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Restoration of mine pit lakes for recreation is going to be very important for the future of Minnesota's Mesabi Iron Range area. As many as 250 lakes will need to be restored in the future along this 100-kilometer-long swath of mining country. Information regarding the organisms that currently inhabit these lakes is important for future restorations to be a success. The focus of this study was to identify the types of aquatic invertebrates present in pit lakes and to identify some of the factors that influence their abundance and diversity. During this study, invertebrate samples were taken along the shore of 10 pit lakes. It was found that there is a significant difference in diversity and species richness among some lakes. Increasing shoreline vegetation coverage was found to have a positive effect on species richness (P < 0.01). Increasing rock size had a negative effect on invertebrate density (P = 0.03). Increasing macrophytes abundance had a positive effect on species richness (P < 0.01) and a negative effect on diversity (P = 0.02). Substrate consisting of a glacial till had a higher species richness than substrates consisting of hard rocks uncovered by mining (P = 0.04). Hopefully, the results of this study can be used to improve these lake systems for future recreational use.

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# Introduction

Mining first began on the Mesabi Iron Range in the early 1890's and is continuing today. Deep, cold oligotrophic lakes have formed in pits where mining has ceased. These pits characteristically have steep sidewalls and have very little littoral area. A previous study of these lakes found that there is not enough prey abundance to support and grow enough trout to a sufficient size for anglers (Tomcko and Pierce 1992). Most of these abandoned pits have not been restored because they closed prior to legislation enacted in 1980 requiring mining companies to reclaim pits prior to closing. Some reclamation efforts, consisting of bank grading/stabilizing, and stocking of Lake Trout Salvelinus namaycush, Rainbow Trout Oncorhynchus mykiss, and Splake Salvelinus namaycush x Salvelinus fontinalis, have been undertaken by the Minnesota Department of Natural Resources and the Iron Range Resources and Rehabilitation Board with mixed success. Further knowledge of the biota of these lakes is needed to successfully rehabilitate them.

Aquatic invertebrates are a very important component of aquatic systems. They have been found to improve water quality, process organic material, and cycle nutrients (Collier et al. 2016). They are an important food source for many terrestrial and aquatic organisms and have been found to have a significant role in transferring primary production to higher trophic levels (Vander Zanden and Vadeboncoeur 2002). Another study found them to be the main food source for trout in lakes (Graynoth et al. 1986). Invertebrates are also used as bioindicators because they possess the necessary traits (Holt and Miller 2010).

The multiple biological rolls of invertebrates and their usefulness in monitoring aquatic systems make them an important factor in pit lake restoration efforts. Therefore, the objective of this study is to assess the composition of benthic invertebrate communities in mine pit lakes and to test for relationships between invertebrate diversity, density, and species richness and various physical and biotic components (substrate size, macrophytes density, shoreline vegetation density, bank stability) in mine pit lakes along Minnesota's Mesabi Iron Range.

# Methods

Mine pit lakes were sampled along Minnesota's Mesabi Iron Range. In total, 61

samples were collected from 10 different lakes. Sampling took place during September and October 2020. The maximum depth of lakes ranged from 9.14 to 135 m and they ranged in size from 3.24 to 108.14 hectares. Sample sites within individual lakes were selected in a way to represent a variety of different combinations of biotic and abiotic factors (substrate type, rock size, macrophytes abundance, shoreline vegetation, and shoreline stability) within that lake.

Sample plots were established using a 0.5 by 1.0 m quadrat placed perpendicular to shore at an average depth of 0.5 m. Samples were collected by sweeping a D-net through the plot multiple times until most of the invertebrates within the plot were collected. The material collected by the net was then condensed for preservation. Rocks and large debris were scrubbed to remove all invertebrates and then discarded. Fine sediments were sieved out and the resulting materials and invertebrates were preserved in alcohol.

Substrate types were determined by observing whether clay/sediment, sand, or rock was most dominant within the sample plot. Rock substrates were categorized further as glacial rock (characterized by round edges and a variety of colors) or mine rock (characterized by sharp edges and a reddish color). Average rock size was recorded as the average diameter of 10 randomly selected rocks within the sample plot. Macrophytes density was determined by estimating the percent of the sample plot covered by macrophytes growth and the dominant type of macrophytes was also recorded.

A plot of 20 m by 25 m, was also established on the shoreline directly above each sample site. The estimated percent of this area covered by some vegetation type was recorded as well as the dominant vegetation type. Shoreline stability was recorded by estimating the percent of this area composed of bare soil.

Sampled invertebrates were identified and counted using a dissecting microscope. A Shannon Wiener Diversity Index coefficient was calculated for each sample. The equation used was  $D(diversity) = (-\sum p_i(ln(p_i)))/ (ln(species richness), where p_i = the proportion of the sample represented by each species sampled. An ANOVA analysis was used to test for differences in invertebrate diversity, species richness, and densities among lakes. Regression analyses were used to test for relationships between invertebrate diversity and substrate type, rock size/type, macrophytes density, and shoreline stability.$ 

#### Results

A total of 42 invertebrate taxa were sampled. Invertebrate diversities ranged from 0.17 to 0.92 with a mean of 0.57 (SD = 0.15). Species richness ranged from 3 to 17 (SD = 3.2) species. Densities ranged from 34 to 11,740 with a mean of 1,484 (SD = 2,029) invertebrates per square meter.

There was a significant difference in invertebrate diversity (P < 0.01; Figure 1) and species richness (P < 0.01; Figure 2) among the 10 pits sampled. No significant difference was found among pits for invertebrate densities (P = 0.09; Figure 3). Substrate types were found to not have any significant effect on invertebrate diversity, density, or species richness. Rock types did not significantly affect diversity (P = 0.99) or density (P = 0.92). Glacial rock type did have a significantly higher species richness compared to mined rock (P = 0.04; Figure 4).

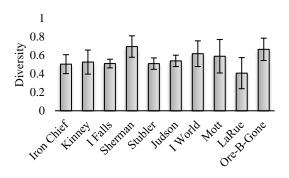


FIGURE 1. Average invertebrate diversity between the 10 pits sampled. Error bars represent  $\pm$  one standard deviation.

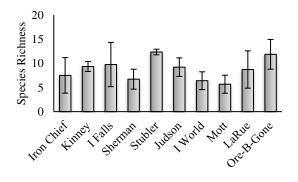


FIGURE 2. Average species richness between the 10 pits sampled. Error bars represent  $\pm$  one standard deviation.

Three multiple regression analyses were used to examine the effect of rock size, macrophytes abundance, and shoreline vegetation density on invertebrate diversity, density, and species richness. The variables recorded did not account for a large part of the variation of diversity, density, and species richness with R<sup>2</sup> values of 0.13, 0.14, and 0.31, respectively. Invertebrate density was significantly affected by average rock size (P = 0.03) but was not significantly affected by vegetation cover (P = 0.12) or macrophyte coverage (P = 0.06; Figure 5). Species richness was significantly affected by vegetation cover (P <0.01) and macrophyte abundance (P < 0.01) but was not affected by rock size (P = 0.24; Figure 6). significantly affected Diversity was by macrophytes abundance (P = 0.02) but was not affected by rock size (P = 0.08) or vegetation cover (P = 0.99; Figure 7).

The pollution tolerance levels, on a range of 1-10, of the 42 taxa sampled had a mean of 5.3 and their distribution is slightly skewed toward lower tolerances (Figure 8). Collectors were the most dominant functional feeding group of the invertebrates collected followed by scrapers. Shredders were the least abundant (Figure 9).

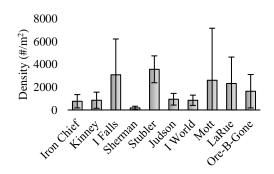


FIGURE 3. Average invertebrate density between the 10 pits sampled. Error bars represent  $\pm$  one standard deviation.

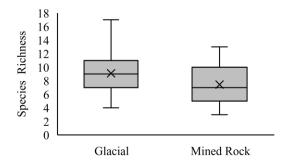


FIGURE 4. Species richness by the two dominant rock types (glacial and mined rock). Glacial rock had a significantly higher species richness (P = 0.04).

### Discussion

The focus of this study was to identify some of factors that affect shoreline benthic the invertebrates in abandoned iron ore mine pit lakes in Northeastern Minnesota. The pits had relatively healthy invertebrate communities with 42 taxa sampled. Mean diversity of all samples was 0.57, mean species richness was 9, and mean density was 1484/m<sup>2</sup>. Densities observed in this study were similar to those of a previous study of two of these lakes where an observed mean of 1485/m<sup>2</sup> was reported (Pierce and Tomcko 1992.). Pollution tolerance levels of all taxa sampled on a scale of 1 to 10 was 5.3 and was slightly skewed toward lower tolerances, indicating that these pits have minimal pollution (Figure 8). Shredders were the dominant functional feeding group at 77%, followed by scrapers at 16% (Figure 9). The study found that macrophytes abundance, shoreline vegetation density, substrate size, and substrate types had varying effects on macro invertebrates.

Macrophyte communities in sample sites were composed of a single species of Chara sp. for all pits except for a single pit, Ore-B-Gone, which had widespread Eurasian Watermilfoil Myriophyllum spicatum. The lack of high macrophyte species richness is likely due to low phosphorus concentrations (Pierce and Tomcko 1992). Macrophytes had a significant effect on both invertebrate diversity and species richness. Interestingly, species richness was positively correlated to macrophytes density, but diversity was negatively correlated to macrophytes density. Lower diversities, as macrophyte beds grew denser, could be a result of lower dissolved oxygen in the understory of the macrophytes bed (Papas 2007). Species richness is likely higher due to the habitat complexity provided by the macrophytes.

Increasing shoreline vegetation coverage was also found to increase invertebrate species richness. This result was similar to recent findings by Blanchette et al. (2020), that showed invertebrate species richness was significantly increased by terrestrial organic inputs in pit lakes formed by abandoned coal mines. There have been several prior studies that have outlined the benefits of debris from shoreline vegetation that can explain this. Evidence suggests that invertebrates use organic matter inputs as habitat (Pope et al. 1999). In addition, organic inputs from shoreline vegetation are known to account for a significant portion of the nutrients in oligotrophic lakes with small watersheds relative to their surface area (France and Peters 2002). Large reductions in dissolved organic compounds and total phosphorus

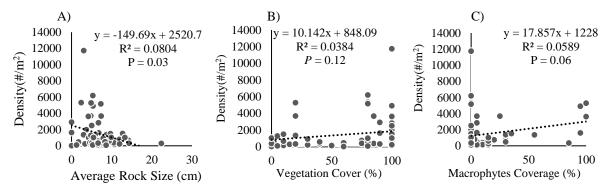


FIGURE 5. Macroinvertebrate densities (#/m<sup>2</sup>) of sample sites as A) average rock size, B) percent shoreline vegetation cover, and C) percent macrophytes coverage increase.

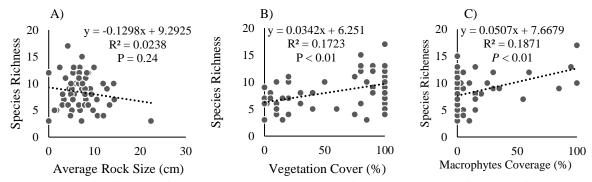


FIGURE 6. Species richness of sample sites as A) average rock size, B) percent shoreline vegetation cover, and C) percent macrophytes coverage increases.

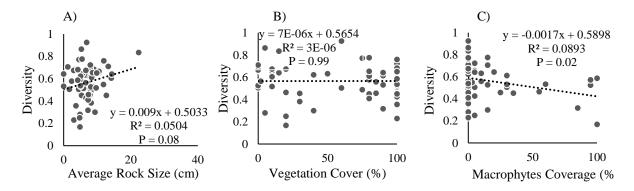


FIGURE 7. Diversity of sample sites as A) average rock size, B) percent shoreline vegetation cover, and C) percent macrophytes coverage increase.

resulting from lack of vegetation near these lakes likely regulate plankton communities (France et al. 1996).

Increasing rock sizes had a significant negative effect on invertebrate density. Some of this could be explained by difficulties in sampling larger rock sizes; however, the results of this study are similar to a previous study that found that mid-sized substrates have the highest invertebrate densities in streams (Williams and Mundie 1978). Substrates originating from glacial material had a significantly higher species richness than substrates uncovered by mining. This can likely be attributed to higher levels of macrophytes growth and terrestrial vegetation growth, which both were observed to lead to significant increases in species richness. This is not surprising since substrates uncovered from mining are largely devoid of nutrients needed for successful vegetation growth (Felleson 1999).

Although data collected in this study was site specific, some observations over the course of this study revealed patterns between whole lake scale attributes and the lake's mean invertebrate diversity. species richness, and densities. Diversities among the 10 pits sampled were found to be significantly different. LaRue pit clearly had the lowest diversity while Sherman pit, and Ore-B-Gone pit had the highest diversities. Sherman pit had low densities of common species which likely is the reason for its high diversity. Ore-B-Gone, however, had relatively high diversity, species richness, and density. It also is the only pit that has two specific invasive species, Zebra Mussels Dreissena polymorpha and Eurasian Watermillfoil. Future studies may want to further examine the effects of these species on this pits' invertebrates.

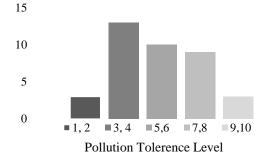


FIGURE 8. Distribution of the pollution tolerance level (on a scale of 1-10) of the 42 taxa identified.

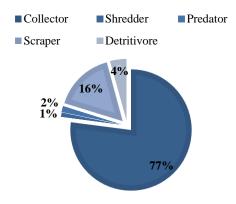


FIGURE 9. Shows the percentage of all invertebrates collected within five functional feeding groups.

Species richness was also significantly different between lakes. The two pits with the highest species diversity (Stubler and Ore-B-Gone) both had very dense macrophyte stands while the three with the lowest species richness (Sherman, Iron World, and Mott) had very limited to no macrophytes present in any of the sample sites. The difference in macrophyte colonization is likely partially responsible for this observed variation among pits' species richness because macrophytes were identified to have a significant effect on species richness.

No significance was detected in mean density among the 10 pits; however, there were some strong patterns observed. The three pits with the lowest densities all had the most unstable walls. Two others with low densities have both been observed to have large fish populations in past MN DNR surveys. Four of the remaining five pits had developed shoreline vegetation well and macrophytes communities as well as noticeably reduced erosion. The last pit which had a higher average density was I Falls pit. This pit stood out, as it had no macrophytes and limited shoreline vegetation. It did, however, have stands of flooded timber throughout and had a stream flowing into it from a larger, well vegetated watershed.

The results of this study indicate that future restoration projects of abandoned mine pits should focus on stabilizing shorelines and establishing healthy terrestrial and aquatic plant communities. Substrates originating from glacial till may be easier to rehabilitate and should be favored for these habitats. Although the results of this study indicate three factors that influence invertebrates, there are clearly several other factors that play a significant role. Future study may want to investigate the roll of these factors on a whole lake scale and try to quantify the roll that allochthonous nutrient inputs affect these pits.

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