9-2-2015

The Effects of Marcellus Shale Gas Drilling Accidents on Wallace Mine Fen in the Moshannon State Forest, Pennsylvania

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The Effects of Marcellus Shale Gas Drilling Accidents on Wallace Mine Fen in the Moshannon State Forest, Pennsylvania

A Thesis

Presented to the Faculty of the Department of Environmental Science and Biology

of the State University of New York College at Brockport

in Fulfillment for the

Degree of Master of Science

Andie Graham

2015

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my family for their support and encouragement throughout my educational journey. I am especially thankful to my better half, Scotty McGough, who made it possible for me to attend college. Scotty supported me, financially and mentally, as an undergraduate at Penn State University, and he quit his job, left his home, and moved out of state so that I could pursue a graduate degree at The College at Brockport, State University of New York. If not for him, none of this would have been possible.

I could not have completed data collection without the help of several awesome field assistants from the Wildlife Program at Penn State: Jackson Martini, Courtney Colley, Steve Zanoni, Kristina Everetts, Mandy Sowers-Marconi, Sean Andrews, Doug Kessler, and Alyssa Knee. Their dedication, hard work, and positive attitudes in the face of black bear and rattlesnake encounters, as well as long, hot days in the muck were extremely helpful and much appreciated. I also thank The College at Brockport graduate students Dave S-K and Becca Bernacki for their assistance with potential study site selection and statistical analyses, respectively.

Water chemistry data, which were instrumental to my project, were provided by Terrance O’Conner, Aaron Furgiuele, Nancy Potts, Ken Undercoffer, and Rick and Marianne Atkinson from the Clearfield County Senior Environmental Corps. The PA Department of Environmental Protection, PA Department of Conservation and Natural Resources, and PA Natural Diversity Inventory provided the appropriate permits and allowed me to conduct research on state-owned land.

I thank Dr. Benjamin Turner at Penn State University for the use of laboratory and field equipment and Dr. Jeffrey Wilcox at The University of North Carolina for conducting tree core analysis. I also thank Dr. James Zollweg at The College at Brockport for his assistance with GIS.

I am extremely thankful to my friend and colleague, Brad Mudrzynski, who I like to refer to as my ‘fourth committee member.’ Brad always took the time to help me with GIS, allowed me to talk through any data analysis issues (and there were many), and taught me how to use PC-ORD software. His encouraging nature throughout this project was much appreciated, and he always helped me focus on the finish line. Cheers!

My committee members, Dr. James Haynes and Dr. Christopher Norment, were incredibly helpful throughout this process, and I especially appreciate their suggestions and editorial comments on my thesis.

Last, but certainly not least, I thank my major advisor, Dr. Douglas A. Wilcox for his constant support, knowledge, patience, and guidance throughout this project. Doug helped me navigate through numerous bureaucratic and scientific obstacles that would have otherwise made this research impossible. His inputs to this project were invaluable, and he has given me so many great opportunities and experiences that will benefit me throughout my career. Not only has Doug been an incredible mentor over the past few years, but he has been really fun to work with! Thank you!

TABLE OF CONTENTS **Pg.**

ACKNOWLEDGEMENTS ……………………………………………………………...i

LIST OF FIGURES ………………………………………………………………vii

LIST OF TABLES ………………………………………………………………ix

LIST OF APPENDICES ……………………………………………………….....x

ABSTRACT ………………………………………………………………………1

INTRODUCTION ………………………………………………………………..3

MARCELLUS SHALE GEOLOGY ……………………………………….....3

HYDRAULIC FRACTURING ……………………………………………6

HYDRAULIC FRACTURING ACCIDENTS ………………………………8

RESEARCH OBJECTIVES ……………………………………………...11

METHODS ………………………………………………………………………12

SITE DESCRIPTIONS …………………………………………………...12

Wallace Mine Fen ………………………………………………...12

Crystal Spring Bog ………………………………………………..13

HABITAT CHARACTERISTICS …………………………………………14

Vegetation …………………………………………………………...14

Pool Size ………………………………………………………..…15

WATER CHEMISTRY ……………………………………………………..16

pH and Specific Conductance ……………………………………16

Alkalinity ……………………………………………………………16

TREE CORES ……………………………………………………………17

FISH ……………………………………………………………………17

AVIAN POINT-COUNTS ..…………………………………………………18

AQUATIC INVERTEBRATES ……………………………………………18

AMPHIBIANS …………………………………………………………………19

Point-Count Surveys ………………………………………………...19

Visual Encounter Surveys …………………………………………...20

STATISTICAL ANALYSES ……………………………………………………...20

HABITAT CHARACTERISTICS ………………………………………….20

Vegetation ………..…………………………………………………...20

Pool Size ……………………………………………………………..21

WATER CHEMISTRY ……………………………………………………..22

pH and Specific Conductance ………………………………………….22

Alkalinity ……………………………………………………………….22

TREE CORES ……………………………………………………………….22

FISH ……………………………………………………………………………23

AVIAN POINT-COUNTS ………………………………..……………....23

AQUATIC INVERTEBRATES …………………………………………….24

AMPHIBIANS …………………………………………………………………25

Point-Count Surveys ………………………………………………...25

Visual Encounter Surveys …………………………………………...25

RESULTS ……………………………………………………………………...25

HABITAT CHARACTERISTICS …………………………………….…25

Vegetation ……………………………………………………………...25

Pool Size ……………………………………………………………..27

WATER CHEMISTRY ……………………………………………………..28

pH and Specific Conductance ………………………………………….28

Alkalinity ……………………………………………………………….29

TREE CORES ……………………………………………………………….29

FISH ………………………………………………………………………...29
AVIAN POINT-COUNTS ……………………………..………………30
2012 ……………………………………………………………………30
2013 ……………………………………………………………………31
AQUATIC INVERTEBRATES ……………………………………………32
AMPHIBIANS ……………………………………………………………33
Point-Count Surveys 2012 …………………………………………..33
Point-Count Surveys 2013 …………………………………………..34
Visual Encounter Surveys …………………………………………...35
DISCUSSION ………………………………………………………………36
HABITAT CHARACTERISTICS …………………………………………36
Vegetation ……………………………………………………………...37
Pool Size ……………………………………………………………….37
WATER CHEMISTRY …………………………………………………..38
pH, Specific Conductance, and Alkalinity …………………………..38
TREE CORES ……………………………………………………………42
FISH ………………………………………………………………………43
AVIAN POINT-COUNTS ……………………………..………………46
AQUATIC INVERTEBRATES ……………………………………………47
AMPHIBIANS ……………………………………………………………50
Amphibians and Habitat Characteristics ……………………………51
Amphibians and Water Chemistry …………………………………..52
Amphibian Biology ……………………………………………………..53
Effects of Contaminants on Amphibians ……………………………58
WHAT HAPPENED AT WMF? ……………………………………………61

THE STATUS OF HYDRAULIC FRACTURING IN THE MARCELLUS SHALE REGION …………………………………………………………64

Ohio ……………………………………………………………………64

West Virginia …………………………………………………………..65

New York ………………………………………………………………65

LITERATURE CITED ……………………………………………………67

FIGURES ………………………………………………………………...78

TABLES ……………………………………………………………...101

APPENDICES …………………………………………………………111

LIST OF FIGURES Pg.

Figure 1. Gas well drilling activity in Clearfield County, PA………………………78

Figure 2. Aerial image of control site, CSB ………………………….…….……….…..79

Figure 3. Aerial image outlining impact site, WMF……………………………………80

Figure 4. Aerial image of CSB showing overlay of vegetation sampling area, and bird and amphibian point-count study design…………………...……………………..81

Figure 5. Aerial image WMF showing overlay of vegetation sampling area, and bird and amphibian point-count study design ……………………………………………82

Figure 6. Aerial image of CSB showing overlay of vegetation quadrants…………..83

Figure 7. Aerial image of WMF showing vegetation quadrants……………………..…84

Figure 8. Aerial image of CSB showing vegetation quadrat placement ……………..…85

Figure 9. Aerial image of WMF showing vegetation quadrat placement …………...86

Figure 10. Aerial image of CSB showing surface and groundwater testing sites…...87

Figure 11. Aerial image of WMF showing surface and groundwater testing sites…..88

Figure 12. Aerial image of WMF showing alkalinity testing location………………89

Figure 13. Aerial image of WMF showing tree core collection locations …………..90

Figure 14. Aerial image of CSB showing fish trap placement in un-named stream...91

Figure 15. Aerial image of WMF showing fish trap placement in Alex Branch ……92

Figure 16. Aerial image of CSB showing aquatic invertebrate sample locations in un-named stream ……………………………………………………………………..…93

Figure 17. Aerial image of WMF showing aquatic invertebrate sample locations in Alex Branch ……………………………………………………………………....94

Figure 18. Aerial image of CSB showing VES sample locations ………………...…95

Figure 19. Aerial image of WMF showing VES sample locations …………….……96

Figure 20. Two-dimensional plot of nMDS ordination of selected taxa from CSB and WMF……………………………………………………………………………97

Figure 21. Two-dimensional plot of nMDS ordination of selected taxa location from CSB and WMF……………………………………………………………………….98

Figure 22. Boxplot of surface and groundwater pH at CSB and WMF…………...…99

Figure 23. Boxplot of surface and groundwater SPC at CSB and WMF………….100

LIST OF TABLES Pg.

Table 1. Water chemistry data collected by the PA DEP in 2009…………………....101

Table 2. Pool size and depth comparison table ………….……………….…...102

Table 3. Kruskal-Wallis Test showing temporal changes in pH between ground water and surface water at CSB and WMF ……………………………………………….103

Table 4. Kruskal-Wallis Test showing temporal changes in SPC between ground water and surface water at CSB and WMF ……………………………………...104

Table 5. Alkalinity from nine sample locations at WMF ………………………….105

Table 6. Summarization of bird-point count surveys at CSB and WMF for 2012 and 2013 ………………………………………………………………………106

Table 7. Summarization of aquatic invertebrates detected at CSB and WMF…..…107

Table 8. Summarization of amphibian point-count surveys at CSB and WMF for 2012 and 2013 …………………………………………………………………...108

Table 9. Total adult amphibians detected at CSB and WMF during 2013 VES …..109

Table 10. Total juvenile amphibians detected at CSB and WMF during the 2013 VES ………………………………………………………………………………110

LIST OF APPENDICES Pg.

Appendix 1. Summary of EOG well violations in Clearfield County, PA between 2009 and 2012 ……………………………………………………………….…..111

Appendix 2. Taxa detected at both study sites during the 2012 vegetation surveys. Taxa in bold were used in the nMDS ordinations …………….…………………112-114

Appendix 3. Data from bird point count observations conducted at four point count sites (two at each wetland), six times during the 2012 breeding season …….......…115

Appendix 4. Data from bird point count observations conducted at four point count sites (two at each wetland), five times during the 2013 breeding season …………116

Appendix 5. Data from macroinvertebrate surveys conducted at four sites (two at each wetland), 22 September 2012 ………………………………..................….117

Appendix 6. The maximum calling code and number of individuals of amphibians detected by date at CSB and WMF during the 2012 point count surveys …………118

Appendix 7. Data from amphibian point count observations conducted at four sites (two at each wetland), six times during the 2012 breeding season ………………...119

Appendix 8. The maximum calling code and number of individuals of amphibians detected by date at CSB and WMF during the 2013 point count surveys …………120

Appendix 9. Data from amphibian point count observations conducted at four sites (two at each wetland), six times during the 2013 breeding season ………………...121

ABSTRACT

In 2009, a Marcellus Shale gas-drilling company, EOG Resources, was fined $30,000 by the Pennsylvania Department of Environmental Protection (PA DEP) after several violations occurred at two well sites located on private land adjacent to Moshannon State Forest in Clearfield County, Pennsylvania. Three separate accidents resulted in the deposition of flowback water and frack fluids into Alex Branch, a small, sandy-bottom stream that flows through Wallace Mine Fen (WMF). Contaminated water also infiltrated the ground upslope from the fen. Water testing conducted by the PA DEP indicated elevated levels of Ba, Sr, Mn, Cl-, TDS, and specific conductance in two nearby springs.

In 2012, I initiated a study to determine the ecological impacts of the accidents on WMF. I used a nearby wetland, Crystal Spring Bog (CSB, actually a fen), as a control and sampled amphibians, birds, fish, vegetation, and aquatic invertebrates at both sites. Tree core samples were collected from five trees at WMF and analyzed using Gas Chromatography Mass Spectrometry (GC/MS). Additionally, I collected water chemistry data from groundwater and surface water locations at both wetlands. Results showed no significant differences in vegetation, birds, fish, or aquatic invertebrates between the two wetlands. The GC/MS did not detect any contamination within trees, suggesting that the contamination may have occurred in short-term discharge events. Through point-count and visual encounter surveys, I detected differences in amphibian diversity and species richness between the sites.

Due to the lack of pre-accident amphibian data, I also used habitat characteristics (i.e., vegetation and pool size) in an attempt to determine if differences

in amphibian populations are a result of the accidents or due to differences in habitat. I used vegetation frequency and percent cover to calculate importance values; those values were then used in an nMDS ordination. No major differences were detected in vegetation between sites; however, the ordination showed that CSB was wetter than WMF. I also compared pool area and depth between the two wetlands using a Mann-Whitney test. Pool area was significantly greater in CSB, but there was no difference in pool depth. Overall, habitat characteristics do not fully explain differences in amphibians between the wetlands and results suggest that the accidents at EOG gas drilling operations decreased amphibian diversity and abundance at WMF.

INTRODUCTION

Marcellus Shale Geology

The Marcellus Shale is a black, organic-rich shale that was deposited about 400 million years ago during the Devonian Period of the Paleozoic Era. At that time, much of the area currently occupied by the Appalachian Mountains was a large depression, with high ridges to the north, east, and west. This depression was covered by a shallow sea known as the Appalachian Basin. The basin covered all or parts of the present-day states of Alabama, Georgia, Kentucky, New York, North Carolina, Ohio, Pennsylvania, Tennessee, Virginia, and West Virginia. To the east of the basin was a mountain range called the Arcadian Mountains (Soeder and Kappel 2009). During the Devonian, sediments eroding from these mountains washed downward and accumulated in the basin, along with organic materials from algae and other microorganisms living in the sea (Mintz *et al.* 2010).

Tropical and sub-tropical weather during the time of the Marcellus deposition made marine phytoplankton abundant in the Appalachian Basin. Dust storms, which were common, caused an influx of nutrients such as nitrates, sulfates, and iron, into the sea. The increased nutrients resulted in algal blooms. This explosion in the algae population quickly used all of the available nutrients and the algae died off. The decomposition of the dead material would have used large amounts of oxygen, causing anoxic conditions, resulting in a dead zone. The abrupt increase and die off in the phytoplankton population provided a large amount of organic material for deposition and accumulation (Laughrey *et al*. 2011). This deposition occurred at a time when tectonic plates were diverging, causing the basin to become wider and deeper and allowing the accumulation of sediments and organic material for millions of years. Through a process called diagenesis, sediments were exposed to high temperatures and pressures, resulting in compaction and sedimentation, which reduced porosity and permeability (Soeder 1988).

Sedimentation continued into the Carboniferous Period and sediments began to compress under its own weight, forming what is known as the black shales of the Devonian. There are ten black shale units within this sequence in the Appalachian Basin; the Marcellus Shale is found at the base, in the Hamilton Group (Roen 1984). The Marcellus Shale layer is found in New York, Ohio, Pennsylvania, and West Virginia (Soeder and Kappel 2009), 1,500 m to 3,000 m below the earth's surface (Myers 2012). The layer is tilted toward the south, resulting in a deeper southern layer and a shallower northern layer; the layer is exposed to the surface near the Finger Lakes region of New York (Soeder and Kappel 2009).

The mineral composition of black shales is quartz, feldspar, mica, clays, sulfides, organic matter, and small amounts of carbonate, phosphate, and accessory minerals. Carbonate in the black shales is generally in the form of calcite and occurs in very small quantities (<1%), except for in the Marcellus Shale layer, which contains large amounts of calcite (up to 20%) (Roen 1984). The heating and compression of the decomposed organic material formed hydrocarbons, including natural gas, which is found in the Marcellus Shale layer. Due to the low porosity and permeability of the Marcellus layer, the natural gas attaches to organic matter within the rock and becomes tightly trapped within the layer (Soeder 1988). This makes the Marcellus Shale an unconventional source of natural gas. Most conventional sources of natural gas are found in permeable rocks with high porosity, such as sandstone. High permeability and porosity allow the gas to consolidate into pockets and makes extraction easier and more cost-effective. Because of the Marcellus Shale’s low porosity and permeability, extraction methods are generally more expensive and labor-intensive (Soeder and Kappel 2009).

Until the recent advent of extraction techniques such as horizontal drilling and hydraulic fracturing (or fracking), extraction of gas in commercial quantities from the Marcellus Shale was not cost-effective (Arthur *et al*. 2008, Zoback *et al*. 2010, Considine *et al*. 2012). These advances, along with rising natural gas prices and increased demand, caused gas drilling activities in the Marcellus region to grow rapidly. In addition, estimates of natural gas deposits in the Marcellus Shale layer continued to grow. In 1980, the National Petroleum Council (NPC) estimated that the layer contained up to 0.6 standard cubic feet (SCF) of natural gas. SCF is one cubic foot of gas at standard temperature and pressure. In the mid-1980s, the Institute of Gas and Technology in Chicago estimated that the layer had 26.5 SCF. The estimate increased again when Englander and Lash (2008) reported that the layer contained 50 trillion cubic feet (TCF). Around the same time, the Chesapeake Energy Corporation (CEC) estimated that the layer contained 363 TCF of extractable gas (Kappel 2010). Kargbo *et al*. (2010) reported that recent production data suggests that extractable amounts may be as large as 489 TCF. These estimates, however, continue to change. Currently, the U.S. uses about 23 TCF of gas annually (Soeder and Kappel 2009). Estimates suggests that the Marcellus layer can produce about 3,100 thousand cubic feet of gas (MCF) per well per day (Arthur *et al*. 2008). At the current rate of consumption, the Marcellus Shale layer might supply enough natural gas for the entire nation for 15 years (Soeder and Kappel 2009).

Hydraulic Fracturing

Pennsylvania has a rich history of gas drilling; it has occurred in the state for decades and has produced about 40,000 gas wells, with about 4,000 new wells drilled annually. Most of these are shallow wells, and gas is extracted from just a few thousand feet below the earth's surface (Swistock 2008). However, since the advancement of new drilling technologies, the Marcellus Shale gas industry is booming in Pennsylvania (Arthur *et al*. 2008, Zoback *et al*. 2010, Considine *et al*. 2012, Trexler *et al*. 2014). In 2005, there were just eight Marcellus gas wells in the state (Brantley *et al*. 2014). That number increased to 1,454 by January 2011, with more than 6,000 well permits issued in the state (Hefley 2011). By 2013, Pennsylvania had over 7,200 Marcellus gas wells (Brantley *et al*. 2014).

Horizontal drilling and hydraulic fracturing have significantly increased the ability of gas companies to tap into the Marcellus layer (Osborn *et al*. 2011); horizontal drilling increases the productivity of a well by maximizing the length of the wellbore through the shale (PA DEP 2012), and fracking increases the permeability of the shale (Myers 2012). Fracking was first used in the 1940s (Zoback *et al*. 2010), but over the years the process has been refined and is now a common extraction technique (PA DEP 2012). This technique involves drilling vertically up to 1,800 m below the earth's surface to reach the Marcellus Shale layer and then drilling horizontally about the same distance. Once drilled, a conductor pipe is installed to prevent the hole from caving in. When drilling is complete, a second pipe that is smaller in diameter is installed. This casing extends below the groundwater table and must be properly cemented to protect groundwater from gas extraction activities. Constructing the well in this manner provides hundreds of meters of vertical separation between groundwater supplies and the production zone. Certain conditions, such as the presence of coal, may necessitate the installation of additional casings. Each casing added goes deeper but decreases in diameter. Once all of the appropriate casings are in place, a production interval is drilled. The production interval may extend up to several hundred meters in a horizontal well and is analyzed electronically to determine the production potential of that area. Once these data are retrieved, a production casing is installed and cemented in the borehole. The production casing is then perforated to allow fluids to enter the formation during the fracking phase and to allow gas to flow into the wellbore during production. Fluids used during the fracking phase, commonly called frack fluids, may vary chemically from site to site (Zoback *et al*. 2010, PA DEP 2012).

Frack fluids are produced by mixing water with a variety of chemicals and sand or ceramic particles, which aid in successfully fracturing the layer and releasing the gas. Frack fluids are comprised of a friction reducer, a wetting agent, biocides, and a scale inhibitor. The friction reducer is a water-soluble polyacrylamide polymer that reduces pumping friction and holds the sand or ceramic particles in suspension. The wetting agent is a surfactant that reduces the water surface tension. This allows easier recovery of the frack water. Biocides, such as dibromo nitrilopropionamide, glutaraldehyde, or dibromosulfamate, are commonly used to control the growth of microorganisms that may interfere with the equipment. A scale inhibitor, such as polyacrylate or ethylene glycol, is used to prevent material deposition on the equipment. Other additives commonly used in frack fluids include a dilute acid solution, stabilizing agents such as citric or hydrochloric acid, and gelling agents such as guar gum, to thicken the solution and assist in material transport (Zoback *et al.* 2010). Sodium chloride and isopropanol are also common constituents of frack fluids (Ritter 2014).

During the fracking phase, up to 37 million liters of frack fluids may be used per well. The water, once used, must be removed from the well and is referred to as flowback water. Major constituents of flowback water are salts, naturally occurring radioactive materials (NORMS), arsenic, benzene, and mercury (Zoback *et al*. 2010). Flowback water may also contain hydrocarbons, aluminum, copper, lead, chromium, zinc, nitrogen compounds, fluoride, bromide, and uranium, as well as barium, calcium, iron, magnesium, manganese, strontium, and dissolved and suspended solids. Once a gas well is in the production phase, additional water is generated and transported in the gas flow. This is referred to as production water. Like flowback water, production water may include surfactants, calcium, magnesium, strontium, barium, iron, and manganese (Keister 2008).

Hydraulic Fracturing Accidents

According to the Pennsylvania Fish and Boat Commission (PFBC), some of PA's highest quality waters are in areas of Marcellus Shale development. There are concerns that Marcellus Shale gas development is putting the state's aquatic and natural resources at risk (PFBC 2012). With increased drilling activity in PA, the number of drilling-related accidents and violations has increased. From January 2008 through August 2011, there were 2,988 violations; 1,144 of those were environmental in nature. Although faulty equipment and lax company policies may be partially at fault, many of these accidents were the result of industry negligence. According to Considine *et al*. (2012), many of these incidents were minor events that resulted in little to no contamination; however, there continues to be a lack of research and data describing the environmental impacts resulting from Marcellus Shale gas drilling accidents (Considine *et al*. 2012) and the potential for environmental impacts is not well understood (Trexler *et al*. 2014).

One geographic area that has had several drilling-related incidents is the land in and around the Moshannon State Forest (MSF). Moshannon State Forest is located in Centre, Clearfield, Elk, Cameron, and Clinton Counties. It covers approximately 77,000 ha and contains three state parks: Black Moshannon, Parker Dam, and S.B. Elliot. Moshannon State Forest is home to several natural areas, springs, and wetlands, and much of the forest is a part of the PA Wilds program operated by the PA Department of Conservation and Natural Resources (DCNR). Several hectares within MSF and adjacent to MSF on privately owned land have been leased for gas exploration and drilling. Private land owned by the Punxsutawney Hunting Club (PHC), which is adjacent to state forest land, has had several new wells drilled by EOG Resources Inc. (EOG) in recent years. EOG currently has 146 active wells in PA; 43 of those are in or adjacent to MSF. Since January 2009, EOG has been fined $516,215 for over 65 well violations in PA. Twenty three of EOGs violations have occurred in Clearfield County, and 22 of those violations occurred at wells located on PHC land. The violations range from administrative to environmental in nature (Appendix 1) (NPR 2014).

In 2009, EOG was fined $30,000 by the PA DEP after several violations occurred at two well sites on PHC land. On 24 August 2009, the PA DEP received a citizen's complaint, which led to inspections of EOG well sites 8H and 9H. At 8H, the PA DEP discovered leakage from a containment pit, which was determined to be the most likely source of "a release of an unknown quantity of materials to the Alex Branch…" Alex

Branch is a small, sandy-bottom stream that drains to Trout Run, a high quality coldwater fishery. The PA DEP tested the headwaters of Alex Branch at two locations, Sykesville Spring and Reeds Spring (Table 1), and found elevated levels of barium, strontium, manganese, sodium, calcium, chloride, TDS, and SPC. At site 9H, PA DEP determined that the poor design of a diffuser at the end of a flowback fluid line resulted in an unknown quantity of flowback fluid being released into the atmosphere, as well as being deposited in a wetland near the site (DEP 2010).

On 14 October 2009, PA DEP inspected site 8H for a second time, as a response to an EOG report of a spill of fluids due to a leaking gasket. The spill consisted of approximately 190 barrels of water mixed with Well Wash 2020 and Ultra Vis; these are the frack fluids used by EOG, the contents of which are undisclosed. There are 159 L in one barrel—this equates to over 30,200 L. According to the PA DEP (2010) report, the fluids were rapidly absorbed into the ground and entered an unnamed tributary to Alex Branch. No water chemistry data are known to have been collected directly after this event.

In December 2009, the PA DEP conducted a cause and effect survey to determine if there were any impacts to the benthic macroinvertebrate community in Alex Branch as a result of the accidents at EOG wells 8H and 9H. Three sites were sampled along Alex Branch; 20-30 m (it is unclear if the site is 20 m or 30 m downstream; both numbers are used in the report) downstream from the spill location (Impact 1), 20 m upstream from where the stream crosses McGeorge Road (Impact 2), and 200 m downstream from where the stream crosses McGeorge Road (Impact 3). A reference site on an unnamed tributary located several hundred meters downstream from where the spill entered the stream was also sampled (Reference). The PA DEP concluded that the benthic macroinvertebrate community was not impacted by the pollution event but also noted that it is "impossible to determine exactly why the macroinvertebrate population did not respond to this event (PA DEP 2010)." Aside from unclear and contradictory sample location information, the PA DEP report also did not use the data from site Impact 2 in the analyses, nor did they give a reasonable explanation as to why these data were omitted.

Research Objectives

The development of the shale gas layer has been in the spotlight in recent years for the potential negative impacts that may occur, and it has been linked to a wide range of environmental issues (Zoback *et al*. 2010). These problems include surface water contamination, groundwater contamination, soil contamination (Zoback *et al.* 2010), soil erosion and siltation, surface water extraction (Adams *et al.* 2011), air pollution (Armendariz 2009), habitat fragmentation (Johnson 2010), increased wildlife road mortality due to increased truck traffic (Bayne and Dale 2011), and impacts on wildlife due to artificial night lighting (Rich and Longcore 2006).

For the purpose of this research, I focused on impacts to a small wetland (described below) resulting from surface and subsurface water contamination. Receipt of contaminated surface and subsurface water from drilling-related accidents on private land in 2009 has been documented in Alex Branch. It is possible, and highly likely due to its location, that a near-by wetland, Wallace Mine Fen (WMF) also received the same contaminated water; therefore, I studied WMF to determine if those accidents impacted the site. EOG gas wells 8H and 9H are located in close proximity to Alex Branch and

WMF; 8H is approximately 1.3 km from the wetland and 0.55 km from Alex Branch, and 9H is approximately 1 km from the wetland and 0.55 km from Alex Branch (Figure 1).

I selected a second wetland, Crystal Spring Bog (CSB, actually a fen), as a control site. I selected this site as a control due to its upslope location (which helps protect it from surface spills), and its similar habitat, underlying geology, and soils.

Vegetation, amphibians, birds, macroinvertebrates, fish, and water quality were sampled at WMF and CSB. In addition, tree core samples were collected from five trees at WMF and analyzed using Gas Chromatography Mass Spectrometry (GC/MS). Unfortunately, baseline data do not exist for WMF; therefore, I used CSB, as well as pre-accident anecdotal information from WMF, to determine any impacts to WMF.

METHODS

Site Descriptions

Both study sites are located in the Susquehanna watershed in MSF, Clearfield County, PA: CSB (41.1257 N, -78.5237 W) (Figure 2) and WMF (41.1824 N, -78.4366 W) (Figure 3). These wetlands are located in the Marcellus Shale region of PA, and the underlying bedrock consists mainly of sandstone and red and gray shale, along with clay, coal, and limestone (Enomoto *et al.* 2012).

Wallace Mine Fen

WMF is a ~8 ha fen that receives groundwater discharge, as well as surface flow from Alex Branch. Alex Branch flows into the wetland in the southwestern corner and flows to the east. The local topography slopes gradually to the east-southeast. The fen is bordered by Wallace Mine Road to the north and McGeorge Road to the west. There are several camps in close vicinity to the wetland that tap into the groundwater for potable water. Quehanna Trail cuts through the wetland in a north-south direction, and a bridge constructed of timber and PVC crosses Alex Branch near the middle of the wetland. WMF is a freshwater forested/shrub wetland and is classified by Cowardin *et al.* (1992) as PSS1/EM1A (Palustine, Scrub-Shrub, Broad-Leaved Deciduous/Emergent, Persistent, Temporarily Flooded), and PSS1A (Palustrine, Scrub-Shrub, Broad-Leaved Deciduous, Temporarily Flooded). An initial assessment of WMF showed that dominant vegetation included *Sphagnum* spp, *Carex* spp., *Eriophorum* spp., *Spirea* spp., *Vaccinium* spp., and *Drosera rotundifolia*. The site also had abundant *Alnus* spp., which borders Alex Branch on both banks at the western portion of the wetland. The entire wetland is surrounded by forest dominated by eastern hemlock (*Tsuga canadensis*) and eastern white pine (*Pinus strobus*), with some scattered red maple (*Acer rubrum*) and American beech (*Fagus grandifolia*). WMF is located downslope from several EOG Marcellus Shale gas drilling operations, including wells 8H and 9H, which is why it may have been exposed to contaminants during the 2009 drilling-related accidents.

Crystal Spring Bog

The control site, CSB, is located approximately 10 km southwest of WMF. This wetland, which is also fed by groundwater and is thus a fen, is upslope from the gas drilling operations that may have impacted WMF. CSB is ~22 ha in size; however, research efforts were focused only in a ~12 ha section of the fen that is state-owned. The site has a very gradual eastward slope, and groundwater that discharges on the northwestern border flows in an eastward direction and forms a small, un-named stream, which eventually flows into Lick Run. The eastern side of the wetland contains an old beaver dam, and the stream drains into a ~133 m x 48 m pool formed by the dam. In addition to groundwater discharge, the wetland receives runoff from sloping topography on the northern and southern sides. The interior portion of the wetland is a freshwater emergent wetland with a Cowardin *et al*. (1992) classification of PEM1E (Palustrine, Emergent, Persistent, Seasonally Flooded/Saturated) and PEM1F (Palustrine, Emergent, Persistent, Semipermanently Flooded). The exterior portion is a freshwater forested/shrub wetland and is classified by Cowardin *et al*. (1992) as PSS1/EM1B (Palustrine, Scrub-Shrub, Broad-Leaved Deciduous/Emergent, Persistent, Saturated), PFO1/SS1B (Palustrine, Forested, Broad-Leaved Deciduous/Scrub-Shrub, Broad-Leaved Deciduous, Saturated), and PFO4B (Palustrine, Forested, Needle-Leaved Evergreen, Saturated). Initial assessment of the site showed that dominant vegetation types included *Sphagnum* spp., *Carex* spp., *Vaccinium* spp., and *Typha* spp. I also noted *D. rotundifolia* and *Sarracenia purpurea* at the site. The forest surrounding the wetland is dominated by eastern hemlock, eastern white pine, blueberry (*Vaccinium* spp.), dogwood (*Cornus* spp.), American beech, and red maple (*Acer rubrum*), with some black gum (*Nyssa sylvatica*) scattered throughout.

Habitat Characteristics

Vegetation

Vegetation was sampled from July to August 2012. A 100 m-radius circle was used to define the boundary of each sample site. I laid out two 100 m-radius circles at each wetland by starting at a center point (near groundwater discharge) and pacing 100 m N, NE, E, SE, S, SW, W, and NW. Upon arrival at each point, I verified the distance with a range finder and took a back azimuth with a compass to verify direction. I flagged each point. Due to the size and shape of both wetlands, not all of the area defined within the circles was entirely within wetland habitat (Figures 4 and 5). I divided each circle into four quadrants, for a total of eight quadrants at each site. Each quadrant was given an identifying code: quadrants "A" through "H" at CSB (Figure 6) and "I "through "P" at WMF (Figure 7). I used a 1x1 m PVC quadrat to sample 15 randomly placed points in each quadrant, for a total of 60 quadrats per circle, 120 quadrats per wetland. Each quadrat was also given an identifying code, dependent upon which quadrant it was located in. For example, quadrats "A1" through "A15" were at CSB, whereas quadrats "I1" through "I15" were at WMF (Figures 8 and 9). All plants within the quadrats were identified to the lowest taxonomic level possible, and percent cover was estimated for each taxa. All data were recorded on data sheets. I used GPS to mark each quadrat location.

Pool Size

While sampling vegetation, any pool, seep, or spring (henceforth referred to as pool(s)) that was located within the 1x1 m vegetation quadrat was measured. Measurements were taken by measuring the length and width of each water body at the widest points. Large pools were measured using a range finder. Depth measurements were taken with a meter stick at the approximate center of each pool. Pools were only measured once, even if they fell into more than one quadrat.

Water Chemistry

pH and Specific Conductance

Water quality monitoring was conducted 29 times at both wetlands from May 2012 to October 2013. I tested the pH and specific conductance (SPC) at five surface water locations at WMF (four in Alex Branch and one in standing water in the center of point-count survey plot 2), and at six areas of groundwater discharge at WMF. The same testing was conducted at CSB (three at groundwater discharge sites and six surface water sites along the un-named stream). I collected pH and SPC data using an Extech Instruments ExStik II pH/Conductivity/TDS Meter, Model EC500. The instrument was calibrated in the morning before every use, using pH buffers 4.01, 7.01, and 10.04. Groundwater sample sites were given the identifying codes “CSGW1” through “CSGW 3” at CSB, and “WMGW 1” through “WMGW 6” at WMF, and surface water sites were named “CSSW 1” through “CSSW 6” at CSB and “WMSW 1” through “WMSW 5” (Figures 10 and 11, respectively).

Alkalinity

On 7 August 2012, I collected nine water samples in and around WMF to test alkalinity. Five of those samples were collected from areas of groundwater discharge: one at Reeds Spring, one at Sykesville Camp Spring, and three at ground water discharge locations in WMF. The remaining four samples were collected from surface water locations: one along Alex Branch at McGeorge Rd., two along Alex Branch in WMF, and one in an area of standing water in the wetland (not groundwater discharge). Water samples were collected in 250 mL plastic bottles, placed on ice, and transported to a laboratory at Penn State University, Dubois Campus. A Hawkes alkalinity titrator, which used 0.16 sulfuric acid and Bromcresol Green-Methyl Red Indicator Powder, was used to determine alkalinity (mg/L $CaCO_3$) of each sample within four hours of collection. In addition, I used GPS to mark waypoints of all sites sampled (Figure 12) and sample locations were given ID codes “ALK1” through “ALK9”.

Tree Cores

Five tree core samples were collected on 29 April 2012 from WMF. I used a hand-held auger to collect cores from eastern hemlocks at breast height (~ 1.4 m off the ground). All trees cored were located around the perimeter of the wetland and were roughly the same diameter. Cores were placed in plastic vials that had been filled with distilled water, and the vials were sealed airtight. Cores were sent to The University of North Carolina--Asheville for analysis using Gas Chromatography Mass Spectrometry (GC/MS) by Dr. Jeffrey Wilcox to identify any potential persistent contaminants and potential organics in the frack fluids that may have been taken up by the trees during the accidents. I used GPS to mark waypoints for the trees sampled and gave them the ID codes “TC 1” through “TC 5” (Figure 13).

Fish

Fish surveys were conducted from July to August 2012 at both wetlands. I used one minnow trap at each wetland. I baited the traps with bread and placed them in Alex Branch at WMF and in the un-named stream formed by groundwater discharge at CSB. Traps were set every morning for 8 d and were checked the following morning. Traps were placed in areas where water was deep enough to cover the trap and where vegetation and logs were present. I used GPS to mark the waypoint for each trap, and ID code were given: “CSB 1” through “CSB 8” were located at CSB (Figure 14), and “WMF 1” through “WMF 8” were at WMF (Figure 15).

Avian Point-Counts

Avian point count surveys were conducted six times at both wetlands from May to July 2012 and five times from April to June 2013. Methods used were adapted from the Marsh Monitoring Protocol (Bird Studies Canada 2008). I surveyed from the center of the same two 100 m-radius circles at each wetland that were used to conduct vegetation surveys (Figures 4 and 5). Surveys were conducted at least 10 d apart in appropriate weather conditions (warm, dry, little to no wind). Surveys were conducted by arriving at the site shortly after sunrise, waiting 1 min for birds to start calling again after being disturbed, and then surveying for 5 min. I identified all individuals to species and recorded the number of individuals. All data were recorded on data sheets.

Aquatic Invertebrates

An aquatic invertebrate survey was conducted at both wetlands in September 2012. Due to difficulty finding appropriate sampling sites at each wetland, only four sites were sampled at each wetland. The un-named stream at CSB is very shallow and narrow, and Alex Brach has a sandy bottom and lacks abundant vegetation and debris and does not provide much habitat for aquatic invertebrates. Each site was kick sampled in 1 min intervals and a D-net was used for collection. The goal was to reach 100 invertebrates per sample site. If 100 invertebrates were not collected at the sample site within 1 h (or after 20 kicks, whichever came first), I moved on to the next sample location. At each wetland, sampling began at a downstream location and moved upstream to avoid recapture of individuals. All invertebrates were identified to the lowest taxonomic level possible, and identification was conducted in the field to avoid mortality. Any invertebrate that I could not identify was listed in the category “Unknown.” I used GPS to mark the waypoints of all sites sampled. Code names were given for each sample location: “CS 1” through “CS 4” were at CSB (Figure 16) and “WM 1” through “WM 4” were located at WMF (Figure 17).

Amphibians

Point-Count Surveys

Amphibian calling surveys were conducted at both wetlands six times from April to June 2012 and six times from March to June 2013. Methods used were adapted from the Marsh Monitoring Program (Bird Studies Canada 2008). Surveys were conducted from the center of the 100 m-radius circles that were defined for vegetation surveys (Figures 4 and 5). I conducted the surveys at least 15 d apart in appropriate weather conditions. Surveys were conducted by arriving at the sites after sunset, waiting 1 min to allow frogs and toads to start calling again after being disturbed, and then surveying for 3 min. All observations were placed into the appropriate calling code categories and all data were recorded on amphibian data sheets. The amphibian calling code categories used were as follows (Bird Studies Canada 2008):

1. Calling code 1 = Calls not simultaneous; individuals can be accurately counted
2. Calling code 2 = Some calls simultaneous; individuals can be reliably estimated
3. Calling code 3 = Full chorus, calls continuous and overlapping; individuals cannot be reliably estimated

Visual Encounter Surveys

I conducted amphibian visual encounter surveys (VES) four times at both wetlands from April to June 2013. I surveyed 32, 10 m^2 plots at each wetland. All plots were evenly spaced along transects at each wetland; however, due to size differences of the wetlands, transects were 25 m apart at WMF and 50 m apart at CSB. This allowed me to cover the most area at each site while keeping the sample size consistent (n=32). Each plot was searched for 10 min by looking in any vernal pools and standing water within plots, carefully turning over any objects (rocks, logs, etc.), and gently searching in sphagnum mounds. All objects moved while searching were placed back to their original position when done searching. In addition to the VES, I searched all vernal pools, groundwater seeps, and areas of standing water at WMF. This was done on the same days as the VES, but data were recorded separately. All amphibians were identified to species. The presence of all species, both adult and juvenile (egg masses and tadpoles only; red efts were counted as adults), was recorded on data sheets. I used GPS to mark the waypoint of all sites sampled at both wetlands (Figures 18 and 19).

STATISTICAL ANALYSIS

Habitat Characteristics

Vegetation

I used vegetation relative frequency and percent cover to calculate importance values (IV) for each taxon found at CSB and WMF. IV were calculated using the following formulas:

$$\text{IV} = \%\ \text{Relative Frequency (RF)} + \%\ \text{Relative Cover (RC), where}$$

$$\text{RF} = \frac{\text{Frequency of Individual Plants}}{\text{Frequency of All Plants}} \times 100,$$

and where

$$\text{RC} = \frac{\text{Average Plant Cover}}{\Sigma\ \text{Average Cover of All Plants}} \times 100$$

Both the RF and the RC are numbers between 0 and 100; therefore, IV are between 0 and 200. Using PC-ORD software (Version 5.0), I used the IV in a non-metric multidimensional scaling (nMDS) ordination with Sorenson distance measure. Not all taxa found at CSB and WMF were used in the ordination. I eliminated any taxa from my analyses that were found in ≤ 5 quadrats and had IV ≤ 20. This was a conservative criterion, but it eliminated 23 species (approximately 40% of the species found) from the analyses (Appendix 2). This criterion was justified, however, because most of the taxa eliminated were upland plants, or had such low IV that they were not major components of the wetland ecosystem. I graphed the axis scores to show the similarities and dissimilarities in species composition between CSB and WMF.

Pool Size

I calculated the approximate area (m^2) using the length and width of each pool. Pool size calculations provide a rough estimate of the amount of water found at each wetland. Due to the unequal sample size between the wetlands, I used a Mann-Whitney test in Minitab (Version 17) to compare the pool area between CSB and WMF. I also used Mann-Whitney to compare pool depth between the two wetlands. In addition, I calculated the proportion of pools found in the vegetation quadrats at each wetland.

Water Chemistry

pH and specific conductance

I analyzed pH data by first converting pH values into [H^+]. Using a Kruskal-Wallis test (IBM SPSS Statistics, Version 22), I used date as the grouping variable to show any temporal trends within sample sites for [H^+] at each wetland. Due to potential differences in water chemistry, I analyzed groundwater and surface water separately. I also used Kruskal-Wallis tests to show temporal trends within sample sites for SPC at each wetland. For this study, I was mainly interested in identifying any outliers in the pH and SPC data, which would indicate a change in the system; therefore, most statistical analyses were not appropriate for these data. Instead, I used boxplots to show any outliers in pH and SPC data.

Alkalinity

Due to the small sample size and low sampling frequency, statistical analyses were not necessary for alkalinity data collected, and only raw data are reported.

Tree Cores

Tree cores were sent to The University of North Carolina--Asheville for analysis using Gas Chromatography Mass Spectrometry (GC/MS) by Dr. Jeffrey Wilcox to identify any potential persistent contaminants and potential organics in the frack fluids that may have been taken up by the trees during the accidents.

Fish

I did not catch any fish during sampling at either wetland; therefore, statistical analyses were not necessary for this parameter.

Avian Point-Counts

Statistical analyses were not used on presence/absence data collected during the avian point count surveys. For the purpose of this research, I was only interested in comparing bird communities between the two wetlands to look for similarities or dissimilarities; therefore, I calculated summary statistics (number of individuals, species richness, etc.), as well as diversity and similarity indices for the surveys conducted in 2012 and 2013. I calculated Simpson's Index of Diversity (SID) for each site sampled using the formula:

$$D = 1 - \left(\frac{\Sigma n(n-1)}{N(N-1)} \right), \text{ where}$$

N = the total number of organisms of an individual species, and N = the total number of organisms of all species. SID is widely used in ecological studies and uses richness and evenness to quantify the biodiversity of a habitat. In addition, I used two community similarity indices, which are used to measure the degree of association (or absence thereof) between samples or communities. I used the Jaccard Index of Similarity (C_J), which is one of the simplest similarity indices and does not take species abundance into account. The C_J was calculated by using the formula:

$$C_J = \frac{A}{A+B+C} \text{ ,where}$$

A = the number of species found at both sites, B = the number species only found at the first site, and C = the number of species found at the second site. I also used Sorenson Index of Similarity (C_S), which is similar to C_J but it gives greater weight to species common to both sites. The C_S was calculated by using the formula:

$$C_S = \frac{2A}{2A+B+C} \text{ ,where}$$

A = the number of species found at both sites, B = the number species only found at the first site, and C = the number of species found at the second site. Both similarity indices rely on presence/absence data (Chao *et al.* 2005), and were used to compare the species composition between wetlands. Analysis methods were adapted from the U.S. Fish and Wildlife Service statistical guide to data analysis of avian monitoring programs (Nur *et al*. 1999).

Aquatic Invertebrates

I combined all of the aquatic invertebrate data for each wetland, which gave me the total number of individuals per taxon per wetland. It is common to use biotic indices, such as Hilsenhoff Biotic Index (HBI) and EPT richness, as a means to assess water quality. Both of these methods are based on a 100-organisim sample or subsample; I was unable to use these indices due to the small number of organisms found at both study sites. Again, I reported summary statistics, as well as SID, C_J, and C_S. Unidentified individuals were eliminated from the analyses.

Amphibians

Point-Count Surveys

Due to the lack of amphibians encountered during the call surveys at WMF, statistical analyses were not used. Instead, I reported summary statistics (number of individuals, species richness, etc.) and the maximum calling code for each species detected during the call surveys. If I could not reliably estimate amphibian numbers (i.e., calling in a full chorus), I eliminated those data from the summary statistics. I also calculated SID, C_J, and C_S for each site sampled.

Visual Encounter Surveys

Due to the lack of species detected at WMF, statistical analyses were not needed and only raw data are reported.

RESULTS

Habitat Characteristics

Vegetation

A total of 56 taxa were sampled in the 240 quadrats. *Sphagnum* spp. was the dominant vegetation found at both wetlands; it covered 76% of CSB and 68% of WMF, and had a maximum IV of 190. The second most dominant species was swamp dewberry (*Rubus hispidus*), which covered 24% of CSB and 35% of WMF and had a maximum IV of 150. Species of Cyperaceae were also common at both wetlands; approximately 19% of vegetation found at CSB and 14% of vegetation found at WMF were sedges. The common sedges also had relatively high maximum IV: *Carex crinita* = 52, *C. folliculata* = 89, *Eriophorum virginicum* = 61, and *Rhynchospora alba* = 115. *Vaccinium* spp. was also quite common, and covered 7% of CSB and 15% of WMF. A total of 34 taxa (with IV > 20 found in > 5 quadrats) were used in the ordination (Appendix 2). The majority of these taxa were found at both CSB and WMF. The only exceptions were *S. purpurea* and *T. latifolia*, which were found only at CSB, and *Alnus* spp., *Juncus canadensis, Muhlenbergia glomerata*, and *T. canadensis*, which were found only at WMF.

The nMDS ordination (Figure 20) showed five distinct groupings of taxa. The first grouping of plants (labeled 1) was found in the drier areas and included *Gaultheria procumbens*, *Botrychium virginianum*, *Amelanchier* spp., *Trientalis borealis, A. rubrum*, *T. canadensis*, *Panicum clandestinum*, and *Kalmia latifolia*. These species grow in upland locations and do not need an abundance of water to grow or survive. Most of these plants were found growing in driers areas of the wetlands, the wetland perimeter, or the forest. The second grouping (2) included *M. glomerata*, *J. canadensis,* and *Alnus* spp. These taxa were only found at WMF but grew throughout that wetland. The third grouping (3) included *Spirea tomentosa*, *C. folliculata*, *C. crinita*, and *Sparganium* spp. These taxa were found growing throughout both wetlands. The fourth grouping (4) included *V. corymbosum*, *V. macrocarpon*, *Aronia melanocarpa*, an unknown grass, *Viola* spp., *Rubus hispidus*, *Sphagnum* spp., *Lycopodium* spp., and *Osmonda cinnomomea*. All of these taxa were found growing with and on mounds of *Sphagnum* spp. at both wetlands. The fifth grouping (5) included *Carex* spp., *D. rotundifolia*, *E. virginicum*, *Juncus brachycephalus*, *J. effuses*, *S. purpurea*, *Scirpus cyperinus*, *Selaginella* spp., and *T. latifolia*. Of these species, *D. rotundifolia*, *E. virginicum*, *J.*

brachycephalus, J. effuses, S. purpurea, S. cyperinus, and *T. latifolia* are all distinctly wetland species with an obligate (OBL) wetland rating, and all need an abundance of water to grow and survive. The *Carex* spp. and *Selaginella* spp. included in this grouping were not identified to the species level, so it is impossible to determine the exact wetland rating of those plants. However, many members of those genera are wetland species. Due to placement of taxa on the axes, I determined that Axis 1 is moisture gradient and Axis 2 is wetland location. This ordination suggests that, overall, CSB is wetter than WMF.

The nMDS ordination of the sample plots A- P (Figure 21) determined Axis 1 to be the moisture gradient, going from wettest on the left to driest on the right. Most of the CSB plots fell on the left side of the grid and most of the WMF plots fell on the right, showing two distinct wetland groupings. Quadrants A from CSB and O from WMF were two exceptions from each wetland that grouped together near the lower right. Both of these quadrants were much drier than the others, and the majority of the plots sampled fell outside of wetland habitat. Quadrants E from CSB and N and I from WMF also grouped together near the right center of the grid. These three quadrants were in areas that covered both wetland and upland habitat, and the quadrats sampled consisted of an equal mix of both habitat types. Like the selected taxa ordination, this ordination also suggests that CSB is wetter than WMF.

Pool Size

A total of 46 pools were found in 40 (~33%) of the vegetation quadrats at CSB. At WMF, a total of 34 pools were found in 32 (~27%) of vegetation quadrats. Generally, the pools at CSB were larger, and there was a significant difference in pool area between CSB and WMF (p=0.0119, W=2122). Pools covered ~6630.57 m^2 of CSB, but only about

56.27 m^2 of WMF. Although the quantity and size of pools were greater at CSB, there was no significant difference in pool depth between CSB and WMF (p=0.8492, W=1883.0) (Table 2).

Water Chemistry

pH and Specific Conductance

Since group variances were unequal, parametric statistics were not appropriate for the water quality analyses. Using date as the grouping variable, a Kruskal-Wallis test conducted in SPSS (IBM SPSS Version 2) showed no temporal changes in [H+] concentrations for any of the water sources (WMF Ground Water (WMGW): $x^2(28) = 24.18$, $p = 0.67$; WMF Surface Water (WMSW): $x^2(28) = 24.48$, $p = 0.66$; CSB Ground Water (CSGW): $x^2(28) = 26.01$, $p = 0.57$; CSB Surface Water (CSSW): $x^2(28) = 23.78$, $p = 0.69$) (Table 3).

There were no differences in the surface water between CSB and WMF, and the pH routinely ranged between 4.31 and 5.45 at each wetland. The groundwater pH was also very similar between the wetlands. With outliers removed, the pH ranged from 4.21 to 5.37 at CSB and 4.12 to 5.42 at WMF; however, there were several outliers at the WMGW site that dropped as low as 3.49. In addition, the WMGW had more consistent pH over time, with the exception of those outliers. These outliers were detected using boxplots of the [H+] data. The boxplots show that groundwater and surface water at both wetlands have pH values that remained relatively consistent over time (Figure 22).

A similar trend was found in the SPC data. A Kruskal-Wallis test showed no significant temporal changed in the SPC for any of the water sources (WMGW: x^2 (28) =

19.42, p = 0.89; WMSW: x^2 (28) = 29.00, p = 0.41; CSGW: x^2 (28) = 26.17, p = 0.56; CSSW: x^2 (28) = 21.92, p = 0.79) (Table 4). With outliers removed, the groundwater ranged between 64 and 95 µS/cm at CSB and between 63 and 92 µS/cm at WMF. The surface water ranged between 36 and 91 µS/cm at CSB and from 34 to 99 µS/cm at WMF. As with the [H+], there were outliers at the WMF groundwater sites, where SPC spiked to 129, 143, 171, and 174 µS/cm. These outliers were detected with boxplots (Figure 23).

Alkalinity

Alkalinity ranged from 0 mg/L $CaCO_3$ to 2.3 mg/L $CaCO_3$ across the nine sites. Due to the small sample size, statistical analyses were not appropriate; however, there appeared to be no major differences in the alkalinity based on water source. Mean alkalinity of groundwater sites was 1.46 mg/L $CaCO_3$ and mean alkalinity of surface water sites was 1.1 mg/L $CaCO_3$ (Table 5).

Tree Cores

Tree core analyses conducted using GC/MS at The University of North Carolina--Asheville did not detect any non-natural organic compounds in the cores.

Fish

I was unable to capture any fish at either wetland; however, other organisms were caught in the traps. At CSB, I captured a total of seven crayfish (family Cambaridae), one diving beetle (order Coleoptera), and one red-spotted newt. At WMF, I captured six crayfish and one green frog.

Avian Point-Counts

2012

In 2012, I detected 31 species at CSB; 15 of those species were found at both CS 1 and CS 2, whereas eight were unique to CS 1 and eight were unique to CS 2. The most frequently observed species at CS 1 was Red-winged Blackbird (*Agelaius phoeniceus*), followed by Cedar Waxwing (*Bombycilla cedrorum*), and Song Sparrow (*Melospiza melodia*). At CS 2, Cedar Waxwings were detected most often, followed by American Crow (*Corvus brachyrhynchos*), Blue Jay (*Cyanocitta cristata*), Song Sparrow, and Chestnut-sided Warbler (*Setophaga pensylvanica*) (Appendix 3). I used these data to calculate the C_J and C_S. The C_J showed a 48% similarity in bird species composition between CS 1 and CS 2 and the C_S showed a 65% similarity (Table 6C). I also calculated the SID for each site; diversity was slightly greater at CS 2 in 2012 (Table 6A).

I found similar results at WMF in 2012. I detected a total of 25 species; 16 were found at both WM 1 and WM 2, six were unique to WM 1, and three were unique to WM 2. The most frequently observed species at WM 1 were Blue Jays and Song Sparrows, followed by American Crow, Common Yellowthroat (*Geothlypis trichas*), Gray Catbird (*Dumetella carolinensis*), and White-breasted Nuthatch (*Sitta carolinensis*). At WM 2, Song Sparrows were the most commonly observed, followed by Gray Catbird, Black-capped Chickadee (*Poecile atricapillus*), and Tufted Titmouse (*Baeolophus bicolor*) (Appendix 3). These data were used to calculate the C_J and C_S. The C_J showed a 64% similarity in species composition between WM 1 and WM 2 and the C_S showed a 78% similarity (Table 6C). Diversity was slightly higher at WM 1 in 2012 (Table 6B).

In 2012, there were 19 species common to both CSB and WMF, 12 species unique to CSB and six species unique to WMF. The C_J showed a 51% similarity in bird species composition between the two wetlands and the C_S showed a 68% similarity (Table 6C).

2013

In 2013, I detected a total of 26 species at CSB. Eleven of those were observed at both CS 1 and CS 2, eight were found only at CS 1, and seven only at CS 2. The most commonly observed species at CS 1 was Red-winged Blackbird, followed by Song Sparrow, Red-eyed Vireo (*Vireo olivaceus*), and turkey vulture (*Cathartes aura*). At CS 2, Song Sparrows were the most frequently observed, followed by American Tree Sparrow (*Spizella arborea*) and Field Sparrow (*Spizella pusilla*) (Appendix 4). These data were used to calculate the C_J and C_S. The C_J showed 42% similarity in species composition between CS 1 and CS 2 and the C_S showed a 59% similarity (Table 6C). As with 2012, the diversity was slightly higher at CS 2 in 2013 (Table 6A).

At WMF, I detected 23 species during the 2013 season. Seventy percent of those species were found at both WM 1 and WM 2, whereas 17% were unique to WM 1 and 13% were only found at WM 2. Black-capped Chickadees and Chipping Sparrows (*Spizella passerina*) were the most commonly observed species at WM 1, followed by Song Sparrow and White-breasted Nuthatch. Gray Catbirds and Song Sparrows were the most frequently observed birds at WM 2 during 2013. Chestnut-sided Warblers, Eastern Phoebes (*Sayornis phoebe*), Chipping Sparrows, and Field Sparrows were also frequently observed (Appendix 4). The C_J showed a 70% similarity in bird species composition between these two sites and the C_S showed an 82% similarity (Table 6C), and both had a SID of 0.94 (Table 6A).

In 2013, there were 18 species common to both CSB and WMF, eight species found only at CSB, and five species unique to WMF. The C_J showed a 58% similarity in bird species composition between the two wetlands and the C_S showed that the similarity was 73% (Table 6C).

Due to the lack of wetland-obligate bird species detected at either wetland, I did not focus my efforts in this aspect of the study and opted not to conduct further statistical analyses.

Aquatic Invertebrates

Relative abundance of aquatic macroinvertebrates was greater at CSB where 87 individuals were identified, with five unknowns. At WMF, 78 individuals were identified, with three unknowns. Taxon richness, however, was lower at CSB (10 taxa) than at WMF (12 taxa). Overall diversity was lower at CSB (SDI = 0.73) than WMF (SDI = 0.81); however, the C_J indicated 83% similarity of taxa between the two wetlands and C_S indicated 91% similarity between the two wetlands (Table 7).

Taxa found at CSB were Oligochaeta (worms), Cambaridae (crayfish), Corixidae (water boatmen), Gyrinidae (whirligig beetles), Notonectidae (backswimmers), Zygoptera (damselflies), Anisoptera (dragonflies), Chironomidae (midges), Ephemeroptera (mayflies), and Tipulidae (craneflies). Midges were the most abundant taxon at CSB and represented 48% of all organisms detected there. The second most abundant taxon was Oligochaeta, which represented 16% of all organisms at CSB. All other taxa found at CSB made up 36% of the overall taxa composition, but no one taxon represented >9% of the aquatic invertebrate population there.

Taxa found at WMF were Oligochaeta, Cambaridae, Corixidae, Gyrinidae, Notonectidae, Zygoptera, Anisoptera, Chironomidae, Plecoptera (stoneflies), Ephemeroptera, Tipulidae, and Amphipoda (scuds). Midges were also the most abundant taxon at WMF and represented 33% of all taxa found there, followed by craneflies (Tipulidae), which represented 24% of taxa. All other taxa found at WMF made up 43% of the overall aquatic macroinvertebrate community sampled, but no one taxon represented >9% of the aquatic invertebrate community there (Appendix 5).

Amphibians

Point-Count Surveys 2012

In 2012, I detected a total of six species at CSB: northern spring peeper (*Pseudacris crucifer*), wood frog (*Lithobates sylvaticus*), American toad (*Anaxyrus americanus*), green frog (*Rana clamitans*), pickerel frog (*Rana palustris*), and bullfrog (*Rana catesbeiana*). Spring peepers, American toads, and green frogs were detected at sample sites CS 1 and CS 2, but wood frogs and bullfrogs were only found at CS 1 and pickerel frogs were only found at CS 2. I was able to estimate number of individuals on each sampling date at both sites for all species except spring peepers; spring peepers could not reliably be estimated on two occasions at CS 2 because they were calling in a full chorus. Green frogs were the most commonly observed species at CS 1, followed by spring peeper and American toad. At CS 2, spring peepers were most common, followed by pickerel frogs. Spring peeper was the only species detected at WMF and was only found at WM 1. The peepers were not calling in full chorus, and I estimated a total of 11 individuals throughout the duration of the 2012 sampling period (Appendix 6, Appendix 7).

Point-Count Surveys 2013

In 2013, I detected a total of five species at CSB: northern spring peeper, wood frog, American toad, green frog, and bullfrog. Northern spring peepers and green frogs were found at both CS 1 and CS 2. American toads and wood frogs were only found at CS 1, and bullfrogs were only found at CS 2. Pickerel frogs were not detected in 2013. Spring peepers were the most commonly observed species at CS 1, followed by American toad and wood frog. Spring peepers were also the most commonly observed species at CS 2, followed by green frog and bull frog. At WMF, only spring peepers were detected in 2013, and only at sample site WM 1. They were not calling full chorus, and I estimated a total of 14 individuals (Appendix 8, Appendix 9).

The mean number of individuals detected at CS 1 decreased from 4.0 in 2012 to 3.0 in 2013 but increased during the same time period at CS 2 from 2.8 to 3.8. The species richness per plot decreased from 2012 to 2013 at CS 1 from 5.0 to 4.0 and from 4.0 to 3.0 at CS 2 but did not change at WM 1. Using SID, I determined that the species diversity was greater at CS 1 in both 2012 and 2013. However, diversity decreased from 0.78 in 2012 to 0.74 in 2013 at CS 1 and decreased from 0.57 in 2012 to 0.54 in 2013 at CS 2. Only one species was detected at WMF in 2012 and 2013; therefore, the SID was zero in both years (Table 8A). When I combined all the data per wetland, I found that the average number of individuals did not change at CSB from 2012 to 2013 (3.42), nor did the cumulative number of individuals change (6.83); however, the cumulative species richness decreased from 6.0 to 5.0 at CSB. At WMF, the mean number of individuals increased from 0.92 in 2012 to 1.17 in 2013. The cumulative number of individuals also increased at WMF from 1.83 in 2012 to 2.33 in 2013; however, the cumulative species richness was only 1.0 (Table 8B).

The C_J showed a 50% similarity in species composition between sites CS 1 and CS 2 during the 2012 season but decreased to 40% similarity for the 2013 season. The C_S showed a similar trend, with a decrease from 67% species similarity in 2012 to 57% in 2013. Only spring peepers were found at WM 1; therefore, species composition between WM 1 and WM 2 was 0% similar during both years. The similarity in species richness between CSB and WMF was not great; the C_J showed that the species composition between the two wetlands were only 16.67% similar in 2012 and 20% similar in 2013 (or 83.33% and 80% difference, respectively) (Table 8C). The C_S yielded similar results; the amphibian species composition between the two wetlands was 29% similar in 2012 and 33% similar in 2013 (or a 71% and 67% difference, respectively) (Table 8).

Visual Encounter Surveys

During the 2013 VES surveys, I detected seven species at CSB: dusky salamander (*Desmognathus fuscus*), red-spotted newt (*Notophthalmus viridescens*), red-backed salamander (*Plethodon cinereus*), spotted salamander (*Ambystoma maculatum*) wood frog, American toad, and pickerel frog. Among adults, red-spotted newts were the most abundant and accounted for 58% of all species detected (11 adult, 4 red efts) at CSB. Nine red-backed salamanders were found, which represented 35% of the amphibians detected at the wetland. One woodfrog and one dusky salamander were also detected. At WMF, I detected two adult redback salamanders and one adult green frog during the VES surveys. One adult green frog was also found during an intense search of the entire wetland (outside of the VES plots) (Table 9).

Egg masses detected at CSB included spotted salamander, American toad, pickerel frog, and woodfrog. The spotted salamander and woodfrog egg masses were particularly abundant, and were detected in 28% and 34% of the plots, respectively. American toad eggs were found in 9% of the plots, and pickerel frog egg masses were found in just one plot. In addition, several American toad tadpoles were found in plot 13. No juveniles were found in WMF (Table 10).

Although I was not intentionally surveying reptiles, I found a northern redbelly snake (*Storeria occipitomaculata occipitomaculata*) at CSB in plot 1, an eastern garter snake (*Thamnophis sirtalis*) in plot 8, and a large snapping turtle (*Chelydra serpentina*) in plot 30. As WMF, I found a timber rattlesnake (*Crotalus horridus*) den in plot 42, and I encountered rattlesnakes on two different occasions during the study (one in plot 42 and one just outside of plot 42).

DISCUSSION

Habitat Characteristics

Studies have shown that some taxa richness (particularly for some species of anurans) can be influenced by vegetation and pool size (Glooschenko *et al*. 1992, Hazell 2003, Garcia-Munoz *et al*. 2010); therefore, I collected vegetation (percent cover) data and pool size (depth and area) data, which is discussed below.

Vegetation

A total of 56 plant taxa were detected in the 240 quadrats. *Sphagnum* spp. was the dominant vegetation found at both wetlands, followed by swamp dewberry (*Rubus hispidus*). Members of the Cyperaceae family were also common at both wetlands, as was *Vaccinium* spp. Although vegetation alone cannot be used to classify the type of fen, the vegetation found at the sites—a variety of sedges, cottongrass, white beakrush, alders, and cattail—are all consistent with the types of vegetation found in a ‘medium’ fen (Tiner 2005).

There were no major differences in plant species composition between the two wetlands, and there appeared to be no impacts to vegetation in WMF from the EOG accidents. This was expected, as the sampling was conducted ~ 3 years after the accidents were detected. Although vegetation composition is similar at each wetland, and both wetlands have groundwater discharge, the results of the ordination suggest that, overall, CSB is wetter than WMF. This is not surprising, considering the local topography of the sites. CSB is flatter, sits between two slopes and has more of a bowl shape, whereas WMF slopes gently to the east, allowing water to flow away from the wetland.

Pool Size

Results indicate a significant difference in pool size between the two wetlands, although the depth of the pools measured did not greatly differ. These data support the results of the vegetation ordination, which shows that CSB is wetter than WMF. Again, due to the local topography, this is to be expected. Regardless of pool quantity and size, WMF is still a wetland with several pools, hydric soils, and wetland vegetation.

Water Chemistry

pH, Specific Conductance, and Alkalinity

Alex Branch is known to be a naturally acidic system, with a pH ranging from 4.7 to 6.0 and conductivity ranging from 19 to 48 µS/cm (PA DEP 2010). These pH and SPC values are fairly consistent with those found at CSB and WMF at both groundwater and surface water sites. The similarity in pH and SPC between the groundwater and surface water sites is to be expected—the surface water sites are in close proximity to the groundwater sites at each wetland. The stream at CSB is formed from groundwater discharge at the site. At WMF, Alex Branch is formed from groundwater discharge off-site, but there are no other surface water inputs, except precipitation, to Alex Branch before it reaches WMF. Also, there are several areas of groundwater discharge at WMF that drain into Alex Branch. Both have the same underlying geology that is dominated by sandstone, shale, clay, and coal. Both have comparable hydrology with similar groundwater and surface water chemistry. The temporal range of pH was also very similar for all of the water sources tested at both sites, and there were no significant water chemistry differences between them.

Although the pH and SPC levels detected at CSB and WMF and the alkalinity levels at WMF seem low, they are relatively common for wetlands in this region. Alex Branch, however, has lower pH and SPC than most streams in this region due to its natural acidity. A study by Trexler *et al*. (2014) found similar pH values in Alex Branch (pH = 4.88) as found in my study; however, it should be noted that of the 26 sites sampled in that study, streams located in watersheds with Marcellus Shale gas drilling activities (including Alex Branch) had significantly lower pH. In fact, Alex Branch had the second lowest pH in their study (Trexler *et al*. 2014).

Water chemistry data, along with vegetation data, allowed me to determine that CSB and WMF are very similar wetlands. Additionally, these data support the idea that both are ‘medium’ fens. Fens are peatlands characterized by inputs of groundwater, and the mineral composition of that groundwater varies depending on the underlying geology. In limestone-rich regions, groundwater pH is high due to high carbonate alkalinity. These fens are typically classified as ‘rich fens’ and are dominated by herbaceous and graminoid vascular plants, as well as mosses (Tahvanainen 2004). In central PA, the underlying bedrock consists of sandstone, shale, and coal, which have few soluble minerals (Fleeger 1999); therefore, pH, SPC, and alkalinity tend to be lower. Additionally, shallow groundwater (often called spring water) has a short residence time and cannot dissolve many minerals found within the rocks (Fleeger 1999). These fens are classified as ‘poor fens’ and are dominated by Sphagnum spp. and ericaceous shrubs (Tahvanainen 2004). A study by Vitt and Chee (1990) found the pH in poor fens to range from 4.5 to 4.8, and Tahvanainen (2004) recorded a pH of 4.2 in a poor fen (2007). According to Tiner (2005), ‘rich fens’ have pH values >6.0, ‘poor fens’ have pH values between 3.5 and 5.0, and ‘medium fens’ have intermediate pH values. Tiner’s description of fen types (2005), supports my classification of both CSB and WMF as ‘medium fens.’

No major differences were detected in any of my water chemistry data. Water quality monitoring for my project began about 2.5 years after the accidents at EOG wells 8H and 9H. Data collected by the PA DEP showed that pH, conductivity, and alkalinity in areas of Alex Branch had already returned to levels consistent with this region by

December 2009. SPC data were also collected by members of the Senior Environmental Corps (SEC), a local conservation group, at two groundwater discharge sites (Reeds Spring and Sykesville Camp Spring) from August 2009 until January 2011. At the Sykesville Spring, SPC was 3200 µS/cm in August 2009 and steadily declined. By November 2009, SPC had decreased to 265 µS/cm, and by April 2010, it was back to levels more consistent with this region (82 µS/cm). A similar trend was found at Reeds Spring: SPC was 5004 µS/cm in August 2009, steadily declined for several months, and was 50 µS/cm in April 2010 (SEC, unpublished data). These data provide some insight into the nature of the accidents. First, because the SPC data collected from groundwater discharge showed higher levels in December 2009 than those collected from surface sites by the PA DEP in December 2009, this suggests that the contaminants from the EOG accidents seeped into the ground, resurfaced as groundwater discharge, and then flowed into Alex Branch. Additionally, SPC levels were back to ‘normal’ ranges by April 2010, which suggests that the accidents were short-term discharge events that did not penetrate into deep aquifers, but instead, infiltrated shallow sub-surface flow. Again, considering the geology of this region, this is a very likely explanation. Due to the rugged landscape, as well as the insoluble minerals found in the sandstone and shale, water does not penetrate deep into the ground and, therefore, has a short residence time in near-surface soils (Fleeger 1999).

Although statistical analyses detected no significant differences in pH or SPC, boxplots showed several outliers at the WMF groundwater sites. On 8 August 2012, pH dropped to 3.57 and the SPC spiked to 143 µS/cm at one area of groundwater discharge on the south side of the fen (WMGW 5). On 6 April 2013, pH dropped to 3.49 and SPC spiked to 171 µS/cm at the same site. I tested the water the following two days: on 7 April, pH had increased slightly to 3.91 and SPC increased to 174 µS/cm, and on 8 April, pH increased to a more ‘normal’ value of 4.24; however, SPC was still relatively high (129 µS/cm). The meter used was calibrated and in good working condition, and none of the other sample sites showed anything out of the ordinary on those particular days.

What caused these abrupt changes in groundwater pH and SPC at this particular site? It is possible that contaminants persisted in the groundwater and were eventually flushed out by a large precipitation event. Due to the underlying geology, this area has a short residence time; therefore, I examined precipitation events that occurred for up to 1 year prior to the change in pH and SPC. I accessed data online from a weather station (ID: MKYPP1) located in Penfield, PA, which is the closest weather station to the study sites. In the months leading up to the first detected change in water quality (8 August 2012), precipitation was well below average; however, a few strong storms dropped a large amount of rain over short time periods. This heavy influx of precipitation may have flushed the system. Before that, from May 2012 back to August 2011, the area received average precipitation amounts, and there were only two large precipitation events (precipitation > 2.54 cm in a 24 hr period). Precipitation was also well below average in January, February, March, and April 2013, and no large precipitation events were recorded in the days or weeks leading up to the change in pH and SPC I detected in April, 2013 (The Weather Channel LLC 2015). Due to the timing of the April 2013 event (6-8 April 2013), it is possible that the combination of precipitation and snowmelt may have contributed to groundwater recharge. If any contaminants persisted below the surface, it is possible that they would have been flushed out during groundwater discharge.

Another possible explanation for the changes in water chemistry is that another gas well accident(s) may have occurred. EOG wells 8H and 9H are located approximately 1.8 km and 1.4 km upslope and to the southwest of WMGW 5, and five other EOG wells are located even closer to WMF. Wells 12H and 49H are approximately 0.95 km from WMGW 5, and wells 14H, 47H, and 48H are about 1 km from WMGW 5; the latter three wells are directly upslope from the groundwater discharge site. No accidents were reported around the times I noticed changes in pH and SPC; however, the wells in question are on privately owned land, are not heavily regulated, and accidents have gone unreported in the past (Marcellus Outreach Butler 2012). In fact, EOG Resources failed to report two of the three accidents that occurred at wells 8H and 9H to the PA DEP. The PA DEP only became aware of those accidents because a local citizen noticed changes in her well water and filed a complaint. Failure to report accidents is PA is all too common. Many companies' safety policies hinge on 'self-policing' (Marcellus Outreach Butler 2012), and accidents often go completely unreported or are reported in an untimely manner. This is due to the lack of regulation in PA. According to Price (2014), this lack of regulation creates situations where accidents are not required to be reported.

Tree Cores

We did not pick up any non-natural organic compounds in our tree core analysis, which supports my hypothesis that the contamination occurred in short-term discharge events.

Fish

I did not catch fish at CSB, and they have not been documented at CSB by the PFBC. Given the lack of fish documentation at the site, as well as the combination of water quality (i.e., naturally low pH, presumably low D.O.) and stream location and size (i.e., shallow, narrow, lacks vegetation and debris, few aquatic invertebrates), it is not surprising that I did not find fish during my surveys of CSB.

I also did not capture any fish in Alex Branch at WMF. Alex Branch is a sandy-bottom stream, but it is larger and deeper than the stream at CSB. Like the stream at CSB, Alex Branch is formed by groundwater discharge, but the main source of the groundwater is discharged well over 2.5 km upstream from the wetland; therefore, D.O. levels are likely much higher in Alex Branch by the time the stream reaches WMF than at CSB. Alex Branch also has naturally low pH (ranging from 4.31 to 5.43) due to the underlying geology; however, some species of fish can tolerate pH at these levels.

According to the PFBC, Alex Branch, along with several other surrounding streams (Little Laurel Run, Pray Run, Roberts Run, Trout Run, and Dixon Run) all support naturally producing populations of trout (PASDA 2013). Brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) are able to tolerate low pH levels (Steiner 2002). Dunson and Martin (1973) reported brook trout survival in Sinking Creek, a stream that originates from Bear Meadows Bog in PA and has pH levels ranging from 4.51 to 5.65. These pH levels are very similar to those in Alex Branch.

Other species in the Susquehanna watershed that can tolerate low pH are creek chub (*Semotilus atromaculatus*), white sucker (*Catostomus commersoni*), and fathead minnow (*Pimephales promelas*). Creek chubs are adaptable to a wide range of conditions

(Steiner 2002), are found in small streams, and according to McMahon (1982), are more tolerant of acidic conditions than many other species. White suckers are one of the most common and widely distributed suckers in PA. They are adapted to many habitats, including headwater streams, and are pollution tolerant (Steiner 2002). In PA, fathead minnows are found in a wide range of environments, including slow-moving streams, and can tolerate low D.O. (Steiner 2000). Unfortunately, there are no PFBC biologist reports available for any of these streams before or after gas drilling operations began in the region, or before or after the gas drilling accidents occurred at EOG wells 8H and 9H. The question remains that if trout were here pre-accident, why have they not returned?

I was unable to determine the exact cause for lack of fish, particularly trout, in Alex Branch. It may be related to low sampling intensity, poor trap placement, or perhaps this particular stretch of Alex Branch does not provide suitable habitat to support fish populations. I cannot rule out the possibility that fish were indeed impacted (directly or indirectly) by the contamination of Alex Branch in 2009 by EOG Resources. According to Weltman-Fahs and Taylor (2013), trout tend to display avoidance behavior and will leave areas contaminated with heavy metals, and heavy metals were released into Alex Branch during the EOG contamination events.

Recent studies evaluate the impacts of unconventional gas-drilling activities on fish. In 2007, fracking fluids released into Acorn Fork Creek, KY resulted in a decrease in pH (from 7.5 to 5.6) and an increase in SPC (from 200 to 35,000 µS/cm). As a result, fish were killed, severely stressed, or displaced for several months (Papoulias and Velasco 2013). Of greater importance to my study is a study by Grant *et al*. (2015) that surveyed several streams in this region, including Alex Branch, Little Laurel Run, and

Trout Run. Results from the study indicated that watersheds in this region with more than three Marcellus gas wells (including Alex Branch) had significantly lower fish diversity than watersheds with two or fewer gas wells. Although studies such as this are few, the effects of metals, as well as other contaminants (such as those found in fracking fluids), on fish have been well-documented. Elevated concentrations of metals can affect fish physiological functions (Evans 1987), growth rates, and reproduction, and can lead to mortality (Mance 1987, Sorensen 1991, Farag *et al*. 1994, Woodward *et al*. 1995). Manganese can accumulate in fish liver, kidneys, gills, and muscle (Javed 2005) and impairs growth and increases mortality (Burger *et al*. 2002). Increased levels of contaminants can also lead to harmful algal blooms, resulting in fish kill. Patino *et al*. (2014) found that high salinity-associated variables, such as SPC, chloride, and sulfate, were the most important factor influencing toxic blooms of golden algae (*Prymnesium parvum*) in inland waters. Golden algae are typically found in marine and estuarine habitats but can also be found in brackish inland waters (Patino *et al*. 2014). Interestingly, in 2009, a bloom of golden algae resulted in a massive fish kill in Dunkard Creek, a 72 km-long stream that spans the PA-WV border. The bloom was caused by high levels of chloride and TDS. The PFBC recorded that the PA portion of the creek suffered the loss of over 42,000 fish (Arway 2013). The WV Supreme Court ruled against CONSOL Energy, a natural resources extraction company that mines coal and drills natural gas, which allegedly discharged pollutants into the stream. Investigators never agreed if the discharge was from gas-drilling operations or coal mine waste (Arway 2013); however, elevated chloride and TDS levels are an indication of drilling operations, not mine pollution (Swistock 2010).

The lack of baseline data, paired with the fact that my research was conducted three years after the contamination events, make it impossible to determine the reason for the lack of fish found in Alex Branch during my surveys. However, the types of contaminants (heavy metals, elevated TDS) discharged into Alex Branch---a native trout stream---are known to have deleterious impacts on fish.

Avian Point-Counts

Although I do not report statistical tests, it is clear that there were no major differences in the bird data collected at the wetlands. Any minor differences in bird species between the wetlands is not attributable to the gas drilling accidents and is likely the result of slight differences in habitat. For example, Red-winged Blackbirds were abundant at CS 1 but not found at CS 2 or WMF. CS 1 has a large patch of *T. latifolia*, which did not occur at CS 2 or at WMF, and the Red-winged Blackbirds were always observed in or near this vegetation. This is not unexpected, as several studies have linked Red-winged Blackbirds to *Typha* use in wetlands (Meanley 1965, Bernstein and McLean 1980, Vierling 1999, Prather and Cruz 2006). Another difference in bird species between the wetlands that can be attributed to habitat is the absence of Canada Geese (*Branta canadensis*) and Mallards (*Anas platyrhynchos*) at WMF. CSB has a beaver dam on the stream below the fen that created a large pond; this explains the presence of Canada Geese and Mallards. Although WMF has several pools, seeps, and springs, none are large enough to host birds of that size. I did not detect Tree Swallows (*Tachycineta bicolor*) or Barn Swallows (*Hirundo rustica*) at WMF, but both were found at CSB. This is likely due to the size and openness of the wetlands; CSB is larger and more open, with fewer large trees and shrubs within the wetland. Swallows prefer these large open and semiopen areas (Peterson 2002). Similarly, Cedar Waxwings, which also prefer open woodlands (Witmer *et al*. 2014), were also detected at CSB but not at WMF.

All of the bird species detected at WMF during the 2102-2013 surveys were forest species, field/grassland species, or habitat generalists, and none of the species detected rely specifically on wetland habitat. This is not unexpected, as the wetland is relatively small and completely surrounded by forested area, as well as some clearings where camps are located. There is nothing in the data to suggest that the few differences in bird species between the wetlands are related to the accidents at EOG wells 8H and 9H.

Aquatic Invertebrates

Overall aquatic invertebrate taxa diversity was slightly lower at CSB (SID = 0.73) than WMF (SID = 0.81). Ten taxa were found at CSB, with midges (Chronimidae) being the most abundant, followed by Oligochaeta. Twelve taxa were found at WMF. Midges were also the most abundant taxa at WMF, followed by craneflies (Tipulidae).

Data collected during my aquatic invertebrate surveys were comparable to stream surveys conducted by the PA DEP in Alex Branch. The PA DEP sampled four sites in Alex Branch upstream from WMF; however, in their final report, they only reported findings for three of the sites (Reference Site, Impact 1, and Impact 3). Similar to my study, the PA DEP found relatively small numbers of all taxa, and these taxa were not equally distributed among sample sites. The genus *Leuctra* (stonefly) was found in the greatest abundance (167 total individuals across three sites), followed by Chironomidae (126 total individuals across three sites), and the genus *Simulium* (39 total individuals across three sites). Although I did not identify to the same taxonomic level as the PA DEP, I found similar taxa in Alex Branch at WMF. Some of the differences in taxa found between my study and the PA DEP study may be related to sampling location and habitat. The PA DEP sampled Alex Branch upstream from the fen in a forested area with dense canopy cover and where the stream is faster moving and has more debris, whereas my sampling was conducted in Alex Branch where it cuts through the fen and is slower moving with less debris.

Although the PA DEP claimed the EOG accidents had no “acute impact to the benthic macroinvertebrate community,” I do not believe their study was thorough enough to come to this conclusion. They only sampled a small reach of the stream, they used the same stream as a reference, they eliminated one of their sample sites from the analysis without explanation, and there were no baseline data with which to compare their study. Additionally, it appears that they did not consider the biology of the organisms they were sampling. Their sampling occurred four months after the first two accidents were discovered and two months after the third accident was discovered, and after water quality had returned to ‘normal’ conditions. According to Kosnicki (College at Brockport, personal communication 2015), this was ample time for recolonization of Alex Branch.

The results of the aquatic invertebrate portion of my study are also inconclusive, because, like the DEP, I did not have baseline data to compare my results and I only had one reference site (CSB) to use as comparison. The timing of my study also makes it difficult to determine whether or not the EOG accident(s) impacted Alex Branch. According to Dr. Ely Kosnicki (College at Brockport, personal communication 2015), fall and winter are not favorable times of year to collect aquatic invertebrates.

Additionally, my sampling occurred about three years after the EOG accidents were discovered.

It is possible that the timing of the accidents may have also influenced the outcome of the studies; the first and second EOG accidents were discovered on 24 August 2009, and the third accident was reported on 14 October 2009. Aquatic macroinvertebrates have very different life cycles depending on the particular species, so it is possible that some were not in an aquatic phase during the time of the pollution. The PA DEP did not identify to the species level, so I don't know what life cycle is shown by the taxa that were found; therefore, I don't know if taxa were in the aquatic or terrestrial stages when the accidents occurred.

Chironomids, which were found in large numbers in my study and the PA DEP study, have a wide range of pollution tolerance, which is species-dependent. They are also the most widely-distributed aquatic insect and have adapted to virtually all types of aquatic habitats (Peckarsky *et al*. 1990). Chironomids were not identified by species, so it is difficult to make conclusions about the type or severity of impact they received. Chironomids can complete their life cycle in <15 days, and one study found that egg-laying was greatest in June (Tronstad *et al*. 2007). If this were the case in Alex Branch, impacts would not have been detected in December 2009, as the life cycle would have been completed before the event, and it is possible that they would have recolonized by the December sampling. Tronstad *et al*. (2007) also found egg-laying to be high in June and August by Odonata (dragonflies and damselflies) and other Diptera; this may explain why they were also detected by the PA DEP in December 2009.

Additionally, the nature of the EOG accidents may not have negatively impacted the aquatic invertebrates in the stream. My research suggests that the contaminants were released in short-term pollution events, so it is likely that contaminants did not accumulate in the sediment of Alex Branch, but instead, were quickly carried downstream. For taxa such as Plecoptera, detected in both my study and the PA DEP study, the eggs settle to the bottom of the water body and attach to the substrate (Peckarsky *et al*. 1990). If contaminants are flushed downstream and not accumulated in the sediment, a negative impact to the taxa would be unlikely.

Overall, low sampling intensity, small stream size, time of sampling, and unsuitable habitat may account for the low numbers of aquatic invertebrates I collected at both sites. Additionally, the majority of taxa found at both wetlands were moderately pollution-tolerant to very-pollution-tolerant, which is fairly common due to the naturally low pH in this geographic region. Without pre-accident data, it is difficult to determine if the accidents that occurred at EGO wells 8H and 9H had negative impacts on the aquatic invertebrate populations. It is important to note, however, that the study of Alex Branch and other nearby watersheds by Grant *et al*. (2015) showed that macroinvertebrate taxa richness in this region was negatively correlated to the number of Marcellus well pads.

Amphibians

Through point-count and visual encounter surveys (VES), I detected significant differences in amphibians between the two wetlands. During the 2012 point-count surveys, I detected a total of six species at CSB and just one species at WMF. In 2013, I detected five species at CSB and just one species (the same as 2012) at WMF. During the 2013 VES, I detected a total of seven species at CSB, which included adults, juveniles, and egg masses. In contrast, I only detected two species at WMF. Additionally, the species found at WMF were uncommon. All point-count and VES were conducted on the same days, in the same weather conditions, so temperature or weather cannot account for these differences.

What can explain these significant differences in amphibians between the two wetlands? Aside from the addition of gas-drilling operations (gas wells, new roads, holding tanks, pipelines), there have been no other major changes in or around WMF since the accidents occurred.

Amphibians and Habitat Characteristics

Separation of plant communities shown in the vegetation ordination showed that, overall, CSB is wetter than WMF. This was also supported by the pool size and depth data. Although this creates some minor habitat differences, WMF is still a wetland with several seeps and springs that create pools. It also has vernal pools in the spring. Nearly 27% of the vegetation quadrats that I sampled had a pool, and the majority of the quadrats sampled were saturated. WMF also has wetland vegetation that is characteristic of a medium fen, hydric soils, and wetland hydrology---all the characteristics needed to be classified a wetland. This shows that, although smaller and drier than CSB, there is still ample habitat for breeding amphibians at WMF.

As an undergraduate at Penn State University, I visited WMF many times, starting in March 2007 (I will refer to these later in the discussion as ‘personal observations’). As I became more interested in wetlands, I visited the site regularly, both pre and post-accident. I often kept track of interesting flora and fauna I encountered on my excursions. Although exact date or location information for every single amphibian sighting are not available, I did encounter spring peepers, woodfrogs, red-spotted newts (both adults and red efts), green frogs, red-backed salamanders, Jefferson salamanders (*Ambystoma jeffersonianum*), and American toads (most on more than one occasion) in WMF between March 2007 and May 2009. Therefore, I don't believe that the drier conditions shown in the ordination and pool data provide a reasonable explanation for these species no longer occurring or occurring in such low numbers in WMF.

Amphibians and Water Chemistry

The current water quality, both groundwater discharge and surface water, is very similar between both wetlands (with the exception of the one area of groundwater discharge at WMF that experienced decreases in pH and spikes in SPC), and these levels are typical of the Susquehanna watershed, as well as other watersheds in this region. In addition, any factors that might influence changes in water quality, such as acid rain and *Sphagnum* cation exchange, would affect both wetlands equally; the wetlands are located in the same region where average rainfall has a pH of 4.3 (PA Angler 2001), and *Sphagnum* is the dominant plant at each. Acidic conditions may restrict amphibian distributions (Wyman and Hawksley-Lescault 1987); however, several studies have shown that many amphibian species have relatively high tolerance to acidity. Freda and Dunson (1986) found that wood frog embryos were very acid-tolerant and could hatch at pH of 4.25; Gosner and Black (1957), Karns (1983), and Pierce *et al.* (1984) found that wood frog hatching was successful at pH levels as low as 4.0; Freda and Dunson (1984) found that bullfrog tadpoles can survive in 4.0 pH and green frog tadpoles can survive in 3.5 pH waters; Mazerolle (2005) found spotted salamanders, red-spotted newts, red-backed salamanders, green frogs, wood frogs, spring peepers, and American toads in

Sphagnum-dominated bogs with a pH fluctuating around 4.0. Additionally, there appears to be a healthy breeding population of several amphibian species at CSB that are not impacted by pH, which is similar at both wetlands, so pH would not be expected to affect the amphibians at WMF.

Amphibian Biology

Other factors examined that potentially explain the difference of amphibian populations between CSB and WMF include seasonal variation, competition, predation, and pool location in relation to canopy cover. Seasonal variation in amphibian populations is not uncommon, and detection probabilities for many species may be low (Storfer 2003). Yearly fluctuations in population size can be dramatic and are often due to factors such as temperature and precipitation (SME 2014). I took this into consideration and conducted point-count surveys in two consecutive years to account for seasonality. In addition, I did not see any major changes in the amphibian population at CSB from 2012 to 2013.

Competition for permanent pools and food may occur, but competition for vernal pools is often limited (Skelly *et al.* 2002). Although competition may occur, it would likely result in one species out-competing another, not the eradication of both.

There is no direct evidence of predation. Additionally, both the vernal and permanent pools at WMF lack predatory fish.

According to Calhoun and deMaynadier (2002), canopy cover around pools is important in keeping water temperatures cool, as well as contributing leaf litter to the pool. Most of the pools found at WMF are located around the edges of the wetland, where some canopy cover is present. Overall, WMF provides much more canopy cover than CSB.

Although these above factors might help explain a population decline, it seems unlikely that any or a combination of these would result in the complete extirpation of several species from the site in such a short time period.

I also examined the biology of individual amphibian species to determine how likely it would have been for those species to be exposed to contamination resulting from the EOG accidents. For example, spring peepers, which were detected in low numbers during my 2012-2013 point-count surveys at WMF, are tree-dwelling frogs that only use standing water to breed and lay eggs. Their metamorphosis may be completed within 100 days of hatching, and juvenile frogs may be completely transformed by mid-summer (Shaffer 1999). Spring peepers, in any stage, are unlikely to have been in the water during the time that the EOG accidents were discovered in August and October, so perhaps they were not affected by the accidents and have naturally low abundance in that area. Without pre-accident data with which to compare, it is difficult to reach any conclusions on this particular species.

During the 2013 VES, I detected one green frog in a pool at WMF formed from groundwater discharge. In 2007 and 2008, green frogs appeared to be one of the most common species found at WMF (personal observations), and were often seen in pools and in Alex Branch. Green frogs are highly aquatic and rarely stray far from water. They breed and lay eggs in water. In PA, this can happen as late as August (Shaffer 1999). Because green frogs are aquatic, they would have been in the water at the time of the

EOG accidents, and due to the timing of their breeding cycle, it is possible that eggs, tadpoles, or juveniles also would have been in the water at that time.

Wood frogs were not detected at WMF during my 2012 or 2013 surveys, but I spotted one adult in June 2007, and I heard them calling in March 2008 (personal observation). Like spring peepers, wood frogs are also terrestrial and only use water for breeding and egg-laying. They are known as ‘explosive breeders’ and are one of the earliest species to breed. When not breeding, adults can be found away from water (Shaffer 1999) in moist, deciduous forests (Regosin *et al*. 2003). Due to their early breeding, it is unlikely that wood frogs (adults, juveniles, or egg masses) would have been in the water in August 2009 when the accidents were *discovered,* but we don’t know exactly when the accidents occurred. Because of their breeding behavior, it is notoriously difficult to catch wood frogs during breeding. At CSB, I heard one wood frog in 2012 and three in 2013. I detected several wood frog egg masses during the 2013 VES surveys at CSB, so if there was a breeding population of wood frogs at WMF, egg masses likely would have been detected. Again, without baseline data and not knowing the exact date of the accidents, it is difficult to say what happened to the wood frogs in WMF.

I found American toad eggs in a pool at WMF in July 2007 (personal observation), but I found no evidence of American toads in 2012 or 2013. Toads are mainly terrestrial and can be found in a variety of habitats. They use water for breeding and egg-laying, which occurs from March until July (Shaffer 1999). It is possible that adults, juveniles, and eggs were in the water at WMF at the time of the EOG accidents.

Two red-backed salamanders were detected during our 2013 VES at WMF. One was found in Plot 1 under a log and the second was found in Plot 17, under a log resting on *Sphagnum*. Red-backed salamanders were detected at WMF pre-accident, but there are no data on their abundance. These salamanders are completely terrestrial; therefore, it seems unlikely that the accidents at EOG would have had any negative impact on the population. The low numbers found could be related to habitat or the difficulty of detecting these salamanders.

Jefferson's salamanders were found at WMF pre-accident (personal observation) but were not found during the 2013 VES. These salamanders are mostly terrestrial, preferring deciduous woodlands near wetlands. Adults migrate to water in the spring to breed and deposit eggs, which attach to underwater vegetation or twigs. The transformation from the aquatic to the terrestrial stage occurs in PA from July to September (Shaffer 1999). Due to the timing of the Jefferson's life cycle, it is possible that young larvae or juveniles were in the water during the time of the EOG accidents; however, it is unlikely that adults were in the water at that time. Pierce *et al*. (1984) refer to Jefferson's salamander embryos as being acid-tolerant, but Freda and Dunson (1986) found that Jefferson's embryos would not hatch in a pH <4.5, and Freda (1986) reported that a pH range of 4.0-4.5 is lethal to the embryos. Therefore, these embryos should be able to survive under normal conditions at WMF but likely would not have been able to withstand the low pH (as low as 3.9) that occurred around the time of the EOG accidents or the pH declines (as low as 3.49) that I detected during my study. Additionally, due to the surrounding habitat, which is dominated by evergreens, it is likely that Jefferson's populations were already low in this area.

Red-spotted newts were detected (both red efts and adults) in 2008 (personal observation) but were not found in 2013. Additionally, adults were detected in 2013 at

CSB. Red-spotted newts have three different life stages: the aquatic larval stage, the terrestrial juvenile stage (red eft), and the aquatic adult stage. Adults breed in spring in the water and eggs attach to submerged vegetation (Shaffer 1999). It is only during the red eft stage that the newt is terrestrial; this is a 2-7 year time period (Rinehart *et al*. 2009) that is spent in damp woodlands (Shaffer 1999). Because much of their life cycle is aquatic, it is possible that both the adults and larvae were exposed to contaminants released during the EOG accidents.

Other species detected at CSB but not found in WMF (pre or post-accident) include: pickerel frog, bullfrog, dusky salamander, and spotted salamander. Small differences in habitat may explain why pickerel frogs, bullfrogs, and spotted salamanders were not detected at WMF. Pickerel frogs are mostly terrestrial and only use water for breeding. In summer, they prefer field and wet meadow habitat (Shaffer 1999). This type of habitat is not found near WMF, so it is expected that pickerel frogs would not be found in WMF. Bullfrogs tend to prefer lakes and ponds (Shaffer 1999), which are not found at WMF. According to Shaffer (1999), spotted salamanders prefer hardwood forests with temporary or permanent ponds and wooded hillsides around ponds. CSB has just that—a wooded hillside above a pond, as well as several temporary ponds. WMF has several small permanent ponds created by groundwater seeps and small vernal pools in spring surrounded by forest but lacks a hillside and large ponds, making it unlikely habitat for spotted salamanders. I also did not detect dusky salamanders or mountain dusky salamanders (*D. ochrophaeus*) at WMF, but the area appears to provide the right habitat--springs and seeps surrounded by forest (Shaffer 1999).

Effects of Contaminants on Amphibians

Habitat characteristics, seasonality, competition, and predation do not explain difference in amphibians between the wetlands; therefore, these differences may be attributed to the release of contaminated water from the EOG accidents at wells 8H and 9H. Because we don't know exactly when these accidents occurred, but only when they were *discovered*, it is difficult to pinpoint how amphibians may have been impacted.

Although I am not able to identify specific impacts, it is known that amphibians have many qualities that make them extremely susceptible to pollutants (Venturino *et al*. 2003, Sanzo and Hecnar 2006, Hopkins 2007, Karraker 2007, Hampton *et al*. 2010, SME 2014). Most amphibians have highly permeable skin, which is important for gas exchange and osmoregulation (Sanzo and Hecnar 2006, Hopkins 2007); they have unprotected eggs and aquatic larval stages (Sanzo and Hecnar 2006); and complex life cycles make amphibians reliant on water (Hopkins 2007, Karraker 2007) for breeding, foraging, and hibernation (Sanzo and Hecnar 2006). Additionally, there is a growing body of evidence to suggest that anthropogenic pollution has contributed to amphibian declines (Carey and Bryant 1995, Rouse *et al*. 1999, Kiesecker *et al*. 2001, Collins and Storfer 2003, Storfer 2003, Pounds *et al*. 2006, Sanzo and Hecnar 2006, Hopkins 2007, Peterson *et al*. 2009, Simon *et al*. 2011). If the amphibian population at WMF was impacted directly by the release of contaminants from the EOG accidents, it is difficult to determine what those effects were because the chemical component of fracking fluid is proprietary, and we don't know exactly what chemicals were released during the accidents. Additionally, different companies use different concentrations of different chemicals, which also makes determining any impacts difficult. An intensive internet search on Well Wash 2020 and Ultra Vis—the products used by EOG Resources—yielded no results, and a phone call to the company to find out more information about the products resulted in a hang-up.

Although there are no studies linking the impacts of fracking fluid, production water, or flowback water to amphibian declines, we do know that amphibians are highly susceptible to some of the pollutants found in water associated with Marcellus Shale gas drilling. Common constituents found in frack fluid, flowback water, and production water may include ethylene glycol, sodium chloride, isopropanol, arsenic, benzene, and mercury (Zoback *et al*. 2010), and some contain aluminum, copper, lead, chromium, zinc, fluoride, barium, calcium, iron, magnesium, manganese, and strontium (Keister 2008). There is much documentation of amphibians as biological indicators of various types of pollution (Venturino *et al*. 2003, DeGarady and Hallbrook 2006, Hyne *et al*. 2009), including the types of pollution that may result from Marcellus Shale gas-drilling operations.

Contaminants detected after the EOG accidents, such as barium, strontium, manganese, sodium, and chloride, have negative impacts on amphibians. High levels of strontium impairs growth and development in larval amphibians (Snodgrass *et al*. 2004, Peterson *et al.* 2009). Sanzo and Hecnar (2006) found that sodium and chloride, commonly found in road salts, affected feeding behavior in tadpoles, reduced survivorship in spotted salamanders, and had a toxic effect on wood frog tadpoles. Snodgrass *et al.* (2008) found that pond microcosms with elevated levels of chloride and metals resulted in 100% mortality of wood frog embryos. Collins and Russell (2009) noted behavioral changes in amphibians exposed to elevated concentrations of chloride, and Van Meter *et al.* (2011) noted that road salts can alter interactions in a pond community. Amphibians are critical components of both terrestrial and aquatic ecosystems, and bio-accumulative effects may negatively impact food webs (Unrine *et al.* 2007). Sparling and Lowe (1996) found that strontium, manganese, and barium are sequestered in the gut coil of tadpoles and suggested that this could be toxic to predators.

In addition to toxicity, the impact of heavy metals and other contaminants on amphibians can be very complex (Adlassnig *et al*. 2013). Multiple contaminants, even at sub-lethal concentrations, may still have lethal results (Carey and Bryant 1995, Roe *et al*. 2006). Exposure to toxicants can delay metamorphosis (Hopkins *et al*. 2000, Hopkins and Rowe 2010), making larvae unable to leave breeding ponds at the appropriate time, leading to desiccation (Carey and Bryant 1995). Exposure may cause behavioral alterations, such as reduced activity levels, as well as the inability to feed, attract mates, or breed. Impaired behavioral responses may also make amphibians more susceptible to predation (Carey and Bryant 1995, Storfer 2003).

Ficken and Byrne (2013) found that amphibian species richness was negatively correlated with concentrations of copper, nickel, lead, zinc, cadmium, and mercury, as well as SPC (a proxy for salinity) and orthophosphate. A similar study by Glooschenk *et al*. (1992) found that the presence of frog species was negatively related to increasing levels of cadmium, nickel, aluminum, zinc, and copper. Karasov *et al*. (2005) reported a decline in anuran species richness as concentrations of cadmium, chromium, and lead increased in ponds.

Although there are few studies highlighting the direct impacts of fracking fluids on amphibians, the impacts of gas and oil mining on biodiversity has been documented

(Gillen and Kiviat 2012), and it is likely that we will see more information published on this topic in the future. It is important to note that the CONSOL Energy accident that killed over 40,000 fish in Dunkard Creek also killed an estimated 6,447 amphibians in the PA portion of the stream alone (Arway 2013).

In addition to direct impacts from the contaminants (i.e., egg and larval mortality), indirect impacts are also likely. For example, if amphibian populations were completely eliminated due to the contamination, a source population would be necessary to help recolonize WMF. According to Rittenhouse and Semlitsch (2007), habitat within 300 m of a breeding pool is considered core habitat for most species. The closest wetlands to WMF are ~375 m to the northeast and ~530 m to the west. In addition to the long distance, reaching WMF would require that amphibians move through an upland forest and cross a road that has heavy gas industry traffic. Amphibians that have to migrate near roads have increased mortality and inhibited dispersal (Rinehart *et al*. 2009), and forest roads can reduce terrestrial salamander movement up to 51% (Gillen and Kiviat 2012). This may help to explain why other taxa, such as birds and aquatic macroinvertebrates, are found in WMF but amphibian numbers are so low.

What Happened at WMF?

CSB and WMF are very similar wetlands. They have similar underlying geology that is dominated by sandstone, shale, clay, and coal. They have similar hydrology; they are ‘medium’ fens with similar groundwater and surface water chemistry. There are also many similarities in biotic composition; no major differences were detected in birds, aquatic invertebrates, fish, or vegetation between the two wetlands. Although it cannot be ruled out entirely, I do not think that the accidents at EOG wells 8H and 9H had longterm negative impacts on most of the parameters studied at WMF. Simply stated, birds and fish are mobile and aquatic invertebrates can quickly recolonize. Due to the underlying geology, groundwater in this region has a short residence time. Contaminated water from the EOG accidents would have quickly passed through WMF and likely would not have seeped in and contaminated the soil in the fen. This explains why I did not detect any contaminants in the tree cores and why I found no major differences in vegetation. Slight differences in vegetation appeared to be related to hydrology.

There were, however, significant differences in the amphibian communities between the two wetlands, despite both wetlands providing suitable habitat for many of the same amphibian species. I think that due to some of the habitat characteristics at WMF, it is not as productive as CSB in regard to amphibians, and it is likely that pre-accident populations were smaller at WMF than at CSB. However, I observed seven amphibian species at WMF pre-accident, so it appears likely that amphibians at WMF suffered from both direct and indirect effects related to water quality during and post-accident.

Water quality data collected by the PA DEP post-accident were obtained only from a few locations. Data were collected at two springs (Reeds Spring and Sykesville Spring) that were easily accessible and not located in WMF, and from Alex Branch where it intersects McGeorge Road, which is also not in WMF. No water chemistry data were collected in the fen (surface water or groundwater) until I began my study in May 2012. Based on the topography, I think that contaminated water was discharged into WMF at more than one location. There are three discharge sites downslope from the gas wells; I think that contaminants were discharged at all three sites, as well as through Alex

Branch. Additionally, it is possible that overbank flooding of Alex Branch occurred. During one EOG accident, over 30,000 L (190 barrels) of contaminants were released. This is a very large volume of water traveling through a very small system. Overbank flooding could have affected terrestrial species such as the redback salamander, Jefferson salamander, and red eft.

The ongoing development and expansion of the Marcellus Shale in this region may lead to range restrictions for terrestrial salamanders due to habitat loss (Brand *et al.* 2014) and fragmentation by access roads, well pads, and pipelines (Gillen and Kiviat 2012). It is also noteworthy that the Marcellus Shale range overlaps almost entirely with the ranges of several amphibian species found at the study sites, such as Jefferson's salamander, red-spotted newts, mountain dusky salamanders, and dusky salamanders. On a larger scale, the extent of the Marcellus Shale overlaps with the ranges of several state or federally rare or threatened flora and fauna in the northeast, including the West Virginia spring salamander (*Gyrinophilus subterraneus*), Cheat Mountain salamander (*Plethodon nettingi*), bluebreast darter (*Etheostoma camurum*), shale-barrens pimpernel (*Taenidia montana*), Northern blue monkshood (*Aconitum noveboracense*) (Gillen and Kiviat 2012).

In addition to the accident that occurred at wells 8H and 9H, I strongly believe that other accidents occurred at EOG wells in 2012 and 2013 due to changes detected in water chemistry data I collected during my study. It is possible that if accidents occurred in 2012 and 2013 that EOG was unaware of the accident(s) or failed to report the accident(s). EOG did not report two of the three accidents that occurred in 2009, and not reporting accidents is common (Price 2014). It is important to note that 8H and 9H are not the only gas wells upslope from WMF; EOG has at least five other wells that are closer to WMF, and three of them are directly upslope from where I noticed spikes in pH and conductivity. Again, due to the geology of this region, subsurface flow has a short residence time. Therefore, I do not believe that changes in water chemistry detected in WMF in 2012 and 2013 are related to the 2009 accidents but, instead, were separate incidents.

Due to the lack of baseline data, we will never fully understand how the gas drilling accidents at EOG wells 8H and 9H affected WMF. According to Brand *et al.* (2014), the lack of baseline data surrounding Marcellus Shale activities hinders the ability to quantify ecological risk accurately, determine future impacts, and develop best management practices. The lack of information about accident location and timing, as well as the propensity to not release information about accidents due to liability and/or confidentially agreements make it difficult to study and understand impacts resulting from gas development (Brantley *et al*. 2014). Additionally, the proprietary nature of chemicals used during fracking makes it difficult to determine how amphibians and other organisms may be impacted by gas drilling accidents. This study underscores the importance of collecting baseline data in areas where hydrofracking is anticipated so that impacts of any future accidents can be evaluated more thoroughly.

The Status of Hydraulic Fracturing in the Marcellus Shale Region

Ohio

Since 2011, a number of gas wells have been drilled into both the Marcellus and Utica Shales in Ohio. The Ohio Oil and Gas Energy and Education Program (OOGEEP) estimated that > 3,400 wells would be drilled into the two layers in Ohio between 2011 and 2016; 625 wells were completed in 2012 alone (OOGEEP 2014). Since fracking began in Ohio, the state has experienced environmental impacts related to Marcellus and Utica shale gas drilling activities such as well blow-outs and spills resulting in fish kills (OEC 2015). Several earthquakes in Ohio have made headlines; a recent study tied these earthquakes to wastewater disposal resulting from fracking activities (Skoumal *et al*. 2015).

West Virginia

From July 2009 to June 2010, over 1,400 wells were drilled into the Marcellus Shale in West Virginia (Myers 2012). Like Pennsylvania and Ohio, West Virginia has also reported problems related to gas-drilling activities, including a massive fish kill in Dunkard Creek (Arway 2013). Between July 2014 and July 2015, over 200 violations were reported in the state. Violations included “Polluting the waters of the state,” “Inadequate containment of pollutants,” “Site runoff,” and “Not abiding by the erosion and sediment plan” (WV DEP 2015).

New York

New York is the only state that has recoverable quantities of oil and gas in the Marcellus Shale that does not actively drill into the layer. The New York State Department of Health (NYSDOH) conducted a review of hydraulic fracturing and recommended that it should not take place New York State. Additionally, the New York Department of Environmental Conservation (DEC) conducted a six-year review on the potential environmental impacts that could result from fracking. The two agencies cited several potential health and environmental issues, such as air impacts that could lead to respiratory health issues, climate change impacts, soil and water contamination, impacts to drinking water, earthquakes, and community impacts (i.e. increased traffic, noise, road damage, etc.), should fracking be permitted in the state. Their studies also revealed many gaps in critical information about fracking. On 29 June 2015, hydraulic fracturing was officially prohibited in New York (NYSDOH 2014).

Fracking is currently banned in New York State; this provides a unique opportunity to collect very detailed baseline data across the state. Colleges and Universities, governmental agencies (state and federal), and non-governmental organizations should utilize this time to collect data in the Marcellus Shale region of NY State, and create a large, easy-to-access, on-line database for sharing that data. In the event that the fracking ban were lifted, NY would then have a database to help demonstrate the effects of Marcellus Shale gas drilling activities.

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Figures

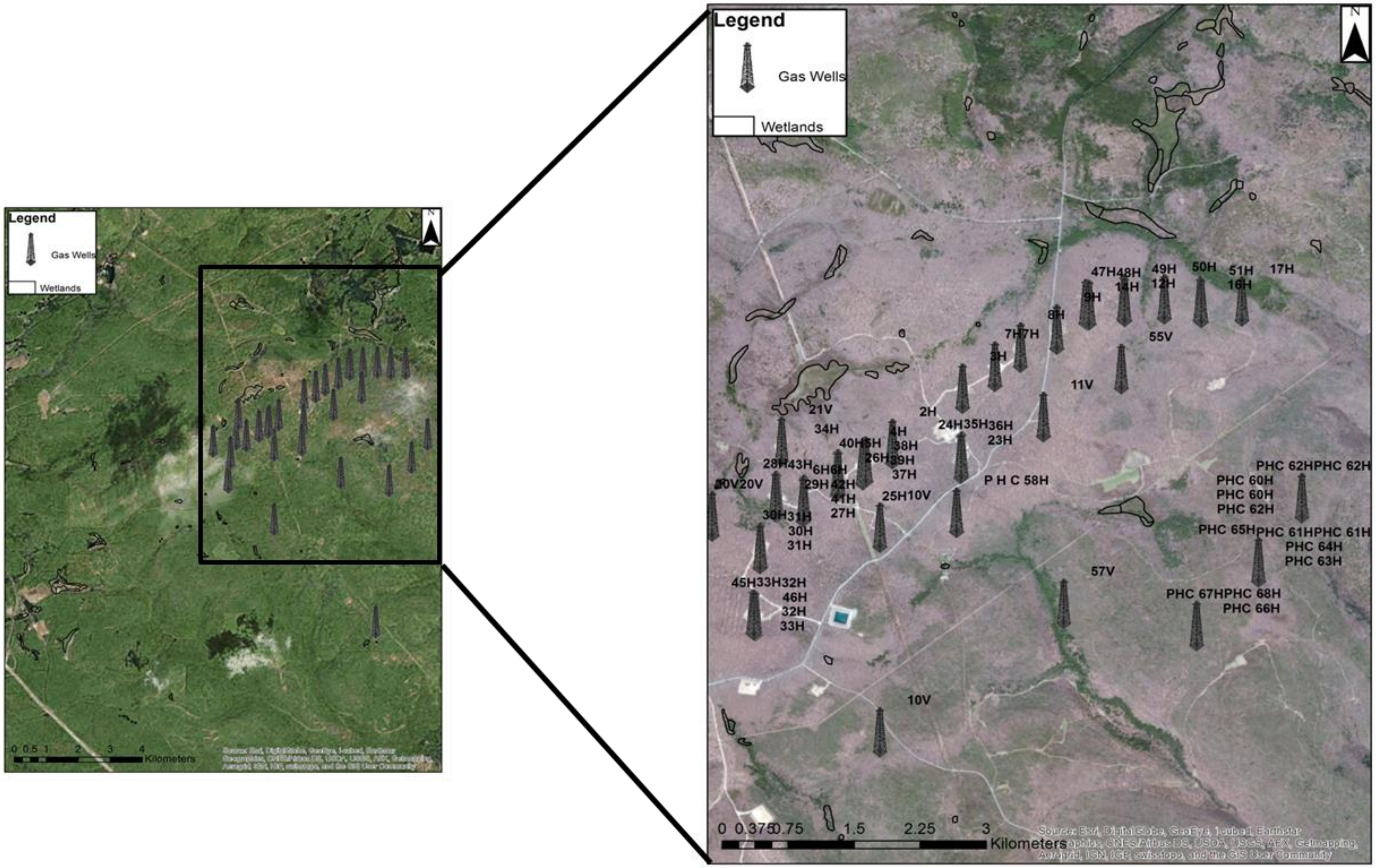

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Figure 1. Portion of Clearfield County, PA, where gas drilling activities have dramatically increased in recent years (left). CSB is shown near the left bottom of the map and WMF is near the top right. The right shows enlarged area near WMF. Several gas wells are located upslope of WMF and are in close proximity to the wetland and streams, such as Alex Branch.

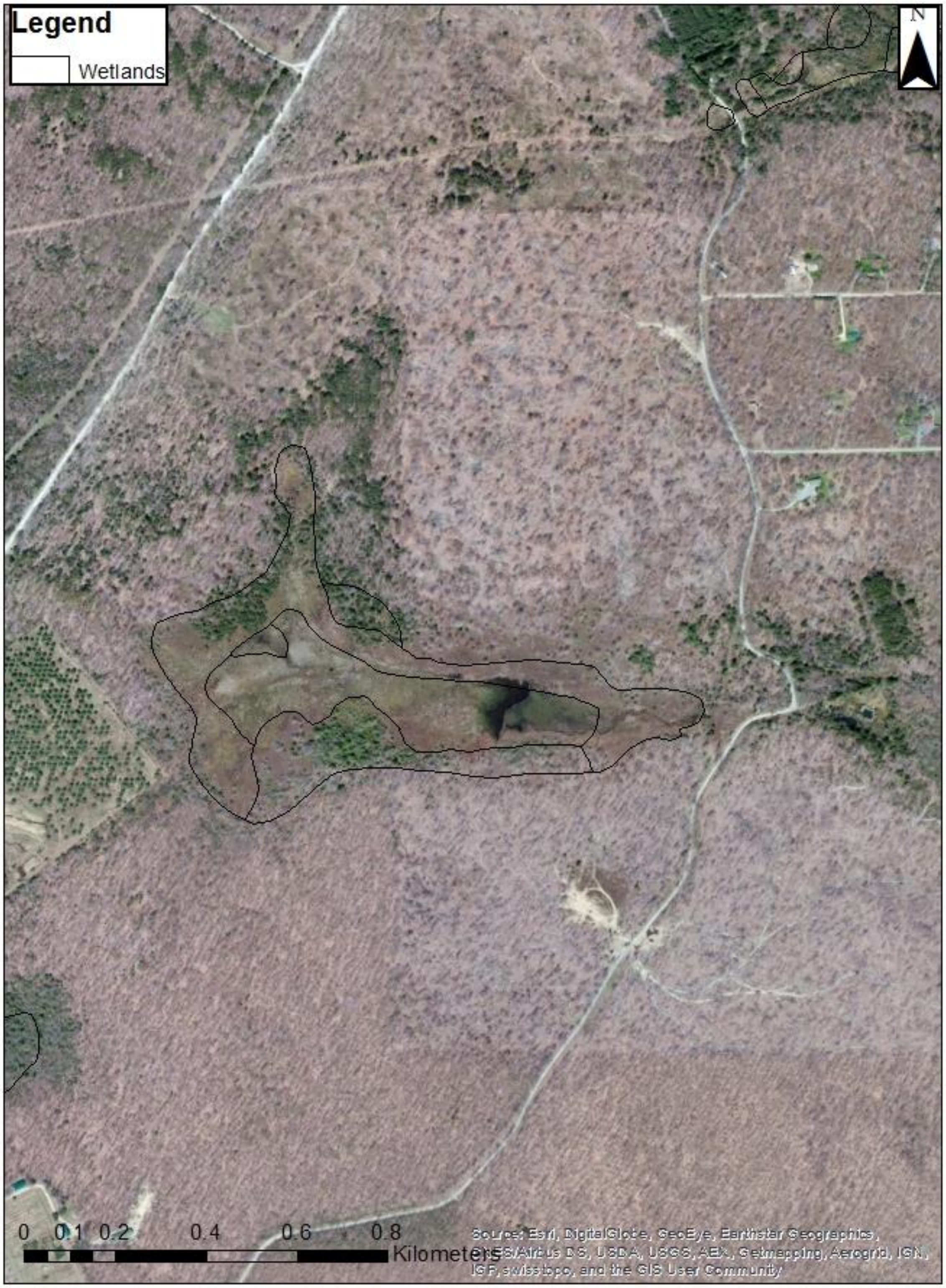

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Figure 2. Aerial image outlining control site, Crystal Spring Bog.

Figure 3. Aerial image outlining impact site, Wallace Mine Fen. Wallace Mine Road is to the north and McGeorge Road is to the west.

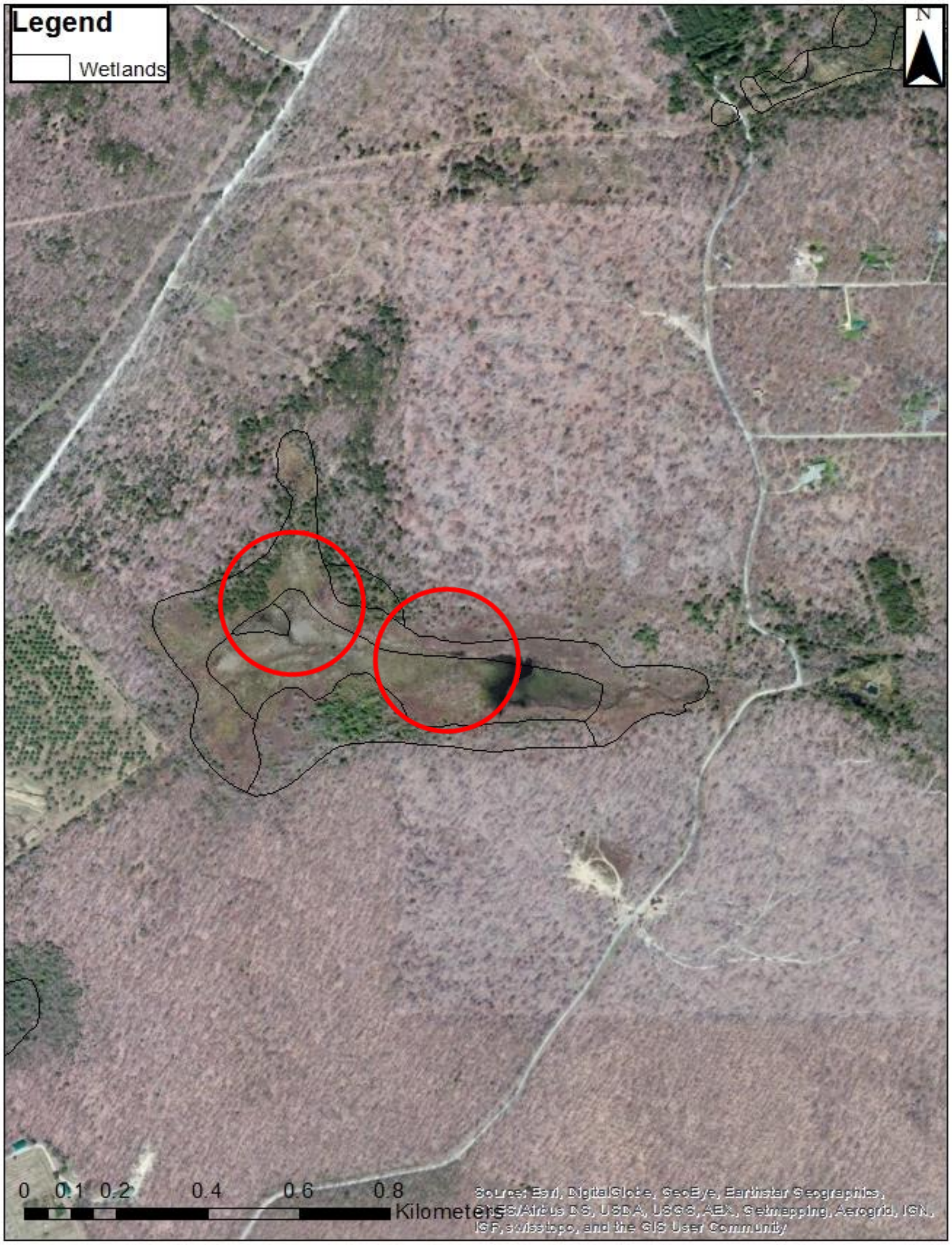
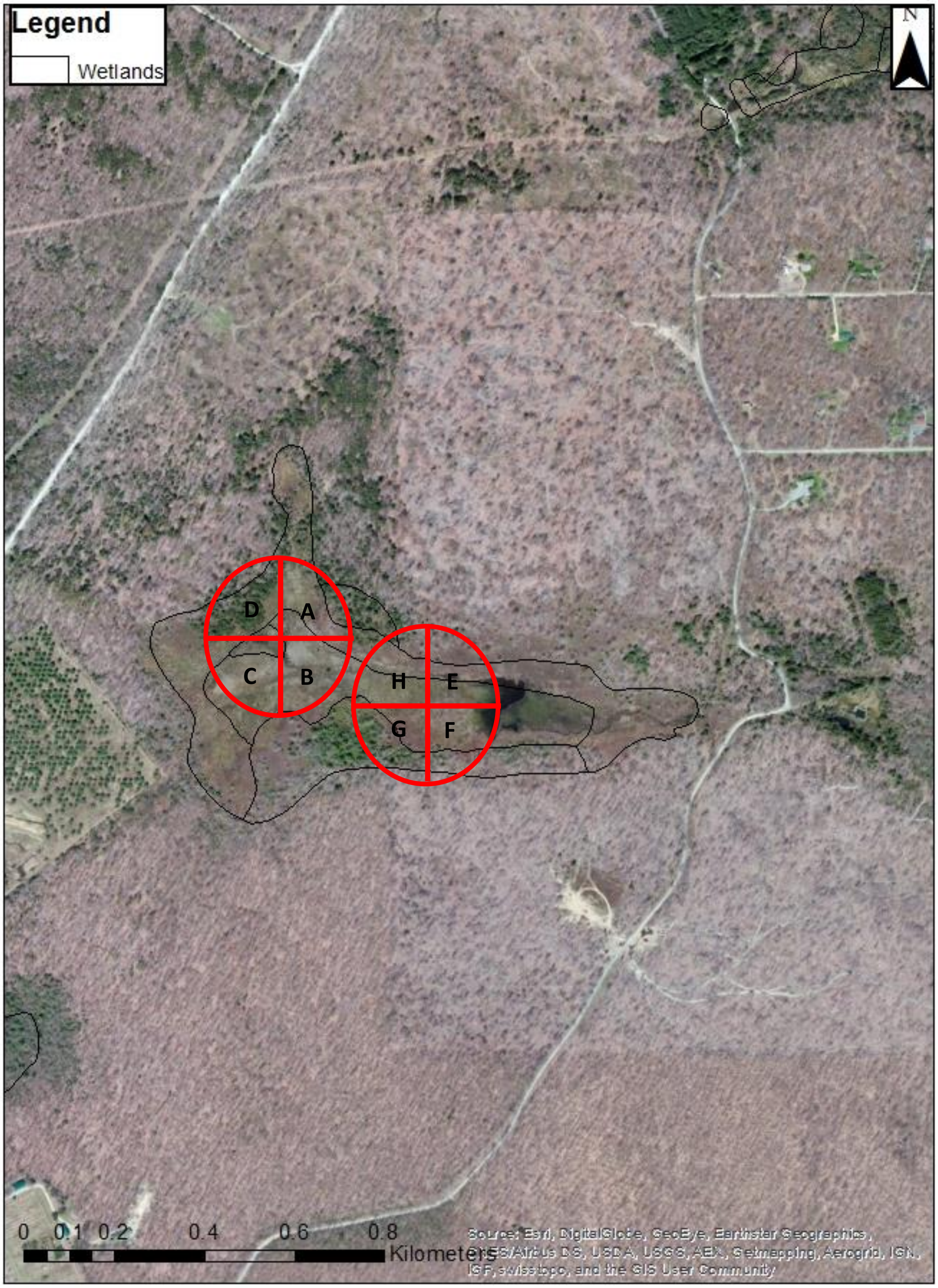


Figure 4. Aerial image of CSB with an overlay of the study design. Red circles indicate the 100 m-radius circles used to define sampling boundaries. All vegetation sampling was conducted randomly within the boundaries of each circle. Amphibian and bird point-count surveys were conducted at the center of each circle, with the edge of the circles used as a boundary.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 5. Aerial image of WMF with an overlay of the study design. Red circles indicate the 100 m-radius circles used to define sampling boundaries. All vegetation sampling was conducted randomly within the boundaries of each circle. Amphibian and bird point-count surveys were conducted at the center of each circle, with the edge of the circles used as a boundary.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 6. Aerial images of CSB with an overlay of the study design. Red circles indicate the 100 m-radius circles used, and quadrants are labeled A-D and E-H.

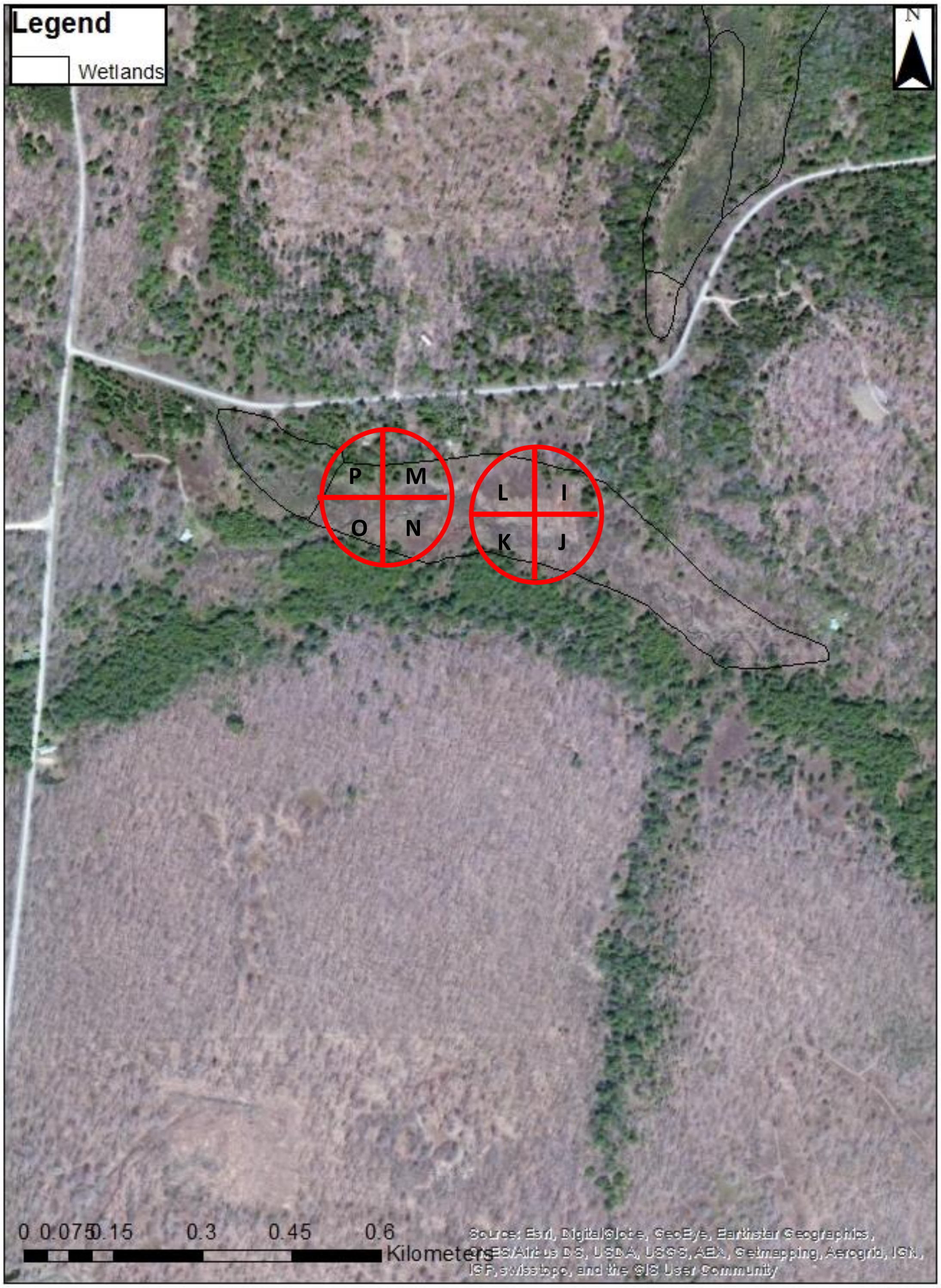


Figure 7. Aerial image of WMF with an overlay of the study design. Red circles indicate the 100 m-radius circles used, and quadrants are labeled I-L and M-P.

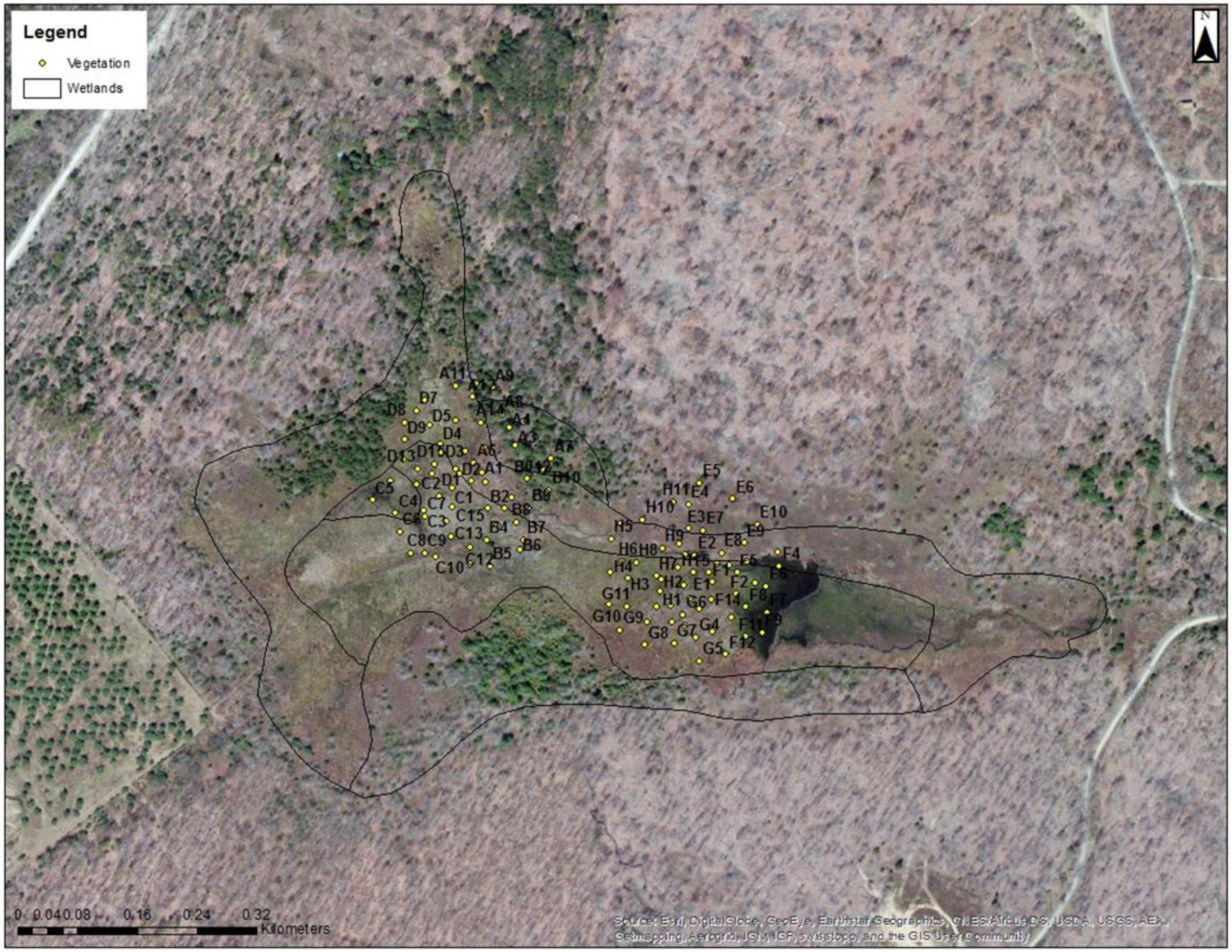
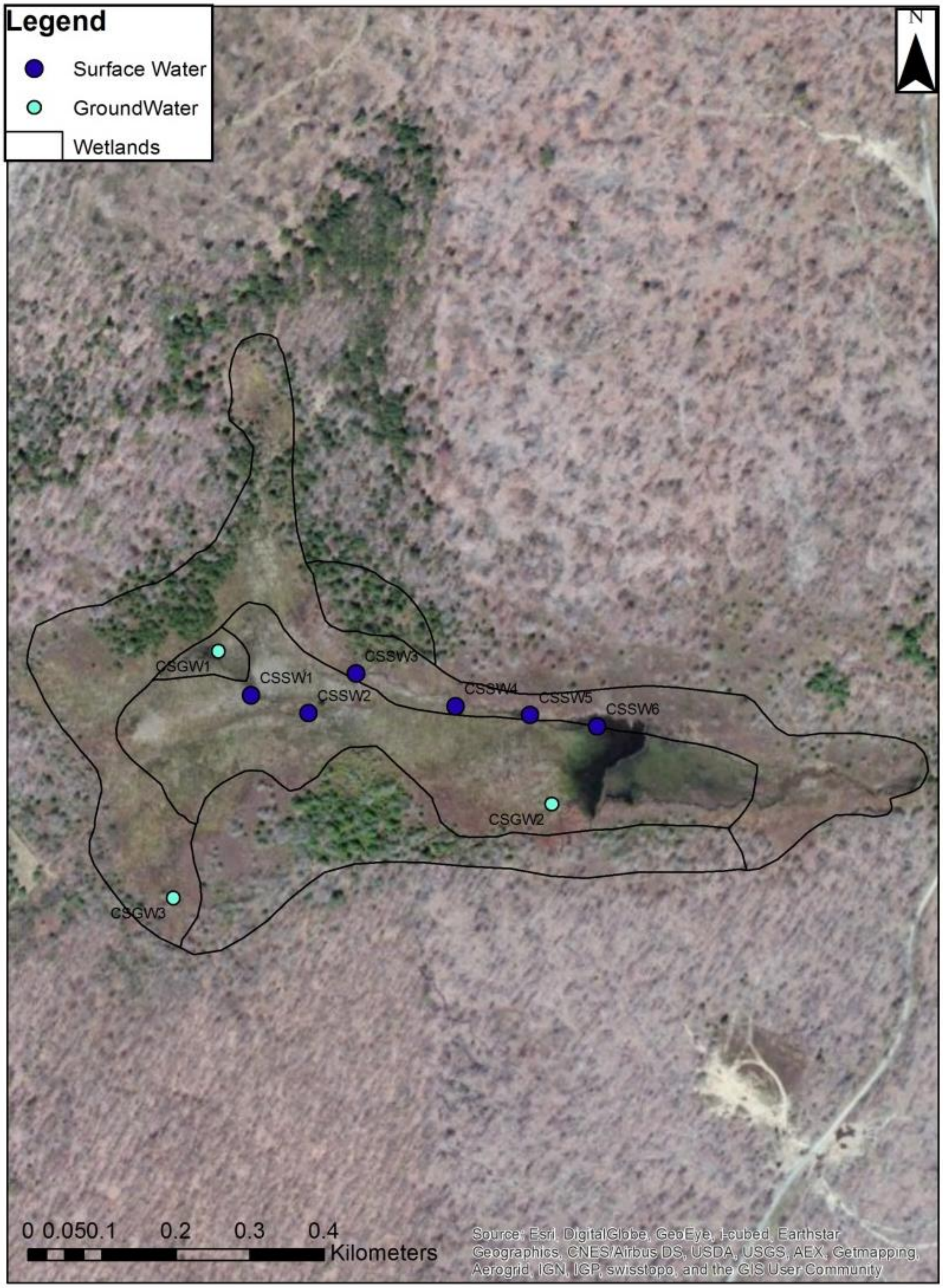


Figure 8. Aerial image of CSB showing vegetation sampling quads. Fifteen $1m^2$ quadrats were randomly sampled within each quadrant, for a total of 60 quads per circle, 120 quads per wetland.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

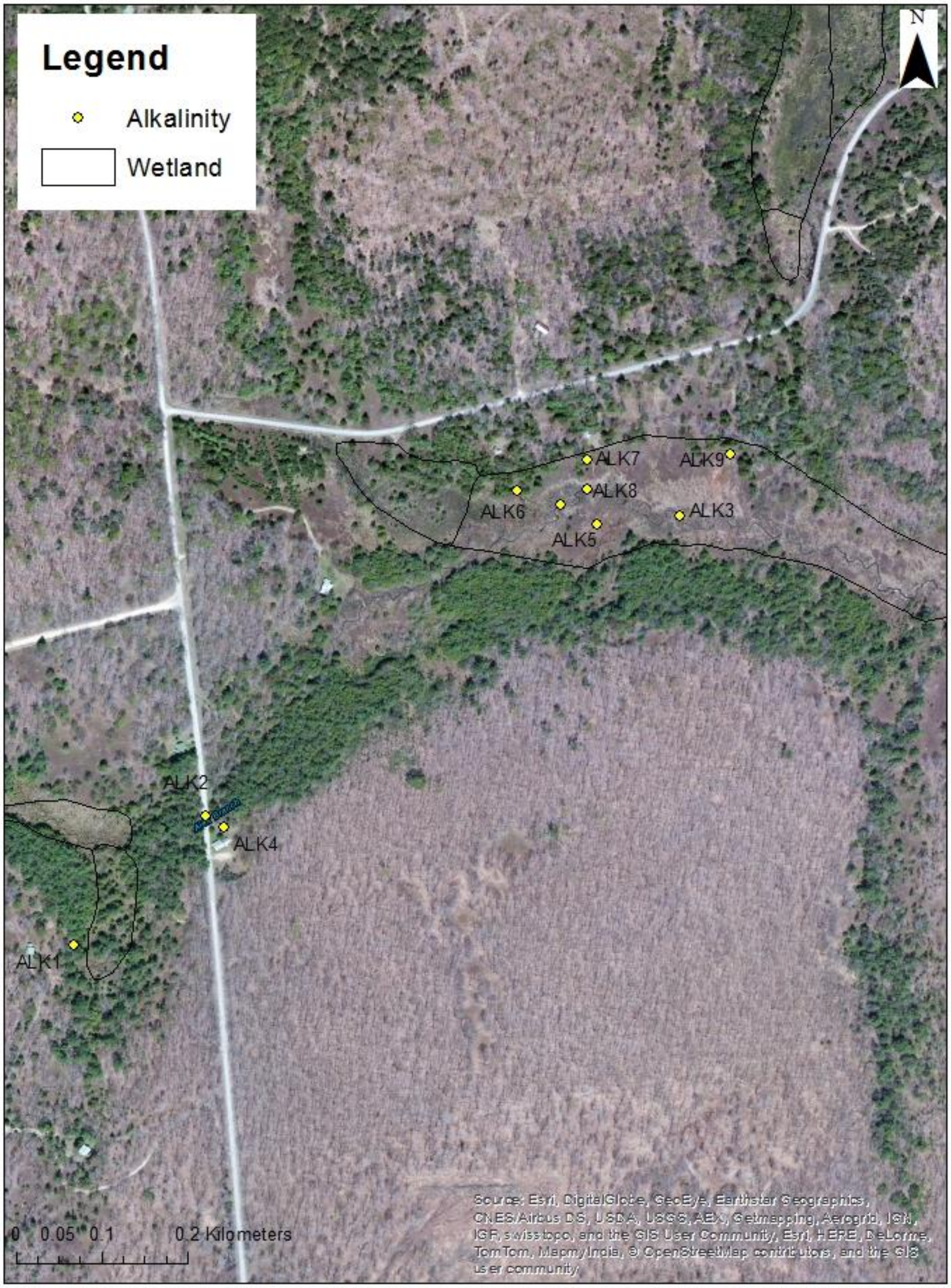
Figure 9. Aerial image WMF showing vegetation sampling quads. Fifteen 1m^2 quadrats were randomly sampled within each quadrant, for a total of 60 quads per circle, 120 quads per wetland.



Source: Esri, DigitalGlobe, GeoEye, i-cubed, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 10. Aerial image of CSB showing location of surface water and ground water collection sites.

Figure 11. Aerial image WMF showing location of surface water and ground water collection sites.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme, TomTom, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 12. Alkalinity sample locations at WMF (collected from Alex Branch and groundwater discharge), Sykesville Spring, and Reeds Spring.

Figure 13. Aerial image of WMF. Tree symbols indicate where tree core samples were collected.

Figure 14. Fish survey locations at CSB. Fish traps were placed in random locations for eight consecutive days in the un-named stream that forms from groundwater discharge.

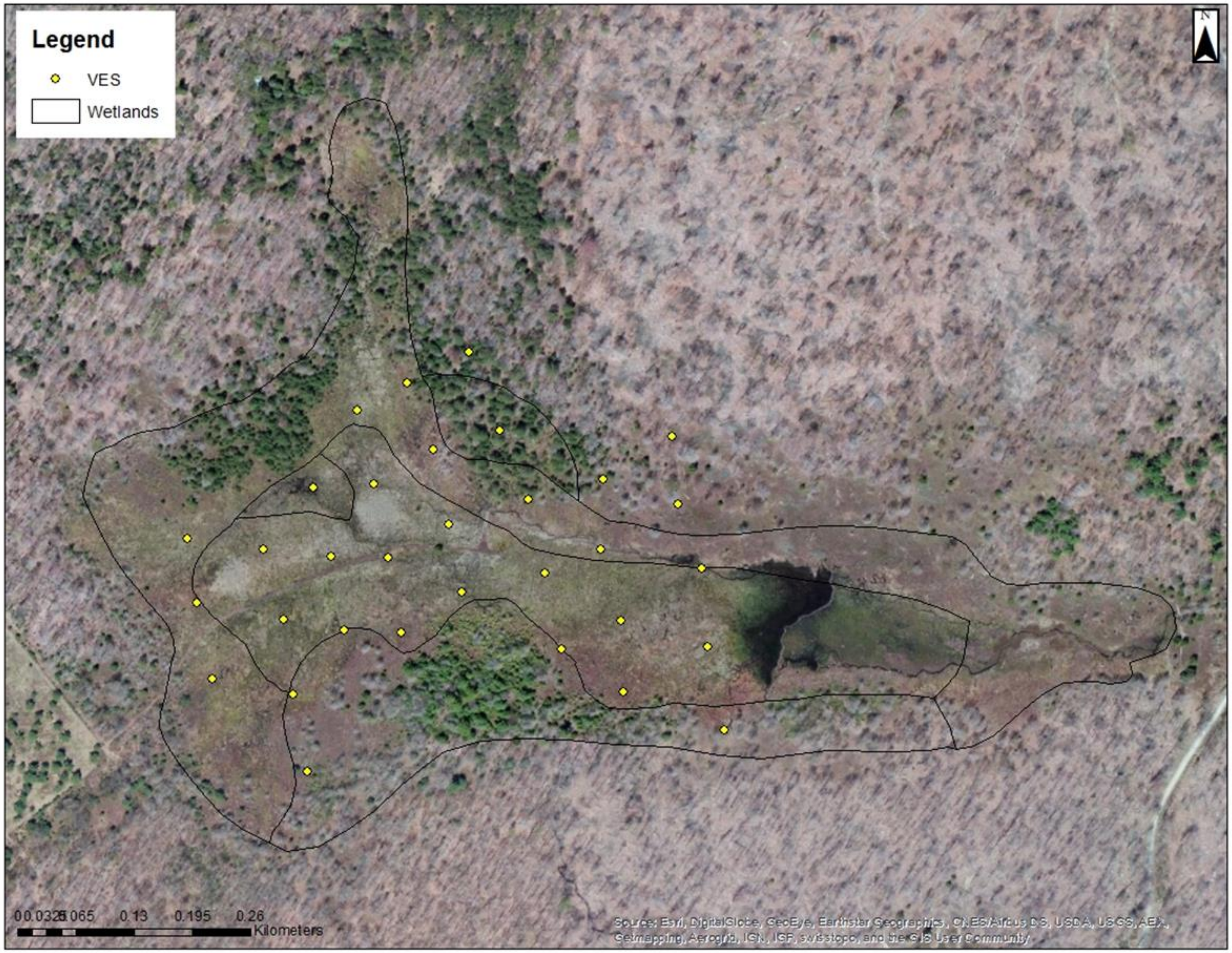
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme, TomTom, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 15. Fish survey locations at WMF. Fish traps were placed in random locations for eight consecutive days in Alex Branch.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme, TomTom, MapmyIndia, © OpenStreetMap contributors, and the GIS User community

Figure 16. Aquatic invertebrate sample locations at CSB. Samples were collected from the un-named stream and identified in the field on 22 September 2012.

Figure 17. Aquatic invertebrate sample locations at WMF. Samples were collected from Alex Branch and identified in the field on 22 September 2012.



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 18. Aerial image of CSB showing the VES sample points. Thirty two 10 m^2 plots were sampled at CSB.

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 19. Aerial image of WMF showing the VES sample points. Thirty two 10 m^2 plots were sampled at WMF.

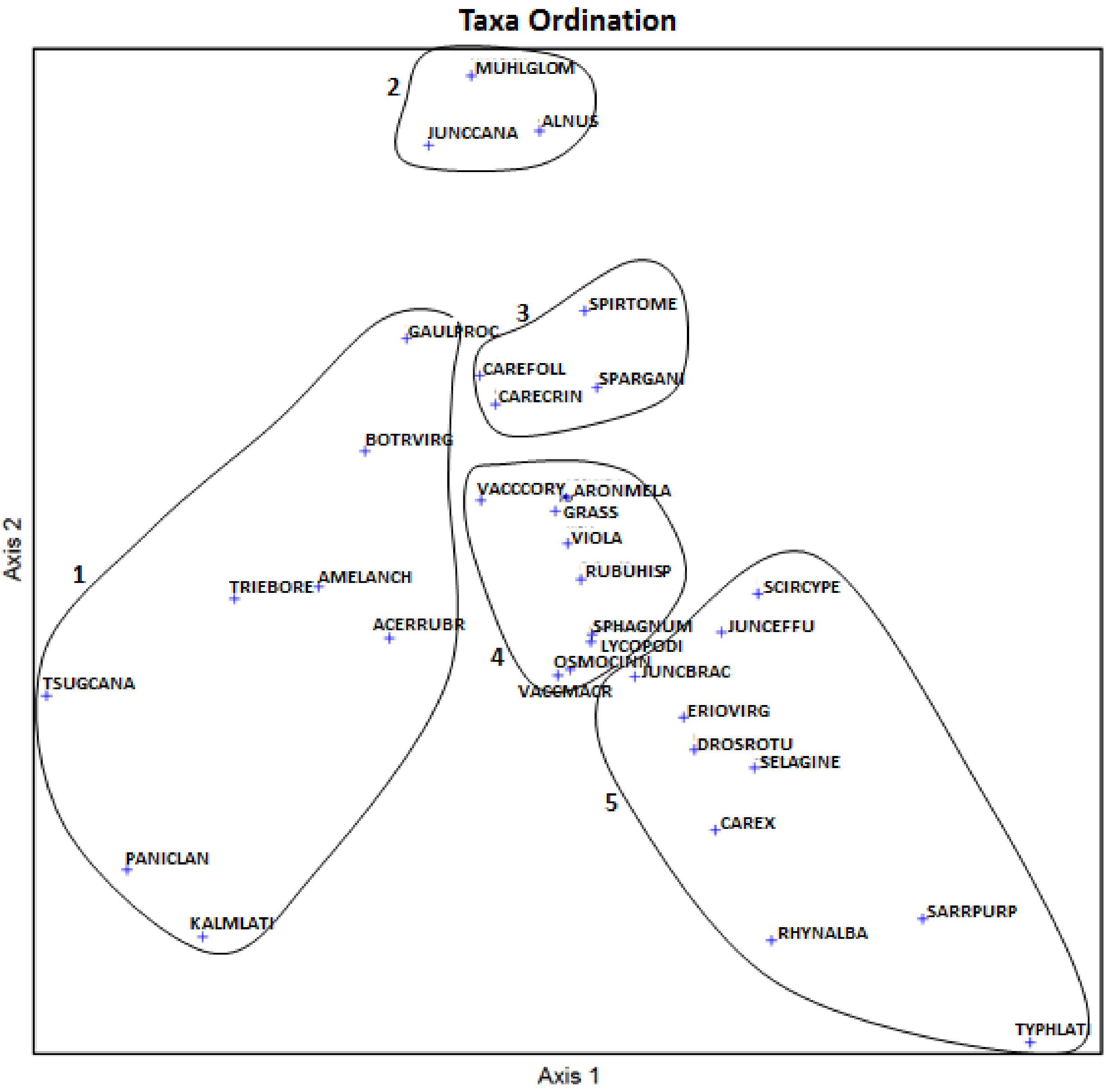


Figure 20. Two-dimensional plot of nMDS ordination of selected plant taxa IV from CSB and WMF (autopilot on, Sorenson distance, no species weighting, final stress = 9.37, final stability = 0, number of iterations = 57). Plant groupings are labeled 1-5: 1 = taxa found growing in the drier areas, wetland perimeter, or forest; 2 = taxa that were only found at WMF but were abundant; 3 = taxa found growing throughout both wetlands; 4 = taxa found growing with and on mounds of *Sphagnum* spp. at both wetlands; and 5 = taxa that are distinctly wetland species with an obligate (OBL) wetland rating or plants that are often found in wet areas.

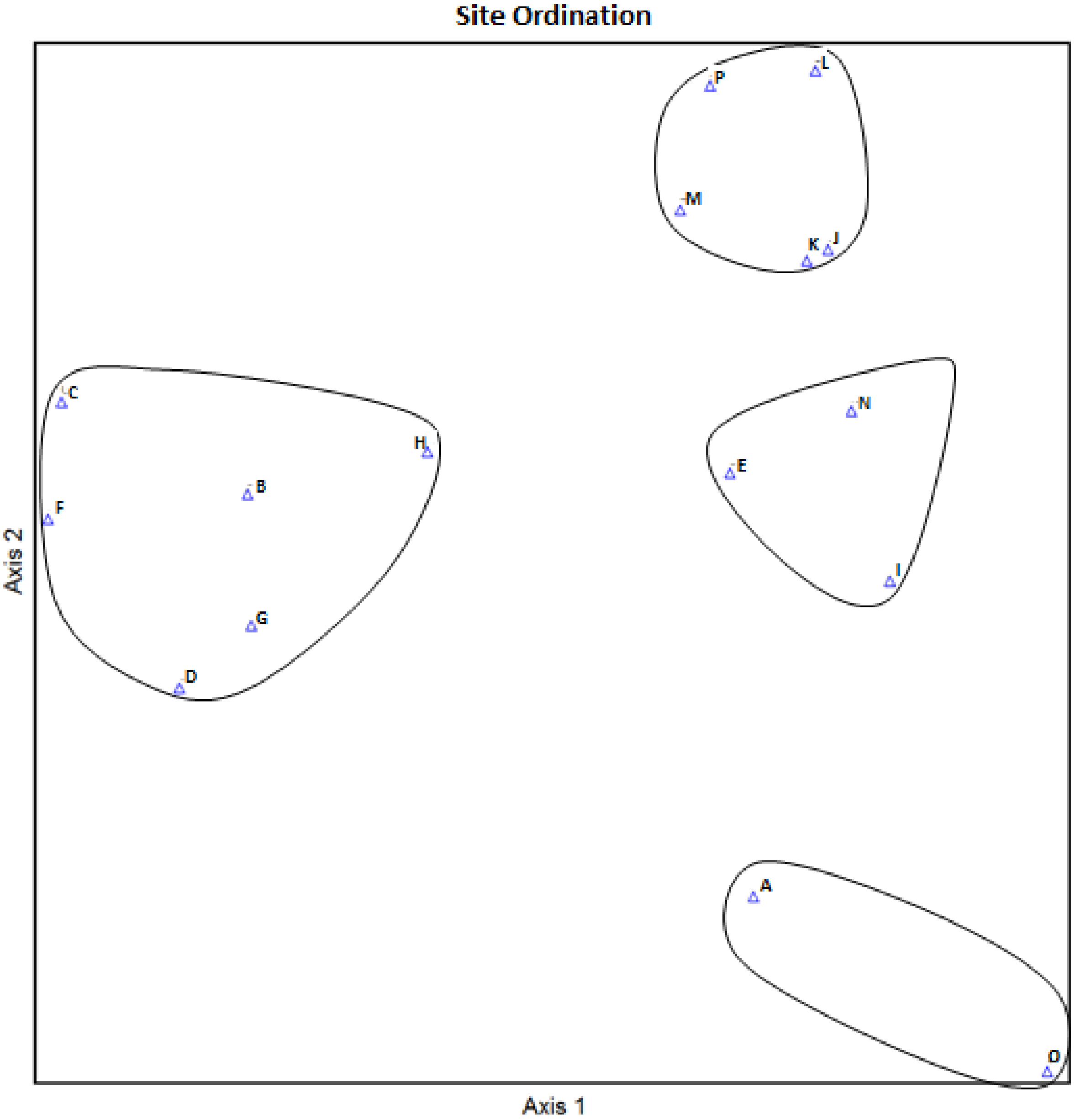


Figure 21. Two-dimensional plot of nMDS ordination of selected plant taxa locations from CSB and WMF (autopilot on, Sorenson distance, no species weighting, final stress = 9.37, final stability = 0, number of iterations = 57).

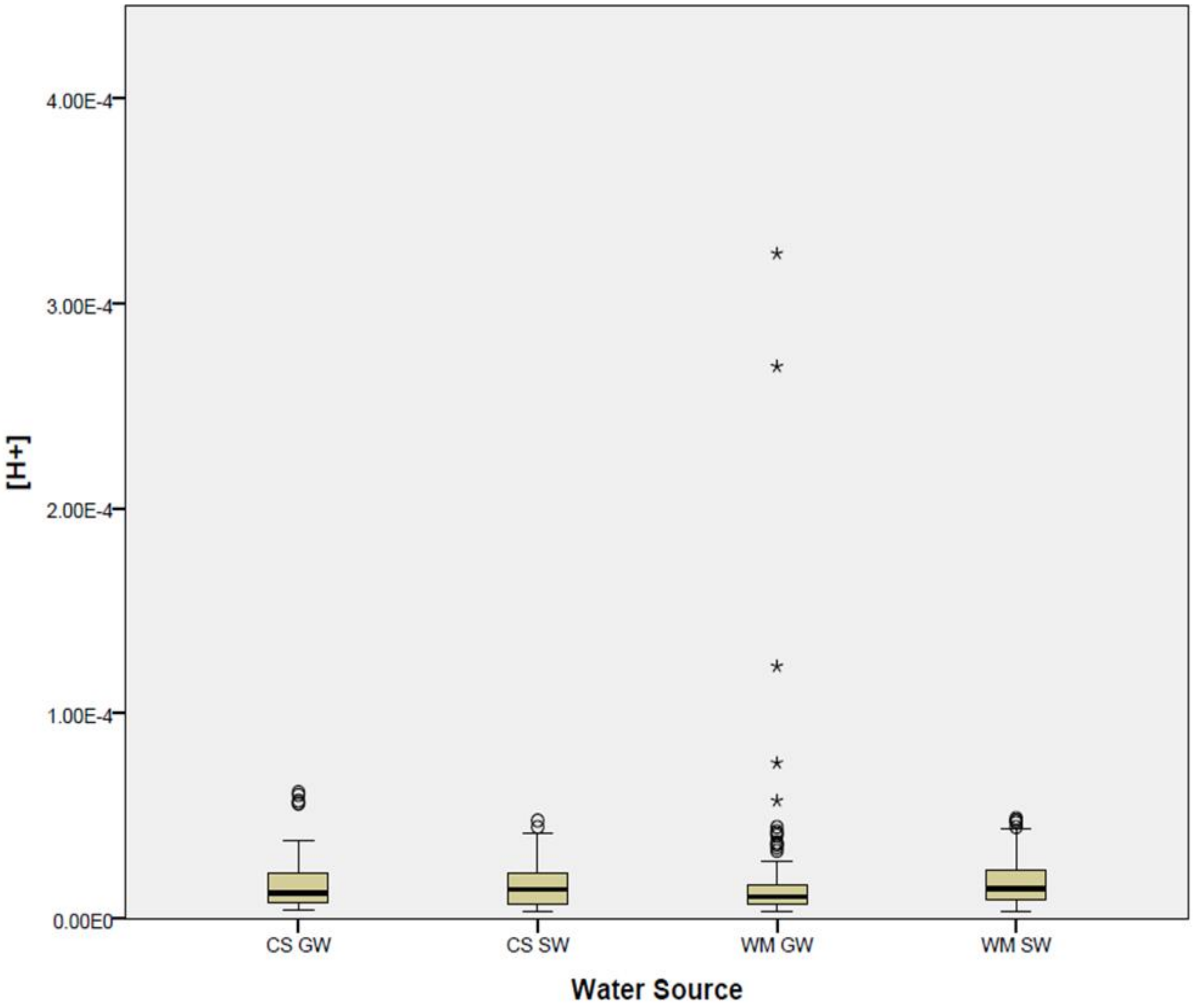


Figure 22. PH values were converted to [H+] and grouped together by wetland and water source to show changes in water quality over time. CS GW indicates groundwater discharge sites at CSB, CS SW indicates surface water sites at CSB, WM GW indicates groundwater discharge sites are WMF, and WM SW indicates surface water sites at WMF.

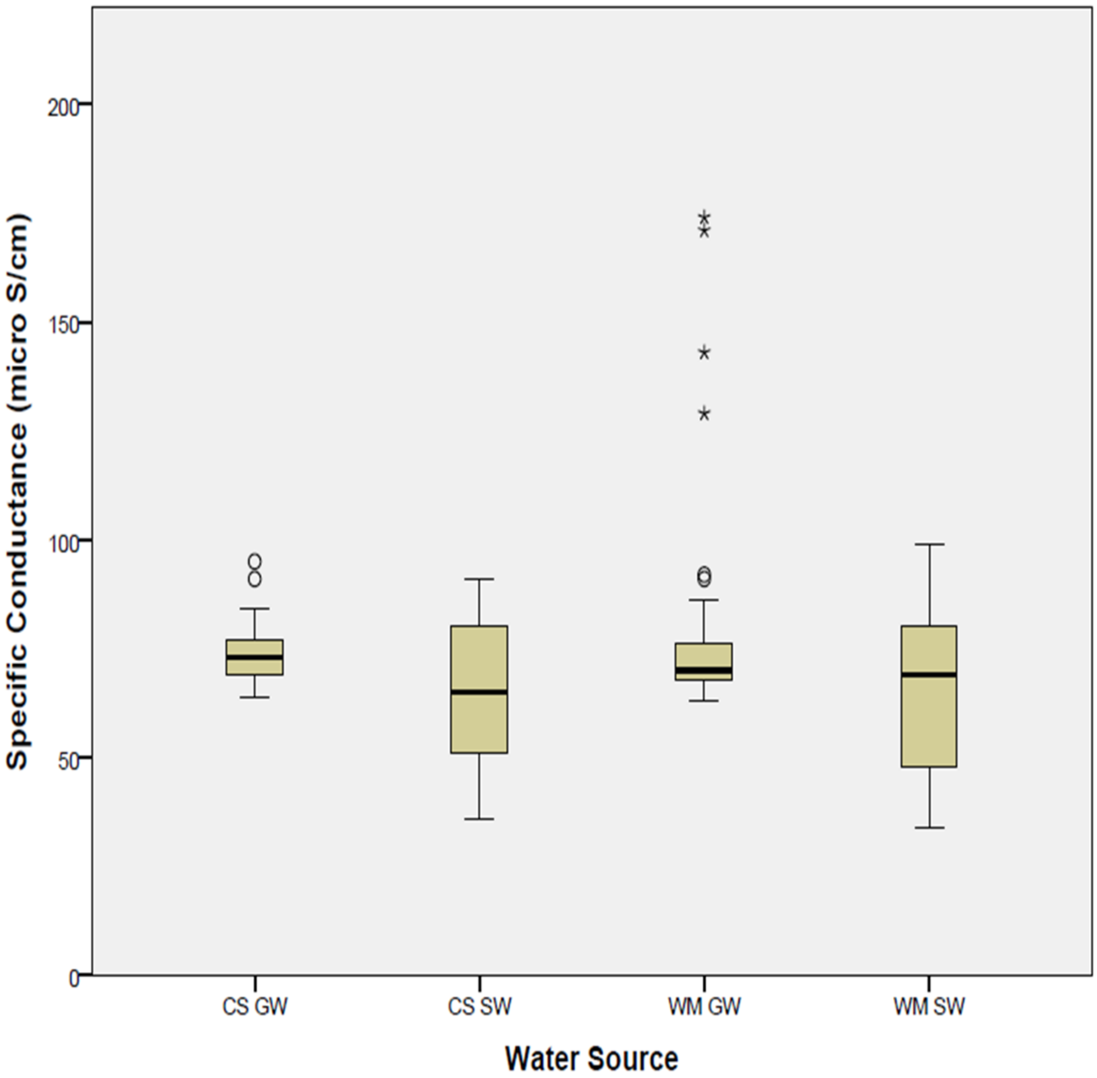


Figure 23. SPC was grouped together by wetland and water source to show changes in water quality over time. CS GW indicates groundwater discharge sites at CSB, CS SW indicates surface water sites at CSB, WM GW indicates groundwater discharge sites are WMF, and WM SW indicates surface water sites at WMF.

Tables

Table 1. Water quality data collected in 2009 (post-accident) by the PA DEP at two springs that contribute to Alex Branch contrasted with safe water quality standards as determined by the US EPA.

	Sykesville Spring	Reeds Spring	EPA Safe Drinking Water Standards
Strontium µg/L	42270	24690	4000
Manganese µg/L	3095	550	500
Barium µg/L	12300	8122	2000
TDS @ 105 C mg/L	3250	1388	<499
Chloride mg/L	1597	600	250
Sodium mg/L	410	195	20
Specific Conductance @ 25C umhos/cm	3200	5004	n/a

Table 2. Pool size and depth data comparing total pool area of CSB to WMF and average pool depth of CSB to WMF. A Mann-Whitney test showed that total pool area at CSB was significantly greater than total pool area at WMF, and that average pool depth did not significantly differ between the two wetlands.

	Total Pool Area (m^2)	Average Pool Depth (m)
CSB	6630.57	0.025
WMF	56.27	0.24
p-value	0.0119	0.8492

Table 3. Using date as the grouping variable, a Kruskal-Wallis Test was used to determine if there were temporal changes in [H+]. Parameters tested were CSB ground water, WMF ground water, CSB surface water, WMF surface water. There were no significant differences (changes in [H+]) over time for any of the four water sources.

Kruskal-Wallis Test on [H+][a]

	CSB Groundwater	WMF Groundwater	CSB Surface Water	WMF Surface Water
Chi-Square	26	25	26	24
df	28	28	28	28
p-value	0.572	0.625	0.552	0.656

a. Grouping variable = date code

Table 4. Using date as the grouping variable, a Kruskal Wallis Test was used to determine if there were temporal changes in SPC. Parameters tested were CSB ground water, WMF ground water, CSB surface water, and WMF surface water. There were no significant differences (changes in SPC) over time for any of the four water sources.

Kruskal-Wallis Test on SPC[a]

	CSB Groundwater	WMF Groundwater	CSB Surface Water	WMF Surface Water
Chi-Square	26	19	22	29
df	28	28	28	28
p-value	0.563	0.885	0.785	0.413

a. Grouping variable = date code

Table 5. Alkalinity results in mg/L of $CaCO_3$ from nine sample locations. For water source, GW indicates groundwater and SW indicates surface water.

Sample Location	Code Name	Water Source	Alkalinity (mg/L CaCO3)
Reeds Spring	ALK1	GW	1.6
Alex Branch at McGeorge Rd.	ALK2	SW	2.3
Alex Branch 1	ALK3	SW	2.1
Camp Spring	ALK4	GW	1.7
Standing Water	ALK5	SW	0
GW Discharge 1	ALK6	GW	1.7
GW Discharge 2	ALK7	GW	1.2
Alex Branch 3	ALK8	SW	0
GW Discharge 3	ALK9	GW	1.1

Table 6. Summarization of bird point-count data for 2012 and 2013; 6a shows the summary data by plot, 6b shows the summary data per wetland, and 6c shows the Jaccard and Sorenson Indices of Similarity between each plot and each wetland.

6a.	Average # Individuals per Plot		Cumulative Species Richness per Plot		Simpson's Index of Diversity	
	2012	**2013**	**2012**	**2013**	**2012**	**2013**
CS 1	11.67	10.8	23	18	0.92	0.92
CS 2	10.33	10	23	18	0.94	0.94
WM 1	10.67	11.8	22	20	0.95	0.94
WM 2	9	10.4	19	19	0.93	0.94

6b.	Average # Individuals per Wetland		Cumulative # Individuals per Wetland		Cumulative Species Richness per Wetland	
	2012	**2013**	**2012**	**2013**	**2012**	**2013**
CS	11	10.4	22	20.8	31	26
WM	9.84	11.1	19.67	22.2	25	23

6c.	Jaccard Index of Similarity (%)		Sorenson Index of Similarity (%)	
	2012	**2013**	**2012**	**2013**
CS 1 and CS 2	48	42	65	59
WM 1 & WM 2	64	70	78	82
CS and WM	51	58	68	73

Table 7. Summarization of aquatic invertebrate data for 2012; 7a shows the summary data by plot, 7b shows the summary data per wetland, and 7c shows the Jaccard and Sorenson Indices of Similarity between the wetlands.

7a.	Average # Individuals per Plot	Cumulative Species Richness per Plot	Simpson's Index of Diversity
CS 1	18	7	0.78
CS 2	21	7	0.71
CS3	16	7	0.88
CS4	32	6	0.54
WM 1	18	9	0.91
WM 2	24	5	0.67
WM3	26	7	0.69
WM4	10	8	0.96

7b.	Average # Individuals per Wetland	Cumulative # Individuals per Wetland	Cumulative Taxa Richness per Wetland
CS	21.75	6.75	10
WM	19.5	7.25	12

7c.	Jaccard Index of Similarity (%)	Sorenson Index of Similarity (%)
CS and WM	83	91

Table 8. Summary of amphibian point-count data for 2012 and 2013; 8a shows the summary data by plot, 8b shows the summary data per wetland, and 8c shows the Jaccard and Sorenson Indices of Similarity between each plot and each wetland.

8a.	Average # Individuals per Plot		Cumulative Species Richness per Plot		Simpson's Index of Diversity	
	2012	2013	2012	2013	2012	2013
CS 1	4	3	5	4	0.78	0.74
CS 2	2.83	3.83	4	3	0.57	0.54
WM 1	1.83	2.33	1	1	0	0
WM 2	0	0	0	0	0	0

8b.	Average # Individuals per Wetland		Cumulative # Individuals per Wetland		Cumulative Species Richness per Wetland	
	2012	2013	2012	2013	2012	2013
CS	3.42	3.42	6.83	6.83	6	5
WM	0.92	1.17	1.83	2.33	1	1

8c.	Jaccard Index of Similarity (%)		Sorenson Index of Similarity (%)	
	2012	2013	2012	2013
CS 1 and CS 2	50	40	67	57
WM 1 and WM 2	0	0	0	0
CS and WM	16.67	20	29	33

Table 9. Total adult amphibians detected at CSB and WMF during the 2013 VES.

Site	Species	4/27/2013	5/11/2013	5/27/2013	6/15/2013	Total
CSB	*Desmognathus fuscus*	0	0	0	1	1
	Notophthalmus viridescens	5	4	3	3	15
	Plethodon cinereus	5	3	1	0	9
	Rana clamitans	0	0	0	0	0
	Rana sylvatica	1	0	0	0	1
					Total Individuals	**26**
WMF	*Desmognathus fuscus*	0	0	0	0	0
	Notophthalmus viridescens	0	0	0	0	0
	Plethodon cinereus	1	0	1	0	2
	Rana clamitans	0	1	0	0	1
	Rana sylvatica	0	0	0	0	0
					Total Individuals	**3**

Table 10. Total amphibian juveniles (egg masses only) detected at CSB and WMF during 2013 VES.

Site	Species	4/27/2013	5/11/2013	5/27/2013	6/15/2013	Total
CSB	*Ambystoma maculatum*	29	19	12	9	69
	Bufo americanus	1	0	3	3	7
	Rana palustris	0	0	3	0	3
	Rana sylvatica	23	32	17	10	82
					Total Juveniles	**161**
WMF	*Ambystoma maculatum*	0	0	0	0	0
	Bufo americanus	0	0	0	0	0
	Rana palustris	0	0	0	0	0
	Rana sylvatica	0	0	0	0	0
					Total Juveniles	**0**

APPENDICES

Appendix 1. Summary of EOG well violations in Clearfield County between 2009 and 2012.

Well Site	Year of Violation	Type of Violation	Description of Violation
3H	2009	Administrative	Failure to post permit number, operator name, address, telephone number in a conspicuous manner at the site during drilling
6H	2009	Administrative	O&G Act 223-General. Used only when a specific O&G Act code cannot be used
7H	2009	Administrative	Failure to post permit number, operator name, address, telephone number in a conspicuous manner at the site during drilling
8H	2009	Environmental Health & Safety	Discharge of pollultional material to waters of Commonwealth
8H	2009	Environmental Health & Safety	Stream discharge of IW, includes drill cuttings, oil, brine and/or silt
8H	2009	Environmental Health & Safety	Discharge of pollultional material to waters of Commonwealth
9H	2009	Environmental Health & Safety	Discharge of pollultional material to waters of Commonwealth
9H	2009	Environmental Health & Safety	Discharge of pollultional material to waters of Commonwealth
23H	2011	Administrative	Failure to report defective, insufficient, or improperly cemented casing w/in 24 hrs or submit plan to correct w/in 30 days
30H	2010	Environmental Health & Safety	Discharge of pollultional material to waters of Commonwealth
30H	2010	Environmental Health & Safety	Discharge of industrial waste to waters of Commonwealth without a permit
34H	2009	Administrative	Failure to post permit number, operator name, address, telephone number in a conspicuous manner at the site during drilling
36H	2010	Administrative	O&G Act 223-General. Used only when a specific O&G Act code cannot be used
36H	2010	Administrative	O&G Act 223-General. Used only when a specific O&G Act code cannot be used
36H	2010	Administrative	Clean Streams Law-General. Used only when a specific CLS code cannot be used
36H	2010	Environmental Health & Safety	Hazardous well venting
36H	2010	Environmental Health & Safety	Hazardous well venting
36H	2010	Environmental Health & Safety	Inadequate or improperly installed BOP, other safety devices, or no certified BOP operator
36H	2010	Environmental Health & Safety	Inadequate or improperly installed BOP, other safety devices, or no certified BOP operator
40H	2009	Administrative	Failure to post permit number, operator name, address, telephone number in a conspicuous manner at the site during drilling
49H	2012	Administrative	Failure to submit well record within 30 days of completion of drilling
49H	2012	Administrative	Drillers Log not on site
Cop 73H-5305	2012	Environmental Health & Safety	Failure to take measures to mitigate spill impact and/or clean up w/in 15 days

Appendix 2. Taxa detected at both study sites. Taxa in bold were used in the nMDS.

Species	Ordination Code	Maximum IV
Acer negundo	ACERNEGU	6.80
Acer rubrum	**ACERRUBR**	**89.67**
Alnus spp.	**ALNUS**	**66.33**
Amelanchier spp.	**AMELANCH**	**35.20**
Aronia melanocarpa	**ARONMELA**	**50.40**
Athyrium filix-femina	ATHYFIFE	7.33
Botrychium virginianum	**BOTRVIRG**	**55.13**
Carex crinita	**CARECRIN**	**52.00**
Carex folliculata	**CAREFOLL**	**88.60**
Carex spp.	**CAREX**	**16.20**
Comptonia peregrina	COMPPERE	14.93
Cornus canadensis	CORNCANA	15.00
Cornus spp.	CORNUS	13.67
Drosera rotundifolia	**DROSROTU**	**88.20**
Dulichium arundinaceum	DULIARUN	6.80
Eleocharis spp.	ELEOCHAR	8.00
Eriophorum virginicum	**ERIOVIRG**	**61.20**
Fagus grandifolia	FAGUGRAN	13.47
Gaultheria procumbens	**GAULPROC**	**72.40**
Glyceria canadensis	GLYCCANA	7.00

Juncus brachycephalus	**JUNCBRAC**	**62.53**
Juncus canadensis	**JUNCCANA**	**21.33**
Juncus effusus	**JUNCEFFU**	**28.73**
Juncus tenius	JUNCTENI	7.00
Kalmia latifolia	**KALMLATI**	**43.20**
Leersia oryzoides	LEERORYZ	14.07
Lycopodium spp.	**LYCOPODI**	**35.93**
Lycopus uniflorus	LYCOUNIF	14.67
Mitchella repens	MITCREPE	6.93
Monotropa uniflora	MONOUNIF	6.87
Muhlenbergia glomerata	**MUHLGLOM**	**82.20**
Nyssa sylvatica	NYSSSYLV	6.73
Osmonda cinnomomea	**OSMOCINN**	**44.07**
Panicum clandestinum	**PANICLAN**	**45.07**
Pinus resinosa	PINURESI	6.73
Pinus strobus	PINUSTRO	20.27
Prunus serotina	PRUNSERO	15.33
Quercus alba	QUERALBA	6.80
Quercus bicolor	QUERBICO	8.00
Quercus rubra	QUERRUBR	13.53
Rhynchospora alba	**RHYNALBA**	**115.47**
Rubus hispidus	**RUBUHISP**	**150.27**
Sarracenia purpurea	**SARRPURP**	**22.73**

Scirpus cyperinus	**SCIRCYPE**	**29.13**
Selaginella spp.	**SELAGINE**	**85.27**
Sparganium spp.	**SPARGANI**	**29.80**
Sphagnum spp.	**SPHAGNUM**	**190.00**
Spiraea alba	SPIRALBA	6.80
Spiraea tomentosa	**SPIRTOME**	**63.47**
Trientalis borealis	**TRIEBORE**	**20.47**
Trillium undulatum	TRILUNDU	6.73
Tsuga canadensis	**TSUGCANA**	**98.60**
Typha latifolia	**TYPHLATI**	**51.87**
Unknown grass	**GRASS**	**40.67**
Vaccinium corymbosum	**VACCCORY**	**112.33**
Vaccinium macrocarpon	**VACCMACR**	**23.93**
Viburnum spp.	VIBURNUM	13.33
Viola spp.	**VIOLA**	**34.93**

Appendix 3. Data from bird point-count observations conducted at four point-count sites, six times during the 2012 breeding season. Survey number refers to the date each survey was conducted; 1 = 5/5/2012, 2 = 5/21/2012, 3 = 5/31/2012, 4 = 6/11/2012, 5 = 6/21/2012, 6 = 7/3/2012. For ease of analysis, all species were given a one or two letter code: A = American Crow, B = American Robin, C = American Tree Sparrow, D = Barn Swallow, E = Black and White Warbler, F = Black-capped chickadee, G = Blue Jay, H = Black-throated Blue Warbler, I = Brown Creeper, J = Canada Goose, K = Chipping Sparrow, L = Common Yellowthroat, M = Chestnut-sided warbler, N = Cedar Waxwing, O = Downy Woodpecker, P = Eastern Bluebird, Q = Eastern Phoebe, R = Eastern Towhee, S = Eastern Wood-peewee, T = Field Sparrow, U = Great-crested flycatcher, V = Gray Catbird, W = Hairy Woodpecker, X = Mallard, Y = Mourning Dove, Z = Northern Flicker, AA = Pileated Woodpecker, AB = Red-bellied Woodpecker, AC = Red-eyed Vireo, AD = Red-winged Blackbird, AE = Red-shouldered Hawk, AF = Scarlet Tanager, AG = Song Sparrow, AH = Tree Swallow, AI = Tufted Titmouse, AJ = Turkey Vulture, AK = White-breasted Nuthatch, AL = Wood Thrush. "A, A" indicates two individuals of species A were seen, "A, A, A" indicates three individuals, "A, B, C" indicates one individual of three species, etc.

Point Count Site	Survey Number	Species Observed	Number Individuals	Species Richness
CS1	1	O, Z, AC, AD, AD, AD, AD, AD, AG, AG	10	5
CS1	2	J, M, M, P, R, AC, AD, AD, AD, AD, AD, AD, AG	13	7
CS1	3	B, I, L, M, O, R, T, T, U, V, AD, AD, AD, AG, AG, AH, AH	17	12
CS1	4	E, L, M, N, N, N, N, N, N, N, T, W, AD, AG, AH,AH	16	9
CS1	5	A, A, B, F, L, M, R, T, V, AD	10	9
CS1	6	C, I, K, K	4	3
CS2	1	K, AG, AG, AK	4	3
CS2	2	A, A, A, L, M, M, M, R, T, X, X, AC, AG	13	8
CS2	3	C, G, G, G, M, N, N, N, N, P, R, T, V, AA, AG, AK, AL	17	12
CS2	4	A, A, A, G, G, G, K, L, M, R, T, U, AB, AG	14	10
CS2	5	B, D, D, L, N, N, N, N, R, AG, AJ	11	7
CS2	6	L, O, T	3	3
WM1	1	A, A, A, E, F, S, V, AA, AJ	9	7
WM1	2	A, A, A, B, L, L, M, M, P, Q, Y, AG, AG, AK, AK	15	9
WM1	3	K, L, Q, S, T, V, AG, AG, AI	9	8
WM1	4	B, B, F, K, M, R, AC, AG, AK	9	8
WM1	5	G, K, L, Q, R, T, V, W, Y, AG, AI, AI	12	11
WM1	6	G, G, G, G, G, G, V, Y, AG, AK	10	5
WM2	1	F, K, S, T, V, V, V, AG, AG	9	6
WM2	2	B, E, M, R, T, V, AG, AG, AK	9	8
WM2	3	F, G, G, G, H, L, T, AG	8	6
WM2	4	A, A, B, F, F, T, V, V, AG, AI, AI	11	7
WM2	5	B, M, Q, V, Z, AE, AG, AG, AI, AI	10	8
WM2	6	A, F, K, S, V, AG, AI	7	7

Appendix 4. Data from bird point-count observations conducted at four point-count sites (two at CSB and two at WMF), five times during the 2013 breeding season. Survey number refers to the date each survey was conducted; 1 = 4/28/2013, 2 = 5/12/2013, 3 = 5/26/2013, 4 = 6/16/2013, 5 = 6/29/2012. For ease of analysis, all species were given a one or two letter code: A = American Crow, B = American Robin, C = American Tree Sparrow, D = Barn Swallow, E = Black and White Warbler, F = Black-capped chickadee, G = Blue Jay, H = Black-throated Blue Warbler, I = Brown Creeper, J = Canada Goose, K = Chipping Sparrow, L = Common Yellowthroat, M = Chestnut-sided warbler, N = Cedar Waxwing, O = Downy Woodpecker, P = Eastern Bluebird, Q = Eastern Phoebe, R = Eastern Towhee, S = Eastern Wood-peewee, T = Field Sparrow, U = Great-crested flycatcher, V = Gray Catbird, W = Hairy Woodpecker, X = Mallard, Y = Mourning Dove, Z = Northern Flicker, AA = Pileated Woodpecker, AB = Red-bellied Woodpecker, AC = Red-eyed Vireo, AD = Red-winged Blackbird, AE = Red-shouldered Hawk, AF = Scarlet Tanager, AG = Song Sparrow, AH = Tree Swallow, AI = Tufted Titmouse, AJ = Turkey Vulture, AK = White-breasted Nuthatch, AL = Wood Thrush. "A, A" indicates two individuals of species A were seen, "A, A, A" indicates three individuals, "A, B, C" indicates one individual of three species, etc.

Point Count Plot	Survey Number	Species Observed	Number Individuals	Species Richness
CS1	1	H, I, L, AC, AD, AD, AD, AD	8	5
CS1	2	F, K, N, N, N, T, T, V, AA, AD, AD, AG, AK	13	9
CS1	3	A, E, AC, AG, AG, AH, AH, AH, AK	9	6
CS1	4	B, B, H, L, AC, AD, AD, AD, AD, AG, AG, AK	12	7
CS1	5	F, F, H, AC, AD, AG, AG, AI, AJ, AJ, AJ, AJ	12	6
CS2	1	C, H, J, J, R, W, AG	7	6
CS2	2	E, M, M, Q, Q, T	6	4
CS2	3	B, C, C, F, F, K, M, Q, T, T, U, V, AC, AG, AG, AK	16	12
CS2	4	C, H, H, N, N, N, N, Q, T, AG, AG	11	6
CS2	5	C, C, E, R, T, U, V, AG, AG, AK	10	8
WM1	1	B, B, F, F, Q, AC, AG, AG, AK	9	6
WM1	2	F, K, K, O, T, T, V, Y, Y, AG, AK	11	8
WM1	3	E, F, F, K, K, Q, R, T, AG, AI, AK	11	9
WM1	4	A, A, A, B, F, F, K, Y, AA, AC, AG, AJ, AJ, AJ, AJ, AK	16	10
WM1	5	C, E, H, K, K, T, V, V, AC, AG, AK, AL	12	7
WM2	1	A, A, E, K, K, M, R, V, AG, AG, AI	11	8
WM2	2	C, K, V, AC, AC, AG, AI	7	6
WM2	3	G, G, G, K, M, M, Q, T, V, AF, AG	11	8
WM2	4	A, K, M, Q, Q, T, T, V, AK	9	7
WM2	5	B, C, C, F, H, M, Q, T, V, V, AA, AG, AG, AI	14	11

Appendix 5. Data from macroinvertebrate surveys conducted at eight sites (four at CSB and four at WMF) on 22 September 2012. For ease of analysis, all taxa were given a one letter code: A = Oligochaeta (aquatic worms), B = Cambaridae (crayfish), C = Corixidae (water boatmen), D = Gyrinidae (whirligig beetle), E = Notonectidae (backswimmers), F = Zygoptera (damselfly), G = Anisoptera (dragonfly), H = Chironomidae (midges), I = Plecoptera (stonefly), J = Ephemeroptera (mayfly), K = Tipulidae (cranefly), L = Amphipoda (scud). "A, A" indicates two individuals of taxa A were seen, "A, A, A" indicates three individuals, "A, B, C" indicates one individual of three taxa, etc.

Sample Plot	Taxa Observed	Number Individuals	Taxa Richness
CS1	A,A,A,C,E,F,F,H,H,H,H,H,H,H,H,J,K,K	18	7
CS2	A,B,C,D,D,E,E,E,F,F,H,H,H,H,H,H,H,H,H,H,H	21	7
CS3	A,A,A,A,B,D,D,D,E,E,F,F,F,G,H,H	16	7
CS4	A,A,A,A,A,A,D,E,G,G,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,H,K	32	6
WM1	A,A,B,C,C,C,F,H,H,H,I,J,J,K,K,K,K,L	18	9
WM2	B,D,D,H,H,H,H,H,H,H,H,H,K,K,K,K,K,K,K,K,K,K,K,L	24	5
WM3	A,A,A,E,F,F,F,G,H,H,H,H,H,H,H,H,H,H,H,H,H,H,I,K,K,K	26	7
WM4	A,A,B,C,D,E,F,F,J,K	10	8

Appendix 6. The maximum calling code and number of individuals of amphibians detected by date at CSB and WMF during the 2012 auditory surveys. The first number represents the calling code (1= calls not simultaneous and individuals can be accurately counted; 2= calls simultaneous, individuals can be reliably estimated; 3= full chorus, calls are continuous and overlapping and cannot be reliably estimated) and the second number represents the number of individuals. Species detected include Northern spring peeper (SPPE), American toad (AMTO), wood frog (WOFR), green frog (GRFR), bullfrog (BUFR), and pickerel frog (PKFR). "NONE" indicates that no frogs or toads were detected during that survey.

PLOT	**3/24/2012**	**4/20/2012**	**5/5/2012**	**5/22/2012**	**6/9/2012**	**6/27/2012**
CS1	SPPE 2-4	SPPE 1-1 AMTO 2-2 WOFR 1-1	AMTO 2-2 AMTO 1-1	GRFR 2-2 BUFR 2-2	GRFR 2-3 GRFR 2-2 GRFR 1-1 BUFR 1-1	SPPE 2-2
CS2	SPPE 3 SPPE 2-2	GRFR 1-1 PKFR 2-2 AMTO 1-1	PKFR 1-1 SPPE 3 SPPE 2-2	SPPE 2-2 SPPE 2-3	SPPE 1-2 GRFR 1-1	NONE
WM1	NONE	SPPE 1-1	SPPE 1-1 SPPE 2-2	SPPE 2-3	NONE	SPPE 1-2 SPPE 2-2
WM2	NONE	NONE	NONE	NONE	NONE	NONE

Appendix 7. Data from amphibian point-count observations conducted at four point-count sites (two at CSB and two at WMF), six times during the 2012 breeding season. Survey number refers to the date each survey was conducted; 1 = 3/24/2012, 2 = 4/20/2012, 3 = 5/5/2012, 4 = 5/22/2012, 5 = 6/9/2012, 6 = 6/27/2012. For ease of analysis, all species were given a code: Northern spring peeper = A, American toad = B, wood frog = C, green frog = D, bullfrog = E, pickerel frog = F. "SPPE" indicates that species A was calling in a full chorus and could not be reliably estimated. "A, A" indicates two individuals of species A were seen, "A, A, A" indicates three individuals, "A, B, C" indicates one individual of three species, etc. "NONE" indicates that no frogs or toads were detected.

Point Count Site	Survey Number	Species Observed	Number of Individuals	Species Richness
CS 1	1	A,A,A,A	4	1
CS 1	2	A,B,B,C	4	3
CS 1	3	B,B,B	3	1
CS 1	4	D,D,E,E	4	2
CS 1	5	D,D,D,D,D,D,E	7	2
CS 1	6	A,A	2	1
CS 2	1	A,A (SPPE)	2	1
CS 2	2	B,D,F,F	4	3
CS 2	3	A,A,F (SPPE)	3	2
CS 2	4	A,A,A,A,A	5	1
CS 2	5	A,A,D	3	2
CS 2	6	NONE	0	0
WM 1	1	NONE	0	0
WM 1	2	A	1	1
WM 1	3	A,A,A	3	1
WM 1	4	A,A,A	3	1
WM 1	5	NONE	0	0
WM 1	6	A,A,A,A	4	1
WM 2	1	NONE	0	0
WM 2	2	NONE	0	0
WM 2	3	NONE	0	0
WM 2	4	NONE	0	0
WM 2	5	NONE	0	0
WM 2	6	NONE	0	0

Appendix 8. The maximum calling code and number of individuals of amphibians detected by date at CSB and WMF during the 2013 auditory surveys. The first number represents the calling code (1= calls not simultaneous and individuals can be accurately counted; 2= calls simultaneous, individuals can be reliably estimated; 3= full chorus, calls are continuous and overlapping and cannot be reliably estimated) and the second number represents the number of individuals. Species detected include Northern spring peeper (SPPE), American toad (AMTO), wood frog (WOFR), green frog (GRFR), and bullfrog (BUFR). "NONE" indicates that no frogs or toads were detected during that survey.

PLOT	3/10/2013	4/7/2013	4/27/2013	5/11/2013	5/28/2013	6/15/2013
CS1	SPPE 1-2	SPPE 1-1/ WOFR 2-3	SPPE 1-1 AMTO 1-2	SPPE 1-1 AMTO 1-1	SPPE 2-2 GRFR 2-2	AMTO 1-1 AMTO 2-2
CS2	SPPE 1-1	SPPE 2-3 SPPE 1-1	SPPE 1-2	SPPE 2-3 BUFR 1-1 GRFR 1-1	SPPE 2-2 SPPE 2-3 BUFR 2-2	BUFR 1-1 GRFR 2-3
WM1	NONE	NONE	SPPE 2-2	SPPE 1-2 SPPE 1-1	SPPE 2-4 SPPE 2-2	SPPE 2-2 SPPE 1-1
WM2	NONE	NONE	NONE	NONE	NONE	NONE

Appendix 9. Data from amphibian point-count observations conducted at four point count-sites (two at CSB and two at WMF), six times during the 2013 breeding season. Survey number refers to the date each survey was conducted; 1 = 3/10/2013, 2 = 4/7/2013, 3 = 4/27/2013, 4 = 5/11/2013, 5 = 5/28/2013, 6 = 6/15/2013. For ease of analysis, all species were given a code: Northern spring peeper = A, American toad = B, wood frog = C, green frog = D, bullfrog = E. "A, A" indicates two individuals of species A were seen, "A, A, A" indicates three individuals, "A, B, C" indicates one individual of three species, etc. "NONE" indicates that no frogs or toads were detected.

Point Count Site	Survey Number	Species Observed	Number of Individuals	Species Richness
CS 1	1	A,A	2	1
CS 1	2	A,C,C,C	4	2
CS 1	3	A,B,B	3	2
CS 1	4	A,B	2	1
CS 1	5	A,A,D,D	4	2
CS 1	6	B,B,B	3	1
CS 2	1	A	1	1
CS 2	2	A,A,A,A	4	1
CS 2	3	A,A	2	1
CS 2	4	A,A,A,D,E	5	3
CS 2	5	A,A,A,A,A,E,E	7	2
CS 2	6	D,D,D,E	4	2
WM 1	1	NONE	0	0
WM 1	2	NONE	0	0
WM 1	3	A,A	2	1
WM 1	4	A,A,A	3	1
WM 1	5	A,A,A,A,A,A	6	1
WM 1	6	A,A,A	3	1
WM 2	1	NONE	0	0
WM 2	2	NONE	0	0
WM 2	3	NONE	0	0
WM 2	4	NONE	0	0
WM 2	5	NONE	0	0
WM 2	6	NONE	0	0