USGS, and SRHCES.

Defining the case of interest (case) establishes the foundation for the rest of the CADDIS process. Also referred to as the subject of the analysis, the case is established by defining and specifying the effects that will be analyzed (e.g., species, age classes, anomalies) and determining the geographical and temporal frame of the effect.

Comparison sites also need to be identified. Comparison sites do not exhibit the noted effect; however, they do not have to be reference quality (U.S. EPA 2010). The details of defining the case are discussed below.

The candidate causes are the stressors that may be responsible for the observed biological effects. Listing these candidate causes further refines the scope of the causal analysis and provides a framework for assembling available data and determining what data are lacking for the causal analysis (U.S. EPA 2010). All participants were involved in developing a robust initial list of candidate causes.

Causal hypotheses were developed into conceptual models by grouping candidate causes together into logical pathways. These models were reviewed and finalized during the second and third workshops. Conceptual models served to illustrate the understanding that some aspects (i.e., candidate causes and modifying factors) of one conceptual model may influence another. All conceptual models are detailed in Appendix A.

In the four months spanning workshops 1 and 2, participants were charged with identifying and organizing data related to the case. If possible, participants were to begin developing analysis worksheets. These worksheets analyze and summarize the different datasets pertaining to the case. Later in the CADDIS process, the worksheets were used as evidence to either support or contradict the different candidate causes.

CADDIS Workshop 2

The second CADDIS workshop was held at the end of January 2015, at the PFBC office in Harrisburg, PA. This workshop began with reviewing and refining the conceptual models and the list of candidate causes. The majority of the workshop focused on reviewing data and analyses compiled since workshop 1, identifying additional data sets and data needs and developing analysis worksheets. All CADDIS participants were encouraged to develop analysis worksheets based on data they collected, researched or were familiar with. Completed worksheets were also vetted by groups comprised of members with experience in the specific discipline (i.e., participants with fisheries background reviewed fisheries-related worksheets, and participants with water quality background reviewed water quality-related worksheets).

When creating an analysis worksheet, ideally the candidate causes and biological response variables are measured at the same sites, during the same time period. The timeframe should capture the most relevant exposure in question. The same variables should be sampled at defined case (subject) and comparison sites. Various plots can be utilized to graphically display the data. Participants were encouraged to not rely solely on statistical differences, but rather a combination of statistical and biological significance. When applicable, the datasets should be analyzed based on their temporal and/or spatial component. Temporal analyses compare data collected before the observed effect (pre-2005) to data collected after the observed effect (post-2005), at the same site. Spatial analyses compare data collected at sites within the defined subject reach to those collected at comparison sites during the same time period.

The next steps identified at the conclusion of Workshop 2 were to make revisions to existing analysis worksheets, to create additional worksheets and to conduct a thorough literature search.

CADDIS Workshop 3

The final CADDIS workshop was held at PADEP's South Central Regional Office located in Harrisburg, PA. The workshop occurred at the end of March/beginning of April 2015, two months after Workshop 2. This workshop addressed the fifth and final step in the stressor identification process: Identify Probable Cause. This was accomplished by weighing the evidence for each candidate cause and comparing the evidence across candidate causes.

Each analysis worksheet was assigned a type of evidence. Seven types of evidence were used: spatial co-occurrence (spatial), temporal co-occurrence (temporal), stressor-response (SR) from the case, stressor-response (SR) from elsewhere, evidence of exposure, evidence of mechanism, and causal pathway (Table 1). A single worksheet can represent multiple types of evidence. Other types of evidence categories are discussed in EPA's CADDIS process; however, only those with available data were used in the Susquehanna and Juniata River CADDIS process.

Table 1. CADDIS types of evidence definitions. Table adapted from http://www.epa.gov/caddis/si_step_evidence.html and Norton et al. 2014

Evidence Type	Definition
Spatial Co-occurrence	The biological effect is observed where the cause is observed, and not where the cause is absent
Temporal Co-occurrence	The biological effect is observed when the cause is observed, and not when the cause is absent
Stressor-Response from the Case	As exposure to the cause increases, intensity or frequency of the biological effect increases and vice versa
Stressor-Response from Elsewhere	At the affected sites, the cause must be at levels sufficient to cause similar biological effects in laboratory or other field studies
Evidence of Exposure or Evidence of Mechanism	Measurements of the biota show that relevant exposure to the cause has occurred, or that other biological mechanisms linking the cause to the effect have occurred

Causal Pathway	Measurements or models demonstrate the occurrence of steps in the causal pathway linking sources to the cause, increased susceptibility of the organism(s), or conditions permitting interaction of the cause and the organism(s).

To weigh the types of evidence, Workshop 3 participants broke out into random groups (i.e. a single group contained members spanning multiple disciplines) based on the types of evidence and assigned a score to each worksheet based on what type of evidence the analyses covered (i.e. spatial, SR, temporal). If a worksheet addressed multiple types of evidence, a score was given for each evidence type. Table 2 defines the type of evidence scoring system with the classification agreed upon by participants in workshop 3.

Table 2. CADDIS type of evidence scoring system.

Score	Description
+++	convincingly supports
++	strongly supports
+	somewhat supports
0	neither supports nor weakens
-	somewhat weakens
--	strongly weakens
---	convincingly weakens
R	refutes
NE	no evidence

By the end of this workshop, all analysis worksheets were reviewed and scored (Appendix C). A consistency score for each candidate cause was agreed upon by all participants, based on the analysis worksheet scores. Lastly, a classification of **Likely**, **Unlikely**, or **Uncertain** was assigned to each candidate cause based on all of the evidence.

Results

The Case

As defined by EPA, “Each causal assessment focuses on a specific impairment or group of similar impairments collectively referred to as the case” (U.S. EPA 2010). The case definition is framed to identify the cause(s) of an observed biological problem in the environment. The temporal frame and geographic range of the observed effect also need to be identified for the case.

Catch per unit effort (CPUE) for both adult and YOY SMB have decreased during the post-2005 era when compared to the pre-2005 era (Figures 4 & 5). Additionally, the SMB population comprised a greater abundance of smaller and younger fish during pre-2005 surveys compared to post-2005 surveys, suggesting a change in population resulting from reduced YOY recruitment (Smith et al. 2015). With this background information in mind, the CADDIS participants defined the case of interest as ***a decrease in abundance of SMB as a result of poor recruitment into the adult SMB population***.

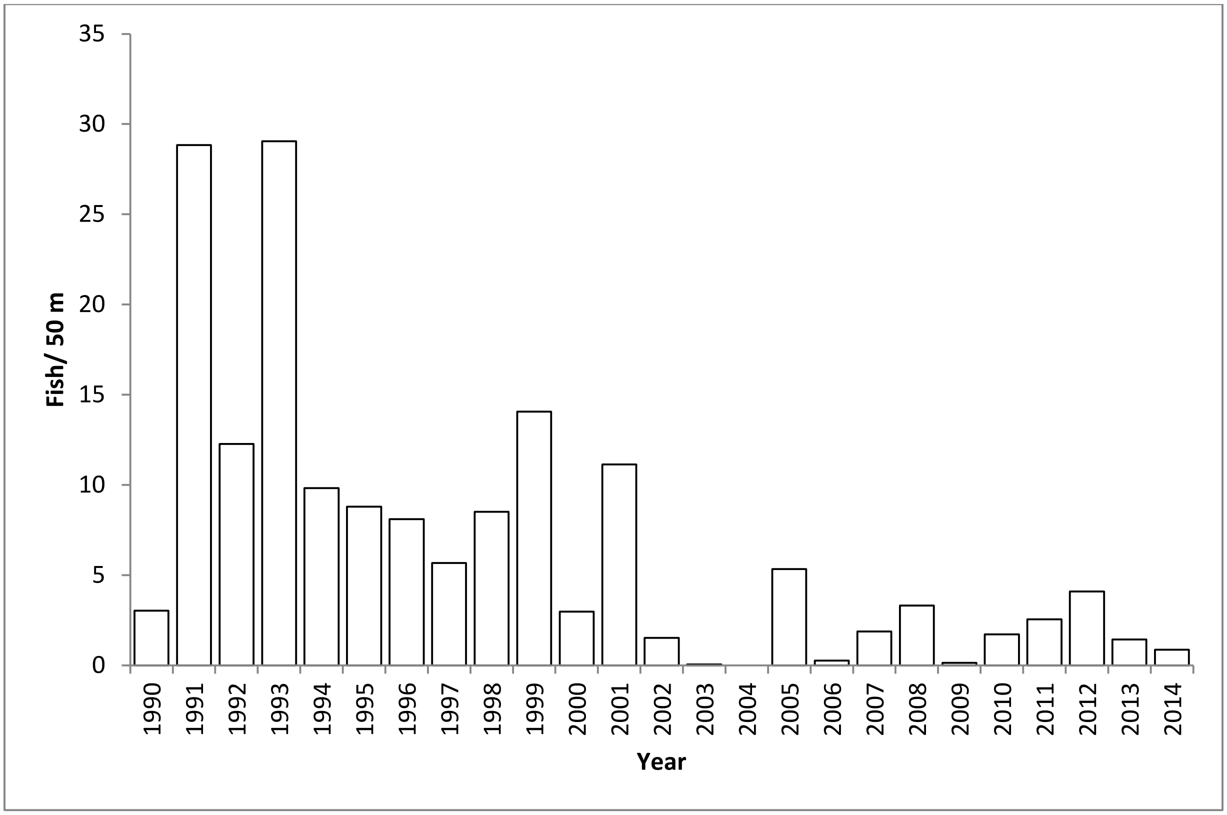

Figure 4. Backpack electrofishing reach-wide composite catch per unit effort (CPUE: fish/50 m) of YOY SMB at the middle Susquehanna River (between Sunbury and York Haven, Pennsylvania)

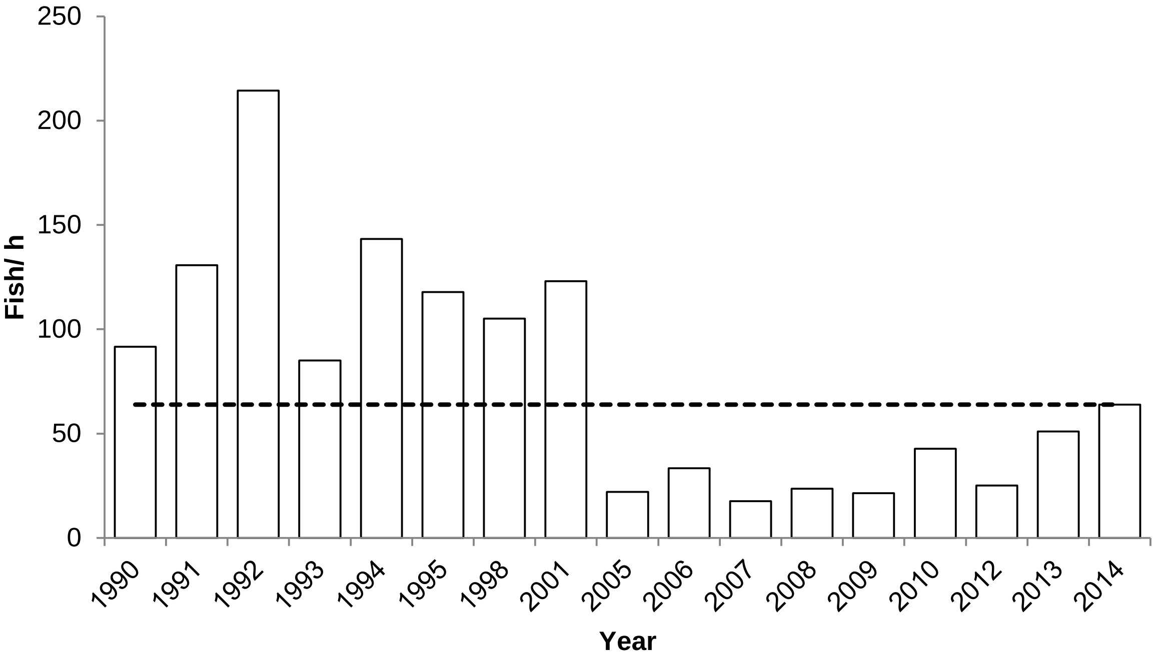

Figure 5. Reach-wide composite CPUE (fish/h) of adult SMB (≥ age 1; white bars) during nighttime boat electrofishing surveys at the middle Susquehanna River (between Sunbury and York Haven, Pennsylvania). Dashed line indicates median CPUE of adult SMB for the period of record at the middle Susquehanna River.

The case definition was agreed upon by all participants in Workshop 1 and confirmed by participants in Workshop 2. The temporal frame of the effect was established as 2005 to the present, since 2005 was the initial year that the decline was recorded. However, some longer term trend data were reviewed in order to elucidate the relationship between several candidate causes and the effect. The geographic range of the subject reaches was identified as the Susquehanna River from Sunbury to York Haven and the Juniata River from Port Royal to the mouth (Figure 6). These were the reaches identified to have a decrease in SMB abundance[1]. Comparison sites were selected to represent streams with stable and sufficiently robust SMB populations without documented instances of SMB population decline. Some examples include the Allegheny River at Franklin, Delaware River at Morrisville, upper Juniata River, upper Susquehanna River, lower West Branch Susquehanna River (Lewisburg), Pine Creek (Lycoming Co.), and Loyalsock Creek. Although there have been confirmed instances of disease at comparison sites, SMB populations have not declined in abundance or experienced changes in length and age structure.

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[1] The Susquehanna River at Marietta sampling location is located approximately 20 miles downstream of York Haven. Although YOY SMB were not sampled here, environmental data were considered to be relevant to the subject reach.

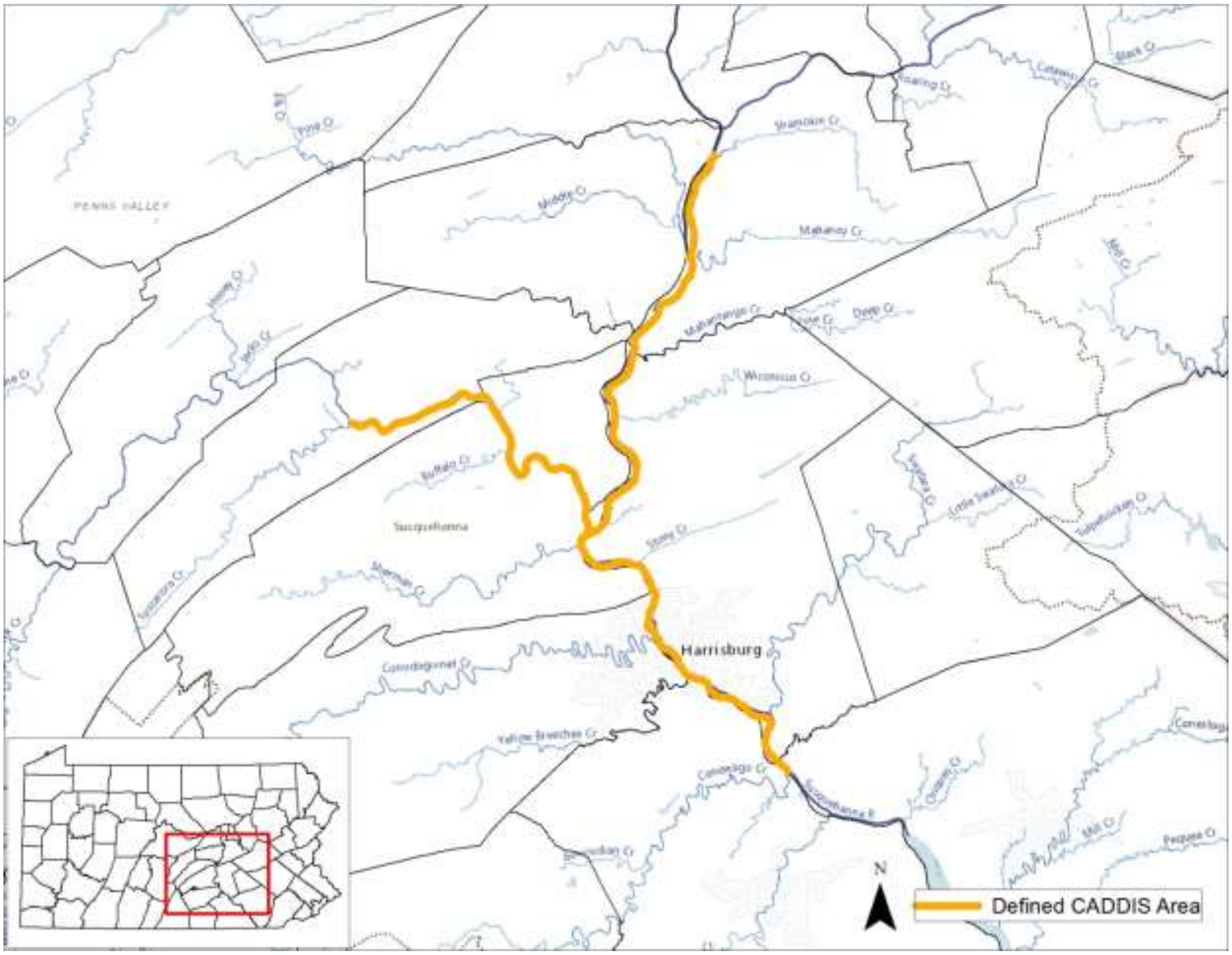

Figure 6. The defined area of concern (subject area) for which reduced SMB recruitment is most prevalent.

Candidate Causes

A total of 14 candidate causes were identified for the decline in SMB recruitment during the first CADDIS workshop. Each candidate cause was thought to be theoretically capable of causing the decline in recruitment through two mechanisms: by directly increasing mortality or indirectly by increasing susceptibility of the fish to disease[2]. Below are the descriptions of each candidate cause, explanation of the data that were analyzed and conclusions reached by the participants. Over 50 worksheets consisting of almost 400 pages of information were used in this evaluation. It is important to note that the following candidate causes were evaluated using data available at the time of

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[2] An exception is that Candidate Cause #13, endocrine disrupting chemicals and herbicides, were only considered to be capable of reducing recruitment by increasing susceptibility to disease.

the third workshop. Consequently, as more data are collected, conclusions made in this report are subject to change.

Candidate Cause 1: Increased late spring/early summer flow

1.1 Cause Description

The temporal pattern of streamflow fluctuation during the spawning period appears to be the most important abiotic factor determining nesting success and failure of SMB in perennial streams (Lukas and Orth 1995). It is well documented throughout the United States that increased stream discharge can result in mechanical nest failure or extrication of young fry from their habitat, increasing mortality and decreasing recruitment (Reynolds and O’Bara 1991, Lukas and Orth 1995, Buynak and Mitchell 2002, Smith et al. 2005). In Pennsylvania rivers, low river discharge during select spring periods tended to correspond with above average YOY catch rates (Lorantas and Kristine 2004). The below average catch rates of YOY SMB in recent years, reductions in catch rates of all sizes of SMB, and catches of SMB ≥ 300mm in the Susquehanna River relative to reference periods have been uncharacteristic of flow related changes evident in other rivers across the state (Lorantas et al. 2012). This candidate cause examines the relationship between SMB abundance and stream discharge and whether temporal and/or spatial changes in the relationship have occurred.

1.2 Evidence (Appendix B: Worksheets 1, 2, 4, and 6)

1.2.1 Temporal co-occurrence

If flows directly affect SMB recruitment, then flows should be different between pre and post 2005. Continuous discharge data from the Harrisburg USGS gage station, from 1990-2014 (Worksheet 1), were analyzed to see if high spring or low summer discharges occurred. There was no difference in discharge values when comparing the earlier time period (Pre-2002) to the later period (Post-2002) (Score = -).

In Worksheet 2, YOY SMB catch rates were compared to pre and post 2005 mean June flow. Regression analysis suggests that while high stream discharges in June still negatively affects YOY SMB abundance, other factors are affecting abundance within subject reaches during post-2005 years with low stream discharge in June (Score = -).

For Worksheet 4, CPUE data of age-1 SMB were compared to pre- and post-2005 mean June flow. Flow does not appear to be the only factor affecting age-1 CPUE (Score = -).

1.2.2 Spatial co-occurrence

If flows directly affect SMB recruitment, then flows would be expected to be significantly different between subject and comparison sites. Worksheets 2 and 4 were similar in that CPUE data were compared to mean June discharges at the following USGS gaging sites: Susquehanna River at Harrisburg (01570500) and Danville (01540500), Delaware River at Trenton (01463500), Juniata River at Newport (0156700) and Mapleton Depot (01563500), West Branch Susquehanna River at Lewisburg (01553500), and Allegheny River at Franklin (03025500). The difference between the worksheets was that Worksheet 2 analyzed back-pack electrofishing CPUE (fish/ 50 m) data for YOY SMB and mean June discharge of the same year and Worksheet 4 evaluated boat electrofishing CPUE (fish/h) data of age-1 SMB and mean June flow for the year that the fish would have spawned (i.e., 2008 CPUE would relate to mean June discharge during 2007).

When comparing subject sites to comparison sites in Worksheet 2, analyses suggest the lower YOY SMB catch rates in subject areas are not being completely driven by discharge, which typically controls YOY SMB mortality. Regression analysis suggests that while high stream discharges in June still negatively affect YOY SMB abundance, other factors are affecting abundance within subject reaches during years with low stream discharge in June (Score = -).

The relationship between age-1 SMB CPUE and previous year mean June discharge (Worksheet 4) has weakened in the post-2005 period for subject sites. In contrast, for comparison sites, the relationship between age-1 SMB CPUE and flow has strengthened for the post-2005 period. This evidence weakens the argument that flow is the major factor influencing reduced recruitment in the subject areas (Score = -).

1.2.3 Causal Pathway

Continuous discharge, temperature, pH, and DO data from near-shore/micro-habitats were plotted to determine the potential sequence of events for SMB pre- through post-spawn and YOY dispersal. This analysis sought to elucidate combined effects between discharge and other water quality parameters within YOY SMB habitats. Data analyzed in Worksheet 6 suggest that discharge and water temperature variability in any given year/season could dictate the magnitude of effect from any candidate cause in the form of a modifying factor. Due to a lack of relevance to direct mortality caused by flow, this worksheet was not scored. Modifying factors and combined effects are presented later in the report.

1.3 Discussion

Both temporal and spatial evidence weaken the argument that increased late spring/early summer flow is directly causing decreased recruitment of YOY SMB since 2005 (Consistency Score = --). This evidence is strong because it is based on continuous discharge datasets. However, low discharges and lack of flushing flows may contribute to stressful water quality conditions such as increased algal growth, increased temperature, and low DO.

Candidate Cause 2: Increased intraspecific competition

2.1 Cause Description

Intraspecific competition is the competition among individuals of the same species for resources (Polis 1981). It is hypothesized that the high density of SMB in the past may have led to a "boom-and-bust" situation where the population was operating above carrying capacity resulting in population crash.

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

2.2 Evidence (Appendix B: Worksheet 3)

2.2.1 Evidence of Mechanism

If intraspecific competition was causing stress and subsequent decline of SMB, then condition measurements should show W_r values less than 80 and/or a significant change at sites or over time. In Worksheet 3, W_r values were generated using a revision of the W_s equation for SMB (Kolander et al. 1993). These values were generated for all adult SMB with individual length and weight data from each river reach. Relative weights of the SMB were consistent over time and space. Subject sites demonstrate that W_r during the study period was at or near the 100 “benchmark”, indicating good fish condition. The W_r data from the middle Susquehanna River and lower Juniata River suggest that SMB condition within subject reaches were not appreciably different than those encountered in comparison reaches during the same time frame (Score = --).

2.3 Discussion

The combination of spatial, temporal and benchmark components weaken the argument that SMB were over-populated and resource limited prior to the 2005 population decline (Consistency Score = -). However, this was the only piece of evidence presented for this candidate cause. Additionally, one limitation of the W_r data is that there are no data between 1998 and 2005 for the subject sites. Therefore, there is no way to determine if W_r decreased just prior to the increased mortality/population declines that were observed starting in 2005.

Candidate Cause 3: Increased interspecific competition

3.1 Cause Description

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

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

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

3.2 Evidence (Appendix B: Worksheet 5)

3.2.1 Temporal Co-occurence

If interspecific competition is causing the decline of SMB, then there would be an expected inverse relationship between SMB and the invasive species after 2005. Worksheet 5 investigated abundance of MS in relation to SMB. The MS population appears to be experiencing exponential growth; however, there is limited data between 1977 and the present within the subject reach (Score = 0).

3.2.2 Spatial Co-occurrence

If interspecific competition is causing the decline of SMB then there would be an expected inverse relationship between SMB and the invasive species within subject sites, but not comparison sites. Worksheet 5 evaluated the measured decline in SMB populations to the exponential growth of MS. It also compared the MS population to other Centrarcidae. The relationship of SMB and MS was inverse to the expected relationship as demonstrated by regressions between MS and Rock Bass, *Ambloplites rupestris* and MS and Redbreast Sunfish, *Lepomis auritus*. These data indicate some correlation; however, they do not explain causality. Additionally, Worksheet 5 only analyzed the potential competition of one invasive species. Worksheet 5 included many community surveys in both wadeable and non-wadeable streams, but the group recommended limiting analysis to only case study sites (i.e., subject and comparison sites) (Score = 0).

3.3 Discussion

More community level data should be collected and a greater number of invasive species relationships need to be analyzed before any conclusions can be reached on this candidate cause (Consistency Score = 0).

Candidate Cause 4: Decreased food quality

4.1 Cause Description

Decreased food quality was addressed by focusing on two specific aspects: fatty acids and thiamine content. An adequate supply of essential dietary nutrients is necessary for survival and well-being (NRC 2011). Essential fatty acids are reported to be important to immune function (Fenton et al. 2013). Arachidonic acid, in the omega-6 (ω6) family of fatty acids, is a precursor for pro-inflammatory eicosanoid (Klurfeld 2008). Teleosts possess many of the same antimicrobial mechanisms observed in mammals (Rieger et al. 2011) indicating the presence of a robust innate immune system (Rieger et al. 2011, Palti 2011, Purcell et al. 2006). When a pathogen invades an organism, the pro-inflammatory response signals the immune system and a cascade of events kill and eliminate the invading pathogen. In a healthy situation, a balance of omega-3 (ω3) modulates or down regulates the inflammatory response. A healthy ratio of ω3 to ω6 is approximately 1:1 to 1:2.5 (NRC 2011).

Another dietary component of interest is the essential nutrient vitamin B_1 (thiamine). Thiamine deficiency has been documented in several aquatic top predator species in association with population declines (Hill and Nellbring 1999, Blazer and Brown 2005, Honeyfield et al. 2008c). In these affected populations dietary thiaminase, an enzyme that destroys thiamine has been involved. The diet of adult SMB is purported to be high in crayfish within the Susquehanna Basin (PADEP unpublished data). A decline in the Susquehanna SMB population may be related to an increase in non-native species such as the Rusty Crayfish, *Orconectes rusticus* and MS. Although it is unknown if these population changes sufficiently overlap temporally, crayfish have been reported to contain thiaminase (Ying and Rutledge 1975) as have *Notropis* spp. (Tillitt et al. 2005). Thus, an increase in SMB dietary prey items containing thiaminase could theoretically lead to thiamine deficiency and population declines as cited in the above examples.

4.2 Evidence (Appendix B: Worksheets 5, 24, and 25)

4.2.1 Stressor-response from elsewhere

If decreased food quality is causing the decline of SMB then there would be nutritional endpoints that meet or exceed levels of concern reported in other cases. Worksheet 24 analyzed the stomach contents of adult SMB to determine their diet and whether potential prey species contained high levels of thiaminase. Adult SMB stomachs contained crayfish (57.5%), fish (28.1%), and other macro-invertebrates (37.1%). Note that total percentages are greater than 100% due to some adult SMB stomachs containing more than one of the three major prey groups. The diet of Susquehanna

SMB is high in crayfish as predicted, but thiaminase activity in non-native and in native crayfish was low and biologically unimportant with respect to causing thiamine deficiency. Thiaminase activity greater than 2.5 µmol/g/min can result in thiamine deficiency. Mean thiaminase in MS was 53.2 µmol/g/min. This high activity would lead to thiamine deficiency assuming that the percentage of the diet reflected the percentage of SMB with fish in their stomachs. However, YOY SMB consuming MS has not been confirmed. Therefore, it was decided the evidence neither supported nor weakened the candidate cause because more research is needed (Score = 0 for direct mortality and 0 for disease susceptibility).

For Worksheet 25, fatty acid ratios were taken from periphyton samples as an indicator of the nutritional quality of the food chain. All fatty acid ratios in the case study were within normal range (ratio of ω3 to ω6 at approximately 1:1 to 1:2.5) thus weakening the argument that unbalanced ratios of fatty acids contributed to SMB declines (Score = -- for direct mortality and -- for disease susceptibility).

4.2.2 Causal Pathway

If thiaminase content of the YOY SMB diet was causing the declines in recruitment, then MS (a species high in thiaminase) would be expected to be a large component of the prey community. Fish community data were collected by PADEP, PFBC, and SRBC in 2008, 2009, 2013, and 2014 using a semi-quantitative sampling protocol for both wadeable streams and non-wadeable rivers. Sites throughout the Susquehanna and neighboring basins were selected based on proximity to the PFBC historic SMB sampling areas (Worksheet 5). Mimic Shiner generally comprised the major percentage of the insectivore guild, especially in the larger river systems. In addition, as the percentage of MS contribution to the insectivore guild increased, the numbers of SMB within their guild (invertivore/piscivore) decreased ($r^2 = 0.40$) (Score = +).

4.3 Discussion

The fatty acid ratios of periphyton are within normal ranges, arguing against this candidate cause (Consistency Score = -). Food sources containing significant thiaminase activity merits continued investigation (Consistency Score = +). Mimic shiner and SMB are thought to hatch at approximately the same time and same general location. Additionally, the overlap of these two species and their inherent size differential may provide opportunity for YOY SMB to consume YOY MS. However, further investigation is needed.

Candidate Cause 5: Decreased egg quality

5.1 Cause Description

The nutritional status and supply of essential dietary nutrients plays an important role in the survival and well-being of fishes (National Research Council 2011). Deficiency of the essential nutrient vitamin B_1 (thiamine) has been documented in several aquatic top predator species in association with population declines (Hill and Nellbring 1999, Blazer and Brown 2005, Honeyfield et al. 2008). Deficient (low) egg thiamine concentrations have been shown to cause fry mortality after hatch in numerous salmonid species and American Alligators, *Alligator mississippiensis* (McDonald et al. 1998, Brown et al. 2005, Honeyfield et al. 2005, 2008). Furthermore, immune dysfunction (T-Cell activity) occurs in fish hatched from eggs containing low thiamine or fish with low thiamine due to poor diet. Dysfunctional T-Cell activity can result in an increase in disease-carrying individuals thus subjecting the population to a greater risk of disease outbreaks and mortality (Ottinger et al. 2012, 2014).

5.2 Evidence (Appendix B: Worksheet 26)

5.2.1 Spatial Co-occurrence

If decreased egg quality is causing the decline in SMB, then there would be a greater prevalence of female SMB ovaries containing low thiamine concentrations at subject sites than comparison sites. Thiamine levels were analyzed in egg tissue of 131 female SMB, which were harvested from 14 sites during the spring of 2014 (Worksheet 26). Within subject sites, 37% of females produced eggs containing less than desirable thiamine levels (8 nmol/g). For comparison sites, only 14% of the females produced eggs with less than 8 nmol/g. Mean egg total thiamine within subject sites was less (9.3 nmol/g) than comparison sites (11.2 nmol/g). However, due to the small sample size and relatively similar mean values for each site, it was determined that more data need to be collected (Score = 0).

5.2.2 Stressor-response from elsewhere

If decreased egg quality is causing the decline in SMB then egg thiamine levels should be as low as or lower than reported levels of concern from other sources. Egg thiamine values greater than 8 nmol/g are fully thiamine replete, thiamine values between 1.5 and 8.0 nmol/g result in fry that are susceptible to secondary effects such as immune dysfunction and overt fry mortality occurs with thiamine levels less than 1.5 nmol/g (McDonald et al. 1998, Brown et al. 2005, Honeyfield et al. 2005, 2008). Analysis of individual SMB egg thiamine values revealed that 28% of the eggs contained concentrations less than 8 nmol/g, but no egg thiamine concentrations were below 1.5 nmol/g. This suggests a potential for immune suppression in approximately 25% of YOY spawned. However, egg thiamine level benchmarks were estimated from different vertebrate species and true benchmarks for SMB are unknown. As a result, more research is needed (Score = 0 for direct mortality and 0 for disease susceptibility).

5.2.3 Causal Pathway

If thiaminase content of adult SMB diets causes thiamine deficiencies in SMB eggs, leading to declines in recruitment, then MS (a species high in thiaminase) would be expected to be a large component of the fish community and the adult SMB diet. Based on fish community analyses, MS generally comprised the major percentage of the insectivore guild, especially in the larger river systems (see Section 4.2.3), and about 28% of adult SMB diet is fish (see Section 4.2.1). In addition, as the percentage of MS contribution to the insectivore guild increased, the numbers of SMB within their guild (invertivore/piscivore) decreased (see Section 4.2.3) (Score = +).

5.3 Discussion

There is potential for poor egg quality in the form of low egg thiamine to cause YOY SMB immune suppression, eventually leading to mortality (Consistency Score = +). Yet, more robust datasets and species-specific thiamine level benchmarks are required to evaluate this cause. Additionally, more research (e.g., T-Cell dysfunction studies) is needed to verify that thiamine deficiencies are leading to YOY SMB having suppressed immune systems.

Candidate Cause 6: Decreased YOY habitat quality

6.1 Cause Description

During the first few months of life, a critical period for survival and development of SMB, YOY occupy near-shore riverine microhabitats characterized by relatively slow-moving and shallow water (Chaplin and Crawford 2012). Decreased quality and quantity of YOY habitat from factors such as high flow, increased algal growth, low DO concentration, high concentration of suspended solids, and high temperatures could result in direct mortality or increased susceptibility of YOY to disease (Smith et al. 2015).

Increased algal growth is commonly associated with increased water temperature and photosynthetic activity. This results in elevated diel variation in DO and pH, and more extreme values of these parameters (e.g., minimum DO and maximum pH values). Due to the lack of quantifiable YOY habitat data and only anecdotal algal growth data, these parameters were used as surrogates for evidence evaluation.

6.2 Evidence (Appendix B: Worksheet 34 and 35)

6.2.1 Temporal Co-occurrence

If decreased YOY habitat quality is causing the decline in SMB recruitment, then surrogate measures for a highly productive environment should be temporally linked to low YOY CPUE years. In Worksheet 35, certain high YOY CPUE years (1991-1994) and low YOY CPUE years (2005-2011) were selected to avoid the confounding factor of high June discharge. These CPUE data were then compared to water temperature, total phosphorus and pH as surrogates for predicting algal growth. Comparisons using nitrogen were also considered; however, data were lacking between the two temporal periods to make conclusions. There was no difference in water temperature, total phosphorus, or pH conditions between high and low CPUE years at the subject sites analyzed (Score = -).

6.2.2 Causal Pathway

Algal blooms may lead to degraded YOY habitat quality by decreasing DO and increasing pH, obscuring substrate and outcompeting macrophytes. Algae blooms were documented in the subject reach using aerial photographs in October 2007 and July 2012 following low flow periods (Worksheet 34, see also Section 11.2.3). The surface blooms were concentrated along shoreline habitats and between islands that would be expected to provide YOY habitat. Additional photographs of a filamentous algae bloom on the Susquehanna River at Rockville were taken by Geoff Smith from the PFBC on August 12, 2012 coinciding with a fish pathology survey where YOY SMB with abnormalities were identified. This evidence suggests a possible link (Score = +); however, there were only two years of documented algal blooms post-2005, and poor recruitment years have been recorded without documented algal blooms. Additionally, there are no baseline data to compare to 2007 and 2012 observations.

6.3 Discussion

Although algal blooms have been observed coinciding with diseased SMB, there were no differences in water temperature, total phosphorus, or pH between low and high CPUE years (Consistency Score = -). However, these conclusions have high uncertainty due to the use of surrogate measurements and anecdotal data. More data that are spatially and temporally related to YOY habitats need to be collected. More importantly, YOY habitat quantity and quality need to be documented in order to properly evaluate this candidate cause.

Candidate Cause 7: Increased water temperatures

7.1 Cause Description

High water temperatures may directly or indirectly affect recruitment of YOY SMB into age class 1+ by stress resulting from an inability to acclimate to warming water temperatures, lower solubility of DO, greater abundance of pathogens, and magnified physiological responses to other stressors (Chaplin et al. 2009, Chaplin and Crawford 2012, Cipriano and Austin 2011, Starliper and Schill 2011).

Juvenile SMB are considered to be more tolerant to high water temperature than adults (Recsetar et al. 2012). Wrenn (1980) suggested that the upper lethal limit of YOY SMB is approximately 37°C; however, water temperatures around 29°C during this life stage may be beneficial for rapid growth and development (Zweifel et al. 1999). Water temperatures during the first summer are positively correlated with year class strength, because energy reserves used to survive the first winter are increased in the summer when food is abundant and the water temperature is most suitable for growth (Horning and Pearson 1973, Clady 1975, Shuter et al. 1980).

Landsman et al. (2011) suggest that egg hatching and YOY larval success is unaffected when water temperatures rapidly (within one hour) fluctuate between -7 to +8°C beyond baseline conditions; however, significant mortality did occur when eggs were rapidly exposed to water temperatures 13°C above baseline conditions. It was also noted that young SMB were slightly more resilient to decreasing temperature changes than increasing temperature changes. Although SMB eggs and larvae may tolerate variable water temperatures, sudden drops in temperature below 14°C during the spring can be lethal due to male nest abandonment (MacLean et al. 1981, Armour 1993).

7.2 Evidence (Appendix B: Worksheets 7, 8, 9, 10, 11, and 12)

7.2.1 Temporal Co-occurrence

If increased water temperature directly affects SMB recruitment, then water temperatures would be expected to be higher for the post-2005 time period than the pre-2005 time period. In Worksheet 7, continuous temperature data were evaluated for one site within the subject area. Analysis suggested no apparent difference in annual high temperatures or variation between the pre-2005 and post-2005 periods. However, the time period for pre-2005 contained only one year (Score = -).

For Worksheet 12, data were analyzed at three subject sites and four comparison sites for long term and short term trends. There were no clear increasing trends within subject sites, but this dataset contained only discrete samples, which are not as robust as continuous data (Score = -).

7.2.2 Spatial Co-occurrence

If increased water temperature directly affects SMB recruitment, then subject sites should experience higher water temperatures than comparison sites. Continuous water temperature data collected by SRBC were graphed for three sites (Worksheets 7-9).

The average temperature was slightly higher at Rockville (subject site) than at Columbia and Danville (in-basin comparison sites) (Score = +).

Discrete transect data were plotted for each site to visually compare bank (or YOY) habitats to main channel habitats (Worksheet 10). Results suggest that water temperatures were not drastically different between the habitat types at each site. Bank habitats did not always have higher water temperatures than main channel habitats, but were variable with slight consistency toward cooler temperatures. However, this worksheet focused on YOY habitat and main channel comparisons. Comparisons of water temperatures between subject and comparison sites were difficult to evaluate with these data (Score = 0).

Long term discrete water temperature data were analyzed at three subject sites and four comparison sites (Worksheet 12). Due to the focus on evaluating long term datasets in this Worksheet, it was difficult to evaluate these data in a spatial context (Score = 0).

7.2.3 Stressor-response from elsewhere

In Worksheet 11, continuous water temperature data were analyzed at several subject and comparison sites to determine the maximum observation, maximum daily ranges, and potential drops below 14°C after the spawn. Daily ranges were a surrogate measure for rapid fluctuations. If daily ranges exceeded 7°C, then further investigation into the magnitude and direction of that fluctuation would be necessary. The maximum daily water temperature was 33.7°C (observed at the Juniata River at Newport) during the two years of continuous record, which was well below the lethal temperature of 37°C suggested by Wrenn (1980). Further, maximum daily ranges did not exceed 7°C at any sites during the two year data record. There were insufficient data to confidently determine if water temperatures dropped below 14°C during or just after spawning. However, no sites had recorded temperatures below 14°C by late May/early June. These data suggest water temperature did not exceed thresholds for thermal stress (Score = -- for direct mortality and - for disease susceptibility).

7.3 Discussion

The evidence weakens the argument that increasing temperatures are causing the SMB recruitment declines. There is no clear trend when comparing water temperature over time at any of the subject stations and the largest increasing trend was observed at a comparison site. There is also no clear evidence to suggest that water temperatures have been exceedingly stressful for the recruitment of SMB. However, much of the continuous data collected were during higher than average to average discharge years and there is a lack of confidence when dealing with discrete datasets. It is important to note that subject sites may be warmer than comparison sites simply because of the physical nature of the Susquehanna River in that section (more wide and shallow than other major drainages, allowing for greater thermal absorption from solar radiation) (Consistency Score = -).

Candidate Cause 8: Increased pH or increased variability in pH

8.1 Cause Description

There is no absolute pH range where all freshwater fishes are unharmed; yet, it is generally understood that there is a gradual decline in tolerance as pH deviates from neutrality (EIFAC 1969, AFS 1979, Alabaster and Lloyd 1980). The acceptable range of pH depends on many other factors, including water temperature, previous pH acclimatization, DO concentration and various ions in the water (McKee and Wolf 1963).

The physiological effects on aquatic life induced by high pH have been studied less than those at low pH (Doudoroff and Katz 1950, Alabaster and Lloyd 1980). It is suggested that the toxic mode of action of high pH is hypertrophy of mucus cells at the base of the gill filaments and destruction of gill and skin epithelium, with possible effects to the eye lens and cornea (Alabaster and Lloyd 1980, Boyd 1990). Due to the effects on gill structure and function, fishes are less able to excrete ammonia (NH_3) through the gills, leading to increased toxicity, even in the absence of high ambient NH_3 (Schaperclaus 1991). The majority of freshwater fishes studied experience harmful effects (lethal or sublethal) at sustained pH values between 9 and 10 (Weibe 1931, AFS 1979, Alabaster and Lloyd 1980).

Rivers frequently exhibit diel fluctuations in pH resulting from photosynthesis, and temporary exceedances of 9.0 pH units occur naturally in shallow and biologically productive waters (Boyd 1990). It is suggested that no harmful effects would be expected in fish experiencing rapid pH changes provided that variations remain within normal ranges (6-9 pH units) (Witschi and Ziebell 1979). Additionally, Wiebe (1931) reported that sunfish, *Lepomis* spp. and Goldfish, *Carassius auratus* survived rapid changes from pH 7.2 to 9.6 (2.4 units); Largemouth Bass, *Micropterus salmoides* from pH 6.1 to 9.6 (3.5 units); and SMB from pH 6.6 to 9.3 (2.7 units).

It is important to note that this section of the report discusses the direct effects of pH. Topics such as the influence of pH on the toxicity of NH_3, dissolved CO_2 and the ratio of dissolved inorganic phosphorous are discussed elsewhere.

8.2 Evidence (Appendix B: Worksheets 13 and 14)

8.2.1 Temporal co-occurrence

If high pH directly affects SMB recruitment, then pH would be expected to be higher during the post-2005 time period than the pre-2005 time period. Discrete samples for pH from PADEP's Water Quality Network (WQN) and EPA's STORET Database were plotted for the years 1990-2014 and a slightly narrower time window of 1995-2014. At the subject sites, a weakly increasing temporal trend in pH was observed (Worksheet 14, Score = +).

8.2.2 Spatial co-occurrence

If high pH directly affects SMB recruitment, then subject sites should experience a greater magnitude, duration, and frequency of high pH compared to comparison sites. PADEP and USGS collected continuous pH data during the spring and summer of 2012 and 2013 (Worksheet 13).There was little difference between subject and comparison sites in the mean duration of pH values above 8.0 and above 9.0. In particular, in 2012 the Delaware River comparison site experienced slightly longer periods with pH values above 9.0 than the subject sites (Score = -).

8.2.3 Stressor-response from elsewhere

Reviews published by the European Inland Fisheries Advisory Commission (EIFAC) suggest that a range of 6.5 to 9.0 pH is likely to be harmless to aquatic life. Excursions above 9.0 pH were observed at both subject and comparison sites (Worksheet 13). However, very little data exist on how SMB are affected by high pH and at which age tolerance may or may not change. For this reason, evidence based on stressor-response relationships was not developed. The influence of pH on ammonia toxicity was considered when evaluating Candidate Cause 10 (NH_3).

8.3 Discussion

The occurrence of high pH values at the comparison sites, which do not exhibit poor SMB recruitment (especially at the Delaware at Morrisville in 2012) weakens the argument that high pH is the cause of low YOY recruitment in the middle Susquehanna River and lower Juniata River (Consistency Score = -). This evidence was based on data from continuous data samplers, which increases confidence. However, the evidence was based on two years of data only, and both years had average or higher than average discharge. Different conditions may occur in lower flow years. In addition, the samplers were deployed in the main channel, and may not represent SMB YOY habitat.

Candidate Cause 9: Low dissolved oxygen concentrations

9.1 Cause Description

Low DO levels can directly kill fish through asphyxiation. Many studies provide evidence that juvenile centrarchids may be among the most sensitive of all warm-water fishes to low DO concentrations. Smallmouth Bass hatchling and larvae survival was observed to be significantly reduced at or below DO concentrations of 4.5 mg/L, (Siefert et al. 1974, Spoor 1984). In laboratory experiments, lethal and sub-lethal effects of reduced DO (i.e., less than 5 mg/L) were, in general, directly related to exposure times, which ranged from hours to days (Mount 1964; Doudoroff and Shumway 1970, Siefert et al. 1974, Spoor 1984).

Like pH, rivers exhibit diel fluctuations in DO because of photosynthesis. As plants, algae and bacteria photosynthesize during the day, they release oxygen. At night photosynthesis ceases but respiration continues to consume oxygen, reducing concentrations. Concentrations of DO are typically lowest just before dawn. In lakes and rivers with high productivity, diel swings can be pronounced (Allan and Castillo 2007).

9.2 Evidence (Appendix B: Worksheets 18, 19, and 51)

9.2.1 Temporal co-occurrence

If low DO directly affects SMB recruitment, then DO concentrations should be lower post-2005 than pre-2005. The SRBC collected discrete DO measurements using a handheld field meter at several areas across the channel, with the median measurement presented. Most data from 1985-2008 were taken in June through September in a time ranging from the mid-morning through early afternoon. Data from 2011 and 2012 were collected in October and November (Worksheet 51). Values before and after 2005 are difficult to compare because of data gaps, but overall are within a reasonable range of each other (Score = 0).

9.2.2 Spatial co-occurrence

If low DO directly affects SMB recruitment, then DO should be lower at subject sites than comparison sites. PADEP and USGS collected continuous DO data from June 1 to July 31 in 2012 and 2013 (Worksheet 18). Data were collected within the main channel at each site and consequently do not capture possible extremes that may exist along the banks. Subject sites tended to have lower minimum DO levels than comparison sites (Delaware River at Morrisville and Susquehanna at Danville). Differences were most conspicuous during 2012 (Score = +).

9.2.3 Stressor-response from elsewhere

If low DO directly affects SMB recruitment, then DO observations within subject sites should be as low as or lower than concentrations of concern reported elsewhere. The continuous data discussed in Section 9.2.2 were compared against a benchmark of 5 mg/L, below which lethal and sublethal effects would be expected (Worksheet 19). Dissolved oxygen did not fall below 4.5 mg/L at any of the subject sites. There were no excursions below 5.0 at any of the Susquehanna River at Harrisburg sites. Dissolved oxygen fell below 5 mg/L only at the Juniata River site and only during 2012 (six events ranging between approximately 0.5 to 3.5 hours). (Score = - for direct mortality and 0 for disease susceptibility)

9.3 Discussion

The lower DO daily minima observed at the subject sites provides evidence that lower DO co-occurs with the low YOY recruitment in the middle Susquehanna River and lower Juniata River. However, the DO levels were not sufficiently low to have produced direct mortality (Consistency Score = -). It is not known whether levels are low enough to increase susceptibility to disease, so this possibility remains (Consistency Score = +). This evidence was based on data from continuous data samplers, which increases confidence. However, the evidence was based on two years of data only, and both years had average to higher than average discharge. Lower DO levels are expected to occur in lower discharge years. In addition, the samplers were deployed in the main channel, and may not represent SMB YOY habitats which have the potential to be different.

Candidate Cause 10: Ammonia Toxicity

10.1 Cause Description

Exposure to elevated concentrations of ammonia is toxic to fish, causing mortality and increase susceptibility to disease (Schaperclaus 1991). Ammonia toxicity increases with increasing pH and water temperature (Ip et al. 2001), which can decrease YOY SMB survival and recruitment to the adult population. Stressful summer conditions, specifically water temperatures exceeding 30°C and pH exceeding 9.0 have been recorded at several locations in the Susquehanna and Juniata Rivers since 2008 (Chaplin et al. 2009, Chaplin and Crawford 2012).

Laboratory methods for surface water samples measure both the un-ionized form (ammonia, NH_3) and the ionized form (ammonium, NH_4^+). The un-ionized NH_3 is much more toxic to aquatic organisms. As temperature and pH increase in the aquatic environment, the proportion of NH_3 to NH_4^+ also increases (Ip et al. 2001). With this understanding, formulas and laboratory measurements can be used to evaluate NH_3 toxicity. Broderius et al. (1985) evaluated the toxicity of NH_3 to early life stages of SMB at varying pH levels. These data provide insight as to whether YOY SMB are generally more or less susceptible to the toxic effects of ammonia.

10.2 Evidence (Appendix B: Worksheet 20, 21, 22, and 52)

10.2.1 Temporal co-occurrence

If ammonia directly affects SMB recruitment, then ammonia concentrations should be higher post-2005 than pre-2005. SRBC collected discrete ammonia samples at the Juniata River at Newport from 1984-2013 (Worksheet 52). No temporal trend was discernable; however, there were too many data gaps to make a confident conclusion (Score = 0).

All NH_3 data collected by the SRBC anywhere within the Susquehanna River Basin were pooled and separated by major subbasins (Chemung River, upper, West Branch, middle, lower Susquehanna River, and Juniata River) and into two time periods, pre-2005 and post-2005. Only data collected from May to September were included. Data were plotted using box plots and analyzed for differences using a non-parametric test (Worksheet 22). Results suggest ammonia values were either significantly higher during the pre-2005 time period or not significantly different; however, this evidence was not scored because it covered basin wide analyses which go outside the case study.

10.2.2 Spatial co-occurrence

If NH_3 directly affects SMB recruitment, then NH_3 should be higher at subject sites than the comparison sites. Discrete total ammonia (NH_3 plus NH_4^+) samples were collected by PADEP and USGS during the spring and summer of 2012 and 2013 at several subject and comparison sites where continuous water temperature and pH sensors were deployed. The discrete total ammonia samples were grouped together at each site and descriptive statistics were calculated (Worksheet 20). Concentrations were similar at subject and comparison sites.

It could be that even at similar total ammonia values, toxicity could be occurring at the subject sites if pH and water temperature increased the proportion of NH_3. This potential was evaluated by comparing the discrete total ammonia measurements against PA chronic ammonia criteria values adjusted for pH and water temperature using the continuous data (Worksheet 20). Discrete measurements were lower than the continuous chronic criteria at both subject and comparison sites (Score = --, see also discussion in section 10.2.3 below).

10.2.3 Stressor-response from elsewhere

If NH_3 toxicity directly affects SMB recruitment, then NH_3 concentrations within subject sites should be as high as or higher than concentrations of concern. Broderius and others (1985) developed total ammonia chronic no-observed-effect levels for SMB at various pH levels. Temperature was held constant throughout the study (at 22°C). For comparison purpose, PA chronic NH_3 toxicity criteria values were derived from formulas in Table 3 of 25 Pa. Code §93.7(a) (Table 3, Worksheet 21).

Table 3. Comparison of chronic no-observed-effect levels in SMB to the PA chronic NH_3 criterion at four pH levels.

pH	SMB Chronic No Observed Effect (mg/L)	PA Chronic NH_3 Criteria (mg/L)
6.60	17.4	2.1
7.25	14.4	1.4
7.83	14.6	0.8
8.68	2.4	0.1

These data suggest that the early life stages of SMB are much more tolerant to the toxic effects of NH_3 than the organisms that were used to develop the PA ammonia criteria, and that the observed ammonia concentrations (Worksheets 20 and 52) are well below levels of concern for YOY SMB (Score= ---).

10.3 Discussion

The body of evidence weakens the argument that high ammonia is the cause of SMB decline; concentrations of total ammonia are similar at subject and comparison sites and are well below no-effect concentrations observed for early life stages of YOY SMB (Consistency Score = ---). The samples were taken in the main channel and may not reflect conditions in YOY habitat during lower flow years. However, the influence of pH and temperature on toxicity was accounted for, and the benchmarks for comparison are highly relevant because they are based on data from early life stages of SMB.

Candidate Cause 11: Increased algal and cyanobacterial toxins

11.1 Cause Description

Cyanobacteria (often called blue-green algae) are photosynthetic organisms that are common in aquatic environments (Quiblier 2013). Cyanobacteria are capable of producing toxins, usually referred to as cyanotoxins, that are harmful to fish (Pavagadhi 2013) as well as other organisms. Cyanotoxins may be classified into three broad toxin groups: cyclic peptides, alkaloids, and lipopolysaccharides (IARC 2010).

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

- Microcystins (MC) – cyclic peptide (IARC 2010) with at least 80 variants known that primarily affect the liver. Most common variant is Microcystis (MC)-LR. Other common variants include MC-YR, RR, LW (Abraxis 2014). Extremely stable and resistant to chemical breakdown – in typical ambient conditions half-life is 10 weeks (Butler 2009). Chronic exposure to MCs can cause serious health problems in animals and humans such as hepatic, gastric and epidermic diseases; neurological impairment; and death (Babica et al., 2006; Leao et al., 2009; Sivonen and Jones, 1999). There is also potential for a negative relationship between condition factor and increasing MC concentrations (Acuna et al. 2012).

- Anatoxin – alkaloid toxin (IARC 2010) with 2 to 6 variants. Typical variants include Anatoxin-a and homoanatoxin-a. Primarily a neurotoxin.
- Cylindrospermopsons – alkaloid toxin (IARC 2010). With 3 known variants. Primarily a liver toxin.
- Saxitoxin – alkaloid toxin (IARC 2010). Commonly associated with marine based Paralytic Shellfish Poisoning (PSP) in humans. However, toxins are also produced in freshwater systems.

11.2 Evidence (Appendix B: Worksheets 15, 23, 32, 33, 34, and 50)

11.2.1 Spatial co-occurrence

If cyanotoxins affect SMB recruitment, then taxa that produce toxins should be more prevalent at subject sites than comparison sites.

PADEP and USGS collected cyanobacterial samples in 2005, 2012, and 2013 from multiple sites throughout the Susquehanna Basin. Identified taxa were associated with cyanotoxin production based on Quiblier (2010). Sites corresponding to 2014 PADEP cyanotoxin fish tissue collection were evaluated for the potential that cyanotoxin producing cyanobacteria may have been present at those sites (Worksheet 32). The analysis suggested that cyanobacteria genera known to produce toxins were observed at both subject and comparison sites at the Susquehanna, Delaware, and Allegheny rivers (Score = -).

This evidence has a degree of uncertainty because the presence of toxin producing cyanobacteria does not necessarily imply that toxins are being produced. Only certain strains within a taxa group possess the genotype necessary for toxin production. Also the environmental factors that stimulate algal toxin production are not well understood for benthic cyanobacteria.

11.2.2 Exposure

Microcystin is known to bioaccumulate in fish (Martin and Vasconcelos 2009). If MC is affecting recruitment of SMB into age class 1+, then MC should be detected in fish livers, and there should be a greater frequency of positive detections or higher MC values in livers at the subject sites than the comparison sites.

PADEP collected liver tissue from adult female SMB during the spring of 2014. Liver samples were analyzed using the enzyme-linked immunosorbent assay (ELISA) method at the PADEP laboratory (Worksheet 33). All sites had at least some positive detection for MC in SMB liver tissue. Liver samples from subject sites were typically similar or lower in MC than all comparison sites. Owing to the limited sampling (only adults in one year), it was difficult to make a determination using this evidence (Score = 0). There was also no exposure evidence available for other algal toxins.

11.2.3 Causal Pathway

Cyanobacteria blooms are often associated with eutrophic or nutrient rich conditions. The evidence described in this section discusses the potential for nutrients and low flow conditions to create the necessary antecedent conditions for blooms. The relationship between nutrients and cyanobacteria blooms is incompletely understood. For example, many cyanobacteria genera are highly competitive at low dissolved inorganic phosphorus (DIP; i.e., orthophosphate) concentrations and have novel ways to acquire organic phosphorus compounds (O'Neil 2012). Additionally, many genera also "display great flexibility in the nitrogen (N) sources they exploit to form blooms" (O'Neil 2012). As discussed above, the presence of toxin producing cyanobacteria does not necessarily mean that toxins are being produced. Toxin production is likely a function of genotype and environmental conditions, and is currently an area of active research. Still, there was evidence of at least some steps in the causal pathway leading to cyanotoxins (Overall score = +).

Increase in Phosphorus

Discrete orthophosphate measurements were downloaded from PADEP's Sample Information System (SIS) and analyzed by USGS to calculate loads and evaluate trends using the ESTIMATOR and WRTDS regression models. There was a large spike in orthophosphate loads at many locations during 2003 and 2004 (Worksheet 15, also reviewed in Worksheet 23). Orthophosphate trends have increased in the last 20 years in the Susquehanna River basin. However, between 2003 and 2012 orthophosphate trends have significantly decreased (Worksheet 15, Score = +).

Increase in Nitrogen

In analyses conducted during Workshop 3 (C. McGarrell, PADEP, personal communication), total nitrogen (TN) levels at the Juniata River at Newport were higher than at the Susquehanna River at Harrisburg and Delaware River at Morrisville. However, the TN levels were just as high in low SMB CPUE years as they were in high SMB CPUE years (Score = 0).

Dissolved Inorganic Nitrogen (DIN):DIP ratios

DIN:DIP ratios were calculated based on discrete data from 2012 and 2013 for two subject sites and one comparison site. The algal taxa lists discussed in Section 11.2.1 were compared to DIN:DIP ratios that have been reported to be associated with cyanotoxin production (Pawlik-Skowronska et al. 2013). The microcystin toxin producing taxa of *Phormidium* spp. and *Microcystis* spp. are present in the subject area and the DIN:DIP ratios are usually above the 23:1 threshold suggested by Pawlik-Skowronska et al. (2013) to favor the production of microcystin. Though *Phormidium* spp. was also identified at the comparison site (Delaware River at Morrisville) the DIN:DIP ratio threshold suggested by Pawlik-Skowronska et al. (2013) is likely met less often there (Worksheet 50, Score = +, with recommendations for continued research).

Decrease in Late Summer Flow

Discharge data from the Harrisburg gage from 1980 to 2014 were used to visually evaluate whether discharge patterns have changed over time and if patterns coincided with the SMB declines (Worksheet 23). The period of 1999 through 2004 is marked by a lack of major elevated discharge events (> 275,000 cfs) coupled with the presence of low discharge events (< 4,000 cfs). From 2005 to the present, elevated discharge events have occurred more frequently. Although the period of low discharge preceded the initial observations of recruitment declines, low recruitment has continued despite frequent extreme discharge events from 2005 to the present (Score = -).

Algal blooms (which may or may not include cyanobacteria) were documented in the subject reach using aerial photographs in October 2007 and July 2012 following low flow periods (Worksheet 34). The surface blooms were concentrated along shoreline habitats and between islands on the river. Additional photographs of a filamentous algae bloom on the Susquehanna River at Rockville were taken by Geoff Smith of the PFBC on August 12, 2012 coinciding with a fish pathology survey where YOY SMB with abnormalities were identified (Score = +).

Increase or Change in Algal Productivity Surrogates

High algal productivity and photosynthetic activity is typically associated with large diel swings in DO and pH, and high temperatures. Values of these surrogates for algal productivity were data collected during years with mean June discharge values below the long-term mean flow (to control for effects of flow on CPUE) (Worksheet 35). There was no difference between periods of time with low YOY CPUE values and high YOY CPUE (Score = -).

11.3 Discussion

The body of evidence for cyanotoxins as the cause of SMB recruitment declines was inconclusive and more research is recommended. Taxa that are capable of producing cyanotoxins were observed at subject sites, and cyanotoxins were measured in livers of adult SMB. However, both of these conditions were also observed at the comparison sites. Additionally, presence of taxa does not necessarily mean cyanotoxins will be produced.

Other evidence comes from evaluating whether conditions that promote cyanobacterial blooms occur at the subject sites. A peak in DIP loads was observed in 2003 and 2004 at subject sites suggesting a possible causal pathway, yet similar spikes were seen at comparison sites as well. DIN:DIP ratios consistent with cyanotoxin production were more frequently observed at subject sites than comparison sites, and algal blooms have been documented during low flow conditions in two separate years. However, neither low flow conditions nor water temperatures expected to promote algal blooms were associated with YOY CPUE. Similarly, diel swings of pH and DO typically associated with algal blooms also were not associated with YOY CPUE (Consistency Score for microcystins = -, other algal toxins = +).

Additional monitoring to document the cyanobacteria species present, determining whether cyanotoxins are present in YOY SMB microhabitats and whether there is evidence of YOY SMB exposure to these toxins is necessary to fully understand the role it may play in the Susquehanna River system.

Candidate Cause 12: Toxic chemicals

12.1 Cause Description

Virtually all anthropogenic chemicals have pathways to the environment. Most chemicals enter aquatic ecosystems through sources including, but not limited to agriculture (Halling-Sorensen et al. 1998), combined sewer overflows (Philips and Chalmers 2009), and wastewater disposal systems (Carrara et at. 2008, Sellin et at. 2009, Phillips et at. 2010).

Toxic chemicals and their degradates can have variable persistence in the aquatic environment and may have stronger affinities to water or sediment (Reif et al. 2012). In recent years, technological advancements have made it possible to quantify most toxic chemicals even at minute concentrations (Snyder et at. 2009). Studies have documented the occurrence of many toxic chemicals in aquatic environments (Kolpin et at. 2002, Loper et al 2007, Blazer et al. 2010, Reif et al. 2012, PADEP 2014a), but the spatial distribution and the complete understanding of their effects on aquatic life generally and SMB specifically, are still in review (Daughton and Ternes 1999, Jorgensen and Halling-Sorensen 2000, Blazer et al. 2014, PADEP 2014a).

12.2 Evidence (Appendix B: Worksheets 37.1, 38, 39, 40, 42, 45, and 46)

12.2.1 Spatial co-occurrence

If metals, polychlorinated biphenyls (PCBs) or other organic chemicals were causing the declines in SMB recruitment, higher concentrations of these chemicals would be expected at subject areas than comparison sites.

Metals

The PA DEP collected discrete sediment and water samples to be tested for metals from both subject sites and comparison sites in 2013 and 2014 (Worksheets 40 and 46). In sediment, the Youghiogheny River at Sutersville, Juniata River at Lewistown, and Conodoguinet Creek at Good Hope had the highest concentrations of metals. In water samples, concentrations were frequently highest at the Youghiogheny comparison site. There is no consistent pattern between prevalence of metals and low recruitment of YOY SMB (Score = -).

Organic toxicants

PADEP collected sediment samples from both subject and comparison sites in 2013 and 2014 (Worksheets 37.1, 38 and 39). Historical pesticides detected were 4,4’-DDD, 4,4’-DDE, chloroneb, cis-permethrin, trans-permethrin, hexachlorobenzene, and trifluralin. There were few discernable patterns in the data; historically used pesticides were detected at many sites, with one or two detections per site per sample when detected. PCBs were detected a total of six times, at very low concentrations. PADEP also sampled for wastewater compounds (samples analyzed by USGS). Compounds were detected at many sites in no discernable pattern. Currently used pesticides were also sampled for (analyzed by USGS) and most were infrequently encountered (Score = -).

PADEP deployed passive water samplers at both subject and comparison sites in 2013 to be tested for a variety of different compounds (organochlorine & currently used pesticides, PCBs, Polybrominated diphenyl ethers (PBDEs, flame retardants), hormones, wastewater compounds, and polycyclic aromatic hydrocarbons (PAHs)), all analyzed by USGS (Worksheets 42 and 37.1). Due to the possible immunosuppression and endocrine disrupting effects, results for hormones and herbicides are discussed under Candidate Cause 13. Pharmaceutical data is currently pending. Results for other toxic substances are presented here.

A large number of compounds were tested and many results were combined by chemical class and mapped to aid evaluation (Worksheet 37.1). PAHs were ubiquitous. Total PBDE concentrations were highest at the Delaware River (comparison site) and

Juniata River (subject site). Concentrations of Dichlorodiphenyltrichloroethane (DDT) and its metabolites were highest at comparison sites. Bromoform was only detected at one subject site (Juniata River at Lewistown). However, due to a limited sample size and lack of striking differences between subject and comparison sites, the evidence for organic toxicants was given a score of 0.

12.2.2 Stressor-response from elsewhere

Chemical concentrations in water quality grab samples were compared to readily available Ambient Water Quality Criteria (AWQC) (i.e., for metals. Criteria for organic chemicals were not available at the time of the workshop). Two sets of samples were compared; the first set drew from PADEP's water quality network for the years 2006-2013 for the Susquehanna River at Marietta and upstream sites (Worksheet 45). The second set of samples encompassed data from 121 sites collected during 2013 and 2014, and included subject sites, nearby tributaries, and comparison sites (Worksheet 46). Aluminum, lead and iron concentrations showed concentration spikes that exceeded chronic AWQC. The evidence was not scored because it is not known how these levels affect SMB. However, given the pH observed in the water column, aluminum and iron would be expected to occur as floc or other particles, rather than as the toxic free ion form.

12.3 Discussion

Concentrations of metals and organic toxicants were not clearly higher at subject sites, arguing against this Candidate Cause (Consistency Score = -). However, the evidence is weak owing to the scarcity of data for organic toxic substances, the low number of samples and sampling years, and the lack of pre-2004 datasets or stressor-response relationships that could be used to derive additional evidence.

Candidate Cause 13: Increased Endocrine Disrupting Chemicals (EDCs) and Herbicides

13.1 Cause Description

EDCs are environmental contaminants that interfere with hormonal regulation. Historically, concern over EDCs has focused on reproductive effects (e.g., Fatima et al. 2007, Kidd et al, 2007, Scholz and Kluver 2009, Kloas et al. 2009, Blazer et al. 2012). Due to the mode of action, EDCs were judged to be unlikely to directly kill YOY SMB. Similarly, herbicides were judged to be unlikely to be directly causing mortality of YOY SMB. However, each of these two chemical categories (including their degradates) has been linked to immune suppression in fish (Ahmed 2000, Kreutz et al. 2010, Rohr and McCoy 2010, Casanova et al. 2011, Milla et al. 2011, Shelley et al. 2012, Rogers et al. 2013). For this reason, the categories were combined. Suppressed immune functionality could lead to mortality and decreased recruitment of YOY SMB by increasing the frequency and severity of disease (see discussion under Candidate Cause 14).

13.2 Evidence (Appendix B, Worksheets 37.1, 38, 39, 42, 43, 44, 48, and 49)

13.2.1 Spatial co-occurrence

If EDCs or herbicides are causing the declines in SMB recruitment, higher concentrations of these chemicals would be expected at subject sites than at comparison sites.

PADEP deployed passive water samplers at 12 sites within and out of the Susquehanna basin in 2013 to be tested for a variety of different compounds (Worksheet 42 and 43: PAHs, wastewater compounds and pesticides were discussed under Candidate Cause 12). Samples were analyzed by USGS. Many results were combined by chemical class and mapped to aid evaluation (Worksheet 37.1). Highest total estrogenicity levels and higher concentrations of hormones were found in samplers deployed in the Juniata River at Newport, and Susquehanna River at Sunbury, Harrisburg and Marietta[3] sites (Worksheet 37.1) (Score = +). The highest concentrations of triazine herbicides were found at the Juniata River at Lewistown and Susquehanna at Marietta sites (Score = +). In a separate study; however (Worksheet 48, Blazer et al. 2014), USGS found higher levels of atrazine in one passive sampler deployed at the Allegheny River at Kittanning than one deployed at Juniata River at Newport, but this evidence was not scored because of the limited sample size.

USGS and SRBC collected discrete water samples under baseflow and stormwater conditions at four subject sites and one comparison site from March through August 2013 and 2014 (Worksheet 44). Atrazine and metolachlor were found at higher concentrations or frequencies of detection at the subject sites (particularly the Juniata River at Newport and Susquehanna at Marietta) compared to the Delaware River comparison site (Score = +).

USGS collected discrete water samples from 12 sites in 2010 (Worksheet 48). There was no consistent pattern in the frequency of hormone detection between subject and comparison sites. In addition, PADEP collected discrete water samples from 29 tributary sites (2013) and tested them for total estrogenicity (Worksheet 49). Samples were

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[3] The Susquehanna River at Marietta sampling location is located approximately 20 miles downstream of York Haven. Although outside of the subject reach, environmental data were considered to be sufficiently relevant to be included.

analyzed by USGS. Estrogenicity was detected with low frequency, but all five sites with detected estrogenicity were in tributaries to the subject reach (Score = 0).

PADEP collected sediment samples from both subject and comparison sites in 2013 and 2014 (Worksheets 38, 39, and 47). Samples were analyzed by USGS. There was no clear pattern in the frequency of detection of herbicides or hormones detected (Score = 0).

13.2.2 Evidence of Exposure

If YOY SMB were exposed to EDCs, then biomarkers of estrogenic exposure should be higher at subject sites than comparison sites. However, the use of measures of reproductive endocrine disruption to assess the condition of YOY SMB is not effective as the fish are not yet mature. Measurements of these biomarkers in adult SMB are used here as surrogates to evaluate whether exposure of YOY to EDCs is occurring.

USGS collected SMB at 16 locations in the basin and outside of the basin from 2007 to 2010 to evaluate reproductive endocrine disruption in adult SMB using plasma vitellogenin and prevalence and severity of testicular oocyte formation in male fish (Worksheet 48, Blazer et al., 2014). Plasma vitellogenin levels were variable within similar bounds in fish collected at the Susquehanna River, Juniata River and Allegheny River at Kittanning sites. However, more male SMB were found to have testicular oocytes (intersex) in the Susquehanna River and Juniata River subject sites, and Swatara Creek, a tributary to the middle Susquehanna River than the Allegheny River at Kittanning (Score = +).

13.3 Discussion

Evidence that water concentrations of potential EDCs and herbicides are higher at the Susquehanna River and Juniata River subject sites than comparison sites supports the argument for this candidate cause. The higher frequency of testicular oocyte formation in adult SMB from the Susquehanna and Juniata rivers lends further support that exposure to EDCs is at biologically meaningful concentrations (Consistency Score = +). However, the evidence is weak due to the low number of samples and sampling years, the lack of pre-2004 datasets and a lack of stressor-response relationships that could be used to derive additional evidence. For this reason, additional research is recommended.

Candidate Cause 14: Increased pathogens and parasites

14.1 Cause Description

As described in the introduction, a high frequency of disease associated with pathogen and parasite infections has been noted in YOY SMB since 2005 (Figure 7). Pathological analysis indicated that the disease was caused by secondary infections by opportunistic bacteria (e.g., *Flavobacterium columnare, Aeromonas hydrophila*) (Smith et al. 2015). As secondary pathogens, these organisms do not typically kill fish directly but manifest as a result of another factor affecting immune function.

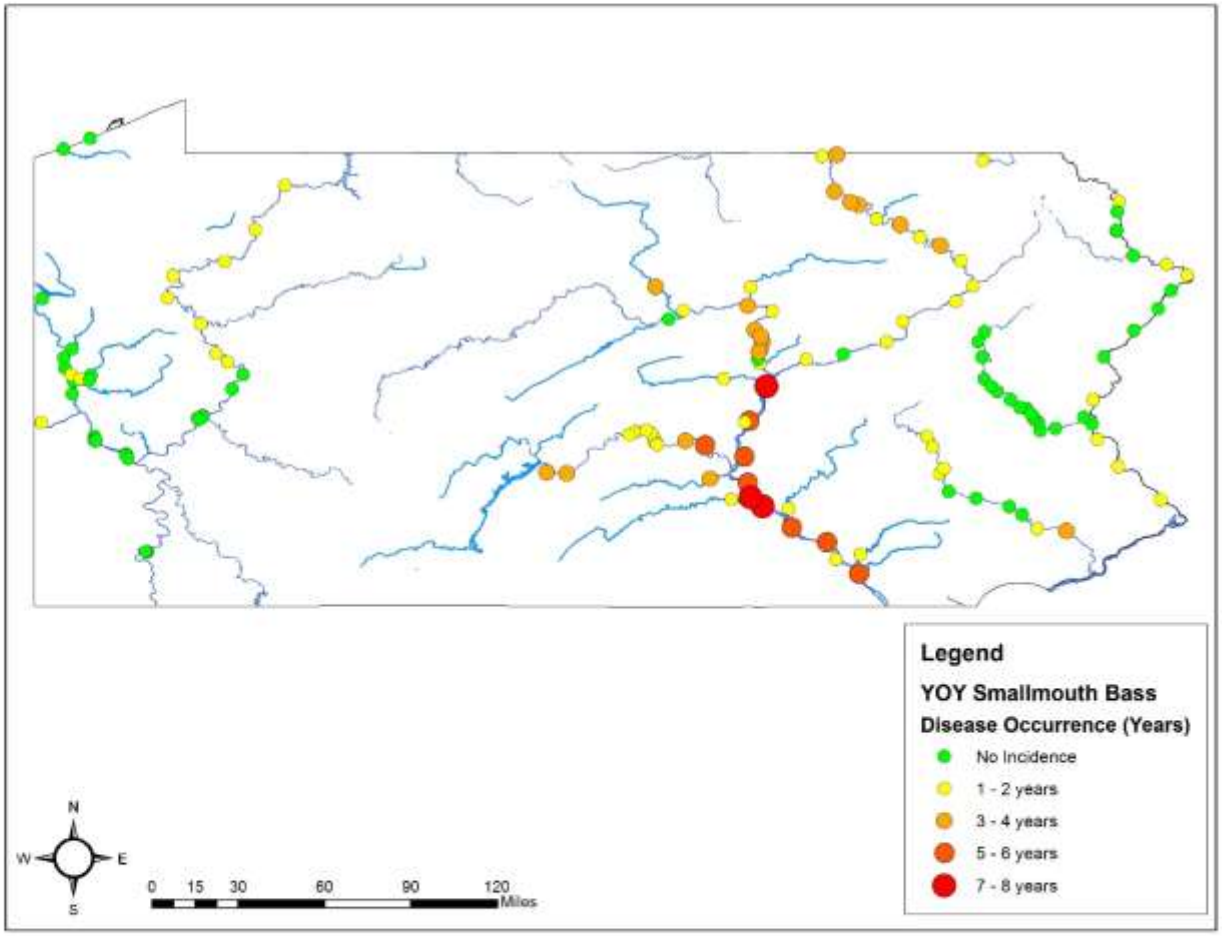

Figure 7. The number of years YOY SMB were collected with obvious signs of disease (lesions, fin erosions, or dead individuals) during backpack electrofishing surveys at major river and warm-water stream locations in Pennsylvania.

Additional pathogens and parasites have been associated with the disease outbreaks (Walsh et al. 2012; Smith et al. 2015). Largemouth Bass Virus (LMBV) has been consistently isolated from moribund SMB specimens collected from the Susquehanna Basin. Clinically diseased fish and apparently healthy fish from the same location have yielded LMBV during past cell culture analysis. Despite testing positive for LMBV, fish did not exhibit the clinical signs of LMBV infection described by Plumb et al. (1996). The repeated detection of LMBV in juvenile SMB specimens from diseased populations and the coincident onset of disease and discovery of LMBV in the Susquehanna River Basin in 2005 (USFWS, National Wild Fish Health Database) necessitates a closer investigation into the potential role of LMBV in causing these annual mortality episodes in juvenile SMB.

An additional concern that is currently being investigated is the presence of the myxozoan parasite *Myxobolus inornatus* (Walsh et al. 2012) within muscle and connective tissue of YOY SMB. Myxozoan parasite infections can be responsible for important economic losses in fisheries and aquaculture industries (Sitjà-Bobadilla 2008, Nehring and Walker 1996, Koel et al. 2006, Hanson et al. 2008, Holzer et al. 2013). Internal and external signs of parasite infections, both trematodes and myxozoans, are commonly observed in the diseased YOY SMB. However, the role that the myxozoan parasites play in YOY SMB mortalities remains unclear. Parasites in general may compromise YOY SMB in a number of ways: high parasite loads may contribute to general stress, as well as immune suppression, resulting in increased susceptibility to opportunistic bacteria; or sites of parasite entry or exit may cause wounds allowing for bacterial entry.

While habitat selection based on higher temperature may benefit YOY SMB metabolically, it also potentially increases the risk of exposure to pathogens. Grant et al. (2003) found that temperatures above 30^{o}C increased the viral replication rate of LMBV and that Largemouth Bass infected with LMBV had higher rates of mortality and higher viral loads at 30^{o}C than at 25^{o}C in laboratory experiments. Similarly, some common bacterial pathogens such as *Flavobacterium columnare* (20 – 25^{o}C, Starliper and Schill 2010*)* and *Aeromonas hydrophila* (25-35^{o}C, Cipriano and Austin 2011*)* share optimal temperature ranges to that of YOY SMB. Therefore, a complex relationship between pathogen and parasite virulence and environmental factors potentially exists.

14.2 Evidence (Appendix B: Worksheets 27, 29, 30, and 31)

14.2.1 Spatial Co-occurrence

If pathogens and parasites affect SMB recruitment then they should be more prevalent at subject sites than comparison sites. Largemouth Bass Virus infected SMB were found at both subject and comparison sites (Worksheet 29). Yet, the sample size available for this analysis was limited and more data are needed (Score = 0). The incidence of the myxozoan parasite infections from histological data of YOY SMB was reviewed for 2013 and 2014 (Worksheet 30). Similarly, it was determined that this parasite occurred at both subject and comparison sites. Although the data demonstrate SMB are exposed to LMBV and the myxozoan parasite, more data are needed to determine if prevalence is greater in subject sites than comparison sites (Score = 0 for LMBV, 0 for Myxozoans, and 0 for other pathogens).

14.2.2 Evidence of Mechanism

If pathogens and parasites were causing the decline in SMB population, then there should be a negative relationship between prevalence and CPUE. The association between prevalence and the next year's age-1 CPUE was calculated using Spearman's rank correlation (Worksheet 31). Although the data used in this worksheet were limited, they demonstrated a strong negative correlation between disease and the following year's age class CPUE (Figure 8, r = -0.90) (Score = + for LMBV, + for Myxozoans, and + for other pathogens).

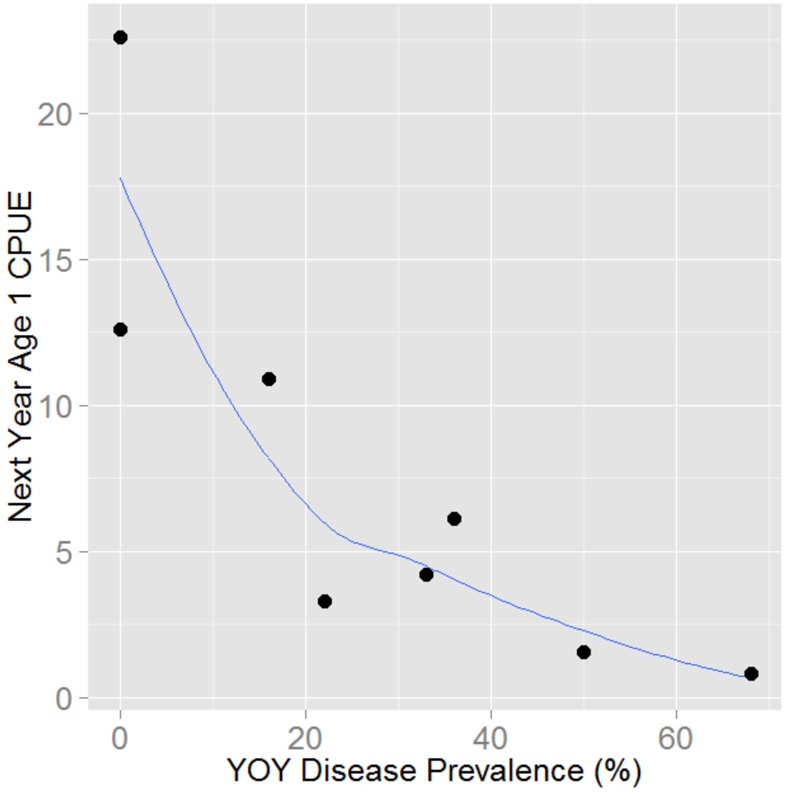

Figure 8. Disease prevalence of YOY SMB during backpack electrofishing surveys compared to age-1 SMB catch per unit effort during night boat electrofishing surveys during the following year at the middle Susquehanna River (Sunbury to York Haven). Line added to aid visualization.

14.2.3 Causal Pathway

If pathogens and parasites are causing the decline in SMB recruitment, then other environmental factors should be allowing for increased prevalence of these organisms, or increasing YOY SMB susceptibility to them. Continuous, physicochemical data (water temperature, pH, and DO) from subject reaches were overlain with results from YOY

SMB surveys (Worksheet 27). There is evidence of stressful water quality conditions occurring prior to the onset of disease at some reaches during some years, but it does not occur consistently. For example, there are incidences where stressful physicochemical conditions occur where no disease is noted as well as instances when physicochemical conditions are not particularly stressful, yet signs of disease were found (Score = 0).

14.3 Discussion

More data on specific pathogens and their virulence are needed. However, there is a considerable linkage between observed disease of YOY SMB and recruitment into the adult population as demonstrated by a high correlation (Consistency Score = +). Currently, there are no consistent patterns in physicochemical data that precede disease onset among reaches and years. Other factors or interactive effects may influence the impact of pathogens and parasites, but more research is needed.

Conclusions

The CADDIS process narrowed the scope of concerns that may be affecting SMB populations in the Susquehanna and Juniata rivers. A summary of each candidate cause score and consistency score is provided in Appendix C. Based on all of the evidence, a classification of **Likely**, **Uncertain**, **or Unlikely** was assigned to each candidate cause (Table 4). Eight candidate causes were not supported by the data analysis and were considered unlikely for directly causing the decline of YOY SMB recruitment. The evidence for eight additional candidate causes was judged to be uncertain. With more data and analysis, these factors could be directly or indirectly implicated in the declines in SMB recruitment (see the section below on Recommendations and Future Research).

Given the current state of available information, the CADDIS process identified EDCs and herbicides (Candidate Cause 13) and pathogens and parasites (Candidate Cause 14) as likely causes for the decline in recruitment of YOY SMB into the adult population (Table 4; Figure 9). It is critical to note that this report only serves to provide information on the current state of data collection and conclusions related to the SMB decline and disease. There are many data that have yet to be analyzed and interpreted which may affect any final conclusions.

Table 4. Candidate cause classifications

Candidate Cause #	Candidate Cause Name
	Likely Causes
13	EDCs and herbicides[1]
14	Pathogens & Parasites with other stressors[2]
	Unlikely Causes
1	High Flows
2	Intraspecific Competition
4	YOY Food Quality: fatty acids
7	Temperature – direct mortality
8	pH
9	Dissolved Oxygen – direct mortality
10	Ammonia (NH_3)
12	Toxic Chemicals: pesticides/PCBs/metals
	Uncertain Causes
3	Interspecific Competition
4	YOY Food Quality: thiaminase
5	Egg Quality
6	YOY Habitat
7	Temperature – increased disease
9	Dissolved Oxygen – increased disease
11	Algal & Bacterial Toxins
14	Pathogens & Parasites alone[2]

[1] The evidence available for herbicides was limited and future monitoring is planned to obtain more data.

[2] The workshop participants concluded that pathogens and parasites were likely interacting with other candidate causes in order to produce the disease. It is uncertain whether they would be capable of doing so alone.

The data used to derive evidence for this report had many good qualities that increase overall confidence in conclusions. Many datasets were collected at both subject sites and comparison sites using the same methods, sampling designs and during the same timeframe. Data that were used to temporally contrast conditions pre- and post- 2005 were particularly valuable. Data for many candidate causes were measured from continuous and passive monitoring systems, increasing confidence that intermittent events were captured. However, much of the intensive and passive sampling was conducted in 2013 and 2014. In these years, the Susquehanna and Juniata Rivers experienced average to above average discharge and data would not necessarily represent conditions during low-flow years. Environmental conditions in years with low flow are of particular interest because low flows in late spring were historically (i.e., pre-2005) associated with strong recruitment of YOY into adult age classes.

A final conceptual model diagram was created to illustrate the overall conclusions of the CADDIS process (Figure 9). The final diagram demonstrates the understanding that uncertain candidate causes may still be playing a role in the decline of the SMB. As more data are collected these candidate causes will be revisited to determine their contribution to the overall SMB population decline.

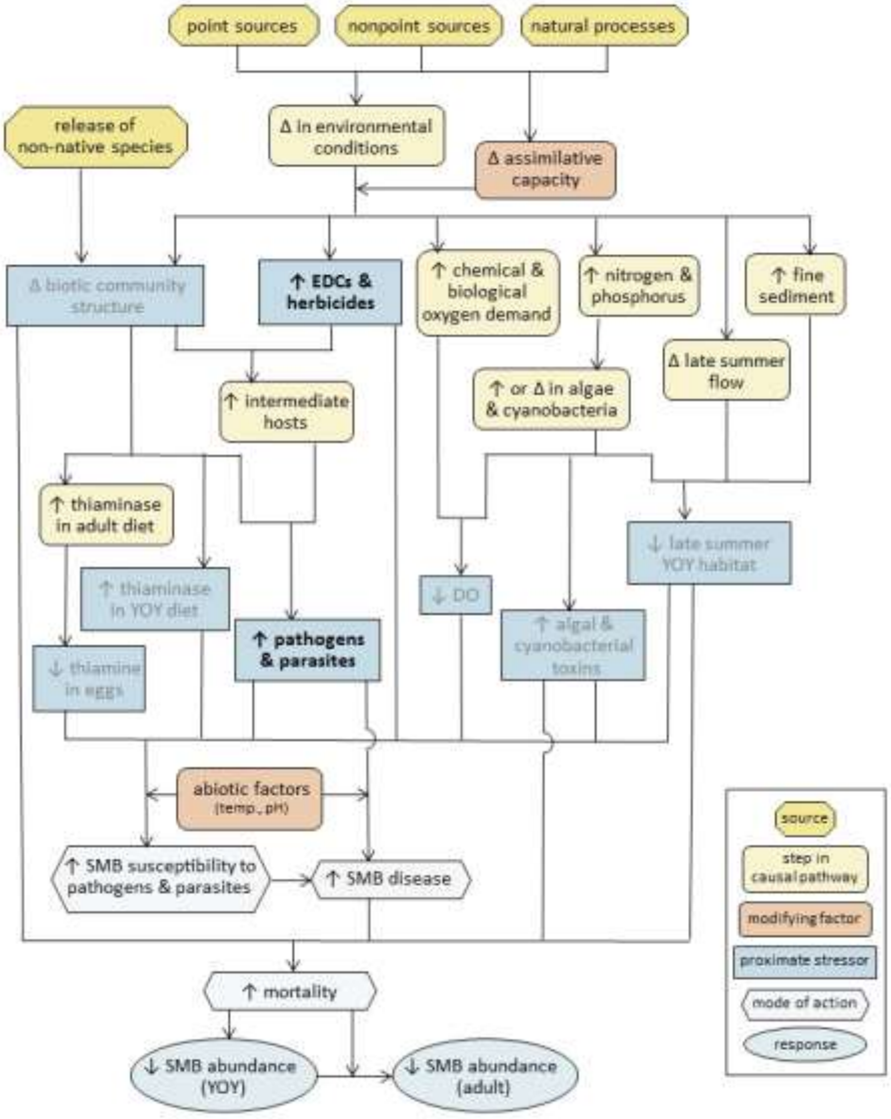

Figure 9. Final conceptual model diagram depicting likely (bold font) and uncertain (gray font) causes in the blue boxes. Further explanation of the legend can be found in Appendix A.

General Discussion

This report represents a large amount of work from many dedicated professionals across multiple agencies and organizations. It is the compilation of the current understanding as it relates to the SMB population decline in the Susquehanna River and will clarify needs for continued research. This report also provides greater transparency on what work has been completed thus far.

Even with these accomplishments, limited time and resources were available for the CADDIS process. Considerable amounts of data were not included because they were still being processed or collected in the field. Consequently, many sub-lethal, complex interaction hypotheses were not fully developed or analyzed. In addition, the process focused on identifying the proximate stressors causing the effects, with less attention paid to sources of those stressors. Many topics including but not limited to water temperature, pH, DO, orthophosphate, and other nutrients were discussed at great length during the CADDIS process, but were not prominently covered by the worksheets or this report. Some of these ideas are discussed in greater detail below.

A changing ecosystem

There is little doubt that the Susquehanna River has experienced water quality changes over the past 20-30 years. Even after the reduction in likelihood of several candidate causes, some parameters are still speculated to affect the health and survivorship of SMB (Chaplin et al. 2009, Chaplin and Crawford 2012, Blazer et al. 2014). However, tracking these effects can be difficult due to variable environmental conditions such as annual discharge (PADEP 2014a) and lack of long-term continuous datasets.

Growing concern over a warming climate and its effect on the landscape and to aquatic life is justifiable (Peterson and Kwak 1999, Najjar et al. 2000). As it relates to this report, increasing water temperatures are not just associated with SMB thermal tolerances, but also with the thermal preferences of pathogens and parasites that inhabit this system (Smith et al. 2015). The projections of increasing temperatures have even more profound implications such as increased precipitation and subsequent changes to stream flow (Najjar et al. 2000). Changes in the magnitude and duration of streamflow are known to cause physical habitat alterations and fluctuations in sediment transport (Poff et al. 1997, Maloney and Shull 2015), and these variations can affect aquatic life (Barbour et al. 1995, Davies and Jackson 2006). Even slight increases in water temperature can affect how chemicals interact with each other. A few relevant examples include an increased proportion of toxic ammonia and increased availability of orthophosphate (Cravotta et al. 2013, Cravotta, unpublished data). Water temperatures in the Susquehanna near Harrisburg, PA have increased between the 1970s and present (Chaplin and Crawford, 2012), potentially amplifying the effect of chemical interactions on aquatic organisms. With this in mind, data are continuing to be collected with greater spatial distribution and frequency than ever before, and a higher priority should be given to continuing these efforts.

Like temperature, pH also potentially affects SMB decline and disease (Chaplin et al. 2009, Chaplin and Crawford 2012, Smith et al. 2015). Although pH is not suspected to have direct effects on the SMB YOY as shown by the CADDIS evidence, there may be indirect effects that have yet to be elucidated. One interesting result from the pH data analysis was the increasing trend in pH levels at several locations across Pennsylvania; in particular, the discernible and relatively recent (since the 1990s) increases in pH at the Allegheny and West Branch Susquehanna Rivers (comparison sites). There are currently several hypothesis for this observed change, which may be working together to cause the observed effect. As Lynch et al. (2007) have shown, hydrogen ion deposition has significantly decreased as a result of the implementation of Title IV of the Clean Air Act Amendments in 1990 (Figure 10). Reductions in hydrogen ion deposition can increase pH, particularly in smaller streams with low alkalinity. Yet, the cumulative effect may very well be perceived in larger systems. In addition, a significant amount of work has been completed in the Allegheny and Susquehanna watersheds specifically addressing acid mine drainage (AMD). As a result, many stream miles have been restored to biologically productive conditions. Abatements, such as the ones developed for the Bennett Branch Sinnemahoning Creek, have significant and far reaching effects on pH levels especially in waters with low buffering capacity (Cavazza and Beam 2010, Baker et al. 2012). Although slightly less intense pH increases are observed in the CADDIS subject sites, it is plausible to conclude that the culmination of restoration activities to both air and water may be contributing.

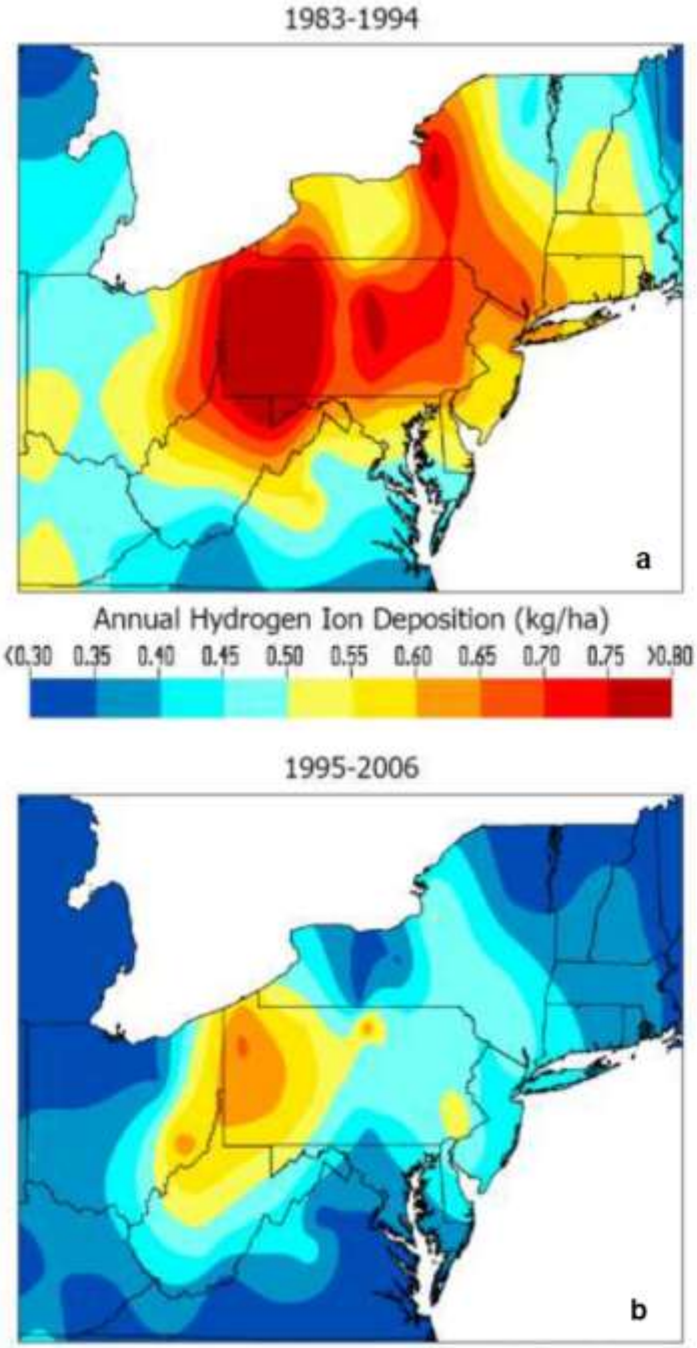

Figure 10. Reproduced from Lynch et al. (2007). Mean annual hydrogen ion deposition across Pennsylvania and neighboring states (a) before (1983-1994) and (b) after (1995-2006) implementation of Title IV of the Clean Air Act Amendments of 1990.

Other sources of concern surrounding the SMB decline include changes in nutrients and sediment loads over time. During the CADDIS workshops, trend analysis on certain nutrient parameters was completed, but not for every constituent. Again, this was primarily due to the limited time and resources available. However, trends in nutrient and sediment loads from the Susquehanna River are available from USGS and SRBC. The two regression models used are the traditional 7-parameter multi-coefficient regression (ESTIMATOR) and the recently developed Weighted Regression on Time, Discharge, and Season (WRTDS) model. Both regression models are similar except for an important distinction in how daily mean discharge is normalized. Comparisons between these two models suggest that WRTDS may be more appropriate for tracking changes in the Susquehanna River (Moyer et al, 2012). For the lower Susquehanna River just below the Conowingo Dam, WRTDS results indicate mostly decreasing nutrient trends with the exception of suspended sediment and total phosphorous (Table 5). Reasons for the increase in suspended sediment and total phosphorous yields from the lower Susquehanna River (impounded sections) are likely due to the loss of dam storage capacity, a decrease in sediment settling, and decreased scour threshold of substrate (Langland 2015, Hirsch 2012).

Unfortunately, published WRTDS flow-normalized trends have not yet been created for sites within the CADDIS subject areas (e.g., Juniata River at Newport, PA), but some statistically significant ($p < 0.05$) ESTIMATOR nutrient trends for the subject sites do exist. While the ESTIMATOR trends suggest that most nutrient and sediment loads from two sites within the subject areas are decreasing (Table 6), there are significantly increasing trends seen with orthophosphate in the Juniata River at Newport and other sites throughout Pennsylvania (PADEP 2014b). However, these substantial increasing trends depicted by ESTIMATOR are likely overestimations at least for orthophosphate. For example, ESTIMATOR trends for the Susquehanna River at Marietta show approximately 35% increase in orthophosphate, whereas WRTDS trends show approximately 2% increase for the same period of record (Langland, unpublished data). Increasing trends in orthophosphate may be due to several factors including increased pH and temperature (Cravotta, unpublished data), increased runoff of phosphorus-saturated soils associated with non-point sources (Langland 2015), and a greater percentage of no-till farming (Langland, unpublished data).

Table 5. Nutrient and suspended sediment trends from WRTDS flow-normalized yields at the Susquehanna River Input Monitoring (RIM) station (just downstream from the Conowingo reservoir) for the time periods 1985 to 2010 and 2001 to 2010. Table modified from Moyer et al. (2012).

Parameter	WRTDS flow-normalized yield			
	1985 to 2010		2001 to 2010	
	Total Change (%)	Slope (%/yr)	Total Change (%)	Slope (%/yr)
Total Nitrogen	-20.8	-0.8	-5.8	-0.6
Nitrate	-14.9	-0.6	-11.5	-1.3
Total Phosphorus	1.9	0.1	18.4	2.0
Orthophosphate	-17.1	-0.7	-2.2	-0.2
Suspended Sediment	86.5	3.5	71.1	7.9

Table 6. Approximate percent change in flow-adjusted trends derived from ESTIMATOR over long term (1992 to 2012) and short term (2003 to 2012) periods. NA indicates either there was no statistically significant trend or there were not enough data to run analysis. Table modified from PADEP (2014b).

Station Name	Trend Period	Total Nitrogen	Nitrate	Total Phosphorous	Orthophosphate	Suspended Sediment
Susquehanna River at Sunbury	Long Term	-87	-69	-36	NA	-86
	Short Term	-25	NA	NA	-61	NA
Juniata River at Newport	Long Term	NA	NA	NA	207	NA
	Short Term	-22	NA	-49	-65	NA

Even more complex changes to the Susquehanna River ecosystem have been the increased abundance and variability of emerging contaminants. These constituents are often challenging to track and understand due to the lack of biologically meaningful endpoints and environmental data. Many of these chemicals are thought to have endocrine disrupting and immunosuppressant effects on aquatic organisms (McMaster 2001, Pal et al. 2010). Emerging contaminants include pharmaceutical compounds, hormones, pesticides, flame retardants, and other organic waste water compounds (Hotchkiss et al. 2008, Diament-Kandarakis et al. 2009). Recent research suggests that even legacy contaminants such as polychlorinated biphenyls (PCBs) and heavy metals may be having estrogenic and immunosuppression effects (Klaper et al. 2006, Datta et al. 2009, Orton et al. 2011).

A greater understanding of these chemicals and their effect(s) on SMB is needed. Mixtures of these chemicals in an aquatic environment can have unpredictable (Sárria et al. 2011) or even additive (Brian et al. 2005) effects. Exposures at low concentrations during critical life stages are suggested to have both lasting immune system and reproductive consequences (Milston et al. 2003, Liney et al. 2005, Vandenberg et al. 2012). Some studies regarding the amount and number of emerging contaminants found in surface waters of Pennsylvania and their effects have been completed (Loper et al. 2007, Reif et al. 2012, Blazer et al. 2014). However, emerging contaminants should be at the forefront of continued research.

Recommendations and future work

The results of the CADDIS process indicate that EDCs and herbicides, as well as pathogens and parasites are likely causes of the SMB decline, but further research is needed. One of the most important next steps is to determine whether the SMB have suppressed immune systems, and identify factors that contribute to immunosuppression. The possible increase of intermediate hosts and other changes within the biological community are critical factors that should not be overlooked. Potential interactions between physicochemical parameters, nutrients, and emerging contaminants, and their relationship to SMB YOY need to be clarified.

A substantial amount of research on these topics is currently being conducted by PADEP, PFBC, USGS, USFWS, Penn State University, and SRBC. Several ongoing studies that were unable to be included in the current effort include egg contaminant analysis, adult and YOY SMB histopathology, evaluation of immune function in wild and laboratory raised SMB, population genetics, movement and habitat selection studies, myxozoan life cycle research, and intense water and sediment collections (V. Blazer, USGS, written correspondence). All of the studies mentioned above currently have results pending and could not be included in this CADDIS process. There are also many studies that have continued to progress during and after the current effort. Those studies include analyzing the susceptibility of SMB to LMBV, determining cyanotoxin levels in YOY habitats, and determining if YOY SMB have diets high in thiaminase. A more extensive list of continuing research may be found in Appendix D.

The CADDIS process provided a chance to consolidate research plans and coordinate future studies. Questions still left unanswered were discussed during the final workshop. These discussions provided many future data needs (Appendix E) such as a greater understanding of conditions within YOY SMB microhabitats, biological endpoints for emerging contaminants, and intermediate host relationships. As future research is concluded, the results will inform resource management decisions for the SMB fishery.

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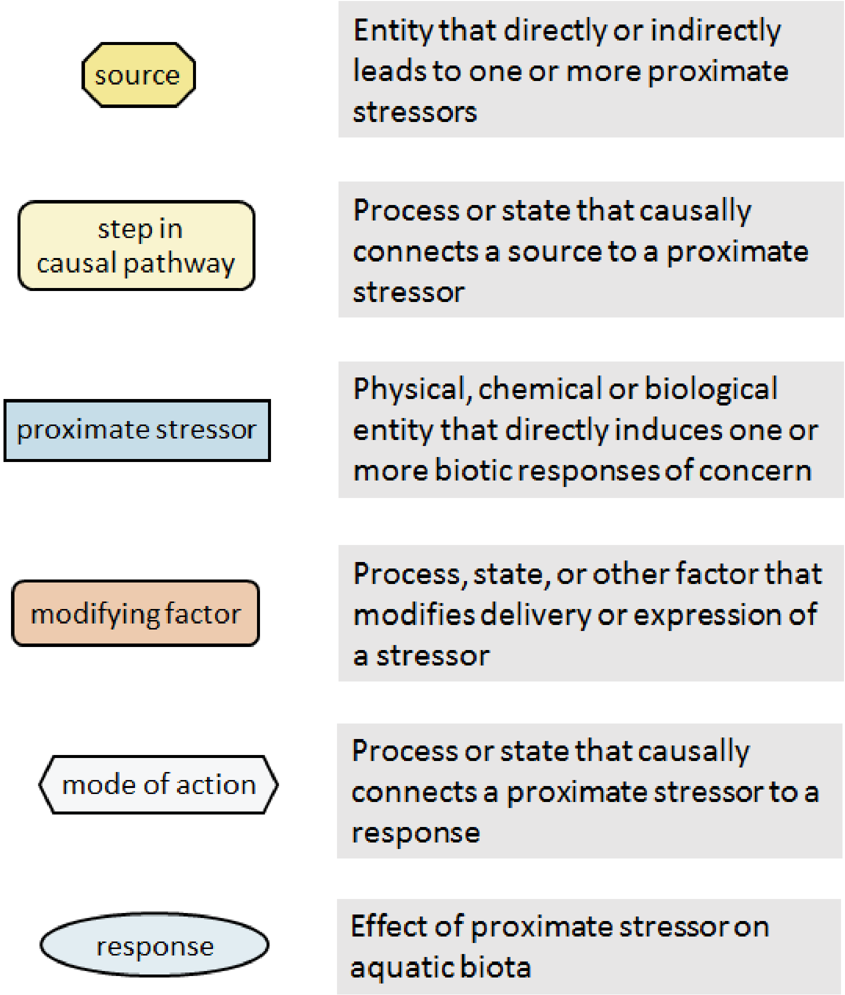
Appendix A: Conceptual Models



source	Entity that directly or indirectly leads to one or more proximate stressors
step in causal pathway	Process or state that causally connects a source to a proximate stressor
proximate stressor	Physical, chemical or biological entity that directly induces one or more biotic responses of concern
modifying factor	Process, state, or other factor that modifies delivery or expression of a stressor
mode of action	Process or state that causally connects a proximate stressor to a response
response	Effect of proximate stressor on aquatic biota

Figure 1. Explanation of the different types of pathways in the conceptual models.

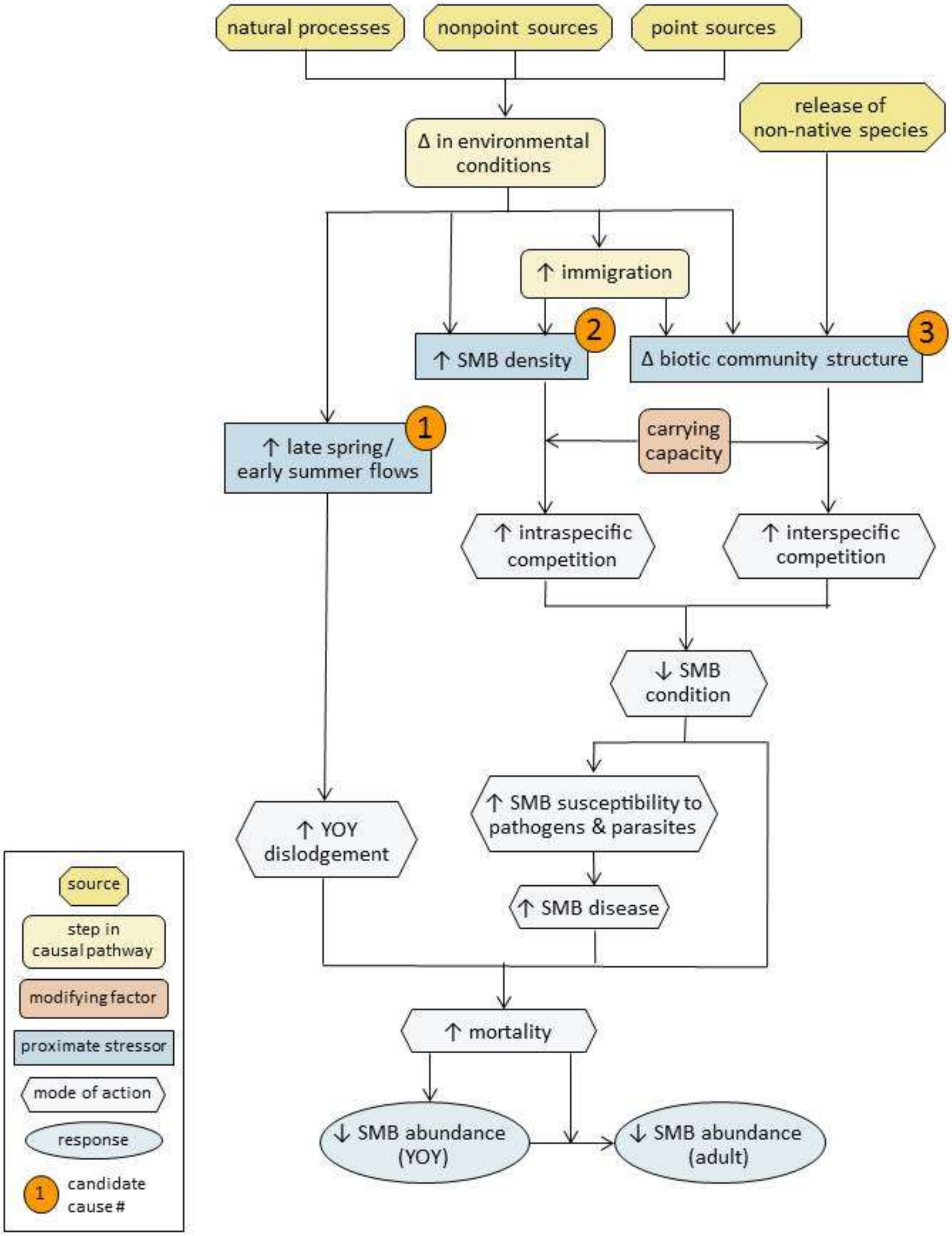


Figure 2. Flow and competition conceptual model for candidate causes 1-3 (Increased flow, intraspecific competition and interspecific competition)

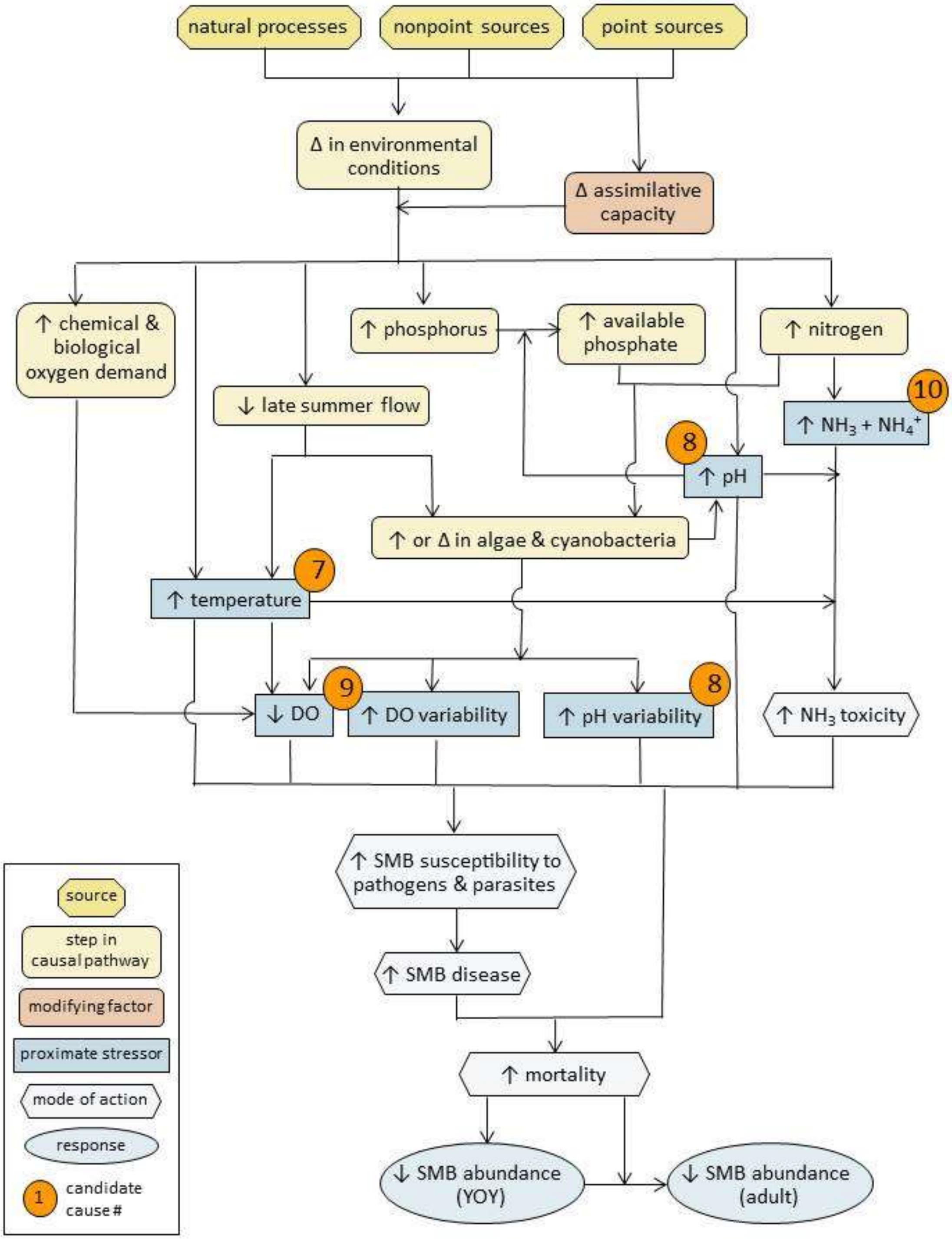


Figure 3. Abiotic conceptual model for candidate causes 7-10 (increased temperature, increased pH and/or increase pH variability, decreased dissolved oxygen and/or increased dissolved oxygen variability and increased ammonia toxicity).

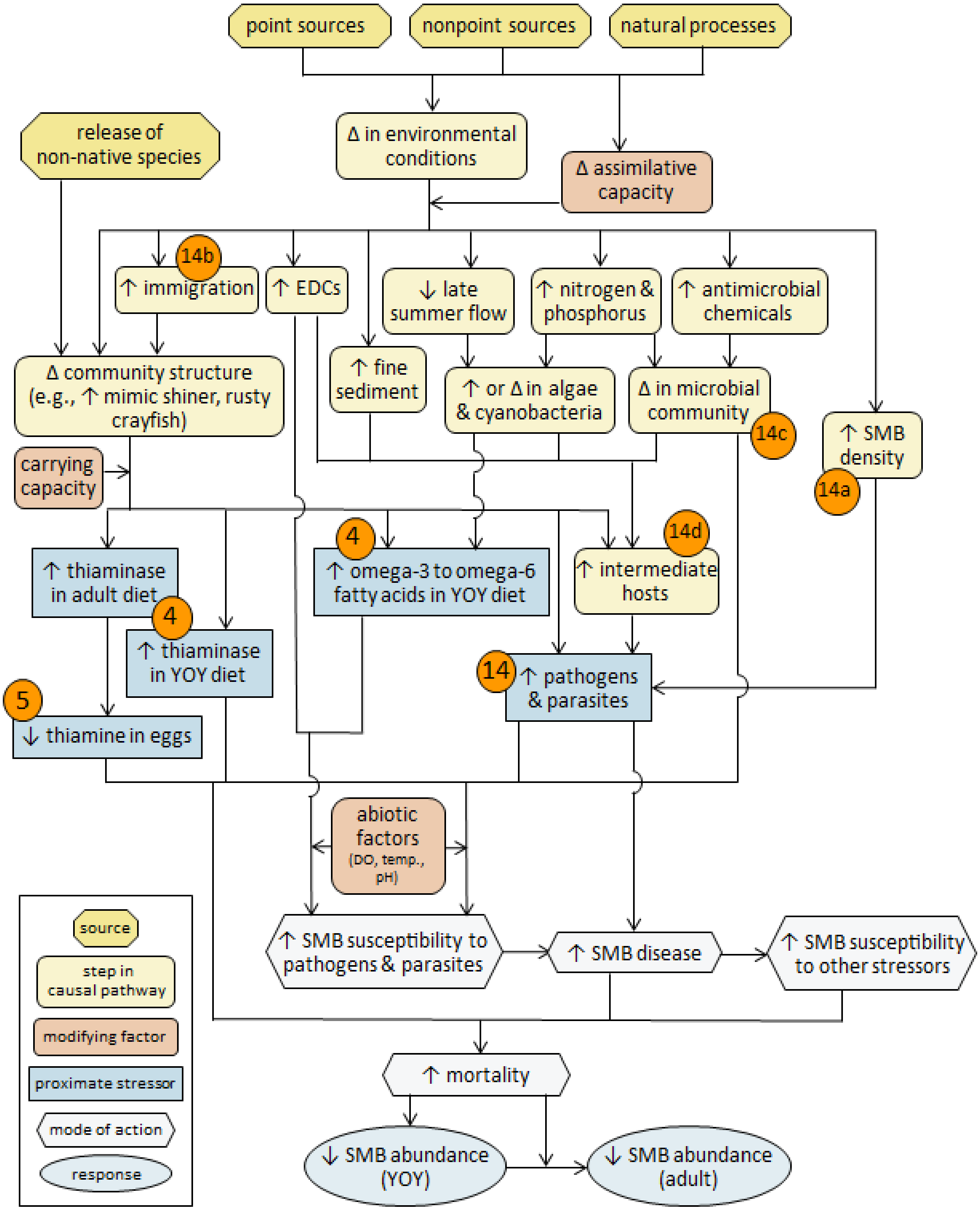


Figure 4. Food quality with pathogens and parasites conceptual model for candidate causes 4, 5 and 14 (decreased food quality, decreased egg quality and increased pathogens and parasites)

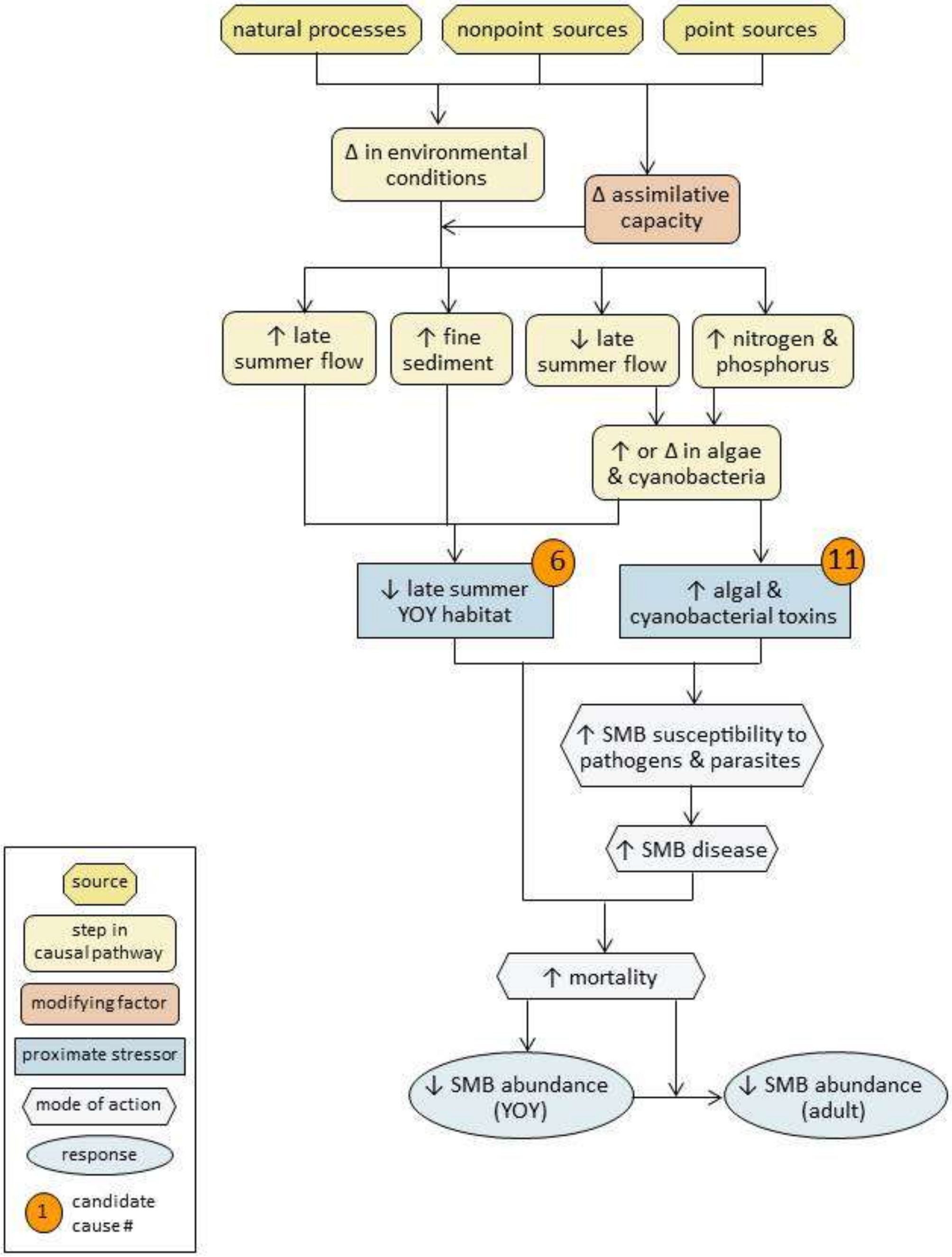


Figure 5. Algal and habitat conceptual model for candidate causes 6 and 11 (decreased late summer YOY habitat and increased algae and cyanobacterial toxins).

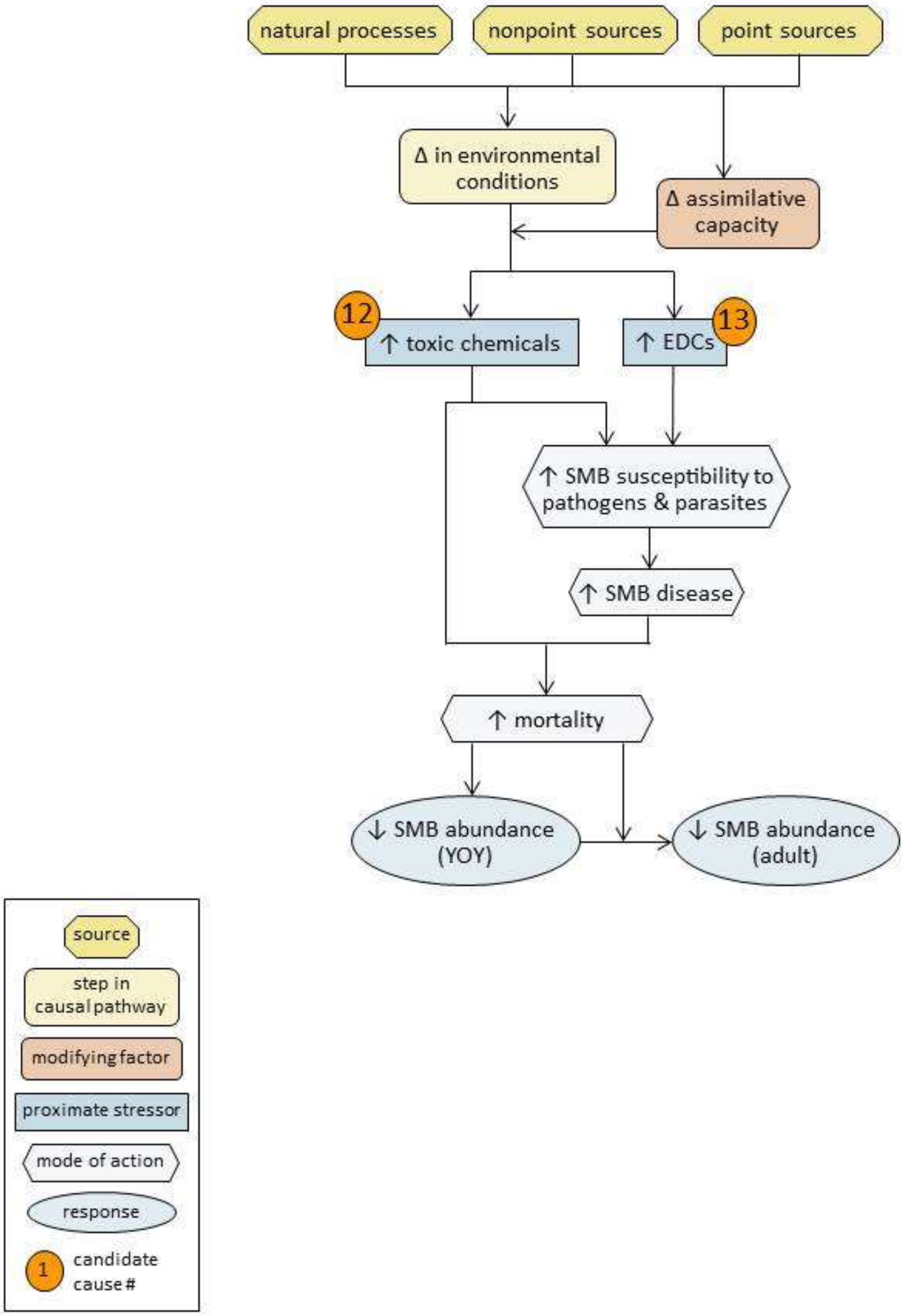


Figure 6. Toxic substances conceptual model for candidate causes 12 and 13 (increased toxic chemicals and increased endocrine disrupting compounds).

Appendix B: Analysis Worksheets

Appendix C: Candidate Cause Scoring Tables

Table 1. Candidate Cause scores based on type of evidence. * indicates that more data are needed to make a confident decision.

	Candidate Cause													
Type of Evidence	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	High Flows	Intra. Competition	Inter. Competition	YOY food quality	Egg quality	YOY habitat	Temp	pH	DO	NH3	Algal & bacterial toxins	Toxic chemicals	EDCs	Pathogens & Parasites
Spatial Co-occurrence	-		0*	-	0		+	-	+	- -	Microcystins: - Others: 0	+	+*	LMBV: 0 Myxozoan: 0 Other: 0
Temporal Co-occurrence	-		0			-	-	+	0	0				
SR from Case	-	-												
SR from Elsewhere				Mortality: Thiamine: 0, Fatty Acids: -- Disease: 0*	Mortality: 0 Disease: 0		Mortality: -- Disease: -		Mortality: - Disease: 0	Mortality: --- Disease: ---		Mortality: Pest/PCBs: NE, Metals: -, Triazines: NE Disease: NE		
Evidence of Exposure											Microcystin: 0, Other: NE		+	LMBV: +, Myxozoan: +
Evidence of Mechanism		--												+ +
Causal Pathway				+	+	+					+			+

Table 2. Final large group consistency scores based off results from evidence scoring (Table 1). * indicates that more data are needed to make a confident decision.

	Candidate Cause													
	1 High Flows	2 Intrasp. Competition	3 Intersp. Competition	4 YOY food quality	5 Egg quality	6 YOY habitat	7 Temp	8 pH	9 DO	10 NH3	11 Algal & bacterial toxins	12 Toxic chemicals	13 EDCs	14 Pathogens & Parasites
Large Group Consistency Final Scores	--	--	0*	Thiaminase: +, Fatty Acids: -	+	-	-	-	Mortality: - Susceptibility: +	---	Microcystin: -, Other: +	Pest/PCBs: -, Metals mortality: --, immune suppression: -, Triazines: +	+	LMBV: ++, Myxozoan: ++, Other: ++

Appendix D: Ongoing Studies in 2015

Geoff Smith (PFBC)

- Investigate the role that parasites are playing. Determine whether they are causing the damage (inflammation) observed in the SMB cells or whether it's related to bacteria. This will be a follow-up to Vicki Blazer's work (the in-situ hybridization they were showing).
- Largemouth Bass Virus Study to determine the susceptibility of SMB to the LMBV.
- YOY and adult microcystin concentration (liver tissue).
- YOY habitat and Anatoxin-a.
- Population genetics study in SMB

Tim Wertz (PADEP)

- YOY Thiaminase: PADEP along with Dale Honeyfield (USGS) will be collecting YOY to see if the Thiaminase circuit is complete. Consider thiaminase activity in YOY as evidence that they are feeding on a thiaminase-positive prey.
- Egg Thiamine
- Investigate additional fish communities and get samples for thiaminase activity among all fish to understand how competition depresses the amount of available thiaminase activity within the system.
- Habitat partitioning of fish communities – seasonal responses in different species for functional group that partition habitat resources

Dale Honeyfield (USGS)

- Immune function study – SMB in the laboratory. It will include thiamine replete and thiamine deficient SMB and then examine immune function in terms of macrophages and bacteria

Josh Lookenbill (PADEP)

- 2015 Sample Collections focused on YOY habitat
    - Water: Herbicides/pesticides, hormones, EDCs, and passive sampler deployments
    - Sediment samples
    - Analyze microcystin in periphyton
    - Study algal communities

Jeff Butt (PADEP)

- Anatoxin-a studies with PFBC
- Seasonal changes in the algal cyanobacteria community

- Resolve algal taxonomy issues between laboratories

Appendix E: Future Research Needs

- Egg Thiamine – Out of basin studies and places with large populations of mimic shiners
- YOY Habitat: water quality data collection to compare these habitats to other areas with incidence of disease
- Effects of fallfish on the fish community
- More EDC work. Target new areas.
- Diet studies – YOY SMB and others
- Literature reviews for algal toxin work
- Telemetry studies to document fish movements
- EDC testing on YOY
- Parasite/Host relationships
- Precisely characterize SMB spawning dynamics (when they spawn and the life cycle dynamics of the YOY)
- Literature review of Atrazine (Stressor response)
- Research on YOY response to parasites (myxozoans, etc.)
- Testing of zooplankton for algal toxin content in the YOY SMB spawning areas
- Fish health monitoring across multiple species at sites with confirmed disease incidence (whole community) and in controls
- Fish health in individual fish - Co-occurrence of disease (with pathogen identification) along with thiamine levels, EDCs, and microcystin in individual fish
- A basin-wide delineation of potential sources of EDCs (point and nonpoint)
- Estimation of the antioxidant pool in diseased and healthy fish
- Biological endpoints and effects of atrazine and other herbicides/pesticides. Biological effects.
- Measurement of immune suppression in the field (YOY and adults)
- Differential cyanotoxin response in SMB versus other species
- Re-evaluation of the data considering the modifying factors (synergistic effects.)
- Identify, map, and characterize YOY habitats
- Identify the intermediate host of myxozoan