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The impacts of Marcellus Shale gas drilling accidents on amphibians in a Pennsylvania fen

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Abstract Gas drilling into the Marcellus Shale play has been linked to environmental issues, including potential impacts on wildlife. In 2009, three separate accidents occurred at two gas well sites in central Pennsylvania, USA that resulted in high levels of contaminants in Wallace Mine Fen and a headwater stream that flows through the fen. We collected water chemistry, vegetation, and amphibian data at the impacted fen and at a control fen in 2012 and 2013 to determine similarity of sites and the impacts of the contaminants. We also reviewed water chemistry reports generated by the Pennsylvania Department of Environmental Protection for data collected shortly after the accidents occurred to provide insight on the nature of the accidents. Ordinations of vegetation data, as well as water chemistry, showed that the two wetlands are similar and dominated by the same plant species and water chemistry. Historically, both wetlands provided habitat for amphibians. However, unlike in pre-accident amphibian data, we detected virtually no amphibians in the impacted Wallace Mine Fen, suggesting that amphibians were possibly negatively affected by gas-drilling accidents.

Keywords Hydraulic fracturing · Marcellus shale · Fen · Amphibian · Contamination

Introduction

Development of the Marcellus Shale gas play by hydraulic fracturing (hydrofracking) has been in the spotlight since the 2000s for the potential negative impacts that may occur and has been linked to a wide range of environmental issues, including habitat fragmentation (Johnson 2010; Kiviat 2013; Brittingham et al. 2014) and impacts on wildlife (Rich and Longcore 2006; Bayne and Dale 2011; Kiviat 2013; Brittingham et al. 2014).

Pennsylvania has a rich history of gas drilling, which has occurred in the state since the nineteenth century and has produced about 40,000 gas wells (Swistock 2008). Since the advancement of new drilling technologies (i.e., horizontal drilling and hydraulic fracturing), the gas industry has expanded in Pennsylvania, particularly in the Marcellus Shale region (Considine et al. 2012; Trexler et al. 2014; Sutter et al. 2015). With increased drilling activity, the number of drilling-related accidents and violations has also increased. From January 2008 through August 2011, there were 2988 violations in Pennsylvania;

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1144 of those were environmental in nature (Considine et al. 2012). The geographic area near Moshannon State Forest in north-central Pennsylvania has had several drilling-related incidents. Many of these incidents were minor events that resulted in little to no contamination (Considine et al. 2012). However, lack of research and data collection describing the environmental impacts resulting from gas-drilling accidents continues to be a problem (Considine et al. 2012), and the potential for environmental impacts is not well-understood (Kiviat 2013; Trexler et al. 2014; Sutter et al. 2015; Campa et al. 2018).

On 24 August 2009, the Pennsylvania Department of Environmental Protection (PA DEP) received a citizen's complaint that led to inspections of two Marcellus Shale gas-well sites, 8H and 9H. At 8H, the agency discovered leakage from a containment pit, which was determined to be the source of "a release of an unknown quantity of materials to the Alex Branch…" (PA DEP 2010a). Alex Branch is a small, sandy-bottom stream that flows through a wetland, Wallace Mine Fen (WMF), and drains to Trout Run, which supports a high quality coldwater fishery (PA Fish and Boat Commission 2020). At site 9H, the agency determined that flowback fluids were deposited in a wetland near the site (PA DEP 2009, 2010a). On 25 August 2009, the agency tested the headwaters of Alex Branch at Sykesville Spring and just below Reeds Spring in Alex Branch and found reduced pH and elevated levels of contaminants and specific conductance (SPC). On 21 September 2009, the agency collected water chemistry samples at a third groundwater-discharge location and also found elevated levels of contaminants and SPC, as well as extremely low pH (3.9) (PA DEP 2009, 2010a) (Table 1).

On 14 October 2009, PA DEP inspected site 8H for a second time in response to a company report of a fluid spill that the company reported 2 days prior. The spill consisted of approximately 190 barrels of water mixed with Well Wash 2020 and Ultra Vis; these are the hydrofracking fluids used by the company, the contents of which are undisclosed. The fluids were rapidly absorbed into the ground and entered a tributary to Alex Branch (PA DEP 2010a). No water chemistry data are known to have been collected directly after this event.

On 30 December 2009, PA DEP collected pH, SPC, and alkalinity data at three sites along Alex Branch and at a small unnamed tributary to Alex Branch. The pH ranged from 4.7 to 6.0, SPC from 19 to 48 µS/cm, and alkalinity was < 5 mg/L. The agency concluded that the water chemistry was not unusual at that time (PA DEP 2010b). Additionally, the complainant who filed the August 2009 complaint collected SPC data from Sykesville Spring and Reeds Spring from August 2009 until January 2011. SPC levels were extremely high at both sites after the accidents but decreased steadily until reaching more typical levels by April 2010.

For the purpose of this research, we focused on impacts to a small wetland, WMF, resulting from the cited surface and subsurface water contamination. Receipt of contaminated surface and subsurface water from drilling-related accidents on private land in 2009 was documented in Alex Branch (Grant et al. 2016; Campa et al. 2018; Ulrich et al. 2018), which flows through WMF, and thus, WMF would have received the same contaminated water. Therefore, we studied WMF to determine if those accidents may have affected the site, selecting a second wetland, Crystal Spring Bog (CSB, actually a fen), as a control site due to its upslope location (which helps protect it from surface spills) and its similar wetland habitat (based on initial site visits), underlying geology (Fleeger 1999; Enomoto et al. 2012), and soils (poorly drained, mesic Typic Fragiaquults on 0–3% slope dominant at both sites) (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx.). This paper reports potential effects on amphibian populations at WMF, with water chemistry and habitat provided by vegetation being similar between WMF and CSB.

Methods

Study sites

WMF and CSB are located in the Susquehanna watershed in Moshannon State Forest, Clearfield County, Pennsylvania, USA (CSB 41.1257 N, − 78.5237 W and WMF 41.1824 N, − 78.4366 W) (Fig. 1). They are in the Marcellus Shale region, and the underlying bedrock consists mainly of sandstone and shale, with clay, coal, and limestone (Enomoto et al. 2012). The resultant groundwater chemistry in the region is highly variable. Historical water chemistry data retrieved from the Storage and Retrieval

Table 1 Water chemistry data collected in 2009 by the PA DEP at three groundwater discharge sites. Sykesville and Reeds Springs contribute to Alex Branch, which flows through Wallace Mine Fen

	Sykesville Spring	Alex Branch (below Reeds Spring)	Groundwater discharge (to Little Laurel Run)
Strontium (µg/L)	24,690	42,270	8680
Manganese (µg/L)	550	3095	411
Barium (µg/L)	8122	12,300	3040
Aluminum (µg/L)	–	–	7880
TDS (@ 105 C mg/L)	1388	3250	876
Chloride (mg/L)	600	1597	331
Sodium (mg/L)	195	195	111
Specific Conductance (@ 25 C µ/cm)	1948	4880	1116
pH	4.2	4.4	3.9

"–" Indicates no data

Data Warehouse (STORET) database of the Environmental Protection Agency (EPA) showed that pH can range from 3.1 to 7.1 and specific conductance from 33 to 515 µS/cm in this region.

Wallace Mine Fen

WMF is a ~ 8 ha fen that receives groundwater discharge, as well as surface flow from Alex Branch. Alex Branch flows from the southwestern corner of the fen to the east (Fig. 1). The local topography slopes gradually to the east-southeast. The fen is bordered by dirt or gravel service roads to the north and west. There are several cabins used seasonally for hunting and recreation that are in close vicinity to the wetland and tap into the groundwater for potable water. An initial assessment of WMF showed that dominant vegetation was typical of poorly mineralized peatlands, including *Sphagnum* spp.

Crystal Spring Bog

CSB is located approximately 10 km southwest from WMF (Fig. 1). This peatland, 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 eastward and forms a small, unnamed stream, which eventually flows into Lick Run. In addition to groundwater discharge, the wetland receives limited runoff from sloping topography on the northern and southern sides. Initial assessment showed that the site is a *Sphagnum*-dominated fen.

Vegetation sampling

We sampled vegetation from July to August 2012. We laid out two 100-m radius circles at each wetland (CSB1, CSB2, WMF1, and WMF2) and divided each circle into four quadrants. We used a 1 × 1 m PVC quadrat frame to sample 15 randomly placed points in each quadrant, for a total of 60 quadrats per circle, 120 quadrats per wetland. All plants within the quadrats were identified to the lowest taxonomic level possible (usually to species), and percent cover for each taxon was estimated every 1% up to 5% and then every 5%. We used relative frequency and relative mean percent cover to calculate Importance Values (IV) for each taxon found at CSB and WMF. With PC-ORD software (Version 5.0), we used the IV in a non-metric multi-dimensional scaling (NMDS) ordination with Sorenson distance measure. Taxa used in the ordination included those found in ≥ 5 quadrats and with IV ranging from 20 to 100 to focus on important wetland taxa (Graham 2015). This was a conservative criterion, but it eliminated 23 species (approximately 40% of the species found) from the analyses, most of

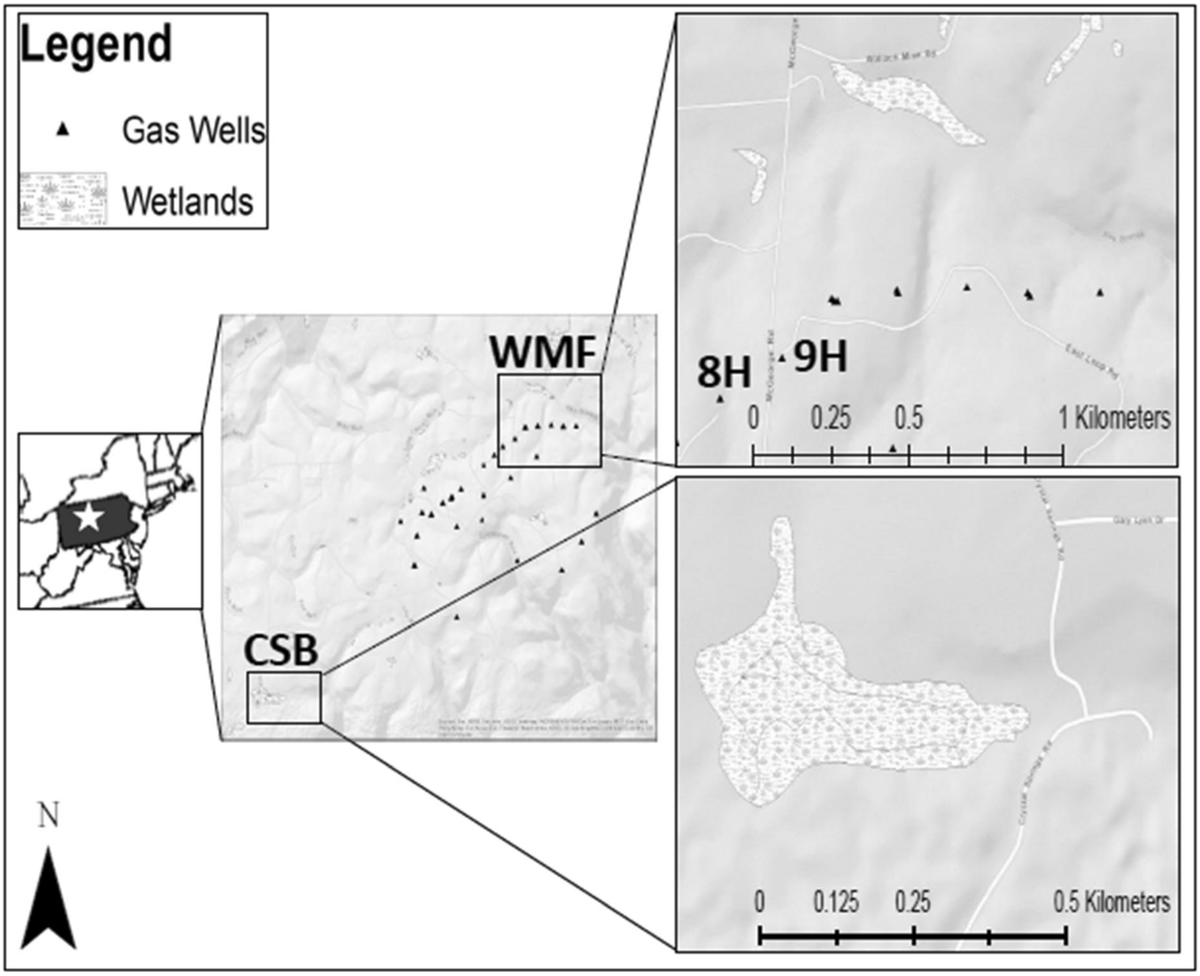

Fig. 1 North-central Clearfield County, Pennsylvania, where gas drilling activities have increased in recent years (left). The enlarged area shows Crystal Spring Bog near the bottom left of the map and Wallace Mine Fen (WMF) near the top right. Several gas wells are located upslope from WMF and are in close proximity to the wetlands and streams, such as Alex Branch

which were upland plants or had low IV (< 20). Using this metric was advantageous to determine how similar the most important vegetation types were at each site. Axis scores were graphed to show the similarities and dissimilarities in species composition between CSB and WMF.

Water chemistry sampling

pH and specific conductance

Water chemistry monitoring was conducted 29 times at both wetlands from May 2012 to October 2013. We tested the pH and SPC at five surface water locations and six areas of groundwater discharge at WMF. The same testing was conducted at CSB at three groundwater discharge sites and six surface water sites along the unnamed stream. Data were collected using an Extech Instruments ExStik II pH/Conductivity/TDS Meter, Model EC500 (precision rating ± 0.01 pH; 2% SPC). The instrument was calibrated each morning before use, using recently purchased Oakton ® pH standards 4.01, 7.01, and 10.04 and Oakton ® low and medium range conductivity standards (84 µS/cm and 1413 µS/cm). We report ranges in water chemistry values at the groundwater and surface water sites located at WMF and CSB.

Pennsylvania Department of Environmental Protection water chemistry data

We reviewed water chemistry reports produced by PA DEP (PA DEP 2009, 2010a, b). Samples were collected by the agency after the three accidents were discovered. They tested the headwaters of Alex Branch at two locations, Sykesville Spring and just below the Reeds Spring discharge at Alex Branch, and found extremely elevated levels of barium, strontium, manganese, sodium, calcium, chloride, TDS, and SPC (Table 1). They also tested groundwater discharge that contributes to Little Laurel Run and found extremely elevated levels of aluminum, barium, strontium, sodium, chloride, TDS, and SPC (Table 1). These data help to provide insight on the nature of the accidents.

Amphibian sampling

Call 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 Coastal Wetland Monitoring Protocol (Uzarski et al. 2017), consisting of auditory surveys that used numerical calling codes to get an estimate of breeding species present at each site (1 = call not simultaneous and number of individuals can be accurately counted, 2 = some calls simultaneous and number of individuals can be accurately counted, 3 = full chorus, calls are continuous and overlapping, and number of individuals cannot be accurately counted). Surveys were conducted from the center of the 100-m-radius circles that were defined for vegetation surveys. Two observers noted the approximate distance and location of each detection in relation to their location to prevent recounting any individuals. We conducted the surveys at least 15 days apart in appropriate weather conditions (i.e., once nighttime air temperatures reached 5 °C, winds $<$ 20 km/h, and no rain to slight drizzle). Surveys were conducted after sunset, waiting 1 min to allow frogs and toads to start calling again after being disturbed, and then surveying for 3 min. Due to the lack of amphibians encountered during the call surveys at WMF, statistical analyses were not used.

Visual encounter surveys

We conducted amphibian visual encounter surveys (VES) four times at both wetlands from April to June 2013 using methods adapted from Campbell and Christman (1982). Thirty-two 10 $\times$ 10 m plots were surveyed at each wetland. All plots were evenly spaced along continuous line transects at each wetland that separated the wetlands into several similar sized sections. Each plot was searched for 10 min by looking in any pools within plots, carefully turning over any objects (rocks, logs, etc.), and gently searching in *Sphagnum* mounds. All amphibians were identified to species, and the presence of all species, both adult and juvenile (i.e., egg masses and tadpoles), was recorded. Two observers moved through the plot in an orderly fashion to avoid recounting any individuals or egg masses, and each plot was surveyed just once per sampling date. Due to the lack of species detected at WMF, statistical analyses were not needed, and only raw data are reported.

Pre-accident amphibian data

Amphibian call surveys were conducted three times from April 2007 to March 2008 near the center of WMF. Surveys were similar to those later described by Uzarski et al. (2017) that were conducted in 2012 and 2013 and used numerical calling codes to get an estimate of breeding species present at the site. Surveys were conducted at least 15 days apart in appropriate weather conditions (defined above). A Time-Constrained Search (TCS) was also conducted in May 2007 using methods adapted from Corn and Bury (1990). The central portion of the wetland was separated into four equally sized sections ($\sim$ 100 m $\times$ 50 m). One of the 100 m $\times$ 50 m sections was randomly selected and searched for 60 min by the primary author and an assistant, for a total of two search hours at WMF. The search was conducted by looking in pools, turning over objects (rocks, logs, etc.), and searching in *Sphagnum* mounds. All amphibians were identified to species. We also report anecdotal observations that were made at the site between April 2007 and March 2009.

Results and discussion

Vegetation

There were no major differences in plant species composition between WMF and CSB, thus suggesting that there were no long-term impacts to vegetation in WMF from the accidents. We sampled a total of 56 taxa in the 240 quadrats across both wetlands. *Sphagnum* was the dominant vegetation found at both wetlands; it averaged 76% cover of CSB and 68% cover in WMF. The second most dominant species was swamp dewberry (*Rubus hispidus* L.), which had 24% mean cover in CSB and 35% cover in WMF. Cyperaceae were also common at both wetlands; approximately 19% mean cover found at CSB and 14% cover at WMF. The common sedges included fringed sedge (*Carex crinita* Lam.), long sedge (*Carex folliculata* L.), cottongrass (*Eriophorum virginicum* L.), and white beak-sedge (*Rhynchospora alba* L. (Vahl)).

The NMDS ordination (Fig. 2a) showed five distinct groupings of taxa. Group 1 included plants often found in upland locations that do not need an abundance of water to grow or survive. Most of these plants were growing in drier areas of quandrants in both wetlands– the wetland perimeter and the adjacent forests. Group 2 included three species only found at WMF but that grew throughout the wetland. Group 3 included four species growing throughout both wetlands. Group 4 included taxa that were growing with and on mounds of *Sphagnum* at both wetlands, most of which are distinctly wetland species with an obligate (OBL) or facultative wet (FACW) wetland rating. Group 5 included three OBL species that were only found at CSB.

The NMDS ordination plotted by study site (Fig. 2b) showed four separate groupings. Group 1 includes plots located at WMF that are dominated by *Alnus incana* ((L.) Moench) and *Muhlenbergia glomerata* ((Willd.) Trin.); these two species were only found at WMF. Group 2 included plots at both wetlands and contained many of the same dominant species. Group 3 included one sample location from WMF and one sample location from CSB. Both sample locations, although at different wetlands, were dominated by eastern hemlock (*Tsuga canadensis* (L.) Carrière) and red maple (*Acer rubrum* L.). Group 4 contains sites that were completely saturated and dominated by purple pitcherplant (*Sarracenia purpurea* L.), white beak-sedge, and broadleaf cattail (*Typha latifolia* L.). These three species were only

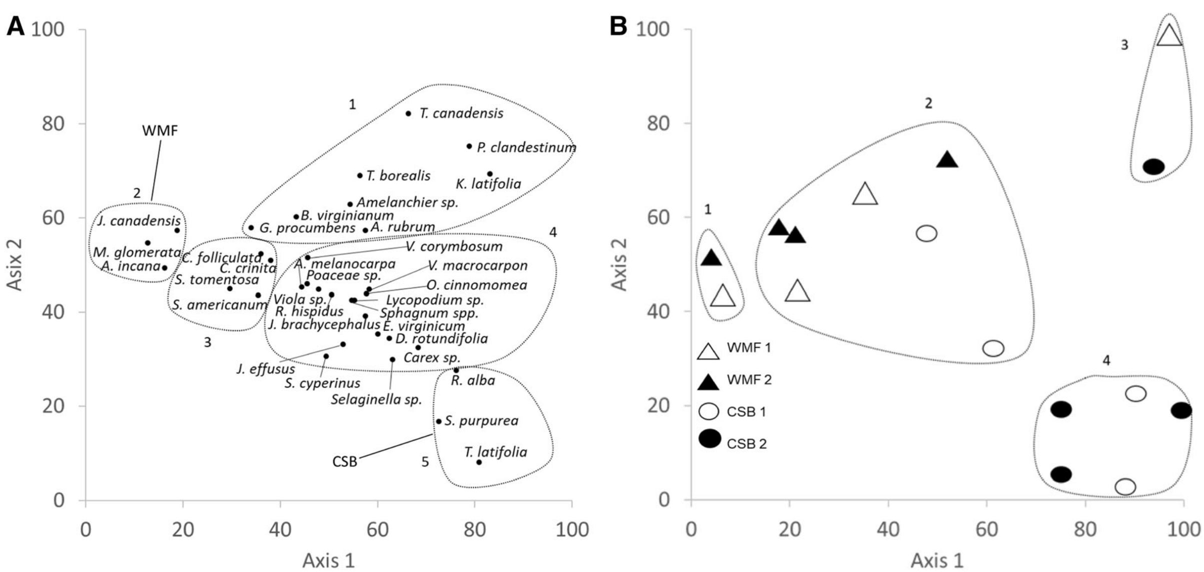

Fig. 2 Two-dimensional plot of NMDS ordination of selected plant taxa IV from Crystal Spring Bog (CSB) and Wallace Mine Fen (WMF) (autopilot on, Sorenson distance, no species weighting, final stress = 9.37, final stability = 0, number of iterations = 80). **a** Plants labeled in groupings 1–5 based on growing location and moisture levels (full taxonomy is shown in an on-line appendix). **b** Sampling sites labeled in groupings 1–4 are based on dominant species found growing in each sample location

found growing at CSB, which helps to explain the separation. The results of NMDS ordination showed that both sites are *Sphagnum*-dominated wetlands with an abundance of sedges and trees such as eastern hemlock and red maple at higher and drier edges.

Water chemistry

Most water chemistry values at CSB and WMF were quite similar, with the exception of one groundwater site at WMF (excluded from reported ranges). Surface water pH at CSB and WMF ranged between 4.3 and 5.4; surface water SPC ranged between 36 and 91 µS/cm at CSB and from 34 to 99 µS/cm at WMF. At CSB, pH at groundwater discharge sites ranged from 4.2 to 5.3; at WMF, it mostly ranged from 4.1 to 5.4. Groundwater SPC ranged between 64 and 95 µS/cm at CSB and between 63 and 92 µS/cm at WMF. At the outlier groundwater sampling site in WMF, the pH dropped as low as 3.4 and SPC increased to as high as 174 µS/cm. These spikes in water chemistry occurred on two separate occasions: August 2012 and April 2013. We accessed online data for a weather station in Penfield, PA, which is the closest station to the study sites (ID: MKYPP1), in an attempt to determine if contaminants persisted in the groundwater and were eventually flushed out by a large precipitation event. Overall, precipitation was well below average. Based on data available, we were unable to conclude if flushing of contaminants from the system resulted in resurfacing as groundwater discharge at WMF.

We also considered additional accidents as the cause of the spikes in water chemistry at WMF. In addition to gas wells 8H and 9H, there are five other gas wells located even closer to WMF; three of those wells are directly upslope from the groundwater discharge site where spikes in pH and SPC were detected. No additional Marcellus Shale gas-drilling-operation accidents were reported; however, the wells in question are located on privately owned land and are not heavily regulated (Marcellus Outreach Butler 2012). It is not uncommon for such accidents to go unreported (Sutter et al. 2015).

Water chemistry data collected in Alex Branch by PA DEP in December 2009 showed that pH, SPC, and alkalinity had already returned to levels consistent with the region a short time after the accidents were detected at the gas wells. Although not quality-controlled, the SPC data collected by the complainant at Sykesville Spring and Reeds Spring corroborated the decreases in SPC reported by PA DEP. Extremely elevated SPC levels (3200 µS/cm and 5004 µS/cm, respectively) in August 2009 decreased steadily at both sites, and by April 2010, levels were 82 µS/cm at Sykesville Spring and 50 µS/cm at Reeds Spring (PA DEP 2010b). The rapid decrease of SPC levels at the springs suggests that contaminants from the accidents seeped into the ground, resurfaced as groundwater discharge, and then flowed into Alex Branch. Additionally, SPC levels returned to more typical 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 subsurface flow. Considering the geology of this region, this is a 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).

Amphibians

Seven species were detected at WMF pre-accident through amphibian call surveys conducted in April 2007 and March 2008. Surveys showed that northern spring peepers (*Pseudacris crucifer* Wied-Neuwied) and wood frogs (*Lithobates sylvaticus* LeConte) were calling in a chorus, suggesting that breeding populations were present pre-accident (Table 2). Adult green frogs (*Lithobates clamitans* Latreille), adult wood frogs, and adult and juvenile red-spotted newts

Table 2 Amphibian call surveys conducted in April 2007 and March 2008 at Wallace Mine Fen indicate that breeding populations of *Pseudacris crucifer* and *Lithobates sylvaticus* were present at WMF pre-accident

Species	April 2007	April 2007	March 2008
Pseudacris crucifer	2–2	3	–
Lithobates sylvaticus	–	–	3

The first number represents the call level code (1 = call not simultaneous and number of individuals can be accurately counted, 2 = some calls simultaneous and number of individuals can be accurately counted, 3 = full chorus, calls are continuous and overlapping, and number of individuals cannot be accurately counted), and the second number represents the number of individuals. The " – " indicates no data

(*Notophthalmus viridescens* Rafinesque) were detected during the May 2007 survey (Table 3). Furthermore, additional observations were made between April 2007 and March 2009 during site visits, and the presence of red-backed salamanders (*Plethodon cinereus* Green), American toads (*Anaxyrus americanus* Holbrook), and Jefferson salamanders (*Ambystoma jeffersonianum* Green) was noted at the site (Table 4).

We detected major differences in amphibian diversity and richness between the wetlands in post-accident sampling. Using call surveys, we detected six species at CSB in 2012: northern spring peeper, wood frog, American toad, green frog, pickerel frog (*Lithobates palustris* LeConte), and bullfrog (*Lithobates catesbeianus* Shaw). Spring peeper was the only species detected at WMF (Table 5). In 2013, we detected five of those species at CSB (not pickerel frog). At WMF, again only spring peepers were detected in 2013 (Table 5).

During the 2013 VES surveys, we detected seven species at CSB: northern dusky salamander (*Desmognathus fuscus* Green), red-spotted newt, red-backed salamander, spotted salamander (*Ambystoma maculatum* Shaw), wood frog, American toad, and pickerel frog. We also detected spotted salamander, American toad, pickerel frog, and wood frog egg masses, and several American toad tadpoles at CSB. At WMF, we found just two adult red-backed salamanders and one adult green frog. No tadpoles or egg masses were found in WMF (Table 6).

Table 3 Time-Constrained Surveys conducted at Wallace Mine Fen in May 2007 in a 2-h search period showed the presence of adult *Lithobates clamitans*, adult *Lithobates sylvaticus*, and adult and juvenile (red eft) *Notophthalmus viridescens*

Species	Adult	Juvenile
Lithobates clamitans	4	–
Lithobates sylvaticus	1	–
Notophthalmus viridescens	2	12

Each number indicates the number of individuals found; the " – " indicates no data

Relations among vegetation, water chemistry, and amphibian abundance

We sought explanations for losses of amphibians at WMF. Aside from gas-drilling operations (i.e., gas wells/well pads; access roads; hydrofracking fluids, and flowback-water holding tanks, pipelines), there have been no other major disturbances near WMF since the accidents occurred. Campa et al. (2018) studied the same watershed and found no evidence of other industrial activities such as acid mine drainage or conventional drilling in the area.

Vegetation and typical water chemistry levels do not explain differences in amphibians between the wetlands, nor do they explain the apparent extirpation of some species from WMF. Viruses, such as Ranavirus, and fungi, such as *Batrachochytrium dendrobatidis* (Bd), both of which cause diseases in amphibians (Blackburn et al. 2015), have been detected in Pennsylvania in recent years (Groner and Relyea 2010; Smith et al. 2016). However, there have been no known detections of Ranavirus or Bd in Clearfield County or in the remote wetlands where our study took place.

A growing body of evidence suggests 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). Amphibians have many characteristics that make them extremely susceptible to pollutants: highly permeable skin, unprotected eggs and aquatic larval stages, and complex life cycles that make them reliant on water for breeding, foraging, and hibernation (Sanzo and Hecnar 2006; Hopkins 2007; Karraker 2007; Hampton et al. 2010). Thus, differences in amphibian abundances could potentially be attributed to the release of contaminated water from the gas-well accidents. We do not know exactly when some of these accidents occurred, but only when they were discovered, so it is difficult to pinpoint how amphibians may have been impacted. Hydrofracking fluids are proprietary, and different companies use different concentrations of different chemicals, so it is also difficult to determine specifically how amphibians may be impacted. An intensive Internet search on Well Wash 2020 and Ultra Vis—the products used by the gas drilling company—yielded no results.

Table 4 Anecdotal observations made during site visits to Wallace Mine Fen between May 2007 and March 2009

Species	May 2007	July 2007	May 2008	July 2008	March 2009
Pseudacris crucifer	C*	–	–	–	–
Anaxyrus americanus	–	E	C, E	–	–
Notophthalmus viridescens	–	–	A (3), J (7)	–	–
Ambystoma jeffersonianum	–	–	A (1)	–	–
Lithobates clamitans	–	–	–	A**	–
Plethodon cinereus	–	–	–	–	A**

Although these data were not collected systematically, they provide insight on species presence pre-accident

The " – " indicates no data

C calling, C* calling in chorus, E egg mass, A adult (# of individuals), A** adult, no information on abundance, J juvenile (# of individuals)

Table 5 The maximum amphibian calling code for all species detected during call surveys at each site in Crystal Spring Bog and Wallace Mine Fen in 2012 and 2013

Plot	Species	Max call code 2012	Max call code 2013
CSB1	*Anaxyrus americanus*	2–2	2–2
	Lithobates catesbeianus	2–2	0
	Lithobates clamitans	2–3	2–2
	Lithobates palustris	0	0
	Pseudacris crucifer	2–4	2–2
	Lithobates sylvaticus	1–1	2–3
CSB2	*Anaxyrus americanus*	1–1	0
	Lithobates catesbeianus	0	2–2
	Lithobates clamitans	1–1	2–3
	Lithobates palustris	2–2	0
	Pseudacris crucifer	3	2–3
	Lithobates sylvaticus	0	0
WMF1	*Anaxyrus americanus*	0	0
	Lithobates catesbeianus	0	0
	Lithobates clamitans	0	0
	Lithobates palustris	0	0
	Pseudacris crucifer	2–3	2–4
	Lithobates sylvaticus	0	0
WMF2	*Anaxyrus americanus*	0	0
	Lithobates catesbeianus	0	0
	Lithobates clamitans	0	0
	Lithobates palustris	0	0
	Pseudacris crucifer	0	0
	Lithobates sylvaticus	0	0

The first number represents the call level code (1 = call not simultaneous and number of individuals can be accurately counted, 2 = some calls simultaneous and number of individuals can be accurately counted, 3 = full chorus, calls are continuous and overlapping, and number of individuals cannot be accurately counted), and the second number represents the number of individuals

Contaminants detected in high concentrations by PA DEP after the accidents, such as barium, strontium, manganese, sodium, and chloride, have negative impacts on amphibians. Strontium impairs growth and development in larval amphibians (Snodgrass et al. 2004; Peterson et al. 2009). A study by Sanzo and Hecnar (2006) found that sodium and chloride affected feeding behavior in tadpoles, reduced survival in spotted salamanders, and had a toxic effect on wood frog tadpoles. Snodgrass et al. (2008) found that

Table 6 Total number of adult and juvenile amphibians found at each wetland during the 2013 visual encounter surveys at Crystal Spring Bog and Wallace Mine Fen

Crystal Spring					Wallace Mine			
Species	4/27/2013	5/11/2013	5/27/2013	6/15/2013	4/27/2013	5/11/2013	5/27/2013	6/15/2013
Ambystoma maculatum	0/29	0/19	0/12	0/9	0/0	0/0	0/0	0/0
Anaxyrus americanus	0/1	0/0	0/3	0/3	0/0	0/0	0/0	0/0
Desmognathus fuscus	0/0	0/0	0/0	1/0	0/0	0/0	0/0	0/0
Notophthalmus viridescens	5/0	4/0	3/0	3/0	0/0	0/0	0/0	0/0
Plethodon cinereus	5/0	3/0	1/0	0/0	1/0	0/0	1/0	0/0
Lithobates clamitans	0/0	0/0	0/0	0/0	0/0	1/0	0/0	0/0
Lithobates palustris	0/0	0/0	0/3	0/0	0/0	0/0	0/0	0/0
Lithobates sylvaticus	1/23	0/32	0/17	0/10	0/0	0/0	0/0	0/0

The first number represents the number of adults; the second is the number of juveniles (or egg masses)

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. 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. Furthermore, Perry (2012) reported dead amphibians in a Bradford County, PA pond after being exposed to hydrofracking fluids.

Heavy metals and other contaminants can also be toxic to amphibians, and the effects can be very complex (Adlassnig et al. 2013). 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 and leading to desiccation (Carey and Bryant 1995). Exposure may cause behavioral alterations, such as reduced activity levels and inability to feed, attract mates, or breed. Impaired behavioral responses may also make amphibians more susceptible to predation (Carey and Bryant 1995; Storfer 2003).

In addition to direct impacts from the contaminants (i.e., egg and larval mortality), indirect impacts are also possible. For example, if amphibian populations were completely eliminated due to the contamination, a source population would be necessary to 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 amphibians to 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 multiple roads can reduce terrestrial salamander dispersal up to 97% (Gillen and Kiviat 2012).

Conclusions

Few data regarding ecological impacts of hydrofacking accidents are available, making our results of special importance. CSB and WMF have similar underlying geology and hydrology; they are fens with similar groundwater and surface water chemistry, and habitat provided by vegetation is similar. Although it cannot be ruled out entirely, we do not think that the accidents at wells 8H and 9H had long-term negative impacts on the vegetation at WMF. However, there were major differences in the amphibian communities between the two wetlands, and there were fewer amphibians in WMF post-accident. Campa et al. (2018) showed that Alex Branch, as well as other streams affected by hydrofracking in this and nearby watersheds, are experiencing long-term impacts. Furthermore, Grant et al. (2016) documented low fish diversity and no aquatic invertebrate scrapers in Alex Branch post-accident. Hydrofracking and the resultant fluids have been linked to the mortality of fish and aquatic invertebrates in streams (Souther et al. 2014) and may alter food webs (Grant et al. 2016). The documented contamination of Alex Branch and surrounding streams as a result of hydrofracking accidents may have disrupted the food web in the watershed, potentially affecting amphibian populations.

Due to lack of extensive quantitative baseline data, we will never understand conclusively how or if gas-drilling accidents at wells 8H and 9H affected WMF. The lack of baseline water chemistry and accompanying amphibian data across the Marcellus Shale play hinders the ability to quantify future ecological risk accurately, determine future impacts, and develop best management practices (Brand et al. 2014). The lack of information about accident location and timing, as well as the propensity for not releasing information about accidents (Souther et al. 2014) due to liability and/or confidentiality agreements, makes it difficult to study and understand impacts resulting from gas development (Brantley et al. 2014). Additionally, the proprietary nature of chemicals used during hydrofracking 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.

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