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A Case Study of a *Prymnesium parvum* Harmful Algae Bloom in the Ohio River Drainage: Impact, Recovery and Potential for Future Invasions/Range Expansion

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Abstract: Inland waters provide valuable ecosystem goods and services and are intrinsically linked to downstream coastal areas. Water quality impairments that lead to harmful algal blooms damage valuable commercial and recreational fishing economies, threaten food security, and damage already declining native species. *Prymnesium parvum* is a brackish water golden alga that can survive in salinities less than 1 ppm and when it blooms it can create toxins that kill aquatic life. Blooms have been documented globally including 23 U.S. states. We report a case study of an aquatic life kill associated with *P. parvum* in Dunkard Creek (WV-PA, USA), in the Ohio River Drainage. We document the immediate impact to aquatic life and responses of the aquatic community ten years post-kill. Most fish species returned within a year. Excellent connectivity to unimpacted tributaries and a river downstream likely aided the reestablishment of most species, although some had not reached pre-kill abundances after ten years. Mussel taxa did not recover despite significant efforts to relocate adult mussels and stocking of host fish inoculated with glochidia; probably due to other water quality impairments. Given the potential for lateral transport of *P. parvum* via industry and natural vectors we conducted an ecological risk assessment mapping the spatial extent of U.S. waters that could be threatened by golden algae colonization and blooms using a national water quality database and a state database. Overall, about 4.5% of lotic systems appeared to have some level of risk of harboring *P. parvum*, making them at risk for potential golden algae blooms in the face of increasing salinization and eutrophication of freshwaters.

Keywords: HAB; golden algae; *Prymnesium parvum*

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1. Introduction

North America has an abundant supply of fresh surface waters which ultimately flow to coastal economic zones. Transported with these fresh waters are nutrients, sediments, pollutants, and contaminants [1–4]. These freshwaters provide drinking water for human populations and livestock and are important economically and as providers of ecosystem goods and services. According to the American Sportfishing Association [5] there are 49.4 million recreational anglers in the United States. Of these, 40.5 million identified as freshwater anglers. These anglers supported 826,000 jobs and had an economic output of $129B USD in 2020 [5]. In addition, commercial fisheries and aquaculture facilities generate additional jobs and economic output. In 2017, commercial fishing supported 1.25 million jobs with $244.1B in sales and $69.2B in value added [6]. Aquaculture production in 2018 generated an additional $1.5B [6].

Fisheries rely on suitable water quality to sustain reproduction, recruitment, and survival. Anthropogenic impacts such as land use changes; oil, gas and mineral extraction; and climate change can contribute to altered aquatic habitat and compromise water quality creating imbalances in nutrients, altered ion balance, and altered pH which can facilitate harmful algal blooms [7–9]. In freshwater such alterations have led to outbreaks of the golden algae *Prymnesium parvum* throughout much of the southern, southwestern, and plains states [8]. Such outbreaks can lead to significant aquatic life kills in lakes, rivers and streams worldwide [10–12]. In one study, *P. parvum* fish kills in Texas were conservatively estimated to have killed 34 million fish resulting in lost revenue from recreational fishing and tourism of $13M [13]. Blooms in Norway were responsible for the loss of 750 metric tons of caged salmon and significant loss to the aquaculture industry [14].

Golden algae are a single-celled brackish, or marine algae that can produce toxins that are lethal to fish and aquatic life. The genus *Prymnesium* is found in coastal or inland waters of all continents except Antarctica [15]. Since 1990, *P. parvum* has spread across the southern U.S. [16] and more recently has been discovered in northern states, particularly in disturbed watersheds [17] (Figure 1). Although their natural range is coastal, anthropogenic disturbance such as mining and hydraulic fracturing can create salty, eutrophic waters allowing *P. parvum* range expansion [18]. Mixotrophic *P. parvum* perform photosynthesis, but when nutrients are limited or out of balance it can produce several toxins that kill fish and other aquatic organisms, as well as algal competitors, which release nutrients that *P. parvum* can consume [8].

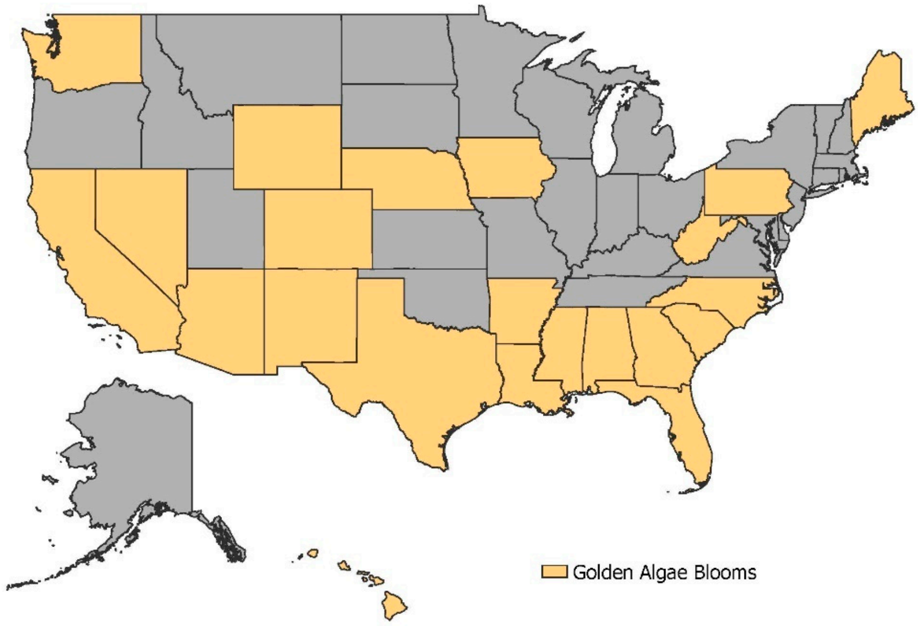

Figure 1. Spatial distribution of *Prymnesium parvum* blooms in the U.S. (yellow shaded states).

Prymnesium parvum have been reported to grow at a range of physiochemical conditions. Early studies [19] found *P. parvum* can live at a wide range of salinity representing nearly fresh to seawater conditions (0.5 to 45.0 PSU), and from 5 to 35 °C. In freshwater, *P. parvum* has been associated with elevated levels of specific conductivity and associated ions such as sulfate and chloride [9,20]. In Texas where *P. parvum* fish kills have become commonplace, damaging fisheries in reservoirs, rivers, and hatcheries, temperatures of 10 −27 °C have been associated with kills [19,21]. Researchers have reported blooms of *P. parvum* at conductivities of 2000 uS/cm (~1 PSU) [9] and at salinity above 0.5 PSU [22,23]. Laboratory studies with the *P. parvum* strain responsible for a fish kill in Dunkard Creek (along the Pennsylvania-West Virginia border) were able to grow at 500–2700 uS/cm (sulfate and chloride concentrations), but a treatment at ~4082 uS/cm was not conducive to growth [20]. Once established in a waterbody, *P. parvum* can produce cysts that can remain dormant until suitable conditions return and under the right conditions they can bloom, potentially creating toxins that will kill most aquatic life [24]. However, blooms do not always result in *P. parvum* producing fish kills. Nutrient imbalances (e.g., unbalanced/low N:P), alkaline waters (pH > 7.0), and high chloride and cation levels (e.g., Ca^{2+} and Mg^{2+}) are cited as contributing to toxicity of *P. parvum* blooms [18,25–27].

While *P. parvum* blooms and associated fish kills have been observed in Texas and other states since the mid-1980s [28] they have only recently become a harmful algae bloom (HAB) of concern in the more northerly states [29]. The geographic distribution of *P. parvum* includes at least 23 states as far north as Maine and Washington and including Hawaii [8] (Figure 1). However, throughout the eastern continuous U.S. and the western corn belt, numerous sites have recorded conductivity sufficiently high (>1000 uS/cm) to support *P. parvum* [30]. We found no published peer-reviewed information on *P. parvum* fish kills and their ecosystem response in more northerly locations. Given that introduction of *P. parvum* is occurring in new locations within the U.S. and numerous sites are already sufficient to support the species, we had two objectives in this paper: (1) to document the impact and recovery of a *P. parvum* aquatic life kill in the Ohio River drainage, and (2) to use existing georeferenced water quality databases to show water quality impairments that could support *P. parvum* and hence, are at risk for colonization and/or fish kills.

Genetic studies of the brackish water *P. parvum* in inland waters of the U.S. showed strains originated in Europe [31]. This suggests invasions have been recent, perhaps naturally via airborne or oceanic currents, or anthropogenically via ballast water or international aquaculture trade [32]. Within the U.S., *P. parvum* is likely spread by airborne currents, birds, and anthropogenic vectors such as recreational vessels, water tankers and drilling equipment [17,33].

2. Dunkard Creek Case Study

2.1. Study Area

We present a case study of an aquatic life kill associated with elevated stream conductivity and a golden algae bloom, from the discovery of the kill through the status of species recovery 10 years later. Dunkard Creek is a low gradient stream (0.05%) that originates at the confluence of the West Virginia Fork and Pennsylvania Fork near the community of Brave, in Greene County Pennsylvania (river kilometer 26.4, Figure 2). The stream meanders back and forth between West Virginia and Pennsylvania before exiting West Virginia for the last time 1.6 km southwest of Mt. Morris, Pennsylvania (Figure 2). For the purposes of this case study, we included the West Virginia Fork of Dunkard Creek in our river kilometer (RKM) structure beginning at the St. Leo mine outfall at RKM 40.3. After leaving West Virginia, Dunkard Creek flows another 32 km through Pennsylvania to the Monongahela River (Ohio River drainage).

2.2. Background

The Dunkard Creek Watershed is heavily influenced by current and legacy coal mining and natural gas fracking activities. Anthropogenic impacts to the Dunkard Creek watershed are evident in water quality data gathered by West Virginia Department of Environmental Protection (WVDEP) since the 1970s (Figure 3) at a station near the confluence of Doll Run (RKM 4.3; Figure 2). Between 15 June 1976 and 17 August 2009 (pre-kill), median specific conductance was 486 uS/cm (mean 677) exceeding the Central Appalachian Region recommended benchmark for impairment of 300 uS/cm recommended by the U.S. Environmental Protection Agency (USEPA) [34]. Coincident with the fish kill, between 09 and 23 September 2009, poor water quality spiked. Specific conductivity (mean 2435 uS/cm) and sulfates (mean 812 mg/L) were significantly higher than the pre-kill and post-kill (19 October 2009–21 December 2010) conditions (Figure 3). Chlorides and pH during the kill were 8–44 mg/L and 7.8–8.9, respectively and not significantly different than other periods.

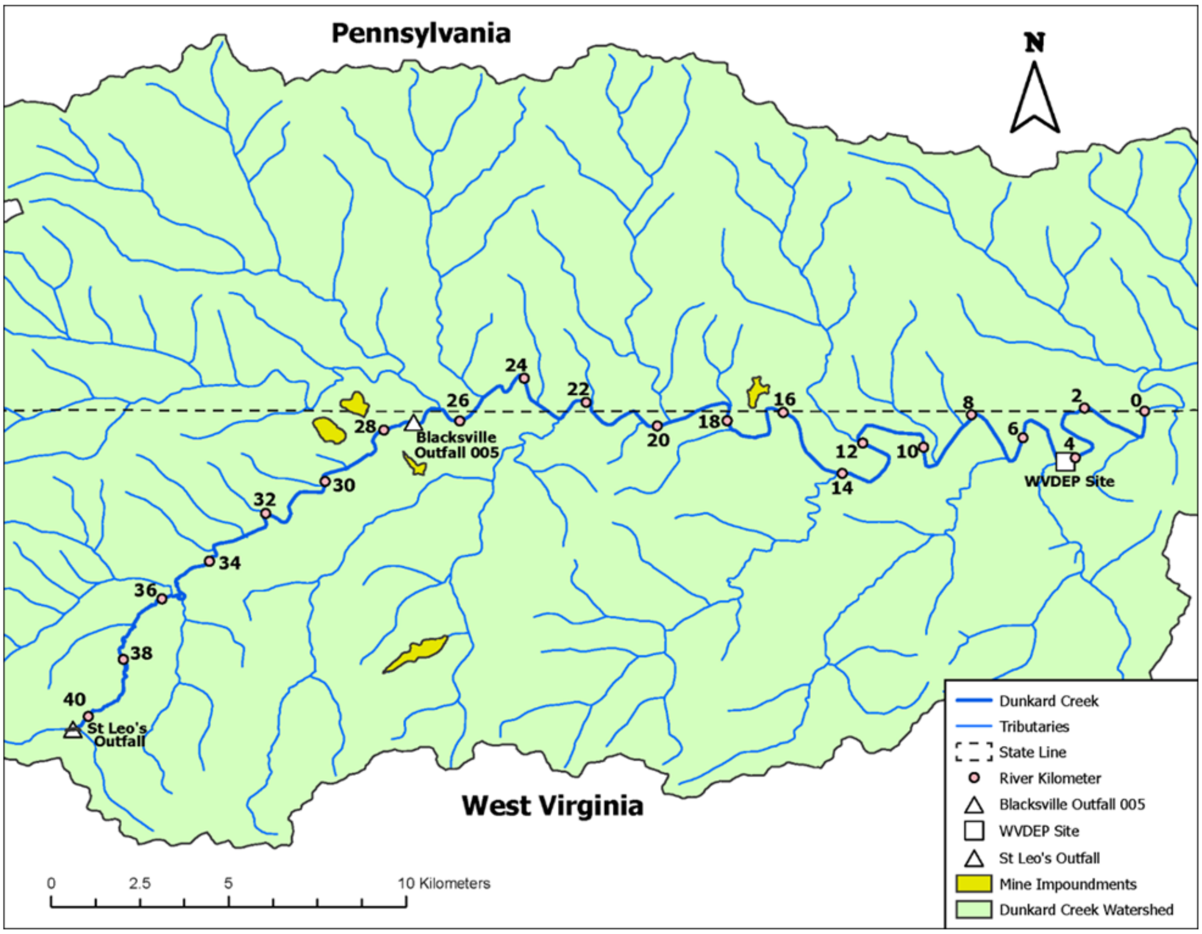

Figure 2. Location of the case study on Dunkard Creek, WV-PA) documenting the aquatic life kill and subsequent recovery over 10 years (2009 to 2019). Included are NPDES permits for surface and ground water discharge from several pumped mine treatment stations (Consol Energy outfall 005 at Blacksville No. 2 mine and Consol Energy St. Leo mine outfall), slurry impoundments, two active deep coal mines (Pentress and Blacksville #2). Distances in river kilometers moving upstream from where Dunkard Creek exits West Virginia for the last time are shown to identify locations related to water quality and fish sampling. A long term WVDEP water quality monitoring station is shown by the square.

The West Virginia Division of Natural Resources (WVDNR) conducted fish surveys on Dunkard Creek 18 times between 1959 and 2009, collecting 44 different fish species. Of these, 13 species were game fishes including Smallmouth Bass *Micropterus dolomieu* and Muskellunge *Esox masquinongy*. These metrics made Dunkard Creek one of the most diverse fish communities in the region for its size.

Prior to the 2009 kill, Dunkard Creek supported the most diverse and abundant freshwater mussel populations in the Monongahela River watershed. From 1919 through 2018, 21 native mussel species were collected. Species such as the Purple Wartyback (*Cyclonaias tuberculata*), Black Sandshell (*Ligumia recta*), and Clubshell (*Pleurobema clava*) were collected only during the early 1900s but were since extirpated. Dunkard Creek also supported one of the last known Snuffbox (*Epioblasma triquetra*) populations in the Monongahela River drainage, which has since been listed as federally endangered following the 2009 Dunkard Creek aquatic life kill.

The Dunkard Creek investigation was triggered by the recording of a very high specific conductivity measure at the Consol Energy outfall 005 at the Blacksville No. 2 mine, hereafter *Blacksville* 005 *outfall* at RKM 27.0 and subsequent reports of dead fish in the vicinity of Pentress, WV at RKM 13.0 (Figure 2). On 27 August 2009, the WVDNR recorded conductivity of over 50,000 uS/cm at the Blacksville 005 outfall discharge into the West Virginia Fork of Dunkard Creek (RKM 27.0). Conductivity was also elevated just upstream of the No. 2 discharge (4266 uS/cm at RKM 35.3 and 2660 at RKM 30.0) and was diluted downstream of the discharge to ~5000 uS/cm. These high conductivities limited electrofishing efficiency, so WVDNR conducted fish surveys using seines and found live fish at all 3 sites and species richness of 19–22 species.

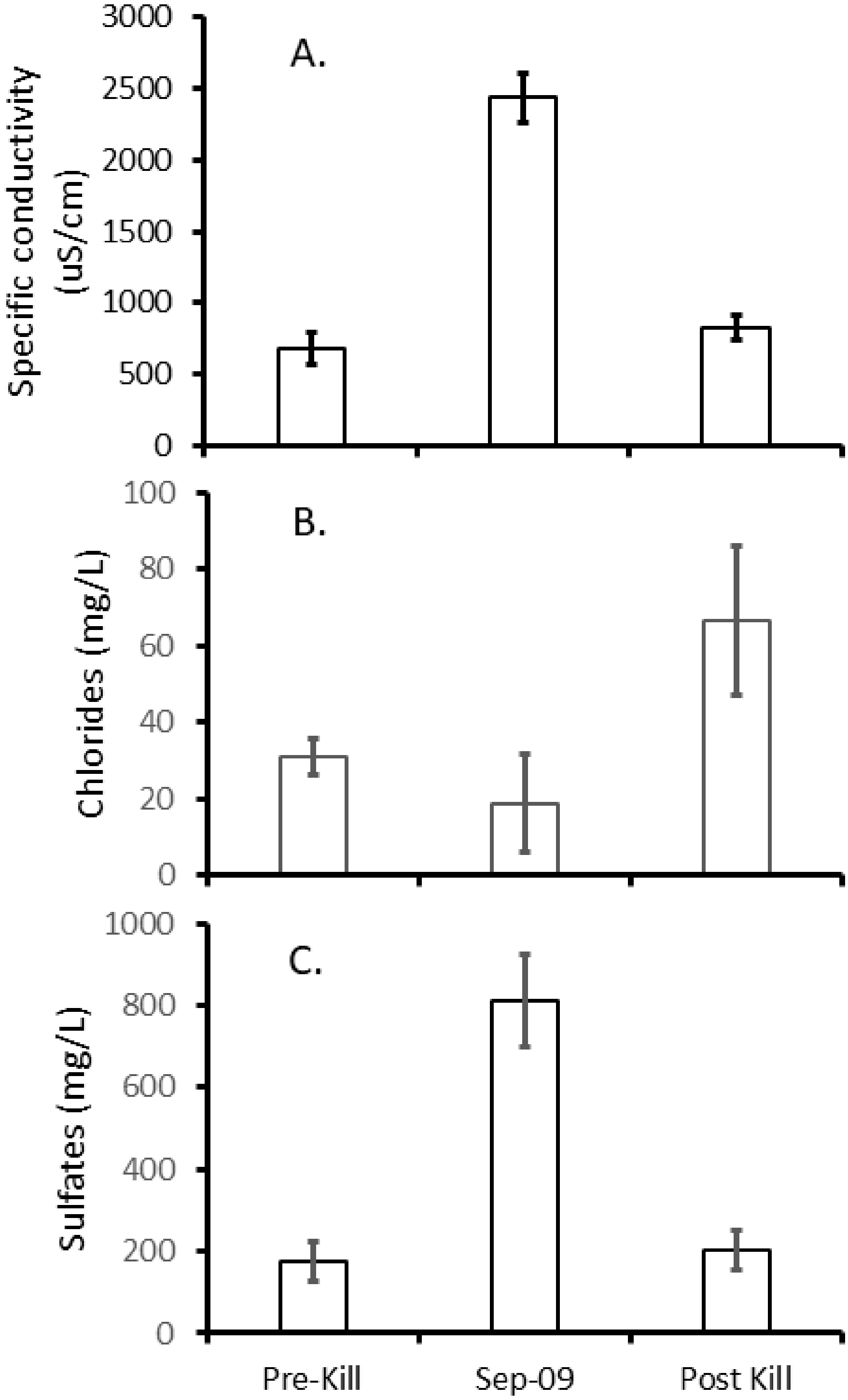

Figure 3. Water quality conditions at RKM 4.3 on Dunkard Creek during the pre-kill period (15 June 1976–17 August 2009), the kill (9–23 September 2009), and post-kill (19 October 2009–21 December 2010). Shown are specific conductivity (**A**), chlorides (**B**) and Sulfates (**C**). Error bars represent 95% confidence limits on mean values.

Dead fish were reported by the public in the vicinity of Pentress, WV (~RKM 13) and confirmed by WVDEP on 1 September 2009, launching the fish kill investigation. Multiple state agencies and the USEPA responded to the water quality and aquatic life kills in Dunkard Creek. Site surveys by WVDNR, on 2–4 September 2009 found dead fish from RKM 24.7 to RKM 1.0 and noted live fish from RKM 12.0 upstream to RKM 35.5. However, between 21–25 September 2009, dead fish were found throughout the West Virginia portion of Dunkard Creek. The fish kill continued into early October. Overall, the WVDNR investigated aquatic life kills at 44 sites with 22 observation days. Pennsylvania Fish and Boat Commission (PFBC) conducted multiple aquatic life kill assessments on reaches within Pennsylvania.

Water quality and a golden algae bloom appeared to play a part in the aquatic life kill. Water quality was measured by the WVDEP, USEPA and by West Virginia University (WVU) during the kill. Specific conductivity was measured at 9 sites by WVDEP from RKM 0–27.0 on 2–4 September 2009. Conductivity ranged from 33,800 uS/cm immediately downstream of the Blacksville 005 outfall (RKM 27.0) to 2280 uS/cm at RKM 0. On 9 September 2009 the USEPA collected water quality information at 10 sites from RKM 4.5 to RKM 27.0. Specific conductivity ranged from 2257–5085 uS/cm in the Dunkard Creek mainstem and was over 18,000 uS/cm in the vicinity of the Blacksville 005 outfall (Figures 2 and 4; Table 1). WVDEP collected water quality measures at 16 sites between 9 to 22 September 2009. WVU collected conductivity measures at 17 sites on 28 September 2009. In general, during September 2009 specific conductivity declined moving downstream from RKM 40.5 (St. Leo mine outfall) until RKM 27.0 where inputs from the Blacksville 005 outfall caused specific conductivity to increase (Figure 4).

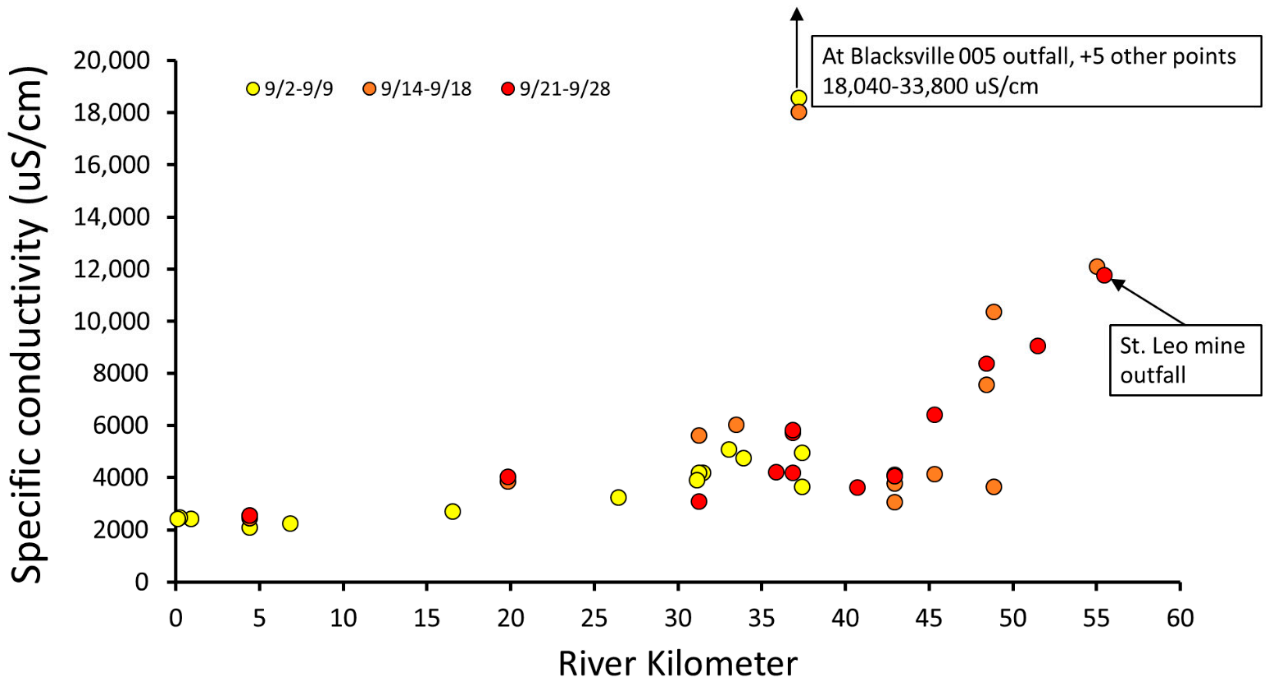

Figure 4. Specific conductivity moving upstream from RKM 0 (where Dunkard Creek exits West Virginia for the last time). Point sources from 2 treated, pumped deep (coal) mine facilities are evident by high specific conductivity at RKM 27 (Blacksville 005 outfall) and RKM 40.5 (St. Leo mine outfall). Five other readings of 18,040 to 33,800 uS/cm at RKM 26.9 and RKM 27.0 are not shown to allow better resolution in the specific conductivity measures at other locations to be shown.

Treated mine water discharge from the Blacksville 005 Outfall (RKM 27.0) and a second site at the St. Leo mine outfall at RKM 40.3 appeared to be the source of high conductivity in Dunkard Creek during this time that produced conditions favorable for *P. parvum* (Figure 4). A fly over by WVDEP detected a golden algae bloom on Dunkard Creek from the confluence with the Monongahela River upstream and throughout the

South Fork of the West Virginia Fork of Dunkard Creek on 18 September 2009. Water samples collected during September identified the bloom as *Prymnesium parvum*. Density of *P. parvum* ranged from 460–345,320 cells/mL at various locations along Dunkard Creek (WVDEP unpublished data). Subsequent tests conducted at the University of North Carolina-Wilmington using *P. parvum* isolated from Dunkard Creek water samples proved it was capable of toxic affects to aquatic organisms [35].

The *P. parvum* cell density required to cause fish mortality varies in the literature, but was certainly exceeded in the Dunkard Creek kill. Sager et al. [27] found *P. parvum* could cause fish kills at densities of 10,000 cells/mL but were more common at 20,000 cells/mL in Texas. Rodgers et al. [36] found toxic effects to Fathead Minnow larvae (*Pimephales promelus*) at 14,000 cells/mL but not at 3800 cells/mL. WVDEP samples collected on 20 September 2009 found *P. parvum* densities of over 94,600 cells/mL at five sites over a 33 km stretch of Dunkard Creek. Pennsylvania Department of Environmental Protection sampled the Pennsylvania waters of Dunkard Creek at 9 sites on 30 September and 1 October 2009 and found 108,000–269,250 cells/mL. They sampled one site on the PA Fork upstream of the WV Fork and found 6250 cells/mL. Collectively these samples provide a glimpse of the cell densities of *P. parvum* present in Dunkard Creek in relation to specific conductivity (Figure 5).

Table 1. Water quality measures recorded by the USEPA at Dunkard Creek on 9 September 2009 during the aquatic life kill investigation. Two sites with RKM listed as "n/a" were tributaries originating in Pennsylvania. Of note are the supersaturated dissolved oxygen measures (bold) at 4 sites suggesting the presence of an algal bloom at least 9 days before a flyover of Dunkard Creek first detected the *P. parvum* bloom.

RKM	Location	Temp_C	Sp.Cond (uS/cm)	DO_mg/L	DO % Sat	pH
5.1	Dunkard Creek upstream of Dolls Run	19.7	2257	8.67	95.3	8.28
12.0	Dunkard Creek in Pentress	20.1	2714	13.93	**154.8**	8.37
19.0	Dunkard Creek in Blacksville	19.5	3259	9.52	104.7	8.2
22.7	Dunkard Creek downstream of Miracle Run	19.4	3911	8.85	97.3	8.13
24.0	Dunkard Creek upstream of Morris Run	20.67	5085	10.36	**117.4**	8.39
n/a	Hoovers Run (tributary to Dunkard)	18.8	770	8.64	92.8	8.45
n/a	PA Fork Dunkard at T309 Bridge	18.95	672	7.88	84.7	8.02
27.1	WV Fork Dunkard downstream of Consol Outfall	21.93	18,570	13.45	**165.8**	8.17
27.0	Consol Outfall 005 WV 0064602	22.64	25,250	8.34	105.3	8.55
26.9	WV Fork Dunkard upstream of Consol Outfall	20.62	4957	11.54	**130.7**	8.13

2.3. Aquatic Life Sampling

Fish kill investigations by agencies estimated 21,360 fish representing at least 20 species in West Virginia and 42,997 of 40 species of fish in Pennsylvania were lost during the kill [37]. To illustrate the damage caused by the *P. parvum* bloom and its subsequent response, we present fish collection data from a site in the mainstem of Dunkard Creek. The WVDNR sampled the Pentress site (RKM 12.6) prior to the fish kill in 1983, 1997, and 2005. It was sampled in 2009 immediately following the fish kill and four times since the kill (2010, 2014, 2016, and 2019). Fish sampling was done by rotenone application in 1983 and 1997 and the West Virginia parallel wire electrofishing method [38] in subsequent years. Due to the differing methods of collection, numbers of fish caught were presented as presence/absence, and standardized to the 2005 abundance for each species collected using the following equation:

$$\mathrm{Ni_{std}} = \mathrm{Ni_x}/\mathrm{N_{2005}}, \quad (1)$$

where $\mathrm{Ni_{std}}$ is the standardized abundance of species i, $\mathrm{Ni_x}$ is the number of species i in year x, and $\mathrm{Ni_{2005}}$ is the abundance of species i in 2005. Using this formula all standardized abundance values in 2005 are 1.0 and values >1 or <1 represent greater or lesser abundance respectively. We also calculated species richness of fishes collected at this site as another metric of community structure.

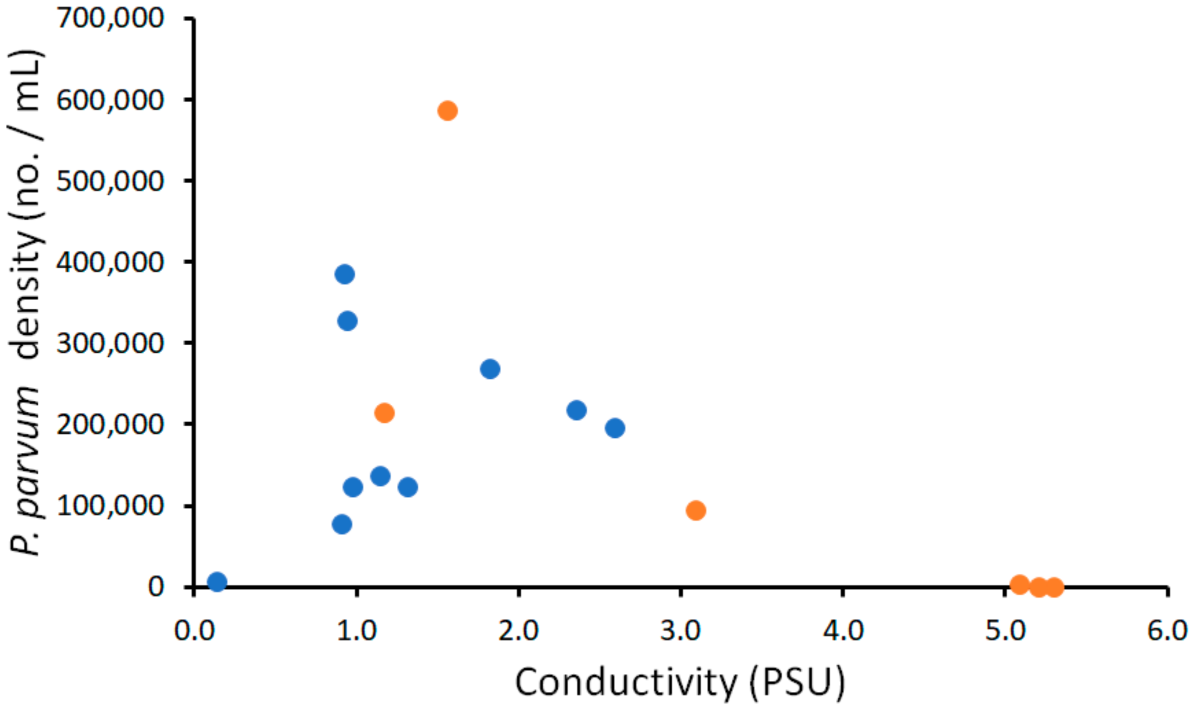

Figure 5. Microscope counts of *P. parvum* cell densities relative to water sample conductivity in waters of Dunkard Creek during the golden algae bloom and associated aquatic life kill in September 2009. Orange symbols represent samples collected by WVDEP and blue symbols were collected in the Pennsylvania waters by Pennsylvania Fish and Boat Commission. Highest cell densities were associated with 1–2 PSU.

Dunkard Creek supported the greatest diversity of freshwater mussels in the Monongahela River drainage prior to the 2009 kill. Fourteen mussel surveys conducted in the West Virginia waters of Dunkard Creek between 1919 and 2018 showed 21 native mussel species present prior to the 2009 kill (Table 2). Data on the mussels prior to 2009 came from a variety of sources including review of museum collections and collections from agencies and environmental consulting firms. Data from 2009 were collected after the kill and documented what had been present at the time of the toxic event. Over 40,000 mussels were estimated killed by the bloom. The WVDNR estimated that 25,000 mussels of 17 species died following the *P. parvum* bloom including the now, federally endangered Snuffbox mussel (*Epioblasma triquerta*) [39]. Pennsylvania estimated 15,323 mussels were killed including several state-listed species [40].

Table 2. Presence (X) and absence of 21 mussel taxa recorded in the West Virginia portion of Dunkard Creek (Monongahela River drainage) from surveys. Extensive surveys immediately following the toxic event in 2009 yielded live or recently dead mussels of 16 taxa. Tagged adult mussels were stocked from 2012–2015, but there has been no recruitment since prior to the kill and declining numbers of tagged mussels since the stocking. Mussel recruitment has not been detected in Dunkard Creek surveys from 2010–2018. Species stocked since 2009 are noted by "S" below.

Species	Common Name	Year						
		1919	1969–1980	1992–1997	2000–2007	2009	2010–2018	Stocked 2010–2018
Amblema plicata	Threeridge	X	X	X	X	X		S
Cyclonaias tuberculata	Purple Wartyback	X						
Epioblasma triquetra	Snuffbox	X	X	X	X	X		
Eurynia dilatata	Lady Finger	X	X	X	X	X		
Fusconaia flava	Wabash Pigtoe	X	X	X	X	X		S
Lampsilis cardium	Plain Pocketbook	X	X	X	X	X		S
Lampsilis fasciola	Wavyrayed Lampmussel		X					S
Lampsilis siliquoidea	Fatmucket	X	X	X	X	X		S
Lasmigona costata	Fluted-Shell	X	X	X	X	X		S

Table 2. *Cont.*

Species	Common Name	Year						
		1919	1969–1980	1992–1997	2000–2007	2009	2010–2018	Stocked 2010–2018
Leptodea fragilis	Fragile Papershell					X		S
Ligumia recta	Black Sandshell	X						
Pleurobema clava	Clubshell	X						
Pleurobema sintoxia	Round Pigtoe	X	X	X		X		S
Potamilus alatus	Pink Heelsplitter	X				X		S
Ptychobranchus fasciolaris	Kidney Shell	X	X	X	X	X		S
Pyganodon grandis	Giant Floater	X	X	X	X	X		S
Simpsonaias ambigua	Salamander Mussel		X	X	X			
Strophitus undulatus	Creeper	X	X	X	X	X		S
Tritogonia verrucosa	Buckhorn		X	X		X		S
Utterbackia imbecillis	Paper Pondshell			X		X		S
Villosa iris	Rainbow-Shell	X	X	X		X		S
Species Richness		16	15	15	11	16	0	15

A settlement with Consol Energy led to the establishment of a restoration plan for Dunkard Creek fish and mussels in 2011 that called for monitoring, as well as tagging and stocking adult mussels and host fish inoculated with glochidia (larval mussels) from nearby watersheds [39]. Mussel monitoring events in 2011, 2015, and 2018 are based on 3 random start methodology with 0.25 m^2 excavations, usually about 100 per site for 4 sites [40]. Visual qualitative mussel surveys were also conducted following excavations (6 × 10 min).

3. Results

3.1. Fish Community Recovery

The events associated with the *P. parvum* bloom in September 2009 resulted in nearly complete mortality of all fishes and mussels in the West Virginia waters of Dunkard Creek (Tables 2–4; Figure 6). A survey conducted a month post-kill resulted in only 19 individual fish collected (Table 3). In contrast, in all other years, the average catch was 5745 fish. Thus, approximately 99.7% of the fish normally at this site were killed. Taxa richness followed similar trends (Figure 6). Ten minnow species were commonly collected and dropped to 3 species in 2009. Sucker species dropped from 5 taxa before the kill to 1. Darters, game fishes and others disappeared after the kill.

Table 3. Presence (1) and absence (0) of fish species collected at the Pentress site on the West Virginia portion of Dunkard Creek. Fish survey data were summarized between 1983–2005 (pre-kill, n = 3), in 2009 following the P. *parvum* bloom, and between 2010-2019 (post-kill, n = 4). Here, Total is the cumulative number of each species collected across all sample dates.

Taxa	Common Name	1983–2005	2009	2010–2019	Total
Darters					
Etheostoma blennioides	Greenside Darter	1	0	1	2508
Etheostoma caeruleum	Rainbow Darter	1	0	1	463
Etheostoma flabellare	Fantail Darter	1	0	1	324
Etheostoma nigrum	Johnny Darter	1	0	1	161
Etheostoma variatum	Variegate Darter	1	0	1	1800
Etheostoma zonale	Banded Darter	1	0	1	1049
Percina caprodes	Logperch	1	0	1	275
Percina maculata	Blackside Darter	1	0	1	74
Minnows					
Campostoma anomalum	Central Stoneroller	1	1	1	5808
Cyprinella spiloptera	Spotfin Shiner	1	0	1	1140
Ericymba buccata	Silverjaw Minnow	1	0	1	329
Labidesthes sicculus	Brook Silverside	1	0	1	85
Luxilus chrysocephalus	Striped Shiner	1	1	1	1796
Notemigonus crysloeucas	Golden Shiner	0	0	0	1

Table 3. *Cont.*

Taxa	Common Name	1983–2005	2009	2010–2019	Total
Notropis atherinoides	Emerald Shiner	1	0	1	388
Notropis photogenis	Silver Shiner	1	0	1	26
Notropis rubellus	Rosyface Shiner	1	0	1	1394
Notropis stramineus	Sand Shiner	1	0	1	4019
Notropis volucellus	Mimic Shiner	1	0	1	3703
Pimephales notatus	Bluntnose Minnow	1	1	1	9598
Semotilus atromaculatus	Creek Chub	1	0	1	273
Suckers					
Carpiodes cyprinus	Quillback	1	0	1	2
Catostomus commersoni	White Sucker	1	0	1	423
Hypentelium nigricans	Northern Hogsucker	1	1	1	1839
Moxostoma anisurum	Silver Redhorse	0	0	1	203
Moxostoma duquesnei	Black Redhorse	1	0	1	167
Moxostoma erythrurum	Golden Redhorse	1	0	1	357
Game Fishes					
Ambloplites rupestris	Rock Bass	1	0	1	88
Ameiurus natalis	Yellow Bullhead	1	0	1	48
Esox masquinongy	Muskellunge	1	0	1	2
Ictalurus punctatus	Channel Catfish	0	0	1	16
Lepomis cyanellus	Green Sunfish	1	0	1	34
Lepomis gibbosus	Pumpkinseed	1	0	1	24
Lepomis macrochirus	Bluegill	1	0	1	120
Micropterus dolomieu	Smallmouth Bass	1	0	1	234
Micropterus salmoides	Largemouth Bass	0	0	1	17
Pylodictus olivaris	Flathead Catfish	1	0	0	11
Other					
Noturus flavus	Stonecat	1	0	1	299

Table 4. Relative abundance of fish species at Pentress on Dunkard Creek from 1983 to 2019. Rotenone was used for collections in 1983 and 1997 while those in 2005–2019 were made with a parallel wire electrofishing technique. To standardize gears, all catches were standardized relative to the 2005 catches. Seven species in 2005 were not collected but were collected in other years. In those cases, the actual number collected in a year are reported as **N* where N is the number collected.

Taxa	Common Name	2005 Base Abundance	1983	1997	2009	2010	2014	2016	2019
Darters									
Etheostoma blennioides	Greenside Darter	82	1.37	6.98	0.00	2.66	2.33	7.37	8.89
Etheostoma caeruleum	Rainbow Darter	49	0.39	2.71	0.00	0.88	0.82	2.16	1.49
Etheostoma flabellare	Fantail Darter	7	17.86	17.86	0.00	2.43	1.43	2.29	3.43
Etheostoma nigrum	Johnny Darter	6	4.00	2.17	0.00	4.17	3.17	3.50	8.83
Etheostoma variatum	Variegate Darter	78	0.32	5.27	0.00	1.71	1.08	5.53	8.18
Etheostoma zonale	Banded Darter	97	0.11	4.64	0.00	0.05	0.28	1.07	3.66
Percina caprodes	Logperch	20	5.15	5.35	0.00	0.60	0.20	1.10	0.35
Percina maculata	Blackside Darter	5	3.40	3.60	0.00	1.60	2.40	1.80	1.00
Minnows									
Campostoma anomalum	Central Stoneroller	1902	0.12	0.62	0.00	0.28	0.22	0.43	0.39
Cyprinella spiloptera	Spotfin Shiner	100	0.00	3.15	0.00	0.21	0.41	3.72	2.91
Ericymba buccata	Silverjaw Minnow	12	0.00	3.92	0.00	4.25	3.67	12.83	1.75
Labidesthes sicculus	Brook Silverside	4	0.00	18.00	0.00	0.00	0.25	2.00	0.00
Luxilus chrysocephalus	Striped Shiner	121	3.98	3.18	0.09	0.70	1.03	1.88	2.97
Notemigonus crysloeucas	Golden Shiner	0	–	–	–	–	–	**1*	–
Notropis atherinoides	Emerald Shiner	0	**385*	–	–	**3*	–	–	–
Notropis photogenis	Silver Shiner	0	–	–	–	–	–	**2*	**24*

Table 4. *Cont.*

Taxa	Common Name	2005 Base Abundance	1983	1997	2009	2010	2014	2016	2019
Notropis rubellus	Rosyface Shiner	135	0.00	3.25	0.00	0.23	1.01	2.52	2.32
Notropis stramineus	Sand Shiner	121	6.10	5.63	0.00	0.77	7.37	7.88	4.47
Notropis volucellus	Mimic Shiner	66	0.00	29.12	0.00	1.88	1.45	8.14	14.52
Pimephales notatus	Bluntnose Minnow	1860	0.71	1.17	0.00	0.12	0.77	0.83	0.55
Semotilus atromaculatus	Creek Chub	17	3.76	3.71	0.00	3.00	2.41	0.94	1.24
Suckers									
Carpiodes cyprinus	Quillback	1	0.00	0.00	0.00	1.00	0.00	0.00	0.00
Catostomus commersoni	White Sucker	34	0.94	0.74	0.00	4.15	3.79	1.15	0.68
Hypentelium nigricans	Northern Hogsucker	240	0.58	0.89	0.00	0.74	1.63	1.17	1.64
Moxostoma anisurum	Silver Redhorse	2	0.00	0.50	0.00	50.50	46.00	0.50	3.00
Moxostoma duquesnei	Black Redhorse	15	0.00	1.07	0.00	0.27	0.67	7.60	0.53
Moxostoma erythrurum	Golden Redhorse	50	0.32	0.54	0.00	0.90	1.66	1.90	0.82
Game Fishes									
Ambloplites rupestris	Rock Bass	8	4.00	4.00	0.00	0.00	0.38	1.38	0.25
Ameiurus natalis	Yellow Bullhead	7	1.71	2.29	0.00	0.14	0.14	1.57	0.00
Esox masquinongy	Muskellunge	1	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Ictalurus punctatus	Channel Catfish	0	–	–	–	–	**1*	**4*	**11*
Lepomis cyanellus	Green Sunfish	1	12.00	14.00	0.00	0.00	0.00	7.00	0.00
Lepomis gibbosus	Pumpkinseed	0							
Lepomis macrochirus	Bluegill	1	112.00	0.00	0.00	1.00	1.00	4.00	1.00
Micropterus dolomieu	Smallmouth Bass	31	1.61	1.29	0.00	0.39	0.45	2.06	0.74
Micropterus salmoides	Largemouth Bass	0	–	–	–	**2*	**12*	**3*	–
Pylodictus olivaris	Flathead Catfish	0	–	**11*	–	–	–	–	–
Other									
Noturus flavus	Stonecat	5	2.40	47.00	0.00	0.60	0.80	4.00	4.00

Fish sampling in 2010 and since have shown that while many of the fish species have returned to Dunkard Creek since the kill, individual species have not necessarily recovered to pre-kill abundance (Table 4). Species richness (the number of individual species found) shows most species could again be found at the Pentress site by 2010 (Figure 6). However, not all individual species have fully recovered (Table 4). As of 2019, game species, which are of the most economic importance, have had lower abundance than pre-kill with the exception of 2016. Among the 8 darter species, 6 have recovered to pre-kill levels. Five of six sucker species and 7 of 10 common minnow species have also recovered to pre-kill abundance or higher (Table 4).

3.2. Mussel Community Recovery

While fish species have returned rather rapidly in Dunkard Creek following the HAB, the mussel community has not (Table 2). The intensive surveys in 2009, immediately following the kill showed 16 species of mussel had been present before the kill. In 2011, the WVDNR drafted a restoration plan that involved the release of inoculated host fish, translocation of adult mussels, and propagation of juvenile mussels. From 2012–2015, over 3000 host fish, mostly Bluegill (*Lepomis machrochirus*, $n > 3300$), but also some Freshwater Drum (*Aplodinotus grunniens*, $n = 56$), were inoculated and released into Dunkard Creek [36]. Glochidia were from *Lampsilis cardium*, *Lampsilis. siliquoidea*, *Lasmigona costata*, *Leptodea fragilis*, *Potamilus alatus*, *Pyganodon grandis*, and *Strophitus undulatus*. Adult mussels of these species were also translocated to the stream; in addition, adult mussels of *Alasmidonta marginata*, *Amblema plicata*, *Eurynaia dilatata*, *Fusconaia flava*, *Lampsilis fasciola*, *Pleurobema sintoxia*, *Ptychobranchus fasciolaris*, *Tritogonia verrucosa*, *Utterbackia imbecillis*, and *Villosa iris* were also translocated to Dunkard Creek. Subsequent surveys of potential monitoring and restoration sites within the impacted area of Dunkard Creek have been conducted. Despite the stocking, subsequent mussel surveys including intensive surveys in 2011, 2015, and 2018 have uncovered only previously translocated adults and there is still no sign of natural recruitment within the West Virginia portion of the stream.

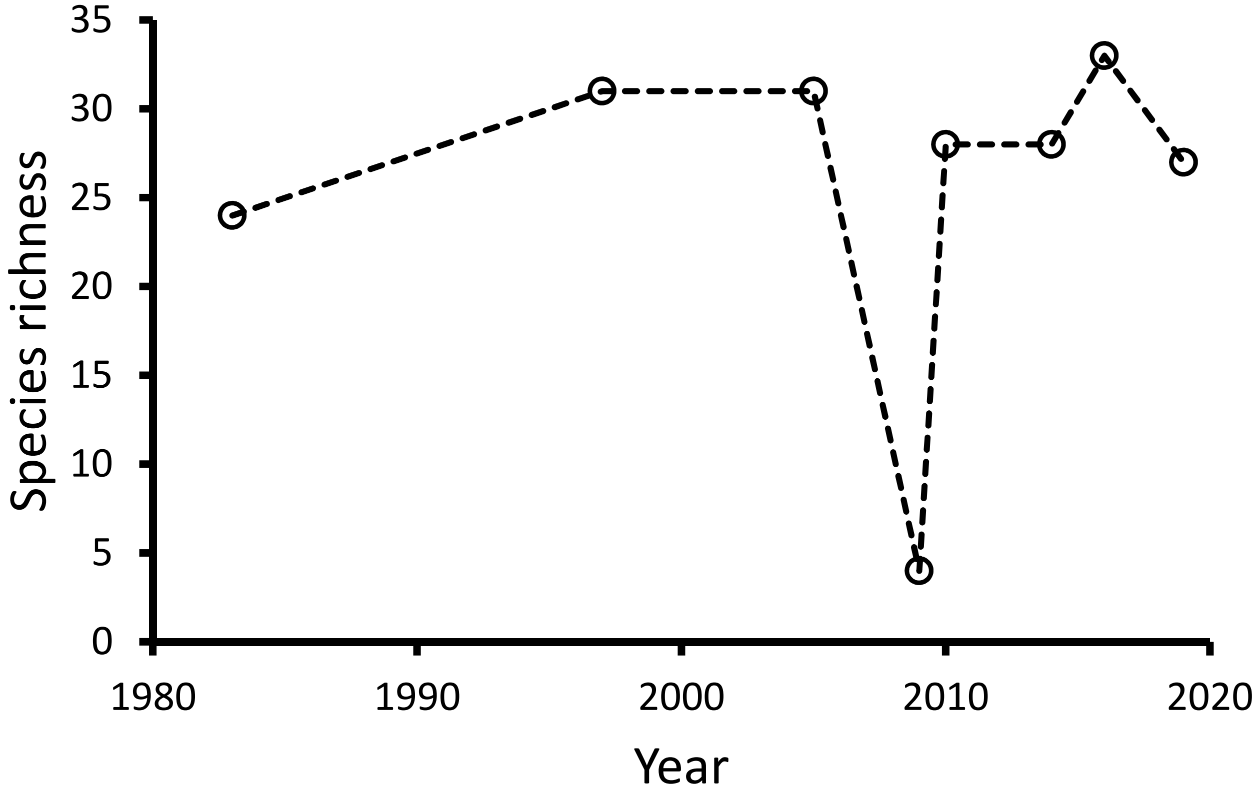

Figure 6. Fish species richness collected at the Pentress, WV site on Dunkard Creek 1983–2019. Only 4 species and 19 individuals were collected in 2009 following the *Prymnesium parvum* bloom.

3.3. *Spatial Analysis of Potential Prymnesium parvum Waters*

3.3.1. Water Quality Criteria and Justification

To understand the potential for *P. parvum* to spread and potentially impact the blue economy in the U.S. we utilized several databases that contained information on key water quality variables that relate to that species habitat requirements. We used the stream and river data from USGS National Water Information system (https://waterdata.usgs.gov/nwis/current/?type=quality; accessed on 3 October 2021) and WQX/STORET (https://www3.epa.gov/storet/wqx/wqx_downloads.html; accessed on 3 October 2021). For the national maps we restricted the data to include the maximum values for the period 1 May to 31 October for 2015–2020 for each site. This was done to capture the most recent warm season water quality and quantity conditions available at a time of year when nutrient imbalances would be expected to occur in most waterbodies [41–43].

We also used a state level database for West Virginia from the WVDEP that includes data from 2015–2020. State data was restricted to maximum values from May–October across years for each site. Together these sources were used to screen physiochemical parameters to identify areas that could support *P. parvum* and thus may be susceptible to future HABs. Our assumption was that if physiochemical values at a site were within ranges supporting *P. parvum* that they could be present, or could become established there, and under favorable conditions could produce a toxic bloom.

Previous research has identified the water quality conditions required by *P. parvum*, and to a lesser extent, the conditions related to toxic blooms. In our ecological risk assessment, we considered specific conductivity to be the primary variable driving habitability of waters. In Dunkard Creek during September 2009 sampling, *P. parvum* were detected at low levels (6250 cells/mL) at 0.14 PSU, but cell densities increased rapidly around 0.9 PSU (about 1600 uS/cm, see Figure 5). A more complete study of Texas Lakes found minimum mean salinities of about 1200 uS/cm specific conductance were associated with *P. parvum* blooms [27]. Based upon this we established a threshold value of 1500 uS/cm conductivity in our evaluation of ecological risk for *P. parvum* in the U.S. lotic waters. If a site in the database exceeded the threshold it was considered at risk for *P. parvum*. The golden algae could exist in systems with lower specific conductivity, but the 1500 uS/cm threshold represented a conservative estimate of ecological risk.

The ecological risk assessment considered other environmental conditions that appear related to *P. parvum* blooms. In a CART analysis of Texas reservoirs, Patino et al. [22] determined that *P. parvum* blooms only occurred when chloride levels exceeded 228 mg/L. Chloride levels in Dunkard Creek during the bloom ranged from 215–702 mg/L, similar to the threshold found by Patino et. al. [22]. Based on this we set the threshold of risk for chlorides at 228 mg/L. *Prymnesium parvum* prefers alkaline waters [18,44,45]. Growth of *P. parvum* at pH < 7.0 depends on the concentration of metal ions with increases in Fe and Mg ions compensating for lower pH [9,44]. Field and laboratory studies have documented increased growth rates of *P. parvum* in alkaline waters ≥ 7.5 [26]. In Dunkard Creek pH levels were typically >7.5 during September 2009. Therefore, we established a threshold of pH ≥ 7.5 for risk of *P. parvum* blooms.

The ecological risk assessment classified sites as No risk, Moderate risk, High risk, or Severe risk for *P. parvum* based upon whether data for a site met thresholds for these three parameters. Sites with maximum specific conductivity <1500 uS/cm were considered as No risk under the water quality conditions recorded in the databases. If a site was ≥1500 µS/cm we considered it at Moderate risk for *P. parvum*. Sites that were ≥1500 µS/cm and either exceeded chloride level threshold (228 mg/L) or had pH ≥ 7.5 were classified as High risk. Sites for which all three criteria thresholds were exceeded were considered at Severe risk for *P. parvum*. We created two spatial maps showing risk levels for *P. parvum* at the national level, and at the West Virginia level using a WVDEP database.

3.3.2. Spatial Mapping of Water Quality to Identify Risk Levels for the Golden Algae *Prymnesium parvum*

The national dataset using only conductivity as criteria for *P. parvum* risk contained data from 52,810 stream/river sites. Of the 52,810 sites, 5217 displayed a maximum specific conductivity of 1500 uS/cm or greater. Thus, 9.88% of all sites reported conductivity high enough to support *P. parvum* between May and October (Figure 7). Of the sites exceeding the conductivity threshold, 3585 sites (6.79% of all sites) exceeded either the pH or chloride threshold and 1036 (1.96% of all sites) were rated as severe risk by exceeding all three thresholds. The majority of Severe risk sites were clustered in Texas, Oklahoma, and the southwestern U.S., although Severe risk sites could be found in 30 of the states (Figure 7).

Applying these same criteria and thresholds to the WVDEP data highlights how potentially under-represented the risk of invasion or presence of *P. parvum* is based on the national datasets (Figure 8). The WVDEP data includes 2358 sites that contain all three of our parameters of interest, conductivity, pH, and chloride. Of these, 2252 (95.5%) were ranked as No Risk and 106 (4.50%) showed conductivity levels greater than 1500 (µS/cm). Overall, 4.0% of sites were evaluated as High or Severe risk in the ecological risk assessment using the state database.

Although the WVDEP-based ecological risk assessment returned a lower percentage of sites considered at risk for *P. parvum* than the national database, more sites in the southern part of West Virginia were identified as at risk. In comparison, the national database included 425 sites in West Virginia, only 8 of which (1.88%) were ranked as High or Severe risk. Thus, the state level data returns over twice the incidence of elevated risk locations than the national data for West Virginia. It is reasonable to assume that other states are housing similar datasets that may be of future use evaluating HAB potential.

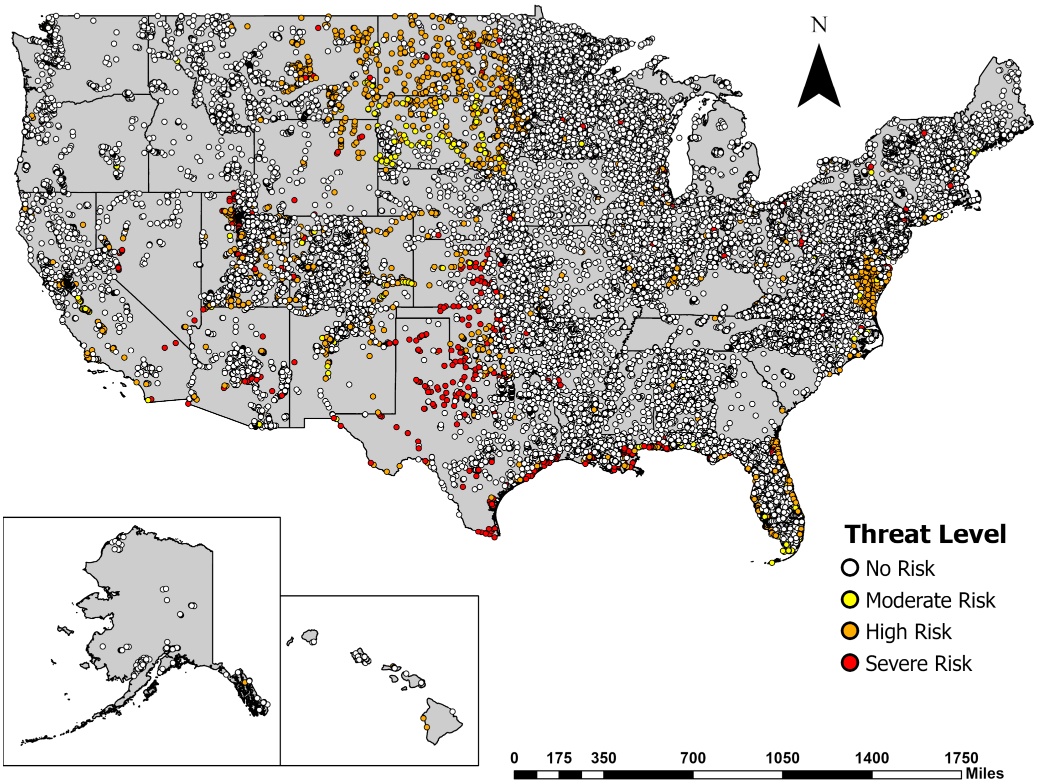

Figure 7. Map of the U.S. showing the ecological risk of *Prymnesium parvum* based upon water quality criteria. Only stations with data for specific conductivity from warm season (May–October) 2015–2020 in the USGS National Water Information system and the WQX/STORET databases were included. Sites with specific conductivity ≥1500 µS/cm were considered as potentially supporting the algae and at Moderate risk. Sites that exceeded 1500 µS/cm and either exceeded chloride level threshold (228 mg/L) or had pH > 7.5 were classified as High risk. Sites for which all three criteria thresholds were exceeded were considered at Severe risk for *P. parvum*.

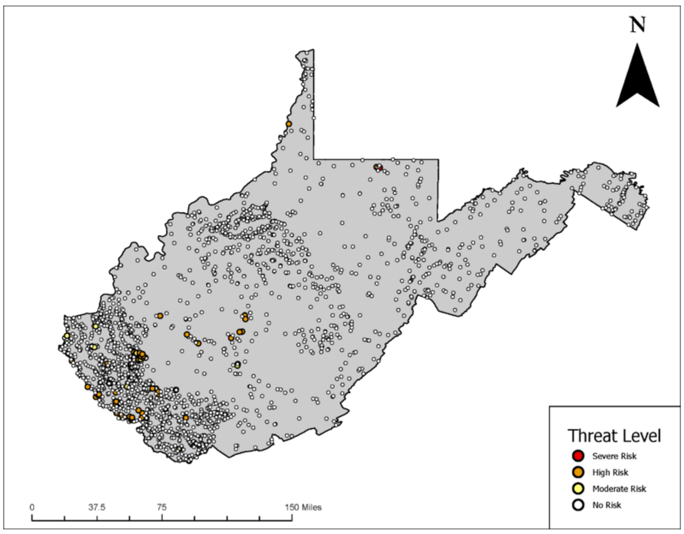

Figure 8. Spatial map of West Virginia showing the risk of *Prymnesium parvum* based upon water quality criteria. Data are from the WVDEP dataset from May–October 2015–2020. Sites with specific conductivity <1500 uS/cm were considered as No risk. If a site met or exceeded 1500 µS/cm we considered it at Moderate risk for *P. parvum*. Sites that exceeded the specific conductivity threshold and either exceeded chloride level threshold (228 mg/L) or had pH > 7.5 were classified as High risk. Sites for which all three criteria thresholds were exceeded were considered at Severe risk for *P. parvum*.

4. Discussion

Previous studies have documented *P. parvum* fish kills in aquaculture [10,46] as well as wild settings in Europe and Scandinavia [11,14,47–49] and blooms have been reported on nearly every continent with the exception of Antarctica [18,27]. However, few studies have documented the loss of aquatic life associated with *P. parvum* kills in North America. Our study of Dunkard Creek is the first published report detailing the impacts and long-term recovery of fish communities from a *P. parvum* kill. It is also the first detailing impacts to mussel communities. Previously, in Lake Texoma, Oklahoma-Texas, Zamor et al. [50] followed the response of the fish community over a one-year period spanning a *P. parvum* bloom. The Lake Texoma bloom completely killed all fish in the treatment cove, but within 6 months after the bloom pre-kill fish composition, abundance, and richness were regained. In Dunkard Creek, many of the fish species were collected again the following year, but not all species have returned to pre-kill abundances ten years post-kill.

Recovery of fish communities can be difficult to determine due to their dynamic nature. However, many studies have shown fish to be resilient following catastrophic events such as drought [51,52], floods [53,54], and toxic spills [55]). Minnows, darters, and juvenile sport fish (i.e., Black Bass) have shown higher rates of recovery than older and larger individuals [53,55,56]. Additionally, those fish with high fecundity and mobility tend to recover from disturbances more rapidly than those fish with low fecundity and low mobility [56,57]. Recovery times are greatly influenced by the location of potential colonizers [58–60]. Fortunately, Dunkard Creek had several tributaries not impacted by the *P. parvum* toxin as they served as refugia for minnows, darters, juvenile bass and smaller suckers. Additionally, Dunkard Creek is a direct tributary to the Monongahela River, which was not noticeably impacted during the kill. It is assumed that the Monongahela River also served as a source for recolonization, specifically for larger, older fish. VanLandeghen et al. [12] documented that long-term declines in relative abundance and size structures caused by *P. parvum* varies between river basins and fish species and can be impacted by repeated kills. Since the Dunkard Creek kill was a singular event caused by *P. parvum* and not by physical alteration of the stream channel or watershed, the fish community is recovering and approaching pre-kill abundances.

Although the Dunkard Creek fish community appears to be recovering, the mussel community has not. In the extensive surveys immediately following the 2009 event in Dunkard Creek, no live mussels were found and recently dead mussels comprising 16 species were found [39,40]. The mussel restoration plan called for transplanting tagged adult mussels and stocking fish that had been inoculated with larval mussels to reestablish populations. Between 2012 and 2015, 3200 adult mussels of 17 species were stocked into seven sites [39]. Thus far these stockings and the stocking of over 3000 inoculated fish hosts have been unsuccessful. In the eleven years since the kill, only tagged-transplanted mussels have been recovered in West Virginia surveys and no recruitment of mussels has occurred since the 2009 kill.

It is unclear why mussels have not recovered in Dunkard Creek. Factors such as the intricate reproductive life history of mussels involving a parasitic stage and water quality may play a role in the lack of recruitment and low survival despite stocking attempts. The majority of these mussel taxa require a fish host for early life stages prior to settlement [61]. Many of the mussel taxa in Dunkard Creek can utilize a wide array of fish species as hosts [62], but some Unionid mussels are more host specific and may only utilize one species of fish [63–66].

The fish community recovery may be necessary to reestablish recruitment of mussels. While the darter community has recovered to pre-kill levels, many of the formerly abundant minnows such as Central Stoneroller *Campostoma anomalum* and Bluntnose Minnow *Pimephales notatus,* have not. Further, larger potential host species such as game fishes have not fully recovered, and larger-bodied fish have larger gill area for glochidial attachment and are likely more mobile than smaller fishes for transfer of mussels upstream. Thus, fish host availability may be a factor in failed recruitment of mussels. However, stocking of adult mussels and fish inoculated with glochidia have failed, suggesting some other factors may be limiting survival.

All life stages of mussels are sensitive to water quality impairments [67,68] (Ausberger, Prosser) including chlorides [69], PAHs, and metals [70,71]. In cage studies in the Allegheny River Pennsylvania, background survival of the Northern Riffleshell *Epioblasma rangiana* was 84% compared with high salinity (>4000 uS/cm) wastewater discharges (2–34% survival) and municipal wastewater outflow (21% survival) [72]. Correlative studies in the Clinch River (Upper Tennessee River basin, VA, USA) examining organic and inorganic contaminant stressors and mussel declines found PAHs and manganese were elevated in zones of mussel decline. Data for PAHs is lacking for Dunkard Creek but is frequently associated with anthropogenic activities [73]. Water quality has improved in Dunkard Creek since the kill, but impairments still exist. As part of the settlement following the kill, Consol Energy was required to develop a desalination plant to treat mine discharges into Dunkard Creek. As a result, WVDEP data shows specific conductivity now rarely reaches 1500 uS/cm and chlorides have not exceeded our threshold values [74].

However, juvenile and adult mussels are sensitive to common agricultural pollutants such as ammonia present in Dunkard Creek. Goudreau et al. [75] found glochidia of the Rainbow Mussel *Villosa iris* 24 h LC50 for ammonia was 0.284 mg/L and Myers-Kinzie [76] reported 48 h LC50 of 0.091–0.142 mg/L for the Fat Mucket *Lampsilis siliquoidea*. In Dunkard Creek at the WVDEP monitoring location (see Figure 2), ammonia total N ranged from 0.10–0.99 mg/L from 2009 until spring 2014. Thereafter, it dropped to 0.02–0.05 mg/L (WVDEP unpublished data). Potassium levels in Dunkard Creek may also be impacting mussels. Total potassium levels show peaks from August through November of 3.0–6.7 mg/L since the kill (WVDEP unpublished data). These levels are less than the 48-h LC50 for juvenile Fat Muckets (28 mg/L) found by Myers-Kinzie [76] and levels reported by Imlay [77] to result in 90% mortality of adults for 45-day exposure of Fat Mucket (11 mg/L). Chronic exposure of 7 mg/L potassium resulted in 100% mortality in Fat Mucket in less than 8 months [77]). Although the potassium levels are greatly reduced in Dunkard Creek since 2014, they could still serve as an additional stressor in a suite of potentially synergistic stressors affecting recovery of mussels [78]. The worldwide declines in freshwater mussel taxa and the lack of recovery following the *P. parvum*-kill in Dunkard Creek underscores the importance of the potential threat to mussel species by *P. parvum*. Many of the inland systems identified as at risk in our spatial water quality maps are also home to diverse and threatened mussel species.

The relative roles of water quality and *P. parvum* in the Dunkard Creek aquatic life kill are difficult to disentangle. Ultimately, water quality impairments associated with discharges from pumped and treated deep coal mines were undoubtedly the root cause of the fish and aquatic life kill on Dunkard Creek by elevating the conductivity in the receiving waters and creating conditions favorable for the brackish water *P. parvum* to grow. Dead fish were found throughout September 2009 but *P. parvum* was not detected until 18 September and confirmed in USEPA water sampled from 20 September [79]. However, field measures taken by USEPA on 9 September showed 4 of 8 sites in Dunkard Creek had oversaturated dissolved oxygen suggestive of an algal bloom [80,81]. Three sites in particular—upstream of the Blacksville outfall 005 (RKM 26.9), downstream of the 005 outfall (RKM 27.1), and at Pentress, WV (RM 12.0) had D.O. percent saturation levels of 130.7, 165.8, and 154.8 respectively. Specific conductivity was also elevated at those same sites (4957; 18,570; and 2714 uS/cm) suggesting that the algal blooms were already underway and dominated by *P. parvum* by 9 September.

The literature suggests that for the most part, specific conductivity, chlorides and sulfates alone were not responsible for the mortality of fish in Dunkard Creek in September 2009. Toxicity tests for most fish species found in Dunkard Creek are unavailable, but similarities among common freshwater fishes lend themselves to extension in this case. Specific conductivity was generally less than 6000 uS/cm throughout Dunkard Creek in September 2009. Four-day LC50 for conductivity in fishes is 4640–7650 uS/cm for Fathead

Minnow [82,83], 5840–7865 uS/cmfor Bluegill [83,84], and up to 10,616 uS/cm for Mosquito Fish (*Gambusia affinis*) [85]. Acute toxicity of chlorides for Channel Catfish and Bluegill (8000 mg/L), and Largemouth Bass (8500 mg/L) [85] were well below the levels observed in Dunkard Creek water samples (215–702 mg/L). Acute 14-day toxicity of sulfates are 10,000 for Channel Catfish and Bluegill, and 11,000 for Largemouth Bass [86] whereas sulfate concentrations in Dunkard Creek during the kill were 754–1180 mg/L. Despite seemingly sub-lethal effects from conductivity, chlorides and sulfates, the impact of the combination of these water quality impairments or other constituent ions associated with the elevated conductivity in Dunkard Creek are unknown.

However, field studies from nearby watersheds help to elucidate the role of elevated conductivity versus *P. parvum* in the Dunkard Creek fish kill. Kimmel and Argent [87,88] examined impacts of a specific conductivity gradient related to treated coal mine discharges upon the fish community of Tenmile Creek in southwestern Pennsylvania. Similar to Dunkard Creek, Tenmile Creek was found to have 42 fish taxa. Sampling 17 stations along the stream, Kimmel and Argent [88] found a threshold of fish community impairment for instream conductivity of 3000–3500 uS/cm. In another study, Kimmel and Argent [89], sampled 10 sites along a 20 km section of Whiteley Creek, a tributary of the Monongahela River near Dunkard Creek. They found specific conductivity of 2400–5400 uS/cm and pH > 8.3. These are similar ranges of water quality values as observed during September 2009 in Dunkard Creek. Fish sampling in Whiteley Creek observed 12 species persisting there that were common in Dunkard Creek both before the kill and in years following the kill. The ability of these fish species to persist in the chronically high salinity of Whiteley Creek suggests they should have survived in 2009 in Dunkard Creek had it not been for the *P. parvum* bloom.

5. Conclusions

Water quality impairments associated with discharges from pumped and treated deep coal mines were undoubtedly the root cause of the fish and aquatic life kill on Dunkard Creek. These discharges elevated the conductivity in the receiving waters creating conditions favorable for the brackish water *P. parvum* to grow and produce toxins. Except in the immediate vicinity of the St. Leo and Blacksville No. 2 outfalls, dilution maintained the conductivity, chlorides, and sulfates at levels less than shown to affect fish species in toxicity tests individually. Identification of the *P. parvum* bloom part way through the fish kill made it difficult to partition mortality related to water quality versus the HAB. However, it is clear that the *P. parvum* bloom completed the aquatic life kill.

P. parvum represent a widespread threat to the biota and economies of inland and coastal waters. Spatial mapping of threat levels for *P. parvum* based upon water quality conditions suggest about 4.5 percent of water quality monitoring sites nationally meet minimal conditions for supporting this golden alga. An analysis of West Virginia state water quality databases found lower incidence of Moderate risk designations, but a higher incidence of High and Severe risk than suggested by the national database for West Virginia. In the U.S., *P. parvum* blooms have already been reported in 23 states throughout the south but extending into the Midwest and as far north as Washington, Wyoming, and Maine [8,27]. Once established in a water body, *P. parvum* can persist until conditions again become available to bloom. These locations can become sources for lateral movements to other waterbodies via wildlife and anthropogenic activities. Worldwide there is concern about increasing salination of freshwaters related to anthropogenic activities such as road deicing salt, land use/impervious surfaces, mining, and climate change [90–94] so local water quality conditions are likely to continue to degrade increasing the likelihood of the spread of golden algae and their blooms that damage freshwater and coastal ecosystems and economies.

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