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Impact Of A Diesel Spill On Macroinvertebrate Communities In Ponds In S.E. Alabama, USA

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Abstract. In this study, macroinvertebrate communities were examined to determine an indication of the water quality in ponds on a wetlands property after a diesel spill. In 2012, a train derailed, and at least 3000 gallons of diesel fuel leached into a small pond of the wetlands. Even after clean-up attempts there remains major concerns that the contamination spread throughout the wetlands. This concern led to the present study. Macroinvertebrates were the chosen focus due to their sensitivity to changes in the environment. Two field trials were conducted in which Hester-Dendy multiplate samplers were placed in ponds to allow macroinvertebrates to colonize on the artificial substrate over the winter months. The macroinvertebrates were classified to family or order to compare taxonomic groups at increasing distances from the affected pond. The results of the study seemed to indicate the presence of pollution, which may be due to the diesel spill. At increasing distances from the spill site, a greater number of moderately pollution tolerant to pollution sensitive macroinvertebrates were found to be present in the samples. However, due to the small sample size collected, further study needs to be conducted to more conclusively evaluate the impact of the diesel fuel spill.

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1. INTRODUCTION

The impact of a diesel spill on macroinvertebrate communities was studied in ponds on a wetlands property in southeastern Alabama owned by the Eason family. On March 28, 2012, the Norfolk Southern Railway experienced a derailment on the western boundary of the Eason family's property. In this derailment a locomotive fuel tank spilled at least 3000 gallons of diesel fuel, which leached into a small pond located alongside the railroad tracks. Because of EPA regulations, the company did an emergency clean-up to remove the free flowing diesel fuel. However, significant levels of polycyclic aromatic hydrocarbons (PAHs) most likely remained in the soil and groundwater adjacent to the property line (L. Eason, written communication). According to the property owner, Lesley Eason (written communication), the railroad company pumped the water from the affected pond into a secondary pond, therefore, increasing the possible spread of diesel pollution through the wetlands. Also, the level of PAH contamination directly adjacent to the surface water is approximately above the screening levels for commercial and industrial risk (L. Eason, written communication). The Eason family is concerned that the contamination spread down the watershed through the wetlands. Lesley Eason (written communication) discussed how the Norfolk Southern Railway did not acknowledge that the diesel spill was within the boundaries of a National Wetlands area, which is required by the Oil Pollution Act and the Clean Water Act.

To determine the possible effects of the diesel spill, macroinvertebrate communities were studied. Freshwater macroinvertebrate communities represent a choice group of organisms for monitoring water quality because of their heightened sensitivity to changes in their environment. Possible environmental changes include physical changes such as temperature, chemical changes such as pollution, and other water quality changes such as dissolved oxygen levels. As the community level shifts in abundance, members of the macroinvertebrate community are measured in response to an environmental change. Not only will this study provide an indication of the water quality after the pollutant was introduced based on macroinvertebrate communities, but it may also imply possible effects on other organisms such as birds and fish. Macroinvertebrates are important members of the wetland ecosystem, because they are both an intermediate between lower and higher levels in the food chain and indicators of pollution (Merritt and Cummins 1996). If the diesel fuel spill has severely affected the macroinvertebrate community and the species that depend on their survival, then the ecosystem may require further attention for remediation.

Wetlands are sites of biological productivity and habitats for plant and animal biodiversity (Steven and Lowrance 2011). Wetland habitats are often transitional areas between terrestrial and aquatic macroinvertebrates with greater number of aquatic macroinvertebrates compared to terrestrial macroinvertebrates (Anderson and Smith 2000). According to Pettigrove and Hoffmann (2005), macroinvertebrates are good indicators of both water and sediment pollution. Many freshwater macroinvertebrates have limited mobility and as a result can live in the same habitat for up to several years (Strayer 2006). Because of their relative lack of migration, the sources and effects of pollution can be found by comparing macroinvertebrate communities. The primary environmental concerns of diesel fuel contamination are the aromatic components alkyl in their environment. Possible environmental changes include physical changes such as temperature, chemical changes such as pollution, and other water quality changes such as dissolved oxygen levels. As the community level shifts in abundance, members of the macroinvertebrate community are measured in response to an environmental change. Not only will this study provide an indication of the water quality after the pollutant was introduced based on macroinvertebrate communities, but it may also imply possible effects on other organisms such as birds and fish. Macroinvertebrates are important members of the wetland ecosystem, because they are both an intermediate between lower and higher levels in the food chain and indicators of pollution (Merritt and Cummins 1996). If the diesel fuel spill has severely affected the macroinvertebrate community and the species that depend on their survival, then the ecosystem may require further attention for remediation.

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The main objective of this study was to assess the effect of the diesel spill on the aquatic macroinvertebrate communities in the affected ponds. Macroinvertebrates were sampled from ponds at increasing distances from the spill site. The prediction was that there would be more pollution tolerant macroinvertebrates closer to the spill site, and increasing numbers of pollution sensitive groups further from the site. This prediction was tested by comparing count data of the macroinvertebrate communities from the ponds on the wetlands property in southeastern Alabama, USA.

2. MATERIALS AND METHODS

Study Site

The site is located at the Eason family's property in Phenix City, Alabama, which is within Russell County. The GPS coordinates for the wetlands property are latitude 32.6166°N and longitude 84.9786°W. According to Lesley Eason (written communication), the area size is approximately 56.68 hectares, of which 44.53 to 48.58 hectares is classified by the U.S. Fish and Wildlife Service as National Wetlands/Southern Hardwood Bottomland. In the late 1800's to early 1900's, clay was extracted and manufactured on the property (Eason, written communication). Because of the mining enterprise, large, spring-fed pits were formed and resulted in the present wetland found on the property. This property is part of a larger wetlands in the Chattahoochee watershed. Since 1919, the Eason family has owned the property and maintained it as a wildlife habitat for more than 60 years.

Collection of Specimens

For the first field trial, macroinvertebrate specimens were collected in 12 Hester-Dendy multiplate samplers in three ponds. Four samplers were placed at each of the three ponds at increasing distances from the diesel spill site and labeled Pond A, Pond B, and Pond C, respectively. Pond A was at the diesel spill site. The samplers were set in place Monday, October 13, 2014, and left to allow macroinvertebrate assemblages to colonize on artificial substrates over the winter months. The samplers were secured with fishing line and tent stakes and left until spring. The samplers were collected Friday, March 20, 2015. For the second field trial, macroinvertebrate specimens were collected using 19 Hester-

Dendy multiplate samplers in four ponds. The samplers were set in place on Friday, September 11, 2015, and left for macroinvertebrates to colonize with three samplers in Pond A, six in Pond B, seven in Pond C, and three in Pond D, respectively. As a modification to the methods, 65-pound fishing line was used, and Pond D was sampled to provide a comparison at a further distance from the spill site. After approximately 22 weeks, the samplers were collected on Saturday, January 30, 2016. The distances from the original spill site increase as follows: Pond A to Pond B is 65 meters, Pond A to Pond C is 321 meters, and Pond A to Pond D is 380 meters. The samples of macroinvertebrate specimens were contained in 70% ethyl alcohol in order to preserve them while sorting and classifying in the laboratory. The macroinvertebrates were classified to a reasonable specificity using the book edited by Thorp and Covich (2001), *Ecology and Classification of North American Freshwater Invertebrates.* The data was analyzed with a Chi-square Test of Independence to compare taxonomic distributions from each field trial and the combined trials.

3. RESULTS

Table 1 shows the data for the number of macroinvertebrates from each classification group and pond site from the first field trial. There was a significant difference between the pond sites and the distribution of macroinvertebrate groups (Chi-square Test of Independence, X^2=76.39, d.f.=10, P<0.001).

Table 1. Abundance of macroinvertebrates collected at ponds near diesel spill on wetlands property in Phenix City, AL. Data collection from the first trial (2015).

Classification (common name)	Pond A	Pond B	Pond C
Chironomidae (Midge Fly Larvae)	48	18	0
Lumbriculidae (Aquatic Worms)	92	3	8
Corbiculidae (Clams)	0	3	1
Euhirudinea (Leeches)	0	1	0
Hyalellidae (Scuds)	0	3	0
Planorbidae (Snails)	0	0	1

Table 2 shows the data for the number of macroinvertebrates from each classification group and pond site from the second field trial. There was a significant difference between the pond sites and the distribution of macroinvertebrate groups (Chi-square Test of Independence, X^2=57.74, d.f.=10, P<0.001).

Table 2. Abundance of macroinvertebrates collected at ponds near diesel spill on wetlands property in Phenix City, AL. Data collection from the second trial (2016).

Classification (common name)	Pond A	Pond B	Pond C	Pond D
Chironomidae (Midge Fly Larvae)	8	34	*	23
Lumbriculidae (Aquatic Worms)	5	9	*	0
Corbiculidae (Clams)	0	18	*	53
Euhirudinea (Leeches)	0	2	*	3
Hyalellidae (Scuds)	0	6	*	21
Plecoptera (Stonefly Larvae)	0	1	*	0

* The Hester-Dendy multiplate samplers could not be recovered.

Table 3 shows the combined data for each trial of the study. There was a significant difference between the pond sites and the distribution of macroinvertebrate classification groups (Chi-square Test of Independence, X^2=268.33, d.f.=18, P<0.001).

Table 3. Abundance of macroinvertebrates collected at ponds near diesel spill on wetlands property in Phenix City, AL. Data collection from both trials (2015 and 2016).

Classification (common name)	Pond A	Pond B	Pond C	Pond D
Chironomidae (Midge Fly Larvae)	56	52	0	23
Lumbriculidae (Aquatic Worms)	97	12	8	0
Corbiculidae (Clams)	0	21	1	53
Euhirudinea (Leeches)	0	3	0	3
Hyalellidae (Scuds)	0	9	0	21
Plecoptera (Stone Fly Larvae)	0	1	0	0
Planorbidae (Snails)	0	0	1	0

In table 4 the macroinvertebrate groups are organized into levels of pollution tolerance for reference.

Table 4. The pollution sensitivity groups for each classification group determined from charts in the laboratory.

Pollution Tolerant	Moderately Pollution Tolerant	Pollution Sensitive
Chironomidae (Midge Fly Larvae)	Corbiculidae (Clams)	Plecoptera (Stonefly Larvae)
Lumbridculidae (Aquatic Worms)	Hyalellidae (Scuds)	
Euhirudinea (Leeches)		
Planorbidae (Snails)		

Table 5 shows the comparison across the pond sites and the count data of the pollution groups for the first field trial. There was a significant difference between the pond sites and the distribution of pollution tolerance of the macroinvertebrates (Chi-square Test of Independence, X^2=29.44, d.f.=4, P<0.001).

Table 5. The distribution of the pollution tolerance groups across the three ponds for the 2015 field trial data.

Pollution Tolerance Group	Pond A	Pond B	Pond C
Pollution Tolerant	140	22	9
Moderately Pollution Tolerant	0	6	1
Pollution Sensitive	0	0	0

Table 6 shows the comparison across the pond sites and the count data of the pollution groups for the second field trial. There was a significant difference between the pond sites and the distribution of pollution tolerance of the macroinvertebrates (Chi-square Test of Independence, X^2=43.301, d.f.=6, P<0.001).

Table 6. The distribution of the pollution tolerance groups across the four ponds for the 2016 field trial data.

Pollution Tolerance Group	Pond A	Pond B	Pond C	Pond D
Pollution Tolerant	13	45	*	26
Moderately Pollution Tolerant	0	24	*	74
Pollution Sensitive	0	1	*	0

* The Hester-Dendy multiplate samplers could not be recovered.

Table 7 shows the comparison across the pond sites and the count data of the pollution groups for the combined data. There was a significant difference between the pond sites and the distribution of pollution tolerance of the macroinvertebrates (Chi-square Test of Independence, X^2=165.38, d.f.=6, P<0.001).

Table 7. The distribution of the pollution tolerance groups across the four ponds sites for the combined data.

Pollution Tolerance Group	Pond A	Pond B	Pond C	Pond D
Pollution Tolerant	153	67	9	26
Moderately Pollution Tolerant	0	30	1	74
Pollution Sensitive	0	1	0	0

4. DISCUSSION

In this study, it was predicted that there would be greater numbers of pollution tolerant macroinvertebrates at the spill site and more pollution sensitive macroinvertebrates at further distances from the site. A significant difference was found across the ponds in distribution of macroinvertebrates. From the data collected in the first trial, there were significantly more pollution tolerant macroinvertebrates in the affected pond, with increasing numbers of moderately pollution tolerant further away. As expected, the only specimens found at the spill site were pollution tolerant. From the data collected in the second trial, there were more pollution tolerant macroinvertebrates in Pond B and Pond D compared to the affected pond. However, the only macroinvertebrates that were found in Pond A were pollution tolerant as expected. Also, there were more macroinvertebrates in the moderately pollution tolerant group in the pond furthest from the spill site as expected. One pollution sensitive, stonefly was found in Pond B. With the combined data, the greatest number of pollution tolerant macroinvertebrates was found in the affected pond. The number of pollution tolerant macroinvertebrates decreased as the distance from the spill site increased. There were more moderately pollution tolerant macroinvertebrates in the pond furthest from the spill site as expected.

Unfortunately, both field trials did not yield a large sample size. In the first trial the small collection was possibly the result of placing the samplers out too late in the year for the macroinvertebrates to colonize on the artificial substrate. Also, out of 12 samplers only ten were recovered because the fishing line had been broken or severed. In the second trial, modifications were made to the methodology to attempt to ensure that the collection would be greater. The samplers were placed earlier in the fall to allow the macroinvertebrates more than sufficient time to colonize the artificial substrate. Also, an increased number of samplers were placed and stronger fishing line was attached. However, out of 19 samplers only nine were recovered. This low recovery could be because of the large amount of rainfall during the winter months that may have washed away the samplers. When searching for the samplers, most of the area was submerged, and even with flag markers on higher ground, some of the samplers were not found. Some of the fishing lines were broken or severed as well. One sampler had tooth marks all over it, which was likely from a beaver. None of the samplers from Pond C were recovered, and therefore, a comparison of results was impossible with this pond for both trials. After researching how to improve data collection for future studies, one study conducted by Turner and Trexler (1997) compared different sampling methods of macroinvertebrates. They found that funnel traps and D-frame sweep nets collected a greater number and range of macroinvertebrates compared to other samplers including the Hester-Dendy multiplate samplers (Turner and Trexler 1997). Also, they found that one method alone could provide misleading data because each sampler typically captured different types and sizes of macroinvertebrates (Turner and Trexler 1997).

The results of this study did indicate a difference in distribution of pollution tolerant macroinvertebrates in relation to distance from the spill site, which could have negative ramifications on the ecosystem of the wetlands. In addition to the diesel spill, another potential source of pollution is a road on the eastern boundary of the property that is adjacent to Pond D. It is possible that the road's close proximity could provide a non-point source for pollution entry into the furthest pond from the diesel spill site and account for the lack of pollution sensitive macroinvertebrates in the pond. Furthermore, the ponds are interconnected because the pathways between the ponds were constructed using bricks (L. Eason, written communication). Due to the holes in the bricks, fluid can leak through and spread between the ponds. Because the overall majority of the macroinvertebrates found at the wetlands as well as the only groups found in Pond A were pollution tolerant, there is an indication of possible harm from the diesel fuel contamination. In the study conducted by Meier et al. (2013), the researchers found that the macroinvertebrate communities were negatively affected by contaminants such as PAHs. The contamination cited was also attributed to harmful effects in the fish assemblages within the same areas that had not been treated for the PAH contamination (Meier et al. 2013). Areas that contained pollution sensitive macroinvertebrate groups were zones where work had been conducted to decrease the sediment contamination (Meier et al. 2013). In Lytle and Peckarsky's (2001) study, they found that a diesel fuel spill severely impacted the invertebrates. There was some recovery after 15 months, but not a total recovery (Lytle and Peckarsky 2001). Often diesel fuel spills are considered to be highly toxic for a short-term period; however, one study found that the diesel had long-term toxicity effects on fish, and the increased bioavailability and toxicity correlated to increased dispersion (Schein et al. 2009). The current study indicated that the macroinvertebrate communities were negatively affected in the area of the diesel spill as indicated by the lack of pollution sensitive groups.

5. CONCLUSIONS

The results of the study did support the predicted effect of the diesel spill on the macroinvertebrate communities in the wetlands. It was found that at increasing distances from the spill site there was more moderately pollution tolerant to pollution sensitive macroinvertebrates. However, because only a small sample size was collected more study and data collection need to be continued at the site. For further research, one suggestion to improve data collection would be to combine sampling methods to ensure that a wide range of macroinvertebrates is targeted. In addition, increasing the number of Hester-Dendy multiplate samplers with improved securing techniques could yield larger sample sizes. Finally, chemical analysis should be done on both sediment and water samples to evaluate what chemicals and concentrations are actually present before remediating the wetlands for contamination.

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APPENDIX A

The full classification to a reasonable specificity are shown in table 1 as well as the count data of specimens from each pond for the combined data of both trials in this study.

Table 1. The raw data for both trials of this study with the classification of the macroinvertebrate specimens and the count data for each of the pond sites.

Macroinvertebrate Classification	Pond A	Pond B	Pond C	Pond D
Phylum Arthropoda Class Entognatha Order Diptera Suborder Nematocera Family Chironomidae	56	52	0	23
Phylum Annelida Class Oligochaeta Order Lumbriculida Family Lumbriculidae	97	12	8	0
Phylum Mollusca Class Bivalvia Superfamily Corbiculoidea Family Corbiculidae	0	21	1	53
Phylum Annelida Class Clitellata Order Euhirudinea	0	3	0	3
Phylum Arthropoda Subphylum Crustacea Class Malacostraca Order Amphipoda Family Hyalellidae	0	9	0	21
Phylum Mollusca Class Gastropoda Superfamily Planorbidea Family Planorbidae	0	0	1	0
Phylum Arthropoda Subphylum Uniramia Class Insecta Order Plecoptera	0	1	0	0