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MICROSATELLITES REVEAL THE POPULATION GENETIC STRUCTURE OF YELLOW PERCH IN MINNESOTA

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Abstract—**When analyzing a population's genetic makeup, looking at variation of individuals and populations is a crucial factor. The management of yellow perch will be aided by this information particularly in regard to stocking or relocation. Microsatellite loci were used to analyze the genetic population structure of yellow perch *Perca flavescens* within and among 29 sampling areas throughout Minnesota's basins. Seven microsatellite DNA loci were evaluated, with heterozygosities ranging from 0.41 to 0.78. The Lower Mississippi basin samples showed higher numbers of alleles per locus and greater expected heterozygosities compared to other basins, with Lake Pepin (PEP) exhibiting the highest average expected heterozygosity at 0.78. Pfeiffer Lake (PFE) also contained unique alleles. Genetic population structure analysis revealed diverse ancestral compositions among populations, with some, like BRS, TNB, and PEP, showing dominant ancestral clusters, while most others had weaker differentiation. Neighbor-joining tree analysis indicated little genetic separation within Rainy River basin populations but significant differences between Rainy River and Minnesota River basin populations. PCoA and FST values suggested four distinct genetic groups: a central/southern Minnesota & Upper/Lower Mississippi basin cluster, a highly divergent Red River basin group, and tightly clustered Rainy River and Great Lakes basin groups. This study provides valuable insights into the genetic structure, diversity, and connectivity of yellow perch populations in Minnesota. The observed variations highlight the importance of considering regional differences in genetic makeup for effective fisheries management. Understanding these genetic distinctions will inform future conservation strategies, stocking decisions, and translocation efforts to preserve locally adapted gene pools and enhance the long-term resilience of yellow perch populations in the face of environmental changes.**

I. INTRODUCTION

Understanding the variation of individuals and populations is essential when researching a population's genetic structure. Deep comprehension of physical distribution, mating behaviors, dispersion, and life history can improve fitness and lead to versatile populations. For instance, an insect mating behavior study showed that females gain offspring with an increased lifespan when mating with multiple partners (Arnqvist and Nilsson 2000). In another study, fruit fly populations were predicted to have a higher fitness when of a polymorphic population rather than two monomorphic populations (Takahashi et al. 2018). That knowledge, in turn, can be used as a tool for directing efficient restoration, conservation, and management strategies. There is an abundance of equipment accessible to physically monitor a species, however, utilizing traditional techniques has limited the capacity of acquiring genetic variation data. An effective remedy for this issue is observing tandem repeats.

In eukaryotic genomes, repetitive sequences of DNA bases are frequently observed within a chromosome. Represented in the non-coding and coding regions of the DNA and ranging in lengths from one to several thousand base pairs, these repetitive sequences are identified as tandem repeats. Tandem repeats are classified based on the length of their repeated motifs and consist of microsatellite DNA, minisatellite DNA, variable number of tandem repeats, and simple sequence repeats (Marina 2020). Two to six nucleotide base pairs in repeating length distinguishes microsatellite DNA. Appearing in great abundances, manifesting high variability rates, and providing rapid data results, microsatellites are heavily utilized in contrast to any other subcategory of tandem repeats (O'Connell and Wright 1997). Proving enormously useful in studies of population structure, genetic mapping, and evolutionary processes, microsatellite DNA serves as a genetic marker for fisheries scientists to research population and community ecology.

Population structure in many species is dynamic and subject to ongoing refinement through genetic research. The yellow perch *Perca flavescens* is one such species for which population structure has been actively investigated. Previous studies have primarily focused on populations from the East Coast to the Midwest regions of the United States (Leary and Booke 1982; Kapuscinski and Miller 2000; Miller

2003; Grzybowski et al. 2010). Miller (2003) reported that spawning groups in Green Bay were genetically distinct from those in Lake Michigan and various inland locations. Similarly, Kapuscinski and Miller (2000) identified significant allelic differentiation among three examined populations. Grzybowski et al. (2010) further revealed greater levels of genetic differentiation than previously documented, based on analysis of 17 populations across the Midwest and East Coast. These studies highlight substantial population structure within yellow perch across its range. Ongoing genetic investigations are necessary to fully characterize the species' genetic diversity and patterns of connectivity, particularly in geographically underrepresented areas.

The purpose of this study was to describe the intra- and inter-population genetic variation in yellow perch populations across the major hydrologic basins of Minnesota. By utilizing highly polymorphic microsatellite DNA markers, this study aims to provide insights into the genetic structure, diversity, and connectivity of yellow perch populations at a regional scale. Such information is critical for informing fisheries management decisions, particularly with regard to stocking, translocation, and habitat restoration efforts. Understanding population differentiation can help managers avoid unintended genetic homogenization, preserve locally adapted gene pools, and enhance long-term population resilience. Additionally, this study contributes to the broader understanding of how landscape features, hydrologic connectivity, and historical management practices may have shaped the genetic structure of yellow perch in Minnesota. Ultimately, the findings will support evidence-based strategies for conserving, maintaining, and potentially expanding yellow perch populations in the face of environmental change and anthropogenic pressures.

II. METHODS

Sample collections. — In the summer of 2022, 20 state natural resource agency personnel collected yellow perch scales from any life stage at random sampling locations. Collections were obtained from 40 sampling locations within Minnesota: Six from Red River of the North basin, five from Rainy River basin, five from Great Lakes basin, five St. Croix River basin, six from Upper Mississippi River basin, six from Lower Mississippi River basin, five from Minnesota River basin, and two from Missouri River basin (Figure 1). The scales were air-dried and stored in envelopes for genetic analysis. Sample sizes from each location ranged from 7 to 38. A total of 29 out of the 40 sampling locations were included in the analysis, as each location had a sample size of 25 individuals or more (Table 1 and 2).

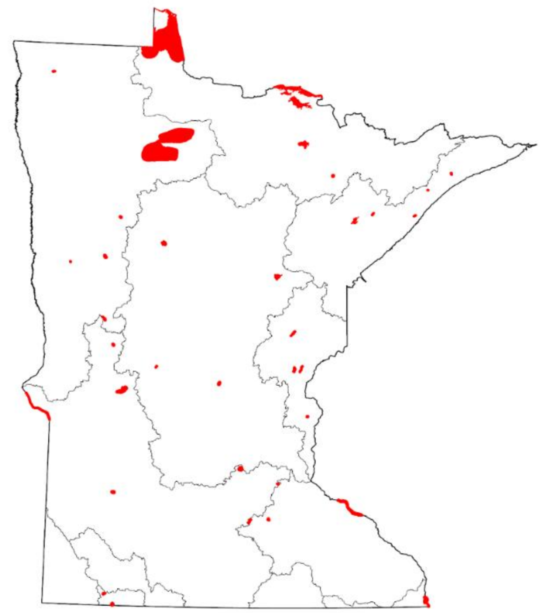

Figure 1. Map of the 40 sampling locations across Minnesota collected by state natural resource agencies in the summer of 2022.

Genetic analysis. — DNA was extracted from scale samples using 300 µL of 5% Chelex (BioRad Research Co., Hercules, California) solution. One to two scales were used for each preparation, lysed overnight, then boiled at 400 ºC for 8 minutes.

Sixteen microsatellite loci previously developed from walleye *Sander vitreus* and yellow perch were evaluated: *Svi3, Svi7* from Eldridge et al. (2002), *Svi4* and *Svi17* from Borer et al. (1999), *Pfla-L2*, *Pfla-L4*, *Pfla-L5*, and *Pfla-L6* from Leclerc et al. (2000), *MPf-2*, *MPf-4*, *MPf-6*, and *MPf-7* from Grzybowski et al. (2010), and *YP6*, *YP13*, *YP16*, and *YP79* from Li et al. (2006). Microsatellite amplification via the polymerase chain reaction (PCR) was performed in a 96-well plate. Each 15 µL PCR reaction contained 0.15 µL of the forward and 0.50 µL of the reverse primers, 5 µL of Chelex extraction as the DNA template, 0.1 µL of GoTaq G2 DNA polymerase, 3 µL of 5X colorless GoTaq reaction buffer, and 0.3 µL of a 0.10 mM deoxynucleotide triphosphate mixture was used for one locus. One primer of each pair was labeled with a fluorescent dye (FAM, PET, VIC, or NET). The following protocol for amplification was used in an Applied Biosystem 2720 thermal cycler: 3 min initial denaturation at 95 ºC; 34 cycles of 30 s denaturation at 95 ºC, 30 s annealing at 50 ºC, and elongation at 72 ºC for 45 s; and a final elongation of 5 min at 72 ºC.

Table 1. Summary of study lakes across the 6 major hydrologic basins in Minnesota. Each entry includes the major basin designation, associated DNR office location, lake name, unique Minnesota Department of Natural Resources (MNDNR) lake identification number, and a corresponding site acronym using throughout the study.

Major Basin	Office Location	Lake Name	Lake ID	Acronym
Great Lakes	Duluth	Bassett	69004100	BAS
Great Lakes	Duluth	Whiteface	69037500	WIT
Great Lakes	Finland	Lax	38040600	LAX
Great Lakes	Finland	Thunderbird	38003100	TNB
Great Lakes	Grand Maris	White Pine	16036900	WPL
Lower Mississippi	Lake City	Lake Pepin	25000100	PEP
Lower Mississippi	Waterville	Gorman	40003200	GOR
Lower Mississippi	Waterville	Volney	40003300	VON
Minnesota River	Glenwood	Minnewaska	61013000	MIN
Minnesota River	Glenwood	Moses	21024500	MOS
Minnesota River	Glenwood	Pelican	61011100	PEL
Minnesota River	Ortonville	Big Stone	06015200	BSN
Minnesota River	Spicer	Tyson	87001900	TYS
Rainy River	Baudette	Lake of the Woods	39000200	LOW
Rainy River	International Falls	Kabetogama	69084500	KAB
Rainy River	Rainy Lake	Rainy Lake	69069400	RNY
Rainy River	Tower	Pfeiffer	69067100	PFE
Red River	Baudette	Bronson	35000300	BRS
Red River	Detroit Lakes	Lee	14004900	LEE
Red River	Detroit Lakes	Rock	03029300	ROK
Red River	Detroit Lakes	Roy	44000100	ROY
St. Croix River	Hinkley	Cross	58011900	CRS
St. Croix River	Hinkley	South Pine	00010001	SP
Upper Mississippi	Atkin	Big Sandy	01006200	BS
Upper Mississippi	Little Falls	Little Sauk	77016400	LLS
Upper Mississippi	Little Falls	Mayhew	05000700	MAY
Upper Mississippi	Park Rapids	Mantrap	29015100	MTP
Upper Mississippi	Shakopee	Crystal	27003400	CRY
Upper Mississippi	Shakopee	Waconia	10005900	WAC

PCR products were visualized in two ways. In order to verify amplification and approximate product size, the products were displayed on a 14 × 16 cm nondenaturing 8% acrylamide gel and stained with ethidium bromide. To score alleles, the results of each individual PCR reaction were pooled and forwarded to the Azenta Commercial sequencing facility for fragment analysis. Genotypes were determined using Geneious Prime software (Biomatters, Boston, MA). Each plate contained a negative control without DNA to identify possible PCR contamination.

Data analysis. — The intra-population genetic variation, using seven polymorphic loci found during the first screening, was quantified as observed heterozygosity (H_o), expected heterozygosity (H_e), and allelic richness, the number of alleles standardized to a common sample size (A_r). Exact tests were used to assess conformity with the Hardy-Weinberg assumptions (Guo and Thompson 1992).

To reduce the potential for false detections of deviations from Hardy-Weinberg equilibrium while conducting multiple comparisons, a sequential Bonferroni correction was applied to Hardy-Weinberg exact test statistics (Rice 1989). A significance level (α) of 0.05 was used across 202 pairwise comparisons among 29 samples (k).

Spatial genetic structure interpopulation was examined using a Bayesian clustering approach in the program STRUCTURE (Pritchard et al. 2000) to identify distinct populations. STRUCTURE was run with 50,000 iterations of burn-in followed by 200,000 iterations to evaluate 1-25 possible populations.

To depict the genetic organization of populations, a neighbor-joining tree was created based on genetic distances in the software program Populations (Langella 1999). Results were visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

In order to represent inter-object similarity in a Euclidean space, a Principal Coordinates Analysis (PCoA) was performed (Gower 1966). The PCoA accounted for the similarity of allele frequencies to locate populations in multi-dimensional space. The add-in software tool GenAlEx (Peakall and Smouse 2012), on Excel 2010, was utilized to perform the PCoA analysis.

III. Results

Microsatellite Variation. — In the preliminary trials, 13 of the 16 microsatellites amplified yellow perch DNA. Out of the 13, 5 loci had monomorphic or minimal variance genetic makeup (*Svi3*, *YP6*, *YP13*, *YP16*, and *YP79*) and one fluorescently colored locus was incompatible due to overlapping size ranges *(Pfla-L2)*. The remaining 7 loci (*Svi4*, *Svi7*, *Pfla-L4*, *Pfla-L6*, *MPf-4*, *MPf-6*, and *MPf-7*) were used to evaluate the genetic variation of the samples.

The sample collections revealed polymorphisms for all seven loci. The one exception included locus *Svi7* at Thunderbird (TNB), which was monomorphic. From the samples in the Lower Mississippi basin, the average number of alleles per locus was 9.0 (range: 6.3–11.9). The average expected heterozygosity per locus was 0.69, with a range of 0.61 to 0.78. These numbers exceeded those from the Red River basin by a wide margin. The Lower Mississippi basin samples showed more alleles per locus and greater predicted heterozygosities than samples from all other basins (Table 2). An exception was observed in the Minnesota River basin, where the number of alleles was comparable, but heterozygosity was slightly lower. In comparison to samples from the Upper Mississippi basin, those from the Minnesota River basin had a similar number of alleles but consistently lower heterozygosity. Lake Pepin (PEP) had a higher level of heterozygosity at all seven loci, with an average expected heterozygosity of 0.78, the highest among all sampled populations.

Table 2. Sample information and genetic diversity measures for 29 locations of yellow perch populations from Minnesota. For each location, values are given for the following: sample size (N), observed heterozygosity (H_o), expected heterozygosity (H_e), and allelic richness standardized to a sample of 29 (A_r).

Sample	ID	N	H_o	H_e	A_r
BAS	1	27	0.59	0.63	4.7
WIT	11	14	0.69	0.64	5.2
LAX	18	35	0.69	0.69	5.6
TNB	20	25	0.41	0.40	3.2
WPL	17	15	0.67	0.58	4.1
PEP	25	29	0.78	0.78	8.5
GOR	3	21	0.65	0.68	6.9
VON	22	27	0.59	0.66	5.4
MIN	5	27	0.64	0.61	6.0
MOS	16	26	0.64	0.66	6.3
PEL	23	27	0.61	0.64	5.9
BSN	19	29	0.66	0.66	6.8
TYS	24	16	0.56	0.56	4.0
LOW	9	23	0.62	0.62	4.9
KAB	29	16	0.69	0.67	5.7
RNY	8	22	0.63	0.63	5.2
PFE	7	24	0.62	0.63	4.5
BRS	15	27	0.57	0.52	3.9
LEE	21	27	0.63	0.63	5.5
ROK	27	21	0.63	0.65	5.7
ROY	13	21	0.58	0.57	4.9
CRS	10	23	0.66	0.66	6.0
SP	14	26	0.64	0.61	4.8
BS	2	21	0.64	0.67	6.1
LLS	4	14	0.68	0.69	6.7
MAY	26	21	0.69	0.68	6.4
MTP	6	15	0.63	0.62	6.1
CRY	12	28	0.67	0.69	5.7
WAC	28	25	0.69	0.66	6.9

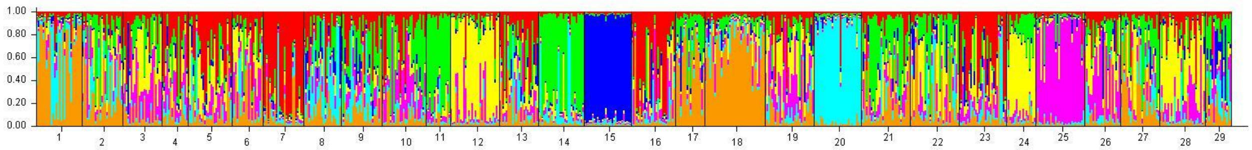

Figure 2A. The individual admixture proportions for 29 distinct populations, generated from a Bayesian clustering analysis without any prior information on sample locations. Each vertical line represents a single individual, and the different colored segments within each line indicate the estimated proportion of that individual's genome assigned to each of the inferred ancestral genetic clusters. The analysis utilized data from seven loci.

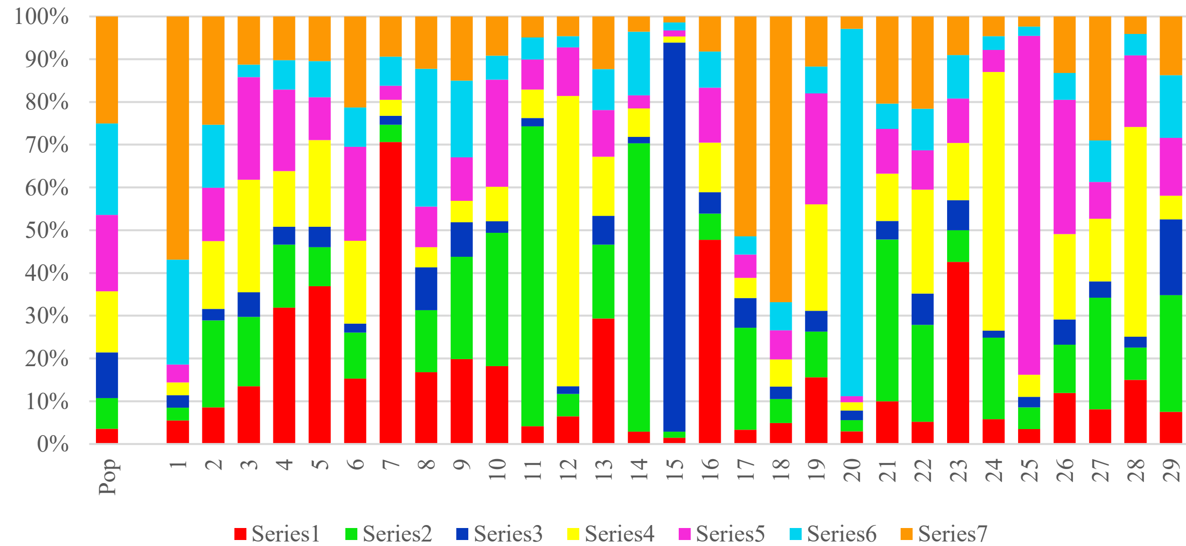

Figure 2B. Illustrates the estimated ancestral proportions for the 29 distinct populations derived from a Bayesian clustering analysis performed without prior location information. Each bar represents a single population, and the colored segments within each bar denote the average proportion of each of the seven inferred ancestral clusters contributing to that population's genetic makeup. The analysis was based solely on genetic data from seven loci, aiming to identify underlying genetic clusters among these distinct populations.

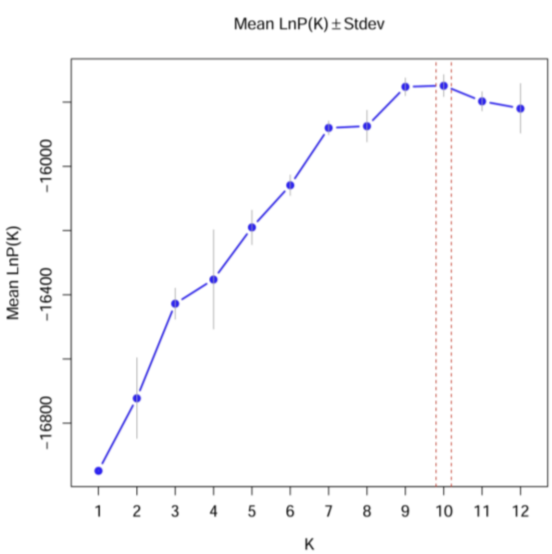

Figure 2C. The mean log-likelihood LnP(K) (± standard deviation) as a function of K, the number of inferred genetic clusters. The analysis was performed using a Bayesian clustering approach in STRUCTURE without a location prior. The line plateaus at 7 showing a plausible K value in accordance with Pritchard et al. (2010).

Only 12 of the alleles in the samples from the 29 samples were specific to a single population. In the Lake Pepin (PEP) sample, *MPf-6* had the greatest frequency of a particular allele at 80.0%. With frequencies ranging from 0.03 to 0.80, the Lake Pepin sample had the most distinct alleles (63 in total). The sample from Lake Pepin contained numerous rare or high-frequency alleles not found all throughout Minnesota. With the exception of *MPf-4* (the locus with the least variation), Pfeiffer Lake (PFE) had alleles at frequencies of 0.16 that were completely absent or only appeared at frequency of 0.01 or less in any sample from the Minnesota River basins. Every basin sample possessed at least one of these common 198 bp alleles.

After sequential Bonferroni correction for multiple tests ($\alpha = 0.05$, $k = 202$ [7 loci × 29 samples]), all loci in all samples complied with Hardy-Weinberg predictions. Eleven percent of the individual tests yielded statistically significant results ($P < 0.05$), and four remained significant after applying Bonferroni correction for multiple comparisons.

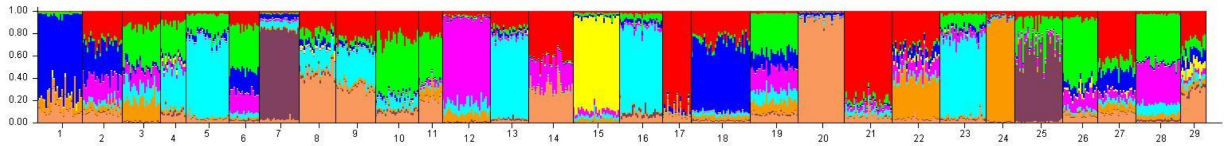

Figure 3A. Individual admixture proportions for the 29 populations (labeled 1-29) inferred using a Bayesian clustering approach in STRUCTURE with a location prior. Each vertical line within a population block represents an individual, and the colored segments indicate the proportion of that individual's genome assigned to each of the inferred genetic clusters. Distinct colors represent different genetic clusters. The analysis was conducted using nine microsatellite loci.

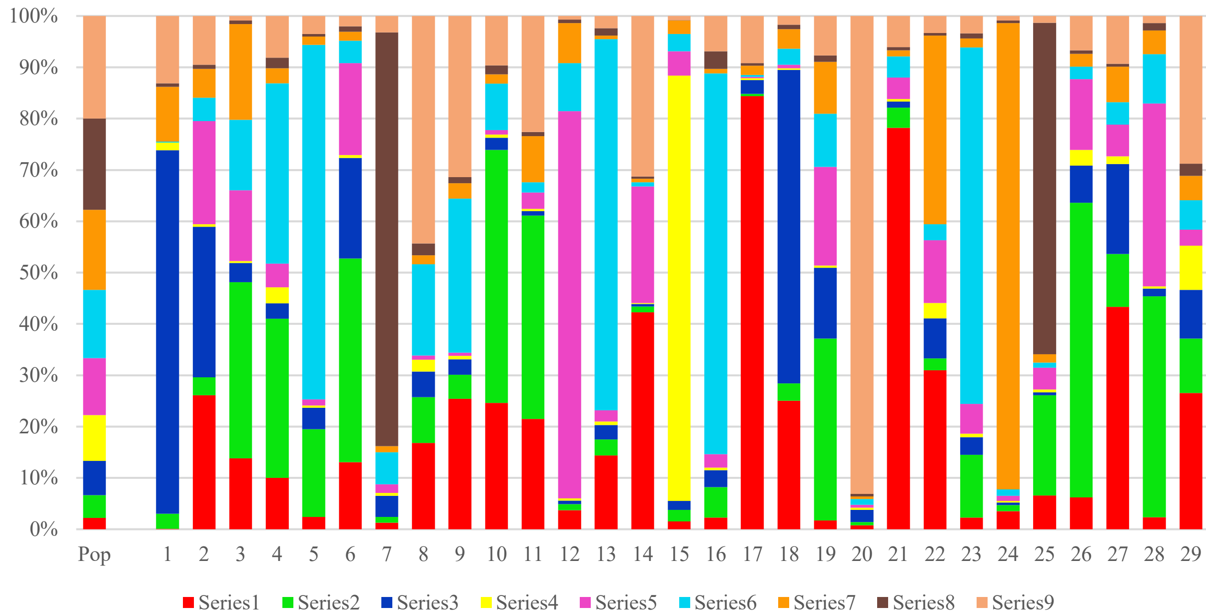

Figure 3B. Each bar represents the average profile for a single population with the colored segments within each bar indicate the average proportion contributed by each of the nine inferred genetic clusters.

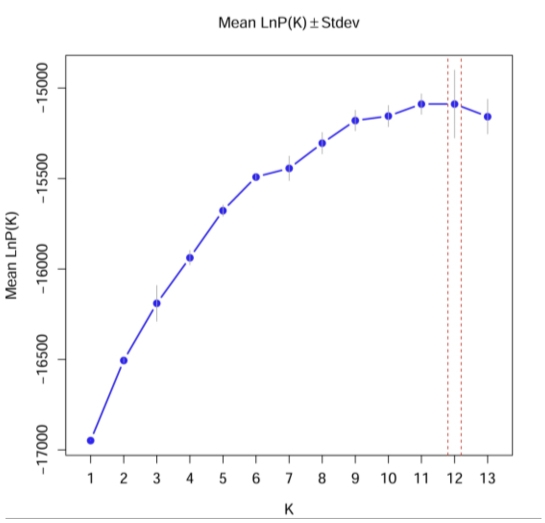

Figure 3C. The mean log-likelihood LnP(K) (± standard deviation) as a function of K, the number of inferred genetic clusters with location prior. The analysis was performed using a Bayesian clustering approach in STRUCTURE. The line plateaus at 9 showing a plausible K value in accordance with Pritchard et al. (2010).

None of the significant deviations originated from the same location or population, and no consistent pattern was observed. There is no evidence that any specific locus or population is systematically out of Hardy Weinberg equilibrium and all data was retained.

Genetic Population Structure. — Populations exhibit diverse ancestral compositions with and without incorporating prior information about the geographic locations of the samples (Figure 2A, 2B, 2C, 3A, 3B, 3C). A handful of populations contain dominant ancestral or population specific clusters (e.g., 15 (BRS), 20 (TNB), and 25 (PEP)), while the majority of the populations contain relatively weak differential structure that was too weak for the amount of markers used to distinguish. No major similarities within watershed populations are observed.

The construction of the tree indicates how similar two groups of samples are genetically (Figure 4). Little genetic separation existed between the Rainy River basin populations. On the tree diagram, the locations within the group were close together (Figure 4).

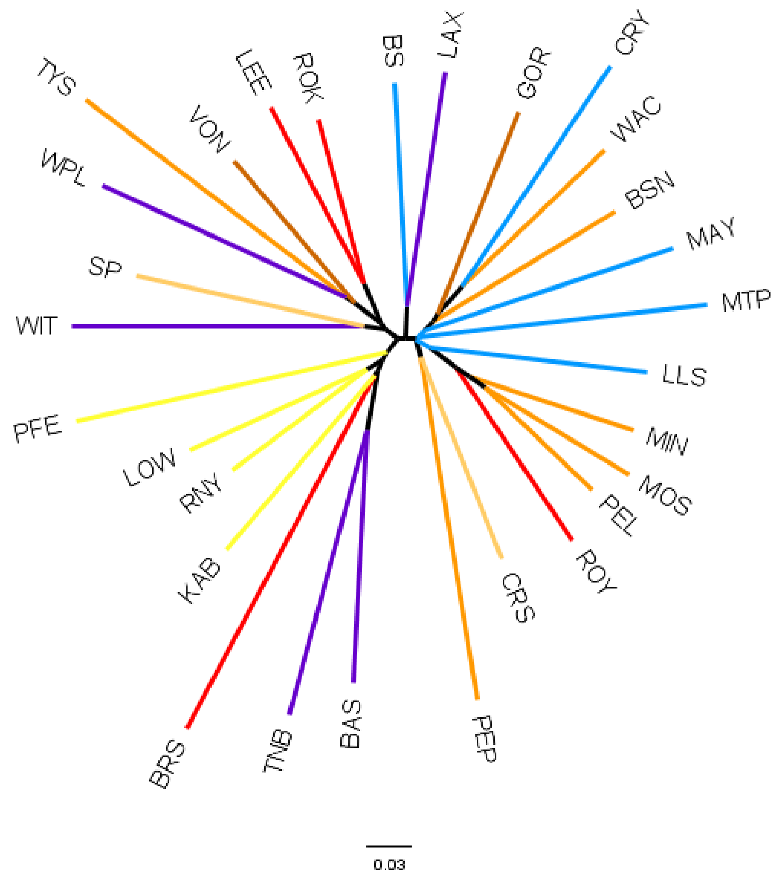

Figure 4. The genetic relationships among the 29 populations, with each line representing a distinct population. The colors of the lines correspond to the major basins from which the populations were sampled, highlighting genetic clustering and potential migratory pathways or divergence patterns associated with geographic origin. The length and angular separation of the lines represent genetic distance, indicating the degree of relatedness and evolutionary divergence between populations.

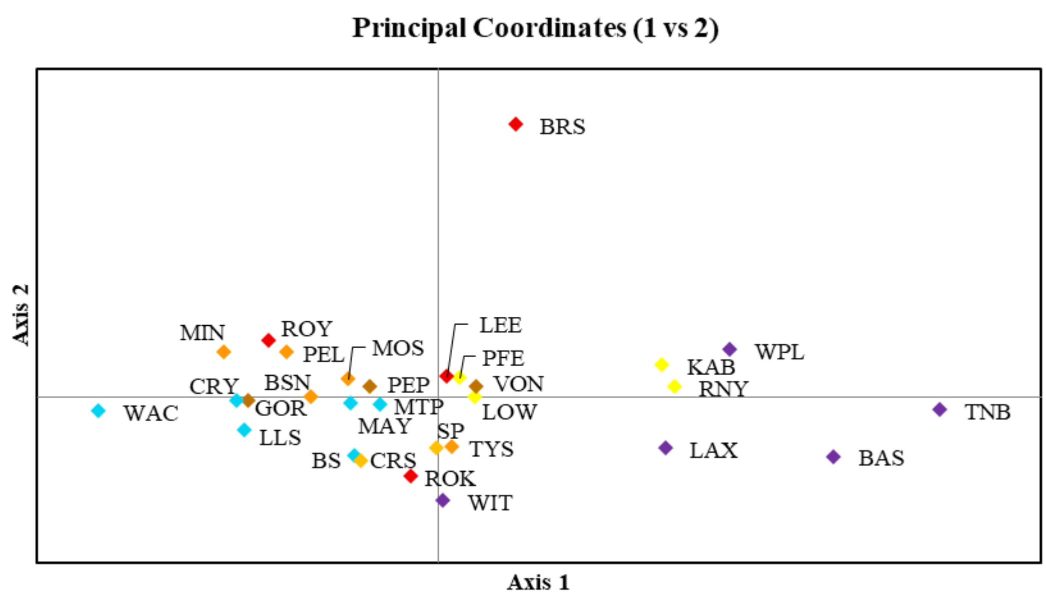

Figure 5. A Principal Coordinates Analysis (PCoA) plot showing the genetic relationships among the 29 populations. The plot is based on the first two principal coordinate axes (Axis 1 vs Axis 2), which explain the largest proportion of the total genetic variation. Each point represents a distinct population, labeled by its acronym and colored according to its major basin of origin. The proximity of points indicates genetic similarity, with more distant points suggesting greater genetic differentiation.

However, the samples from Rainy River basin were distinct from those from the Minnesota River basin. The genetic distances between pairs from Rainy River basin and Minnesota River basin (e.g, RNY-MIN = 0.042, RNY-MOS = 0.047) were comparable to those between pairs from Rainy River basin and Great Lakes locales from various drainages (e.g, RNY-WIT = 0.059, LOW-WIT = 0.079). The Rainy River basin sampling locations were grouped on a different branch from the Minnesota River areas on the tree diagram (Figure 4). All samples, including those taken inside and outside of each basin, were divided into two main groups (Figure 4).

The PCoA plot combined with the FST values (not shown) suggests that there are four distinct main genetic groups (Figure 5). One branch includes the large, dense cluster spanning left to central part below Axis 2, which includes samples from the Central/Southern Minnesota and Upper/Lower Mississippi basin locations. These populations exhibit low FST values among themselves representing a major contiguous genetic lineage. The second group observed is the Red River basin which are genetically highly divergent from other populations, with FST values with other basins ranging up to 0.096 to 0.106. The third and fourth groups include the Rainy River basin cluster and the Great Lakes basin cluster which are both tightly clustered and contain low FST values within their own groups.

IV. Discussion

The present study aimed to characterize the intra- and inter-population genetic variation of yellow perch across major hydrologic basins in Minnesota using highly polymorphic microsatellite DNA markers. Our findings reveal significant genetic structuring among yellow perch populations within Minnesota, consistent with the dynamic nature of population structure observed in many species (O'Connell and Wright 1997). The observed differences in allelic richness and heterozygosity among basins, particularly the higher genetic diversity in the Lower Mississippi basin and the unique genetic signature of Lake Pepin (PEP), underscore the importance of regional-scale genetic assessments for effective fisheries management. These insights are crucial for informing decisions related to stocking, translocation, and habitat restoration, ensuring the preservation of locally adapted gene pools and enhancing long-term population resilience against environmental changes (Arnqvist and Nilsson 2000; Takahashi et al 2018).

The substantial population structure identified in Minnesota yellow perch aligns with previous research on *P. flavescens* across its broader range. Miller (2003) reported genetic distinctiveness between spawning groups in Green Bay and Lake Michigan, while Kapuscinski and Miller (2000) found significant allelic differentiation among populations. More recently, Grzybowski et al. (2010) revealed even greater levels of genetic differentiation across Midwest and East Coast populations. Our study extends these findings by demonstrating distinct genetic groups within Minnesota's hydrologic basins, such as a couple of the highly divergent populations within the Red River basin group and tightly clustered Rainy River and Great Lakes basin groups. The relatively weak differentiation observed in some populations, despite the use of highly polymorphic microsatellites (Marina 2020), suggests potential gene flow or historical connectivity that warrants further investigation.

The identified genetic groupings and varying levels of heterozygosity have direct implications for the conservation and management of yellow perch. The presence of distinct genetic lineages, particularly the highly divergent Red River basin populations, suggests these groups may represent unique evolutionary units requiring specific management considerations to prevent genetic homogenization. Conversely, the contiguous genetic lineage observed across Central/Southern Minnesota and Upper/Lower Mississippi basin locations implies a greater degree of connectivity or shared ancestry, which could influence decisions regarding regional stocking programs. Understanding these genetic patterns can help managers avoid practices that might inadvertently dilute local adaptations or reduce overall genetic diversity, thereby supporting the long-term viability of yellow perch populations (Leary and Booke 1982).

While this study provides a foundational understanding of yellow perch genetic structure in Minnesota, certain limitations and avenues for future research exist. The use of seven microsatellite loci, while informative, may not fully resolve all subtle genetic differentiations, particularly in populations exhibiting weak structure. Future studies could benefit from an increased number of highly polymorphic markers, such as single nucleotide polymorphisms (SNPs), to provide higher resolution genetic insights (Yin et al. 2025). Additionally, incorporating environmental variables and landscape features (e.g., riverine connectivity, dam presence, historical stocking records) into spatial genetic analyses could further elucidate the factors driving the observed population structure. Continued genetic monitoring, particularly in geographically underrepresented areas, will be essential to track changes in genetic diversity and connectivity in response to ongoing environmental and anthropogenic pressures.

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AGE CLASS IS NOT RELATED TO SIZE OR COMPOSITION OF HOME RANGES AMONG SUBURBAN WHITE-TAILED DEER DURING PARTURITION

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***Abstract*—How *w*hite-tailed deer use the landscape in an urban environment can help us understand the effects of urbanization on deer populations. White-tailed deer form observable matriarchal hierarchies in studied populations. This study examines the matriarchal structure, home range size, and home range composition of 25 collared white-tailed deer within the city limits of Bemidji, MN. The results found no significant difference between age class and home range size or composition. However, movement metrics from yearling and mature age classes were more similar than the juvenile age class females. I propose this is because social acceptance between mature females was higher for yearlings than juvenile females in our study area.**

I. Introduction

The social structure of female white-tailed deer (*Odocoileus virginianus*) typically consists of related individuals (Miller et al. 2010). Older females typically are the matriarch of these groups, and the rest of the social group is typically formed of successive generations. Males are typically not a part of these groups.

The fawning season is a time when female white-tailed deer behavior is observed to change. During the 6 weeks before and 18 weeks after parturition, parturient white-tailed deer have smaller home range sizes and less sociality than they do throughout the rest of the year (Bertrand et al. 1996, Ozoga et al. 1982). When parturient female home ranges are decreasing, there is simultaneously an increase in segregation between other parturient female home ranges (Schwede, Hendrichs, McShea 1993). Older parturient females often undergo less movement during the nutritionally stressful time of lactation and have smaller, more aggressively defended home ranges which suggests they are occupying higher quality habitat (Holland et al. 2024, Ozoga et al. 1982).

A high density of individuals can conceal the reduction in home range size typically observed surrounding parturition (Bertrand et al. 1996). This is likely due to home ranges already being too small to noticeably shrink, as average home range size decreases with a higher population density. In multiple instances, deer in a suburban environment have been recorded having smaller home ranges than those in forested or agricultural environments (Kilpatrick & Sophr 2000). This is likely to prevent mis-imprinting of the fawns on other nearby deer (Lent 1974; Ozoga et al. 1982). Additionally, deer in a suburban setting move further away from residential areas during the summer and into undeveloped land instead (Kilpatrick & Sophr 2000). This could be because of an abundance of natural food sources in undeveloped areas during the summer, and the presence of human supplemented food sources like bird feeders that get visited more often in the winter (Kilpatrick & Sophr 2000).

The objective of this study was to determine whether the white-tailed deer population in Bemidji have an observable matriarchal structure during parturition and fawn rearing (May-August). I hypothesized that a matriarchal structure would be statistically observable, with older females occupying different habitat than younger females. Elements of 25 collared white-tailed deer home ranges were analyzed with the age of the deer to determine the presence of a matriarchal structure.

II. Study Area

The Study Area was limited to the city limits of Bemidji, MN. Bemidji is located at 47.4716° N, 94.8827° W in Beltrami County. Bemidji's population as of the 2022 census is 15,946 people. Bemidji averages 27 inches of rainfall per year and lies in the temperate climate zone. Dominant forest types within the study area are mixed deciduous forest and coniferous forests. Common deciduous forest species in Bemidji include basswood (*Tilia americana*), paper birch (*Betula papyrifera*), silver and sugar maple (*Acer saccharinum* and *Acer saccharum*), and trembling aspen (*Populus tremuloides*). Common coniferous tree species in Bemidji include Eastern white pine (*Pinus strobus*), red pine (*Pinus resinosa*), white spruce (*Picea glauca*), and balsam fir (*Aibes balsamea*).

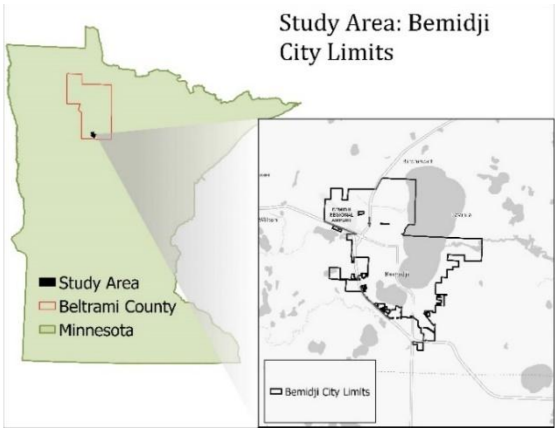

Fig. 1. Map of Bemidji city limits in Beltrami County, Minnesota.

III. Methods

The Bemidji Deer Project was created to study the effects of harvest pressure on white-tailed deer within the city limits of Bemidji, MN. As a component of the research project, 25 female white-tailed deer were collared. Location points were collected for 25 deer from May 2nd to August 30th (120 days) of 2024. GPS coordinate points were automatically taken approximately every 6 hours.

I estimated the home ranges by building a Minimum Convex Polygon (MCP) around the points marked on the satellite collars. Using the National Landcover Data (NLCD) 2019 raster layer, I calculated the landcover proportions of each home range. I simplified the landcover data into 4 categories: Developed, Open, Forested, and Wetland. I then calculated the amount of fragmentation by road using the GIS Fragmentation Index (GISfrag) method from Ripple et al. (1991). This method entails creating a distance raster layer where each cell has a value that is equal to its distance from a road. Then, the mean of the cell values within a home range polygon is taken, which gives the GISfrag value for a specific home range.

To conduct the statistical analysis, I first standardized the variables by subtracting the mean from each measurement then dividing by the standard deviation of the measurement. Those variables being amount of Open landcover, Developed landcover, Forested landcover, Wetland landcover, Total home range size, and the GISfrag value for each home range. I used a polynomial ordinal regression model in "R" to test for significance between home range elements and age classes of yearling (1 year), juvenile (2–3 years), and mature (4+ years).

IV. Results

My results indicated that there was no significant relationship between age classes and any of the analyzed home range elements (Table 1, Fig. 2). However, the yearlings and mature females appeared to have more similar home range composition when compared with juveniles. Mean home range size for mature females was 2091.06 km^2, for juvenile females was 4756.50 km^2, and for yearling females was 2223.00 km^2.

TABLE 1. OUTPUT FOR THE POLYNOMIAL REGRESSION MODEL USING HOME RANGE COMPOSITION TO PREDICT AGE CLASSIFICATION IN FEMALE WHITE-TAILED DEER.

Habitat Element	Estimate	Standard Error	Z Value	Pr(>|z|)
Open	1.120E+02	9.496E+03	0.01	0.99
Developed	7.394E+01	6.335E+03	0.01	0.99
Forested	1.567E+02	1.333E+04	0.01	0.99
Wetland	1.519E+02	1.293E+04	0.01	0.99
Road	-3.308E-01	3.490E-01	0.95	0.34
Range Size	-4.444E-02	9.496E+03	0.13	0.89

V. Discussion

A statistical analysis yielded no conclusions for a matriarchal structure within the Bemidji deer population. This is in contrast to other research in non-urban environments that have observed matriarchal structures within a population (Holland et al. *In press*, Ozoga et al. 1982, Schwede et al. 1993). Despite the lack of statistical evidence, there are some distinct similarities between the habitat elements of yearling and mature deer. Among all measured variables, they appeared to be more closely related to each other than they were with the juvenile age class (Fig. 2). Mature females and yearling females had more similar and smaller home range sizes than juvenile females.

I propose that mature females are tolerating yearlings more than they are tolerating juveniles, possibly having more aggressive interactions towards them than they do yearlings. The smaller home range sizes of mature and yearling females suggests that they were occupying higher quality habitat than juveniles.

Schwede, Hendrichs, McShea (1993) found that there was no significant difference between age class of previous offspring regarding association frequency with their mother during the fawning season. However, this study also found that in previous offspring juveniles had larger average distances to their mother than yearlings.

As this study established home range boundaries using MCPs, activity centers and home range cores could not be established, nor analyzed over time. This could have limited the findings of social organization of the white-tailed deer, or the lack thereof. More research could be done that targets home range cores and activity centers to better understand the social structure of the urban deer population in Bemidji.

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VII. Appendix

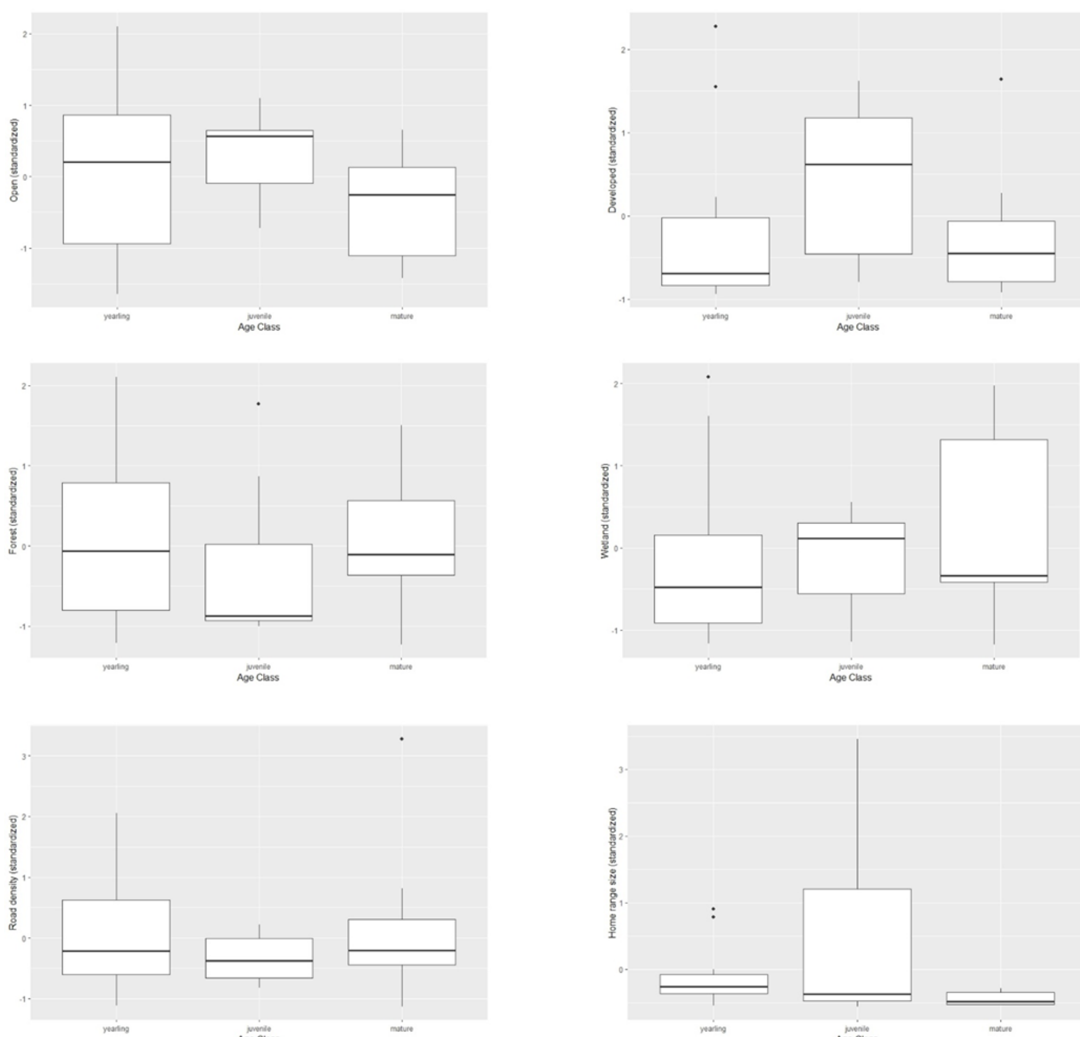

Fig. 2. Box plots comparing home range characteristics between age classes of yearling (1 year old), juvenile (2–3 years old), and mature (4+ years old).

GROWING DEGREE DAYS COMPARED TO GROWTH OF WALLEYE AND SAUGER FROM DIFFERENT SYSTEMS

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***Abstract*—Walleye *Sander vitreus* and Sauger *Sander canadensis* exist in most river and lake systems across the state of Minnesota and have historically been a highly sought-after species. Variable water temperatures play a significant role in the growth of cold-blooded organisms such as walleye and sauger. Therefore, the objective of this study is to test for a relationship between growing degree days (GDD) and the growth of walleye and sauger from Lake Pepin, Leech Lake, and Lake of the Woods. Data for growth and age was readily available and was sourced from MN DNR lake survey database from 2006 to 2023. Growing degree data was sourced from Weather Underground. A regression test was performed on the data, and Akaike Information Criterion (AIC) scores were generated. This study conducted four regressions tests on the data set. There is a clear upward trend showing a positive correlation between GDD and growth, indicating that as GDD increases so will growth. Additionally, a consistent correlation between growth and year was observed with Leech Lake and Lake of the Woods following a clear trend overtime, while Lake Pepin differed. Growing degree days and year indicated a correlation between Leech Lake and Lake of the Woods while Lake Pepin again differed. These findings highlight a significant relationship between both GDD and year, and growth and year. This variation may also be influenced by several factors such as forage species, nutrient loading, and geographic location.**

I. INTRODUCTION

Walleye and sauger have historically been one of Minnesota's top sport fish (Olson 1958). Metabolic rate of fish is largely determined by the ambient temperature of their environment (Chezik et al. 2014). Therefore, variable water temperatures play a significant role in the growth of cold-blooded organisms such as walleye and sauger. The optimal temperature for growth of walleye and sauger is 4.62 °C (Honsey et al. 2023).

Fish growth is defined as the increase in size usually measured by length or weight. Fish growth only happens within certain temperature ranges, of which the upper and lower temperature thresholds are well established for many species. Walleye and sauger begin to grow at a minimum temperature of 4.62 °C (Honsey et al. 2023), while the upper limit for growth is 30 °C (Hasnain et al. 2013).

Air temperatures have been increasingly surrogated for surface water temperatures (Chezik et al. 2014). Air temperatures also show a high correlation with surface water temperatures (Chezik et al. 2014), with historical air temperature databases being more extensive and easier to find. Growing degree days is described as the thermal opportunity for growth and development (Chezik et al. 2014). Therefore, the objective of this study is to test for a relationship between growing degree days and growth of year one walleye and sauger from Lake Pepin, Leech Lake, and Lake of the Woods.

II. METHODS

Lake Pepin is the smallest of the three systems, with a surface area of 188.55 km^2. It is one of the few lakes with a major river flowing through it. Due to its connection to the Mississippi river, Lake Pepin drains a significant portion of the state. Leech Lake is the second largest system in this study, covering a total surface area of 451.33 km^2. The largest system included in this study is Lake of the Woods, with a surface area of 3,849.92 km^2.

The growth data was taken from the Minnesota DNR Large Lake Database which uses gillnets to sample multiple systems for game fish such as walleye and sauger (MN DNR 2025). The growth data was readily available and was taken from the large lake database. The data was broken down by the system it came from, year of capture, age of fish, and length of fish. The analysis for growth of walleye and sauger were pooled. This is because sizes generally do not differ until later in life.

The growing degree day data was collected from Weather Underground (2025) which is a website with historical weather data. Air temperatures were obtained from local airports near the systems. The airports used were the Red Wing Regional Airport for Lake Pepin, Warroad International Airport for Lake of The Woods and, Bemidji regional airport for Leech Lake.

Maximum monthly temperatures and minimum monthly temperatures were used to calculate growing degree days. Monthly maximum and minimum temperatures were input into the equation (Equation 1). The equation then output the amount of growing degree days for given a month. Any negative GDD was input as a zero. Growing degree days were then summed for a given year. With both maximum and minimum temperatures being represented as Tmax and Tmin with Tbase being represented as the temperature at which walleye and sauger begin to grow. Equation one shows the formula for growing degree days (Uphoff et al. 2013).

$$GDD = \left[\frac{T_{\max} + T_{\min}}{2}\right] - T_{\text{base}}$$

Equation 1: This equation was generated to calculate growing degree days (Uphoff et al. 2013). Growing degree days represented as GDD and temperature represented as Tmax and Tmin and temperature at which walleye and sauger start to grow represented as Tbase.

Four regression tests were performed on the data set with each regression focusing on a different section of the data. The series of models tested were length as a function of GDD with lake as an additive, length as a function of GDD times lake, length as a function of lake, and length as a function of GDD (Table 1). Akaike Information Criterion (AIC) scores were generated. AIC score is a number used to determine which model is best supported, the lower the AIC score the better.

TABLE. 1. MODELS REPRESENTING LENGTH (MM) WITH GDD (2006-2023) AND LAKE FROM LAKE PEPIN, LEECH LAKE, AND LAKE OF THE WOODS.

Model	AIC
Length ~ GDD + Lake	318.5
Length ~ GDD * Lake	320.5
Length ~ Lake	332.4
Length ~ GDD	364.3

III. RESULTS

The results show a clear relationship between GDD and length at age one. With ranges for both being listed below. Lake Pepin's ranges were 210 to 362 mm and 74 to 86 growing degree days. Leech Lake's ranges were 178 to 366 mm and 52 to 70 growing degree days. Lake of the Woods ranges were 128 to 416 mm and 44 to 69 growing degree days.

The best supported model was length as a function of GDD with lake as an additive variable (AIC = 318.5; Table 1). The fitted model was plotted using slope of GDD and length from each system (Figure 1). This shows a positive relationship between length and GDD (Figure 1).

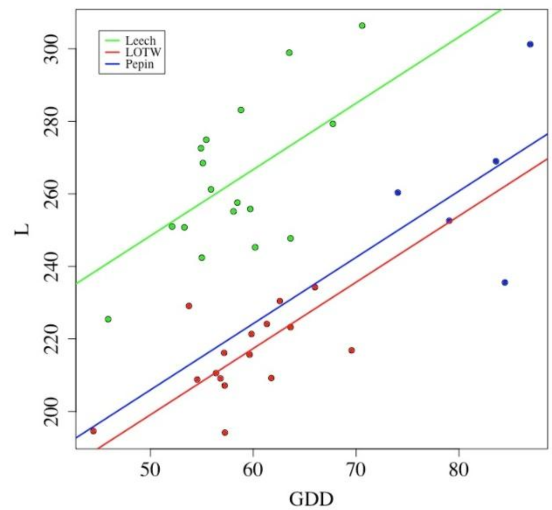

Fig. 1. Length (L; mm) plotted against growing degree days (GDD) from 2006 to 2023 for Lake Pepin, Leech Lake, and Lake of the Woods. Data was collected from MN DNR lake survey data base.

The growth data consists of lengths from 2006 to 2023. The data shows a correlation between GDD and growth for all years and between systems (Figure 2). The relationship is shown in years such as 2009 which shows a sharp drop for both GDD and growth in Leech Lake and Lake of the Woods (Figure 2 and Figure 3). While Lake Pepin shows an upward trend in growth (Figure 2). This trend is also shown in GDD compared to year suggesting a relationship (Figure 3).

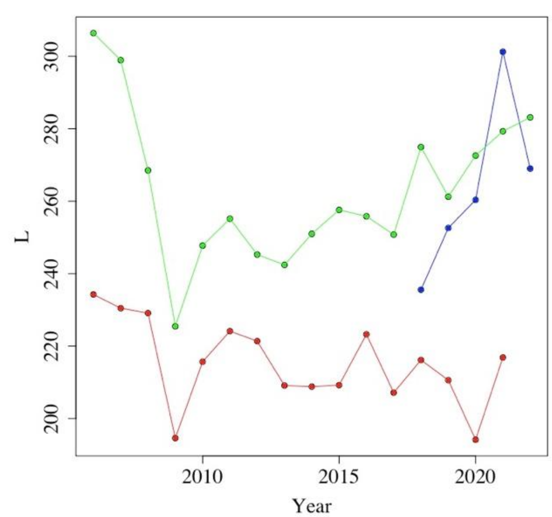

Fig. 2. Length (L; mm) plotted against year from 2006 through 2023 for Lake Pepin, Leech Lake, and Lake of the Woods. Data was collected from MN DNR lake survey data base.

GDD plotted over year shows a correlation between the amount of growing degree days for certain years (Figure 3). This correlation can be seen as a warming or cooling trend (Figure 3). There is a clear correlation between Leech Lake and Lake of the Woods. Growing degree days for both systems are nearly identical. While Lake Pepin differs with an increased amount a growing degree days over time (Figure 3).

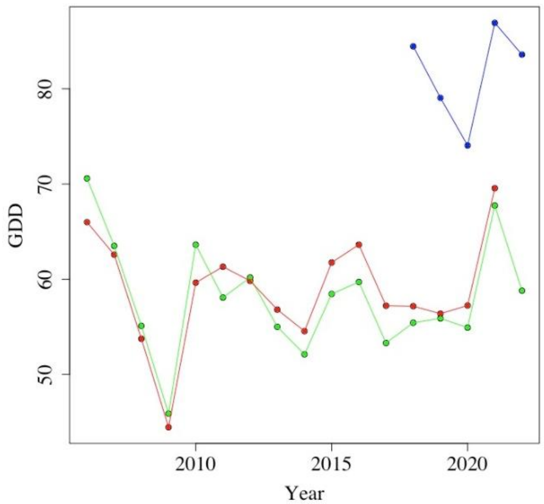

Fig. 3. Growing degree days (GDD) plotted against year from 2006 to 2023 for Lake Pepin, Leech Lake, and Lake of the Woods. Data was collected from MN DNR lake survey data base.

IV. Discussion

There is a clear positive relationship between growth and growing degree days. This correlation suggests that GDD may affect growth of cold-blooded organisms. The positive relationship between GDD and growth may be down to an increase in water temperature. This increase in temperature should increase a fish's thermal opportunity for growth and development (Chezik et al. 2014). There is evidence to suggest that as growing degree days increase fish may experience higher growth rates (Uphoff et al. 2013).

There is a consistent correlation between growth and year that was observed. The correlation could be influenced by the air temperature. Air temperatures are increasingly being surrogated for surface water temperatures (Chezik et al. 2014). With this correlation being seen in Leech Lake and Lake of the Woods with length data seeming to follow one another (Figure 2). This trend is also seen with GDD and year with Leech Lake and Lake of the Woods (Figure 3). This relationship between the two can be seen in multiple years. 2009 showed a sharp decline in GDD and growth suggesting a relationship.

The correlation between growing degree days and year is very interesting. There is a clear correlation between Leech Lake and Lake of the Woods, while Lake Pepin stands on its own. This close correlation maybe because of their close geographic location. Lake Pepin again differs from the other systems with a higher amount of GDD. Perhaps this is because Lake Pepin is further south than the other two systems. This also reveals an interesting relationship with growth of walleye and sauger as it seems to follow the amount of growing degree days in each year.

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COMPARISON OF HOOP NETS TO TRAP NETS IN SAMPLING PANFISH

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***Abstract*—Panfish including bluegill *Lepomis macrochirus* and black crappie *Pomoxis nigromaculatus* are gamefish species of significance found in Minnesota. Hoop nets and trap nets are two types of gear that can be used to sample these fish. Fisheries managers and anglers alike are interested in the best tactics to capture panfish. The behavior of fish, including bluegill and black crappie, change throughout the sampling season and make it difficult to assume accurate representations. Trap nets have historically been adequate in sampling near shore populations, but questions surrounding the gear have allowed other options to be pursued. The objective of this study is to compare the effectiveness of hoop nets to trap nets by analyzing the difference in catch per unit effort (CPUE), length, and proportional size distribution (PSD) of panfish. Nets were set within two weeks of each sample period. Captured panfish accounted for 96.5% of the total catch between the two nets. Black crappie CPUE significantly increased ($P < 0.01$) with hoop nets while bluegill CPUE was not significantly ($P = 0.30$) different by net type. The average lengths for bluegill captured in hoop net were longer than those captured in trap nets. Bias in gear selectivity may have produced different catch rates and size structures for each net and species. Further studies will be needed to accurately estimate the differences in each nets ability to sample panfish.**

I. Introduction

Sampling nearshore gamefish has shown to be effective in measuring population dynamics when using certain gear (Miranda and Boxrucker 2009). Depending on the type of system, gear used, and state of seasonality, certain species can be targeted to sample (Porta et al. 2020). The physical and dimensional attributes of a net can limit the ability to catch as well as create a bias (McIrney and Cross 2020). Trap or modified fyke nets have been used to capture panfish and other large nearshore species that are selective towards cover-seeking and mobile fish (Bonar et al. 2009). Hoop nets are primarily used in capturing mobile species in lotic systems but also have captured species in impoundments and lentic systems (Long et al. 2017). A suitable method of precision in specific sampling is preferred when targeting certain aspects.

Panfish including bluegill *Lepomis macrochirus* and black crappie *Pomoxis nigromaculatus* are gamefish species of significance found in Minnesota. They are the third most sought-after fish in the state (USFWS 2011). Panfish are targeted by anglers year-round and are one of the most widespread groups of fishes in Minnesota. A creel survey on Lake Osakis in 2021 found sunfish and black crappie to be the most sought-after species and the highest amount of harvested catch (Rydell 2021). Fish behavior of these species can change seasonally through abiotic and biotic factors such as temperature, spawning habits, and prey availability (Baumann 1972). Panfish have primarily been sampled for management using trap nets (McIrney et al. 2020).

The ability to capture panfish at the appropriate time can be crucial to understanding their behavior as well as correctly measuring the population (Flammang et al. 2016). It is more beneficial to target nearshore species earlier in the year but before spawning activity to ensure the most unbiased representative sample (McIrney 2020). As the season progresses fish move into deeper water and do not always provide enough numbers to measure population metrics (Long et al. 2017). Other tactics of sampling with different gear such as hoop nets have included bycatch of these species (Flammang et al. 2016). In order to be most effective in effort expounded, the gear selectivity should be closely monitored to understand what the most efficient method is. Therefore, the objective of this study is to compare the effectiveness of hoop nets to trap nets by analyzing the difference in catch per unit effort (CPUE), length, and proportional size distribution (PSD) of panfish.

II. Methods

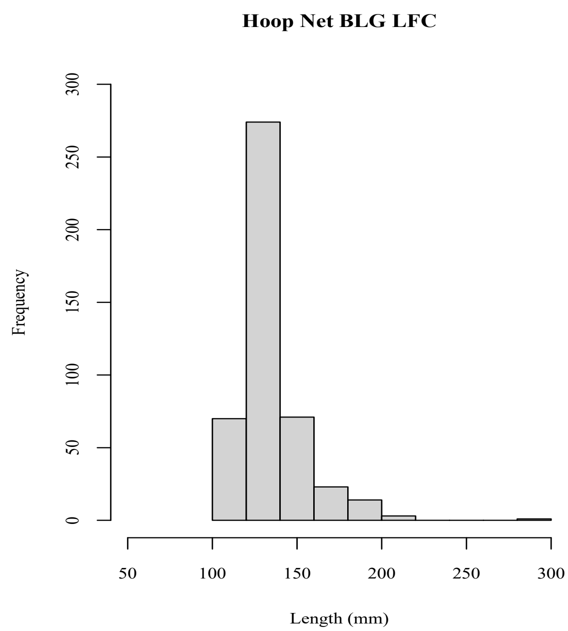
This study was conducted on Stella Lake located in south-central Minnesota. Stella Lake is a 242 ha body of water with a maximum depth of 22.9 m. Fish were sampled in two periods, one for hoop nets and the other for trap nets. Hoop nets were used to capture fish from July 29th to August 2nd, 2024. Trap nets sampled fish from August 13th to August 14th, 2024.

Fish were sampled using standard hoop nets and standard trap nets. Hoop nets consisted of seven 0.8 m hoops with a 25 mm mesh and total length of 3.4 m. Hoop nets were set in tandem by tying the cod end of the first net to the bridal of the second net. Small mesh bags baited with cheese were attached to the inside ring of each hoop net. Trap nets were standard double frame with 19 mm mesh. Trap nets were set with a single net by configuring the lead end to shore.

Sites for the hoop nets were determined by an effort to sample a diverse mix of habitats. Hoop nets were set both parallel and perpendicular to shore. Depths of the hoop nets ranged to sample below the maximum depth of a trap net but above the minimum oxygen threshold to ensure fish health. A total of twenty sites were selected and set with tandem hoop nets. Nets soaked for a period 24 hours and were retrieved. Fish from the first net of the tandem were sorted into one side of a live well. Fish from the second net of the tandem were sorted into the other side of the live well. Each net was recorded as separate data from a singular site.

Captured fish were then identified by species, counted, and released. The target species, black crappie and bluegill, were measured by total length in mm, counted, and released. Hoop net depth, time, and position in the water were recorded. Sites for trap nets were selected randomly from historical locations. A total of nine trap nets were set. Nets soaked for a period 24 hours and retrieved. Fish were then identified, measured, and released.

All analysis was completed through Microsoft Excel and program R. PSD was based on proposed target size structure values from Gablehouse (1984) for both target species with 95% confidence intervals (CI). Catch rates of each net type were evaluated by running a statistical analysis t-test on relative abundance by CPUE and size structure by length groups of black crappie and bluegill. Length frequency analysis was used for bluegill in both net types.



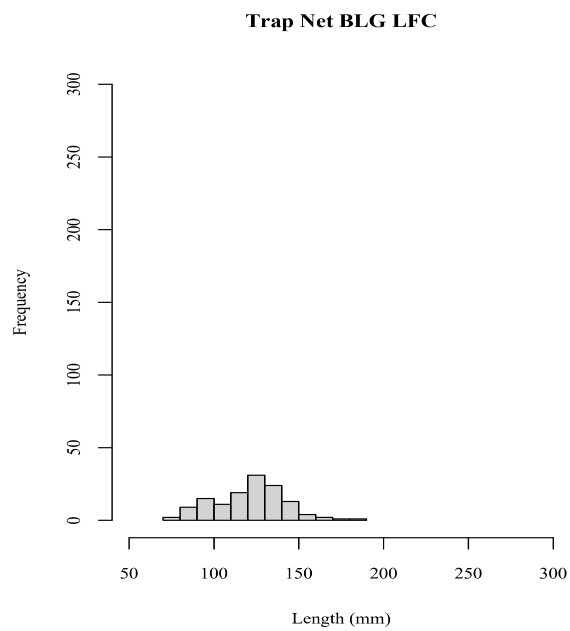


Fig 1. (Left) The relationship between length and count of bluegill captured in hoop nets from Stella Lake during July/August 2024 shown as a length frequency analysis. (Right) The relationship between length and count of bluegill captured in trap nets during sample period shown as a length frequency analysis.

III. Results

Hoop nets captured a total of 528 fish with bluegill and black crappie making up 96.5% of the catch. Trap nets captured 177 fish with bluegill (no black crappie captured) making up 74.6% of the catch. A total of 7 different fish species were captured for hoop nets while trap nets 12 different fish species.

Lengths of bluegill captured in hoop nets ranged from 112 to 284 mm and the mean ± SD length was 135 ± 18.4 mm (Figure 1). Bluegill PSD of quality size fish in hoop nets was 14.9 with a 95% CI [10.2, 19.6] (Figure 2).

Trap nets captured bluegill with lengths ranging from 72 to 189 mm with a mean ± SD length of 122 ± 20.5 mm (Figure 1). Bluegill PSD of quality size fish in trap nets was 6.2 with a 95% CI [2.0, 10.3] (Figure 2). Hoop nets had a significant relationship with longer lengths for bluegill than trap nets ($P < 0.01$). The lengths of black crappie captured in hoop nets ranged from 145 to 380 mm and the mean ± SD length was 258 ± 56.9 mm. Black Crappie PSD of quality size fish in hoop nets was 72.2 with a 95% CI [57.7, 87.0] (Figure 2). PSD is not applicable to black crappie in trap nets since the gear failed to sample the target species.

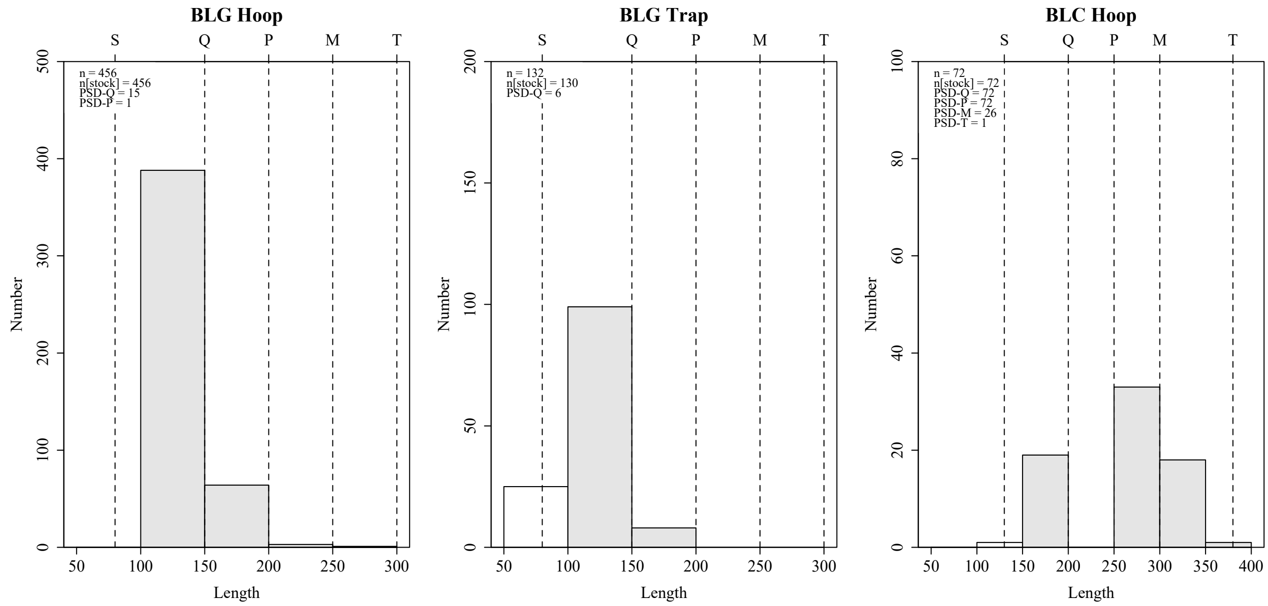


Fig. 2 Proportional size distribution (PSD) for bluegills and black crappie in hoop and trap nets from Stella Lake during July/August 2024. The dotted lines represent the minimum length for proposed target size of each species (stock, quality, preferred, memorable, and trophy).

Hoop net CPUE for black crappie was 3.6 fish/net and ranged from capturing 0 to 19 fish per net. Trap nets did not sample black crappie. Black crappie CPUE had a significantly higher catch rate for hoop nets ($P < 0.01$). Hoop net CPUE for bluegill was 22.8 and ranged from capturing 3 to 95 fish per net. Trap net CPUE for bluegill was 14.6 and ranged from capturing 3 to 34 fish per net (Figure 3). Bluegill CPUE was not significantly influenced by either net ($P = 0.30$).

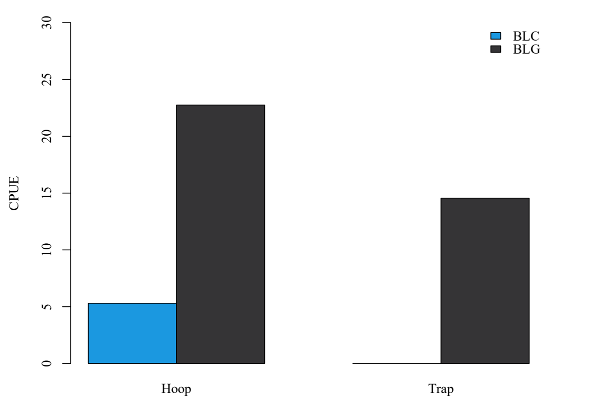


Fig. 3 A comparison of catch per unit effort for both target species across each net type from Stella Lake during July/August 2024

IV. Discussion

When deciding to select gear for appropriate sampling, biologists need to be cautious of possible bias when finding research questions. This study showed significant differences in size and numbers of fish captured similar to literature (Flammang et al. 2016). Hoop nets captured more numbers of black crappie and had a better size structure than trap nets. Bluegill CPUE was similar in both net types, but longer lengths were found in hoop nets. The bias of gear selectivity is not as clear.

For comparing the different gear types with the target species lengths, there are certain differences. When looking at the bluegill population, the PSD was low for both types of gear with hoop nets having a small advantage and more overall fish caught. Due to the minimal number of fish larger than quality length, it is apparent that the number of quality size fish is not different for this system. This similarity could be attributed to a high density and slow growing population for bluegills in this system. The black crappie population cannot be successfully evaluated with only one type of gear capturing the target species. It is worthwhile to not when examining the PSD of black crappie in hoop nets, there is a larger number of fish over preferred sized than there is below quality size.

The effort expounded to set each net type was equal. Hoop nets were set in tandem at twenty locations whereas trap nets had nine single sets. Although CPUE of bluegill was not significant for net types with different effort, literature showed hoop nets to be more efficient in sampling panfish with less effort (Flammang et al. 2016). Mesh size also with hoop nets having a larger mesh than trap nets. The longer lengths of fish captured in hoop nets could be attributed to this where literature (Flammang et al. 2016) also found bluegill to have the largest PSD in hoop nets compared to other net types. Hoop nets were also able to be set at greater depths and around submerged aquatic vegetation. This was thought to be able to sample a greater ensemble of fish although trap nets had a greater species richness.

There are several differences between the nets that may have produced different catch rates and size of fish caught. The size of the net structure, size of the throat constriction, and bait used are ways that may produce bias. Modifying the nets to be more similar in these ways should be considered to help reduce potential bias when sampling. Further studies will be needed to accurately show the differences in hoop nets compared to trap nets in capturing panfish.

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THE GROWTH OF YELLOW PERCH IN RELATION TO ENVIRONMENTAL FACTORS

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***Abstract*—Yellow perch *Perca flavescens* is one of the major fishes in northern Minnesota. These fish are important to the ecosystems they live in but, these ecosystems are subject to change at anytime if the factors present themselves. Research was done in the past that studied water temperature, air temperature, climate events, and human activity showing their effect on fish growth. This study looks at the same four factors that influence the ecosystem in Lake Bemiji during the month of September in the years of 2017-2024. Each factor is compared to the average total lengths of yellow perch to test for a correlation between them to see how each factor impacts the growth from year to year. The only factor that indicated significance was age-3 water temperature ($P = 0.05$). Age-3 air temperature produced results that were non-significant ($P = 0.14$). Both age-0 water and air temperature also produced non-significance ($P = 0.94$, $P = 0.79$). Age-0 weather and docks exhibited non-significance as well ($P = 0.99$, $P = 0.43$). Age-3 of the same factors produced results that were non-significant ($P = 0.44$, $P = 0.18$).**

I. INTRODUCTION

Yellow perch *Perca flavescens* play a large role in the ecosystem of Lake Bemidji. This role includes being a major prey fish and a popular game fish. This means there is an abundance of yellow perch in the lake to keep up with the many roles. Thus, the population needs specific habitat conditions to reach these numbers. Factors that can influence these conditions include but are not limited to water temperature, air temperature, human activity, and storm events. Each of these factors can directly influence the growth of yellow perch and the change of one of the factors can influence a change within another. The change of these factors can decrease overall growth and could cause death, depending on the shift.

Many studies in the past used these factors as an impact on growth. Researchers found the optimal water temperature for yellow perch is 24 – 28 °C, any lower or higher indicated reduced conditions (Tidwell et al. 1999; McCormick 1976). Because of the optimal range of temperatures yellow perch need this suggests that their behavior could also change, such as feeding habits and reproduction periods (Jeppesen et al. 2010). Growth of yellow perch can also be affected through the air temperature. Results of an experiment on largemouth bass *Micropterus salmoides* showed the best climate was accumulated degree-days over 10 °C (McCauley and Kilgour 1990). Because air temperature can influence thermal habitats, the fish that are able to adjust to the temperatures will continue to grow as usual, but the fish that cannot adjust will experience decreased growth (Hill and John 1990). Though yellow perch do grow better in consistent temperatures (King et al. 1999) the shift could greatly affect them. Changes to these temperatures could be linked to extreme weather events caused by climate change.

Native species in a system can suffer from extreme weather events through fluctuating temperatures affecting growth. (Ilarri et al. 2022; Macusi et al. 2015). Due to the changes of environment from extreme weather events different conditions appear in many waterbodies and change population demographics (Spurgeon et al. 2020). Changes in waterbodies can also be due to human activity such as residential development along lakeshores. Heavy development along lakeshores can cause decreased growth in fish species and makes some species less productive (Schindler et al. 2000). Although some organisms can grow faster under these conditions because of different temperatures and lower competition (Barrett et al. 2010). Fish growth can also be pointed towards fisheries management and how fish are used as a resource. Fished populations experience increased growth and increased recruitment, which introduces a balance within the population. Unfished populations show the opposite but have balanced natural mortality and recruitment.

Because these factors have the potential to affect fish growth, correlations to freshwater fish populations need to be made. This study's main purpose is to correlate the growth of yellow perch within Lake Bemidji to water temperature, air temperature, storm events, and human activity.

II. Methods

This study was done on Lake Bemidji using multiple different resources to collect necessary data. The device used to collect water temperature data was a YSI device that measured water temperature (C°) at 4, 11 and 16 m until the device hit the bottom. The data used in this study was the first four meters of temperatures at each predetermined site during the last two weeks of September. Yellow perch were captured using three different nets. Seine nets were either 15 x 1.2 m or 16 x 1.2 m with 6 mm mesh. Two seine hauls were pulled at six randomly selected sites perpendicular to the shore 50 m away or as far as two people could go without breaching their waders. Fyke nets were 1.2 x 1.8 m, 12 m lead, and 10 mm mesh. Gill nets were about 14.6 m long and 1.8 m high. The sizes of the mesh varied from 9.5, 12.7, 15.9, 19.1, 25.4, or 31.8 mm. One fyke and gill net was set on different dates at the six random locations on the lake.

The measurement of fish growth was the comparison of total length (TL (mm)) across the collection years of age-0 and age-3 yellow perch. Measurement of each fish was taken by using a measuring board and pinching the tail to get a total length measurement. Air temperature data was found at the National Weather Service website (NOAA 2025). Weather information was found at the National Centers for Environmental Information (NOAANCEI 2025). This site provided data about weather events that occurred during the study month. These weather events are any type of storm that could occur around this time of year, such as thunder or lightning storm. Human activity was measured through estimates of docks on and along the lake, this data was found on an ArcGIS website of Bemidji (Esri 2025). This site had arial images of Bemidji every three years, these years were 2017, 2020, and 2023. Estimates provided a gradual increase in dock amounts over the given years, so the gaps in the data were approximated.

Water temperature, air temperature, and weather information consisted of data from 2017 through 2024 during the months of September. All factors used the same metric of total length to measure growth to correlate each factor using a regression analysis.

III. Results

Average age-0 lengths through 2017-2024 during September ranged from 55-68 mm. Average age-3 lengths during the same times ranged from 195-221 mm. Average water temperature from this time frame ranged from 16.73 – 20.19 °C.

The comparison of yellow perch total length to water temperature shows enough evidence that water temperature is not a significant factor to growth at age-0 (P = 0.94). Age-3 comparison indicates that water temperature is a significant factor at this stage (P = 0.05; Figure 1).

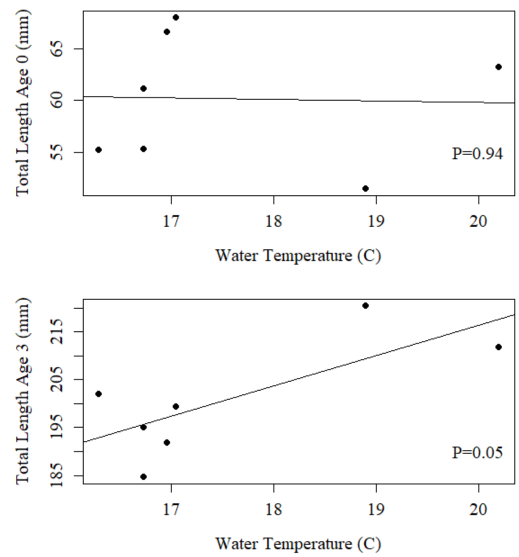

Fig. 1. The comparison of water temperature to age-0 (Top) and age-3 (Bottom) yellow perch total length through 2017-2024 during September

The air temperature comparison resulted in non-significance in both age-0 (P = 0.01) and age-3 growth. (P = 0.21; Figure 2). Average air temperature ranged from 55.5 - 65.1 °C.

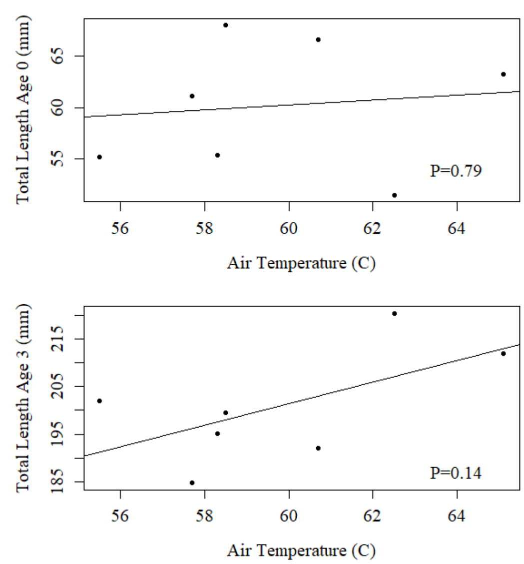

Fig. 2. The comparison of air temperature to age-0 (Top) and age-3 (Bottom) yellow perch total length through 2017-2024 during September

Both age-0 and age-3 comparison to the amount of weather events showed no significance to growth. Age-0 had a p-value of 0.99, age-3 had a p-value of 0.44 (Figure 3). The total amount of weather events in September ranged from 0 – 2.

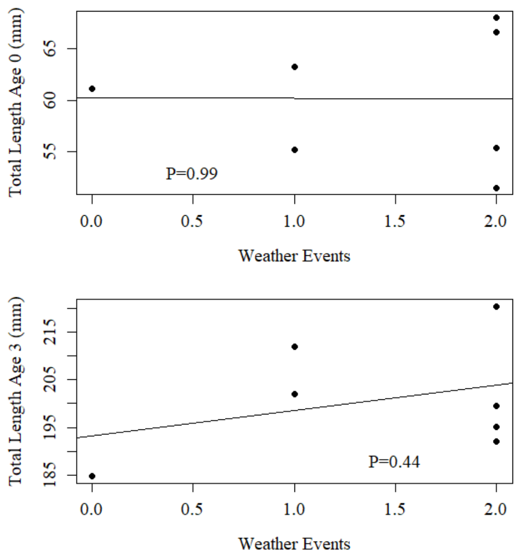

Fig. 3. The comparison of age-0 (top) and age-3 (bottom) yellow perch total length to the amount of weather events through 2017-2024 during September

The number of docks in the lake showed no significance to the total lengths of age-0 and age-3 yellow perch. Age-0 had a p-value of 0.43, age-3 had a p-value of 0.18 (Figure 4). The estimated number of docks ranged from 100 – 273.

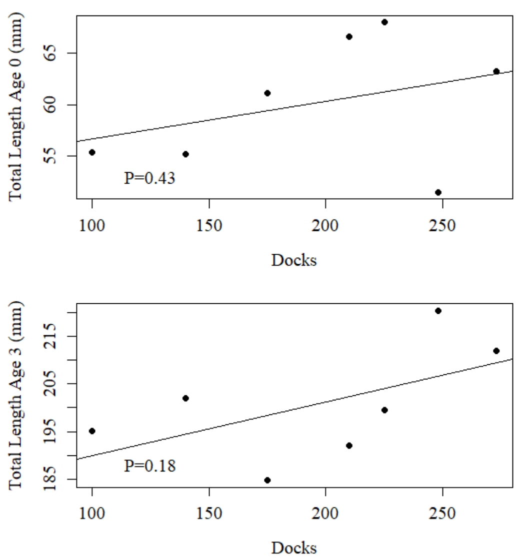

Fig. 4. The comparison of docks in and around the lake to the total lengths of age-0 (top) and age-3 (bottom) yellow perch through 2017-2024

IV. Discussion

One of the key findings of this study is that only the age-3 water temperature comparison indicated significance to growth. Age-0 water temperature showed much higher non-significance at 0.94. The difference in significance between age-0 and age-3 yellow perch could have been because of the optimal water temperature for growth could have shifted as the fish grew older. Although the average water temperature was lower than the previously studied optimal temperatures reported by Tidwell (1999) and McCormick (1976). Resulting in possible slower growth around this time of year. Air temperature could have also changed the thermal habitats of yellow perch shifting optimal growth temperature. Though the air temperature comparison showed non-significance in both age-0 and age-3 yellow perch. In this case the age-3 yellow perch comparison was closer to significance at 0.14.

Another key finding is that the amount of weather events and the number of docks in the lake has no significance to the growth of yellow perch in both age classes. Weather events were unlikely to have significance due to the short time frame of data that was used. Only using weather events during September may have not affected the growth of yellow perch enough to show significance in the analysis. Although weather events can change population demographics in a lake (Spurgeon et al. 2020) this would most likely be multiple events over an extended period of time. The number of docks produced results that were non-significant due to the docks themselves not impacting the ecosystem enough to cause change. This could have been because the development along the lakeshore was not as heavy as other areas in the state (Schindler et al. 2000). Though age-3 indicated that docks were closer to significance at 0.18. Although the docks themselves may cause little change near where they are placed, this is not enough the cause change in yellow perch. One thing that may cause change near the docks is the human development along the lakeshore as well as the people that own and use the docks (Schindler et al. 2000). This could be in the form of garbage, introduction of invasive species by boat, or general use by humans. This could impact the growth of yellow perch in a negative way.

Overall this study can provide data when monitoring yellow perch populations in lakes or when raising yellow perch in a hatchery. It could also provide data on how different factors influence yellow perch growth and could be intergrated into a management plan. It’s important to understand how many different factors can influence an animal’s growth , no matter how small the factor may seem. This is important because one factor could be the leading factor in that animal’s growth. Many of these factors can be changed very easily causing change within the animal for better or worse.

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A SAMPLE OF FISH COMMUNITIES IN BEAVER PONDS OF NORTHERN MINNESOTA

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***Abstract*—The North American beaver *Castor canadensis* is an ecosystem engineer and a keystone species to the environment. Beaver inhabit ponds and construct dams to build ponds that provide habitat for certain fish. Little information is provided on the species of fish that live in beaver ponds. The objective of this study is to sample the fish communities in beaver ponds while comparing the effectiveness of two styles of minnow traps. Twelve beaver ponds were selected in the Bemidji area to be sampled. The quantity and species caught in each style of trap were recorded to compare effectiveness of each trap and a sample of the fish community. A total of 15,453 fish were caught, seven different species and possible hybrids. Between the two trap types used, there appeared to be a significantly higher catch in the clover leaf trap type.**

I. Introduction

The North American beaver *Castor canadensis* is a relatively common and abundant semi aquatic mammal across most of the United States and especially Minnesota. Beaver are ecosystem engineers and their impact on the landscape has been known to increase species richness (Rosell et al. 2005). Bodies of water in which beaver inhabit are crucial for a wide variety of both terrestrial and aquatic systems. Fish communities rely on beaver heavily for their role in controlling water levels. In open water created by beaver in unconnected peatland by stopping water from draining, it was found fish colonize the pond quickly (Ray et al. 2004). Little research and sampling have been done on what species of fish live in beaver ponds. Beaver pond fish communities hold a spot in the ecosystem and show further importance of the presence of beaver.

Beaver ponds have a wide range of variance of size, hydrology, and depths that may influence fish communities. Factors in beaver ponds such as low oxygen, low water levels, and the changes of water temperatures across the year may also be limiting to fish (Collen and Gibson 2001). While some ponds may be void of fish inhabitants, others may have abundant fish life with little information about species present. (Williamm and Magnuson 1982) suggest bodies of water with low oxygen during times of winter, such as beaver ponds, consist of species of small bodied fishes.

Northern Minnesota presents a great opportunity to sample the different complexities of beaver ponds through the abundant flowages, ponds and small streams in the area. None of the ponds have shown documentation on the species of fish that are present, leaving fish populations relatively unknown in the ponds inhabiting fish. The objective of this study was to sample the fish communities in ponds that were or are currently inhabited by beaver.

II. Methods

Beaver ponds were chosen from a criteria system to determine which ponds would be sampled. The first of the criteria was that the pond had to contain beaver sign such as a recently active beaver lodge. The pond also had to be less than 10 acres in area of open water and could not contain a name in accordance with MNDNR (2024). Lastly the pond could not have visible water flowing through the system at the time samples were taken and could not be a part of a river system. Using this criterion, a total of 12 ponds were selected and sampled.

Ponds were sampled 8 October 2024 through 1 November 2024. A total of six minnow traps were set on each pond. Of the six traps, three were clover leaf style and the other three were cylinder style traps. Clover leaf and cylinder traps were placed interchanging along the bank with at least 20 ft between the traps. The minnow traps were baited with a handful of dog food and placed in water deep enough to completely submerge the traps.

Traps were checked and removed the following day they were set. The fish in the traps were removed, species and the quantity of fish were recorded. Fish in the *Chrosomus* genus that were caught and could not be accurately identified in the field was represented as *Chrosomus* sp. in the data. The data recorded from the different styles of traps will be compared to determine the effectiveness of the styles of traps along with a sample of the fish community.

The data that was recorded was broken up into four different methods to analyze the catch. A pie chart was created to show the total number of species compared to the total number of fish caught. To show differences in style of trap, a bar graph was created to show the number and species that was caught in each style of trap. To supplement the comparison in style of trap, an NMDS plot was created to show the differences in the trap types in regard to the number of fish and species caught in each. To plot differences of fish communities between the ponds, another NMDS plot was created.

III. Results

A total of 15,453 fish were caught during sampling with six different species recorded. The six species included central mud minnows *Umbra limi*, brook sticklebacks *Culaea inconstans*, northern redbelly dace *Chrosomus eos*, finescale dace *Chrosomus neogaeus*, brassy minnows *Hybognathus hankinsoni*, fathead minnows *Pimephales promelas* and unidentified fish in the genus *Chrosomus* (Figure 1).

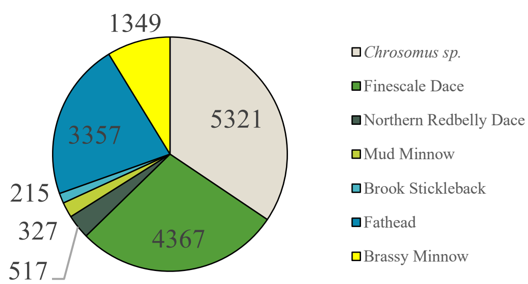

Fig. 1. Northern Minnesota ponds containing recent beaver inhabitance were sampled in the fall of 2024. The pie chart represents the number of each species caught (7) in comparison to the total number of fish caught (15,453) between clover leaf traps and cylinder traps.

The clover leaf trap type overall caught higher quantities of fish in comparison to the cylinder trap type. All species had a higher catch rate in the clover leaf trap compared to the cylinder trap. Species caught in lower numbers remained to have a higher catch rate in the clover leaf traps (Figure 2). In another analysis of the difference in trap type catch, it also appeared to have a difference in trap catch when taken account for species, total number of fish, and the pond (Figure 3).

Eleven of the twelve ponds that were sampled contained fish. Pond number nine failed to sample any fish. Although most ponds contained a similar species richness, they differed in overall fish density and evenness of species. The fish communities that were sampled appeared to have a difference depending on the pond that they were sampled from (Figure 4).

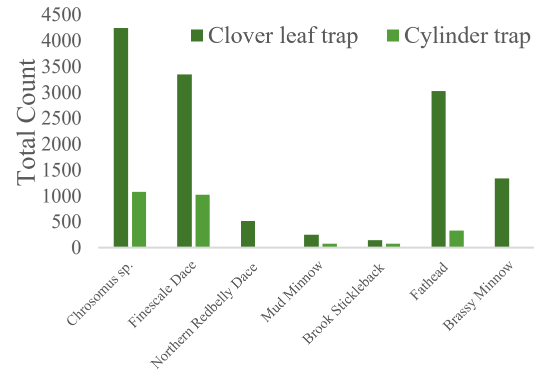

Fig. 2. Northern Minnesota ponds containing recent beaver inhabitance were sampled in the fall of 2024. The bar graph represents the number of each species caught in comparison to the clover leaf trap and cylinder trap.

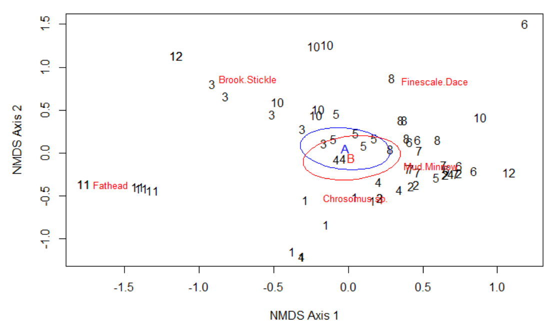

Fig. 3. Northern Minnesota ponds containing recent beaver inhabitance were sampled in the fall of 2024. The NMDS represents the proximity of species and number caught between trap A (clover leaf trap) and trap B (cylinder trap).

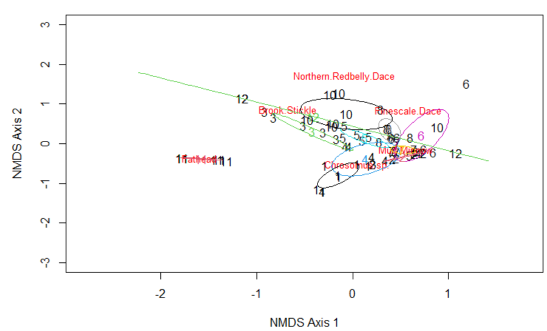

Fig. 4. Northern Minnesota ponds containing recent beaver inhabitance were sampled in the fall of 2024. The NMDS represents the proximity of fish communities among the twelve ponds excluding pond nine due to lack of catch.

IV. Discussion

A large portion of the fish caught consisted of finescale dace *Chrosomus neogaeus* and Northern Redbelly Dace *Chrosomus eos* and were often present in the same pond that was sampled. Past literature has demonstrated that the finescale dace and northern redbelly dace hybridize to create a *C. neogaeus* x *C. eos* with intermediate characteristics and can happen frequently (New 1962). It was originally thought the hybrid populations were only female and males were never recorded. In a study done on two populations of hybrids, it was found the reason for an all-female population was because a form of asexual reproduction and can happen with only *C. eos* or *C. neogaus* present (Goddard et al. 1998). Although in a recent study, evidence of male hybrids and backcrossing with the parental species were found and changed the original thinking on the hybrids (Schultz 2025). The ecology of the hybrids, *C. eos*, and *C. neogaeus* is complex and identifying a hybrid from either parental species is difficult. Although a *C. neogaeus* x *C. eos* hybrid has never been recorded in Minnesota, it is plausible to occur given both species are present and seemly abundant. It is also possible during this study hybrids were present and were either misidentified or simply identified as a *Chrosomus* species. Further research could be done to either confirm or deny the presence of hybrids in Minnesota.

The presence of fish seemed to be abundant throughout the ponds that were sampled. The communities sampled consisted entirely of small bodied fishes. The beaver ponds that were sampled were relatively small, shallow and all most likely experienced periods of hypoxia. Small bodied fish such as the cyprinids and mudminnows use less oxygen than larger fish and make the small bodied fish more suitable to the small ponds with less oxygen (Klinger et al. 1982). Another possibility that would explain the small body size of fish caught is the connectivity of the waterbodies that were sampled. In shallow systems with an outlet or inlet, species of fish can travel to areas of higher oxygen (Magnuson et al. 1985). The ponds chosen for the study were relatively closed systems that would not allow larger fish to escape the winter hypoxia. Beaver ponds that do connect with larger bodies of water may have varying fish communities and possible seasonal changes in the community.

To further build a map of the fish communities in beaver ponds at a larger scale, more geographic regions should be sampled. Regions with differing substrate types, elevations, or even beaver densities may result in differing fish communities. Areas with much lower beaver densities and less natural ponds containing beavers in different geographic locations would most likely contain different species compared to Northern Minnesota. In the Ohio river basin, areas of differing glacial activities have resulted in predictable fish communities (Jacquemin and Pyron 2011). Areas of differing past glacial events may play a role in the communities that can be found in the beaver ponds. Glaciers may limit the distribution of many species and create a predictable map of fish assemblages. It may be difficult to construct a consistent map that would cover a whole geographic area. Small systems such as beaver ponds hold more variability and less stability than many of the other large lakes and rivers (Jackson et al. 2001). Further sampling of a large area and different geographic zones of beaver ponds could be done to determine a better picture of fish communities that is more broad than northern Minnesota.

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SIZE STRUCTURE OF BLACK CRAPPIE AND BLUEGILL IN NORTHERN, EASTERN, WESTERN, AND SOUTHERN MINNESOTA

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***Abstract*— Fish size structure is an important factor in a fishery population. Panfish such as black crappie *Pomoxis nigromaculatus* and bluegill *Lepomis macrochirus* play a major role in the health of fisheries. Five lakes from southern, northern, eastern, and western Minnesota were selected for analysis. Data were collected using standard sampling methods, including gill nets, electrofishing, and fyke nets. Length-frequency distributions were created to assess size structure in each region. Relative abundance was measured using catch per unit effort. Data analysis included a one-way ANOVA to compare Proportional Size Distribution (PSD) values and the distribution of fish sizes among regions. Chi-square tests revealed a significant regional difference in size distribution for both species ($P < 0.001$). Bluegill in the South region shows higher proportions of smaller fish, while black crappies in the West region showed higher proportions of larger individuals. The findings suggest regional variation in panfish population structure, which may influence fisheries management strategies in each individual region.**

I. Introduction

Panfish play a significant role in the overall health of fisheries. In addition to ecological importance, they also provide considerable recreational and economic value. According to a 2014–2015 statewide mail survey, anglers targeted panfish on 27% of their fishing trips—more than any other species or group (Hansen and Wolter 2017). The increased interest among anglers adds pressure on panfish populations, making effective management strategies crucial to sustaining healthy fisheries.

The size of panfish populations influences both genetic diversity and population health. Panfish include rock bass *Ambloplites rupestris*, sunfish *Lepomis* sp., crappies *Pomoxis* sp., and yellow perch *Perca flavescens*. Size structure analysis is one of the most used tools in fisheries assessment (Neumann and Allen 2007). Length-frequency data offers valuable insight into fish population dynamics and can help identify issues such as inconsistent year-class strength, slow growth, or excessive mortality (Anderson and Neumann 1996). These data are often used alongside other assessment tools, such as catch per unit effort.

Fisheries managers may use several strategies to address panfish population issues, especially when large year classes dominate. Common management practices include liberal harvest regulations, netting removal, selective poisoning to reduce overly dominant year classes, intensive piscivore stocking, and supplemental feeding (Hansen and Wolter 2017). Bag limits may also be adjusted to prevent the overharvesting of larger adults, maintaining a healthier population structure.

Size structure analysis can also provide regional insights, helping managers identify beneficial or detrimental trends. The objective of this study was to assess the size structure of panfish populations in lakes across northern, eastern, western, and southern Minnesota. The results are intended to highlight regional differences in fishery health and identify areas that may require management attention.

II. Methods

This study was conducted across four regions of Minnesota: North, East, South, and West. Five lakes were selected from each region based on known populations of bluegill *Lepomis macrochirus* and black crappie *Pomoxis nigromaculatus*, as well as similarities in lake size to ensure comparability. Data was obtained from the Minnesota Department of Natural Resources (MN DNR) Lake Finder resource, which provides comprehensive fish population data collected through standardized sampling methods (MN DNR 2024).

Sampling methods used by the MN DNR include standard gill nets, trap nets, and electrofishing. Gill nets, typically 76.3 meters in length, are used to sample species such as Walleye *Sander vitreus* and northern pike *Esox lucius*, while trap nets are effective for capturing bluegill and other small-bodied fish (MN DNR 2024). Electrofishing is employed to sample fish in shallow, nearshore habitats and provides a representative sample of the fish community (MN DNR 2024).

To assess the size structure of bluegill and black crappie populations, length-frequency distributions were created for each lake. Fish were grouped into 50 mm size classes, consistent with standard practices in fisheries research (Anderson and Neumann, 1996). The proportional size distribution (PSD) index was then calculated to evaluate size structure using the formula:

$$PSD = \left(\frac{Number\ of\ fish\ \geq Quality\ length}{Total\ number\ of\ fish\ \geq Stock\ Length} \right) x\ 100$$

The PSD index provides a standardized measure of the proportion of quality – sized fish in a population, allowing for the comparison across regions (Anderson & Neumann, 1996).

Data analysis was performed using R software. Chi - square tests were used to assess the differences in size class distributions.

III. Results

The overall PSD for all regions indicate that a majority of the panfish populations falls within the smaller size categories (Figure 1). Specifically the range between 0 – 127 mm. This range includes roughly 45% of the total catch. The secondary majority fall into the 132-178 mm range, this range includes 40% of the total catch.

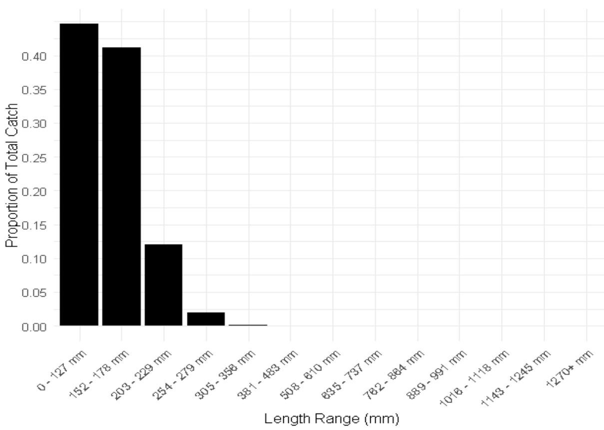

Fig.1. Overall proportional size distribution (mm) of both bluegill and black crappie combined across all sampled Minnesota lakes. Frequency is presented as a percentage of total catch. The distribution shows a dominance of smaller size classes (0-127 mm), with a sharp decline in larger individuals.

Regionally there were some noticeable differences. The North and West regions displayed the highest proportion of fish in the 132-178 mm range, with both regions having nearly 50% of their total catch in this size category. The East region had the highest catch in the 0 – 127 mm range with over 50% of its total catch in this range. This was closely followed by the South region where 49% of its fish fell in this range. The on commonality between all the regions that the class sizes beyond the 203 mm were not represented across all the regions.

There was a significant difference in bluegill size distributions among region ($\chi^2(12) = 4812.4$, $P < 0.001$; Figure 2). The South region displayed notably high frequencies of smaller individuals in the population (0 – 127 mm), while larger size classes were noticeably uncommon in all regions (Table 1).

TABLE 1. CHI SQUARE RESULTS FOR BLUEGILL SIZE DISTRIBUTION ACROSS FOUR MINNESOTA REGIONS (NORTH, SOUTH, EAST, WEST). SIZE CLASSES ARE GROUPED INTO 50 M INTERVALS. SIGNIFICANT DIFFERENCE WAS OBSERVED AMONG REGIONS, INDICATING VARIATION IN POPULATION STRUCTURE.

Region	0-127 mm	128-178 mm	179-229 mm	230-279 mm	280-355 mm
North	3232.204	2926.486	335.6872	29.0117	5.610752
South	8653.039	7834.593	898.6792	77.66816	15.020729
East	4134.191	3743.159	429.365	37.10778	7.176504
West	7599.565	6880.762	789.2686	68.21237	13.192014

There was a significant difference in black crappie size distributions across the regions (χ^2 (12) = 1891.7, $P < 0.001$; Figure 2). The South region contained the highest proportion of individuals in the 179-229 mm range, suggesting a population dominated by mid-sized fish. In comparison, the West region showed the greatest frequency of fish in the 230-270 mm class, indicating a stronger presence of larger presence of larger individuals (Table 2). These findings reflect substantial regional variation in black crappies population structure.

TABLE 2. CHI-SQUARE RESULTS FOR BLACK CRAPPIE SIZE DISTROBUTIONS ACROSS FOUR MINNESOTA REGIONS. THE FREQUENCY OF INDIVIDUALS WITHIN EACH SIZE CLASS REVEALS A SIGNIFICANT REGIONAL SIZE DIFFERENCES IN SIZE STRUCTURE, IN PARTICULAR THE 179-229 MM AND 230-279 MM RANGES

Region	0-127 mm	128-178 mm	179-229 mm	230-279 mm	280-355 mm
North	406.92	440.17	455.06	102.64	8.21
South	2416.45	2613.93	2702.37	609.51	48.74
East	1804.78	1952.27	2018.32	455.23	36.4
West	180.85	195.63	202.25	45.62	3.65

IV. Discussion

This study identified significant differences in size class distribution for both bluegill and black crappies across regions. Bluegill in the southern region of Minnesota were generally smaller, while those in the western region included more individuals in the mid-to-large size classes. This suggests potential regional differences in growth conditions or harvest pressure. Black crappie size distributions showed similar trends, with the southern region having more fish in the mid-size length ranges and the western region showing a higher proportion of larger individuals.

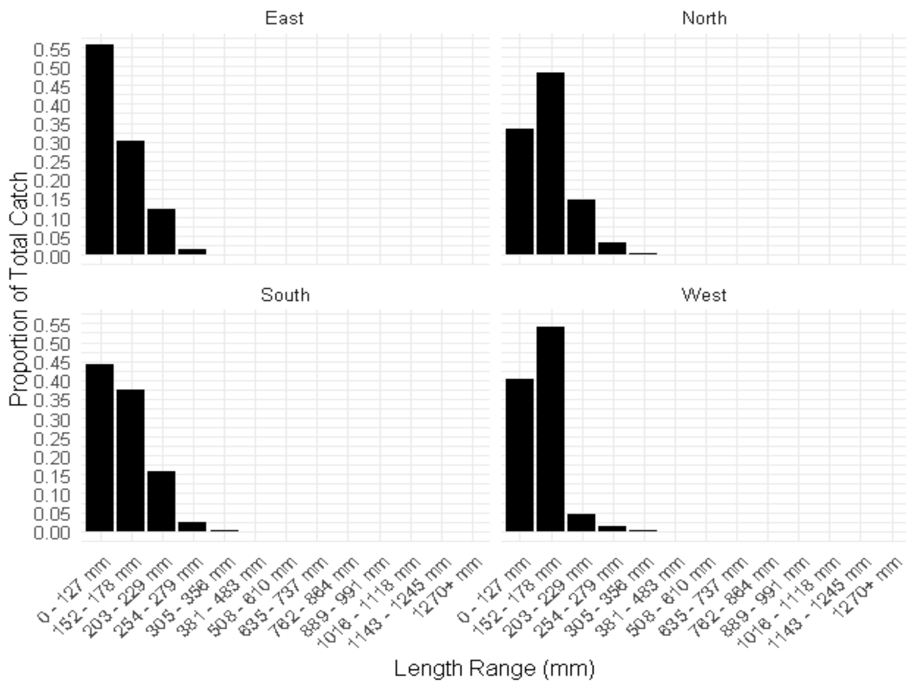

Fig. 2. Proportional size distribution by region (mm) of bluegill and black crappie combined across all sampled Minnesota lakes by region (North, East, South, West). Each region's distribution highlights the differences in population structure, with the South region showing a higher percentage of smaller individuals and the West region displaying more mid-to-large sized fish.

These patterns may reflect regional environmental or anthropogenic factors influencing size distribution in both species. Previous studies have shown that angling pressure can significantly affect the size structure of bluegill populations, often resulting in a dominance of smaller individuals in heavily fished areas (Rypel et al. 2015). Similarly, the implementation of minimum length limits (MLLs) for black crappies has produced mixed outcomes. Some lakes exhibited improvements in size structure, while others showed no significant change—possibly due to factors such as noncompliance or variability in recruitment (Isermann et al. 2007).

These results emphasize the importance of localized fisheries management to maintain balanced size distributions and support sustainable fish populations. Tailoring management strategies to region-specific conditions—such as adjusting harvest regulations or implementing targeted habitat management practices—may be necessary to address the observed disparities. Future research should further investigate the ecological and human-driven factors contributing to regional differences in panfish size structure.

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THE EFFECTIVENESS OF THE MINNESOTA BUFFER LAW AND IT'S IMPACTS ON PEOPLE

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In January 2015, the then-governor of Minnesota Mark Dayton announced new legislation that would require 50-foot vegetation buffers along all waterways in Minnesota; this legislation would come to be known as the Minnesota buffer law, exact requirements being "perennial vegetative buffers of up to 50 feet along lakes, rivers, and streams and buffers of 16.5 feet along ditches" (Albert, 2017; MN Board of Water and Soil Resources [MN BWSR] 4). A buffer in the context of vegetation and natural resource management is a strip of vegetation around a body of water "designed to intercept stormwater runoff and minimize soil erosion" (DNR Waters, 2007). The Minnesota buffer law specifically targets nitrogen, phosphorus, and sediment. The deadline for compliance for public waters was November 1st, 2017, and the deadline for public ditches was November 1st, 2018 (MN BWSR 4). Landowners could apply for an eight-month extension to install buffers or an approved alternative (Albert, 2017)

The buffer law came about due to reports published by the Minnesota Pollution Control Agency that stated that the water quality in Minnesota watersheds was below standard. One report specifically stated that "In watersheds dominated by agricultural and urban land, half or fewer of the lakes fully support the standard for swimming because of phosphorus" and that watersheds in areas dominated by agriculture " tend to have high levels of nitrogen, phosphorus, and suspended solids" all of which are known to be harmful to aquatic life (Minnesota Pollution Control Agency [MPCA] 3, 2015). Aquatic life was not the only victim of low water quality. Interviews with Minnesota residents conducted by The Pioneer Press in 2015 found that many Minnesotan residents felt unsafe drinking their tap water due to high levels of nitrates (Magan, 2015). Magan further draws attention to the fact that many Minnesota cities such as Hastings and Cold Spring had to invest several million dollars into systems to reduce nitrate levels, such as drilling new wells and upgrading water treatment facilities (Magan, 2015). Minnesota's low water quality was harming wildlife, people, and costing cities and private owners money.

Something needed to be done about the state of Minnesota's water quality; the buffer law was one response. Today, 98% of land next to Minnesota waters is in compliance with this law (MN BWSR 4), but just how effective has it been?

This paper will discuss the impact and how effective the buffer law has been on improving water quality in Minnesota, as well as how the law has impacted Minnesota residents.

I. Environmental Impact

The use of buffers as a method of managing water quality and protecting wildlife has been in use in North America since the 1960s, and even longer than that in other parts of the world (Richardson et al, 2012). Originally, required buffers came about in response to concerns about the effect of forestry practices on water quality and fish habitat. Since buffers have been used as a method of water quality and aquatic life management for decades, there is much known about how they can improve water quality. Vegetation buffers are good for trapping sediment that contains pollutants and preventing topsoil from being blown into the water (DNR Waters, 2007; Albert, 2017). Buffers can also reduce shoreline erosion, capture stormwater runoff, and provide habitat for wildlife (DNR Waters, 2007; Al-Kaisi, 2020). The specific way that a buffer is planted or placed can be used to fulfill different management goals; some reduce soil erosion and damage to plants caused by wind and others can be used to facilitate the ease of machine use in fields (Al-Kaisi, 2020). In the case of the buffer law, the intent was to prevent pollutants in runoff from getting into surface water, which is what buffers are most commonly used for.

The deadline for public waters to be in compliance was 2017, so as of 2024 it has only been seven years since the buffers were installed. In the 2015 report sent out by the MPCA that showed Minnesota waters were impaired, it was also stated that regardless of the exact action taken, it would likely take 20-30 years for the water to be clean; “It took decades to pollute lakes and streams, and it will take many years to restore impaired waters” (MPCA 3, 2015). In the 7 years the buffer law has been in effect, there has not been significant change to Minnesota water but the MPCA continues to monitor Minnesota lakes, streams, and groundwater for contaminants and other health risks. In accordance with the Clean Water Act, the MPCA evaluates all Minnesota waters and determines which fail to meet standards and thus belong on the Impaired Waters List every two years (MPCA 4, 2022). Chloride and nitrogen pollution are two concerns, but a study on Water Quality trends by Minnesota Go that used data from the MPCA found that although the amount of lakes polluted with chloride had a severe increase of 20 lakes in 2012 to 47 in 2014, the rise slowed down, with there being 50 in 2020 (Minnesota GO, 2021). The main way both Chloride and Nitrogen gets into surface water is from road and agricultural runoff (Minnesota GO, 2021), so vegetation buffers could aid in capturing that runoff before it gets to water (MN BWSR 4).

There is not much data to be found that specifically studies how the implementation of the buffer law has affected Minnesota water quality since 2017, but there is a study published by the MPCA in May 2015 that shows the difference in the health of aquatic communities between sites that did have buffers and those that didn’t. This study found that “The greater the percentage of stream channel that is buffered upstream of a monitoring site, the better the health of the aquatic life” (MPCA 5, 2015). The health of aquatic life can be used as an indicator of water quality, so the more intact a buffer was, the better the health of the aquatic life community and the better the water quality.

The MPCA states that “good progress has been made” in reference to the reduction of phosphorus and ammonia (MPCA 2). There are a multitude of ways that Minnesota natural resource agencies and organizations monitor water and what they monitor for. It is not only state and federal agencies that monitor water, but also citizen organizations that then send their data to agencies. Nitrates, chloride, pesticides, and fertilizers are only some of the pollutants that are monitored (Minnesota Department of Natural Resources). The MPCA has monitoring sites on rivers all across the state that have been taking samples from 2001 onward, and this data is processed and published on an interactive map so the public can view the trends on river nutrient levels such as phosphorus, nitrates, and sediment. This data shows that while there are many sites that do detect a trend, when a trend was detected it was a general rise in nitrates, and a general decrease in phosphorus and sediment (MPCA 1). While this data does not include buffers as a factor, it does show a change in water quality.

Minnesota uses a watershed approach to monitor water, which means the MPCA tests each of Minnesota's 81 watersheds every 10 years on a staggered cycle, so that in a given year there are multiple watersheds beginning or ending their cycle (MPCA 3, 2015). Through this method of monitoring and what is known about the timeline of expected results from the buffer law, it may be a few more years before any real results are seen. Buffers can be effective, but they have not been widespread in Minnesota long enough to say with certainty just how effective that is.

II. IMPACTS ON PEOPLE

Around 51% of land in Minnesota is agricultural (MN BWSR 1), and water runs through much of that land in the form of rivers, streams, or man-made waterways. The USDA reported that there were 67,400 farms in Minnesota in 2022. Around 36% of those farms make $10,000 or less from the sale of agricultural products, and 30% make between $10,000 and $100,000 annually (United States Department of Agriculture). Considering this, it is no surprise that the buffer law was met with pushback at the time of its announcement. At the time the law was announced, many farmers spoke about how they did want to improve water quality but were unhappy about the lack of compensation (Market to Market, 2017). For certain farms, complying with the buffer law meant converting acres of land from agriculture to buffer. For the 36% of farms that make less than $10,000 annually, that loss of land is not insignificant.

A “taking” occurs when the government takes private property. A property is considered to have been “taken” whenever the owner can no longer enjoy it. A typical example of a taking is when all or some of a piece of real estate is condemned by the state so a road can be widened or built. In these cases, it is easy to see that the government has literally taken the property from the owner. Under Article 1 Section 13 of the Minnesota constitution, “Private property shall not be taken, destroyed or damaged for public use without just compensation” (Dyson, 2006). “Just compensation” is generally the fair market value of the land, and the amount is agreed on by buyer and seller (Minnesota Department of Transportation, 2015). One of the accepted definitions of public use as further defined by the statute is “the mitigation of an environmentally contaminated area” (Dyson, 2006), which includes any area “in which more than 50 percent of the parcels contain any substance defined, regulated, or listed as … pollutant, contaminant, or toxic substance, or identified as hazardous to human health” (Minnesota Legislature, 2024). Water quality is a human health concern, as it affects drinking water, fishing, swimming, and what we use to water crops

(U.S. Government Accountability Office; MPCA 3, 2015).

The most common issue is over value, and the parties frequently disagree over how much money needs to be paid to the landowner. In this case there is no argument over how much money is to be paid to the landowner that loses the use of their land to create buffers. The buffer law does not provide any payment to landowners. The farmer cannot farm the buffer area, thus losing income. The loss experienced by the farmer is not just profit but also reduction in the fair market of the property.

I reached out to Charles Seykora, a Real Estate lawyer that represents farmers that are directly affected by the buffer law, and he was able to provide me with numbers to show just what a farmer loses by being in compliance. To quote him, "a fifty-foot buffer on either side of a ditch that has been designated as having "public waters" will require the farmer to not plant on 2.83 acres of land in every 40 acre quarter-quarter that a farmer owns. This could be up to 7 percent of the land owned in a quarter-quarter" (Seykora, 2024). The average value of farmland in Minnesota is $6,450 per acre. The farmer loses the income from the crops that could have been grown on the land that is now buffer, and the farmer loses money if he sells the property due to the reduction in usable acreage.

Some farmers already had buffers of some kind on their land, and so didn't need to make drastic changes or give up a significant amount of acreage to get into compliance (Market to Market, 2017). In a video published by Market to Market, it was suggested that the costs of the buffer law would have less of an impact on livestock farmers and farmers who maintain both crop and livestock, than it would on crop only farmers, as the buffer vegetation can still be used for grazing and hay production (Market to Market, 2017). The grazing of buffer vegetation and hay production is not explicitly prohibited, though farmers do need to work with local Soil and Water Conservation staff to create a management plan to stay in compliance with the law (MN BWSR 2). While farmers can still use the land in a buffer under certain circumstances i.e. grazing and haying, they cannot plant crops on that land and thus cannot make profit off of it.

To gain different perspectives on the impact the buffer law has on Minnesota residents, I reached out to a few lawyers for comments. In addition to Charles Seykora, Timothy Erb, A Certified Real Property Law Specialist, brought up how it is not just farmers that take on the costs, but any person that has property with public water. To quote him "Beyond the initial costs of planting or establishing the buffer… costs to residential builders and developers may also include various contractual and ongoing maintenance costs… Although the maintenance costs themselves are also unlikely to be material, the residential builder and developer will not typically agree to remain obligated to maintain the buffer area once sales are complete…costs related to ongoing maintenance of buffer areas may often be passed on to homeowners that live in developments with buffer areas." (Erb, 2024). The costs come in the form of mowing, potential re-seeding, and other landscaping maintenance costs.

The buffer law itself does not contain any compensation for farmers to make up for the land lost from being in compliance, but there have been multiple suggested remedies to this in the years since the buffer law came to be. None of these suggested compensations have been put into effect. One suggested compensation was a $50-per-acre tax credit, which had support but was not legalized (Orenstein, 2019). Another suggestion from Torrey Westrom was a bill that would have the state paying farmers rent prices for the tillable land used in the buffers, and yet another suggestion from Representative Paul Torkelson was a "tax credit worth the full property taxes a farmer would pay on the land used for a buffer" (Orenstein, 2019). All of these proposals had some level of support from other government officials and the public, though none have been put into effect. Some form of tax credit or tax break seems to be the popular choice. In the article by Orenstein, Rep. Paul Marquart is cited for saying that the main reason no tax break on buffer land was passed is due to a different tax break on school building bonds (Orenstein, 2019).

Though there is nothing within the law itself, there are a few programs that farmers can apply to to receive compensation or alternative solutions. Buffers do not work for every landscape (Albert, 2017), so landowners that wish to seek an alternative can apply to the Minnesota Agriculture Water Quality Certification Program (MAWQCP). MAWQCP allows landowners to establish alternative practices, such as retention pools, that fit the land better than a buffer would but provide the same amount of water quality protection (MN BWSR 3). In the case of establishing an alternative practice, the landowners would need to work with local Soil and Water Conservation Districts to get approval. MAWQCP then reviews the water quality of the given site, and the landowners can use being certified through MAWQCP as a way to show that they promote good water quality (Minnesota Department of Agriculture).

To receive financial compensation, landowners can apply to the Conservation Reserve Program (CRP), though applicants must have "environmentally sensitive land" (USDA Farm Service Agency). Landowners that are eligible for CRP receive annual payments or cost assistance to convert land into native vegetation cover and buffers (USDA Farm Service Agency).

But, if a landowner does not qualify for CRP, does not experience the school building bond tax break, or is not able to enroll in MAWQCP, then they are stuck paying taxes on land that they cannot use because all methods of compensation or finding alternative practices are separate from the buffer law itself. Landowners are tasked with finding a way to deal with this law on their own.

III. Conclusion

There is evidence that buffers do improve water quality, aquatic habitat, and prevent soil erosion, but more time is needed before results are seen in regards to the effectiveness of the Minnesota buffers. There are countless water monitoring efforts being made across the state, and current available data suggests that while water quality is improving with a decline in phosphorus, it is degrading with higher levels of nitrates and chlorides. Whatever impact the buffers have will be clearer in the coming years.

The buffer law itself, while made with the intent to improve and protect the environment, does not adequately provide compensation to landowners who lose either potential income by not being able to farm the land used for the buffer, or who pay to maintain them. By Minnesota law, there should be "just compensation" for land that is taken for public use, and considering that maintaining good water quality benefits everyone, the buffers are public use. Time will tell the effectiveness of the buffers, and if there will eventually be a change that provides landowners with "just compensation".

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AQUATIC INVERTEBRATE POPULATIONS IN ROADSIDE WETLANDS IN RELATION TO WATER CHARACTERISTICS

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***Abstract*—The continual habitat destruction and draining of wetland systems makes the need to understand the ecology of roadside wetlands all the more necessary. Roadside wetlands provide valuable habitat to countless aquatic invertebrates, which can provide insight into the health of the wetland system due to their diversity. The objective of this study was to analyze the populations of aquatic invertebrates in roadside wetlands in relation to water characteristics such as pH, salinity, conductivity, water depth and temperature, and dissolved oxygen. This was done by collecting samples of invertebrate populations from roadside wetlands, in addition to data on water quality characteristics using an aquatic D-frame dip net, YSI multi-parameter water quality meter , and a handheld water quality testing meter. The results as shown by the NMDS graph are that the water quality characteristics that are significantly related to invertebrate community structure are dissolved oxygen (P = 0.017) and pH (P = 0.028). On average, sample locations had low dissolved oxygen and low pH. These samples contained communities that were made up of Planorbidae, Chironomidae, *Hylella,* and other groups that are known to live in low water quality conditions. In addition, invertebrate groups were found to fulfil a range of ecological niches such as Sphaeriidae, Coenagrionidae, Ptychopteridae, and Libellulidae. Roadside wetlands are a valuable habitat for aquatic invertebrates, and the results of this study can be used to intentionally manage roadside wetlands for aquatic invertebrates.**

I. Introduction

As climate change continues to alter valuable ecosystems, it becomes more important to understand the role that people play in the creation and alteration of our habitats and ecosystems (Davidson 2014; Tatariw et al. 2021). An estimated 87% of wetlands have been lost due to human activity since the 18th century, with inland wetland reduction being more severe than that of coastal wetlands (Davidson 2014). An estimated 33% of global wetlands have been lost since 2009 (Hu et al. 2017). It is well known that wetland ecosystems provide numerous services both ecologically and economically. A wetland system will reduce the risk of floods, provide habitat for wildlife of all levels, capture and transform chemicals such as nitrogen and phosphorus, recharge groundwater, and provide various recreation opportunities (Kent 2000). As wetlands continue to be altered, man-made systems have been created to make up for the lost ecological and hydrological services, including stormwater systems such as constructed wetlands and roadside ditches (Gold et al. 2019; Tatariw et al. 2021). While the intended purpose of roadside ditches is to manage stormwater runoff and retain pollutants, they have proven to be capable of a level of nitrogen removal approximate to that of natural wetlands (Tatariw et al. 2021).

In addition to being capable of nitrogen removal, for a roadside ditch to truly mimic the function of a natural wetland, the roadside ditch must also be able to provide habitat. While roadside wetlands and other wetland systems that exist in urban settings are often overlooked, they still act as valuable habitat and provide habitat for a wide range of creatures, including invertebrates (Palta et al. 2017). Invertebrates are known to be good indicators of ecosystem diversity and health, due to their size, group diversity, and sensitivity (Weaver 1995). With respect to the health of the ecosystem, the invertebrate population can also be an early indicator of environmental stressors (Weaver 1995; Borges et al. 2021). Within a wetland, invertebrates act as a trophic link between vegetation and animal life, and are an important food source for fish and waterfowl (Gleason et al. 2018; Wrubleski and Ross 2011). A healthy invertebrate population generally reflects a healthy wetland (Wrubleski and Ross 2011; Luell 2020).

Because of the diversity among invertebrates, to get a complete picture of a wetland's health it is necessary to understand which invertebrates are present and why. When it comes to habitat preference, freshwater invertebrates can generally be sorted into multiple broad categories (Bouchard 2004). Some are more likely to be found in areas with heightened water flow, such as order Ephemeroptera and order Trichoptera, while other orders prefer slow water flow, such as order Diptera (Quesada-Alvarado et al. 2020).

An additional method of categorizing freshwater invertebrates is based upon water pollution tolerance -

species that cannot tolerate pollution, species that can somewhat tolerate pollution, and species that can tolerate pollution (Luell 2020; Bouchard 2004). Invertebrates can also be categorized according to functional feeding groups, which are based on how an organism acquires food (Luell 2020). For example, order Ephemeroptera belongs to the group that cannot tolerate pollution, and this information in conjunction with Ephemeroptera being found in areas of high water flow indicates that if Ephemeroptera is found in a wetland or stream, that location most likely has high oxygen and low pollution (Bouchard 2004; Luell 2020). This logic can be applied to all aquatic invertebrates found in wetlands. By collecting a sample of the aquatic invertebrate population within a wetland and identifying specimens down to the family level, valuable information can be inferred about the health and quality of that wetland.

The objectives of this study are to provide an overview and analysis of aquatic invertebrate populations in roadside wetlands located off the major roads in Bemidji, as well as test for a correlation among invetebrate commuity sturcture and water quality characteristics such as pH, conductivity, salinity, total dissolved solids, and water temperature.

II. Methods

The data for this study was collected from September to mid-October 2024. A total of 16 samples of invertebrates and water quality measurements were taken from roadside wetlands along major roads in and around Bemidji, MN (Figure 1). For the purposes of this study, a roadside wetland was defined as any location that had wetland vegetation, at least 2.54 cm of standing water, and was no more than 9 m away from a paved road. Once a location that fit these criteria was selected, the area that had water and vegetation closest to the road was selected for sampling. If there were multiple potential sampling sites that fit these criteria, the site that was the easiest to get to on foot was chosen.

The same process for taking samples was used at each site. To avoid disturbing the sediment, water quality readings were taken first using a YSI multi-parameter to measure temperature and dissolved oxygen and a handheld water quality testing meter to measure pH, conductivity, salinity, and total dissolved solids. Then an aquatic D-frame dip net was used to collect invertebrate samples in the same location the water quality samples were taken. A D-net was used for its ability to be used in habitats of varying sediment types. Using tweezers and buckets, invertebrates were removed from the vegetation and sediment brought up by the D-net and placed into labeled containers containing 70% ethanol. To take water depth measurements, the handle of the D-net was vertically dropped into the water and sediment. The measure recorded was the length to which the handle entered the water with no additional force beyond the drop.

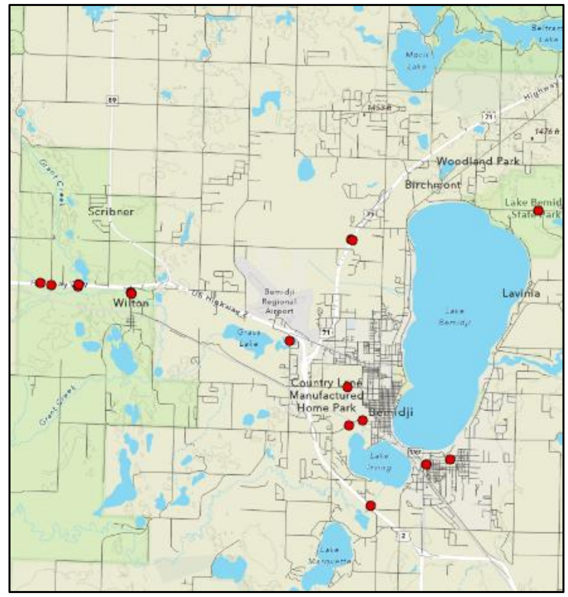

Fig. 1. Map of all 16 locations that were sampled for this study. Samples were taken in September-October 2024. Map created using ArcGIS pro, basemap provided by ArcGIS.

Various taxonomic keys were used to identify invertebrates down to the appropriate taxonomic level i.e., order, family, or genus (Bouchard 2004; Morse et al. 2020; MNDNR 2007; MNWHEP 2021). The identifications were recorded on a data sheet along with the water quality measurements.

Once the data was entered into an excel spreadsheet, R was used to create a nonmetric multidimensional scaling graph and determine significance of water quality measurements. Envfit was used to identify the water quality characteristics that were correlated to aquatic invertebrate community structure. The average values of water quality characteristics were calculated, as well as the most to least abundant invertebrate groups identified in the samples. Using coordinates taken at each site, ArcGIS was used to create a map of sampling locations.

III. Results

In total, 1,608 aquatic invertebrates were collected and identified (Figure 2). The largest number of invertebrates in one sample was 526 at site 16, and the lowest amount was 9 at site 2. The most abundant family was Chironomidae, present in 9 samples. Sample 16 had 506 chironomids, making up the bulk of invertebrates in the sample. The most common family was Planorbidae, which was present in all samples except sample 2. Trichoptera and Ephemeroptera were only present in samples 8 and 14 with dissolved oxygen values of 7.24 - 9.11 mg/L, in addition to sample 13 which contained one Trichoptera and had a dissolved oxygen of 1.54 mg/L.

Samples 8 and 14 also contained the greatest number of different families, with sample 14 containing 15 different families and sample 8 containing 21. These samples were the highest in dissolved oxygen overall. In total, there were 36 different groups collected, most being identified down to the family level. Most samples had 5-7 different families present.

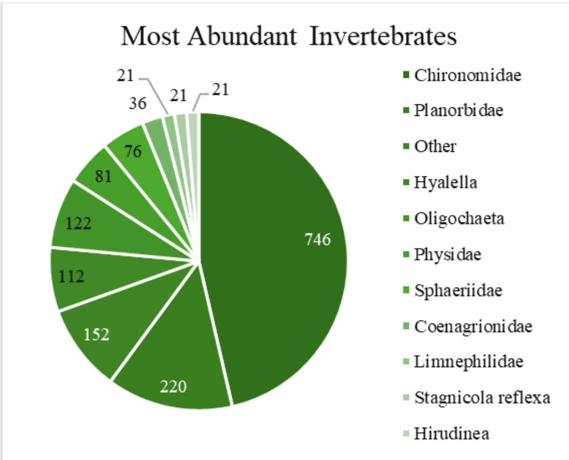

Fig. 2. Most abundant invertebrates found overall, other consisting of groups that were 20 individuals or less. Invertebrates were collected from wetlands along paved roads in the Bemidji area in September to late October 2024.

Dissolved oxygen (*P* = 0.017) and pH (*P* = 0.028) were found to be significantly related to invertebrate community structure (Figure 3). The impact of salinity (*P* = 0.046), conductivity (*P* = 0.046), and total dissolved solids (*P*=0.041) on the invertebrate community are nearly identical and not significant to community structure. Water temperature (*P* = 0.51) and water depth (*P* = 0.29) were also not significantly related to community structure. Most samples had low dissolved oxygen, low pH, and elevated salinity, conductivity, and total dissolved solids (Table 1). The average values of the water quality characteristics indicate that sampling locations were slightly acidic and had low dissolved oxygen.

Even though salinity, conductivity, and total dissolved solids were not significant overall, samples 3, 4, 10, and 13 had the highest values of salinity, conductivity, and total dissolved solids. The invertebrate communities in these samples were made up of groups that have been discussed as living in low water quality conditions. Samples 3 and 4 contain the only Ptychopteridae found during this study, and Ptychopteridae have a very high tolerance level (Bouchard 2004; Morse et al. 2020). Increased salinity is known to cause a decrease in dissolved oxygen, as is the same for conductivity and total dissolved solids. Sample 10 contains the highest number of Planorbidae, and sample 13 contains the highest number of Oligochaeta and Hirudinea. As salinity, conductivity, and total dissolved solids increase, the invertebrate community composition becomes more focused on species that can tolerate low quality water.

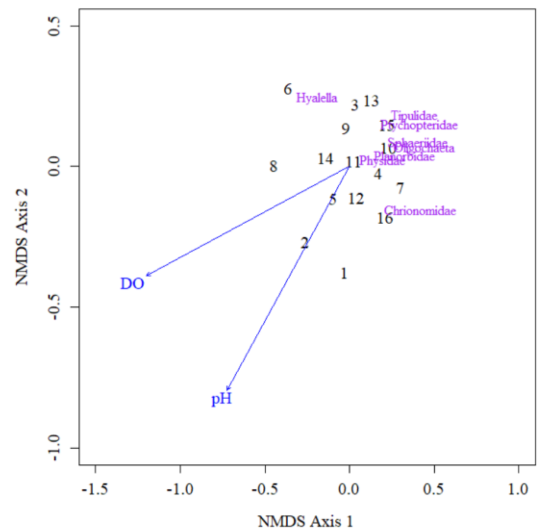

Fig. 3. Nonmetric multidimensional scaling graph showing similarity of samples. Numbers represent the sample identification number. Vectors represent the value of water quality samples of dissolved oxygen (DO) and pH. Samples were collected from wetlands along paved roads in the Bemidji area in September to late October 2024.

TABLE 1. AVERAGE VALUES OF WATER QUALITY CHARACTERISTICS, MEASUREMENTS TAKEN FROM WETLANDS ALONG PAVED ROADS IN THE BEMIDJI AREA IN SEPTEMBER TO LATE OCTOBER 2024.

Water Quality Characteristics	Average	Standard Deviation
pH	6.35	0.28689
DO (mg/L)	5.27	2.54723
Water Temperature (°C)	11.85	5.32152
Conductivity (mS/m)	568.16	267.807
TDS (mg/L)	404.81	199.248
Salinity (mg/L)	312.13	165.291
Water Depth (cm)	42.672	6.03733

IV. Discussion

The objective of this study was to examine roadside wetlands and determine if there was a relationship between invertebrates found and water quality characteristics. It was determined there was a correlation between water quality and number type of invertebrate found. The most common families found in the samples, Planorbidae and Physidae, are known to tolerate a wide range of habitat types and oxygen levels. They are lunged snails, and as such can handle low oxygen conditions well (Bouchard 2004). Similarly, the most abundant family in the gathered samples Chironomidae is known to inhabit any water body of any quality (Bouchard 2004). When living in low-oxygen environments, Chironomidae will present a red color due to hemoglobin for oxygen storage, and many red Chironomidae were found during this study. The sample locations can be considered low oxygen, which is supported by the presence of Planorbidae and Chironomidae. Another group present in most samples is Oligochaeta, and like Chironomidae, Oligochaeta are known to be able to live in a large habitat range, especially polluted waters and low oxygen areas (Bouchard 2004).

The third most abundant invertebrate group collected in the samples was *Hyalella* (Fig. 3), though 59 of the 112 individuals collected were from sample 14. *Hyalella* are known to be very tolerant of low water quality (Bouchard 2004), though sample 14 had high dissolved oxygen and a pH of 6.3. While various Trichoptera and Ephemeroptera were found, majority of collected invertebrates belong to groups that tolerate low water quality.

As an example, Chironomidae, Tipulidae, Corixidae, Planorbidae, Sphaeriidae, Coenagrionidae, Ptychopteridae, and Libellulidae are only a few of the families identified in the samples that tolerate low water quality. The above families represent a range of functional feeding types, from grazers, predators, filterers, and gatherers (Luell 2020, Bouchard 2004). This study collected a range of invertebrates from different orders that occupy different ecological niches.

The water quality measurements taken showed that most sample locations were low in oxygen. Natural wetlands are low in oxygen due to factors such as soil and vegetation composition (Wrubleski and Ross 2011), so these sample sites having low oxygen and low oxygen tolerant invertebrates make them analogous to natural, larger wetlands. The only sample locations that had invertebrates that required high oxygen and are not tolerant to low water quality were the locations that had flowing water, samples 14 and 8. All other locations were stagnant, and majority had biofilm and large amounts of loose organic sediment. All locations had wetland vegetation as that was a selection criterion, though wetland size was highly variable.

The results of this study show that roadside wetlands provide habitat to a diverse range of aquatic invertebrates. Despite commonly being overlooked, roadside wetlands are valuable ecosystems, though often created as a byproduct of construction (Palta et al. 2017). The water is slightly acidic and low in oxygen, and the invertebrates found reflect that. Though the water may be low quality, and the insects found within mostly flies and snails, roadside wetlands are still valuable ecosystems.

Intentionally managing the upkeep of roadside wetlands may benefit areas that have experienced extreme wetland loss, such as urban areas. Knowing that roadside wetlands can host a wide range of invertebrates while having low water quality can be beneficial for future work in wetland restoration.

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MODELING WILD RICE BED HEALTH: A DUAL METRIC APPROACH FOR BED STRENGTH AND INVASIVE IMPACT

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***Abstract*—Wild rice (mannoomin; *Zizania palustris*) is an aquatic grain that grows in slow-moving rivers and shallow bays throughout northern Minnesota. In Headquarters Bay on Leech Lake, wild rice has served as a cultural staple for Indigenous communities for centuries. However, its population has declined in recent years due to multiple environmental issues. One potential contributor is Eurasian watermilfoil (*Myriophyllum spicatum*; EWM), an invasive species that forms dense mats capable of outcompeting native aquatic vegetation, including wild rice. EWM was first recorded on the southern shoreline of Leech Lake in 2005 and has since become widespread. This study aimed to determine whether wild rice has declined and identify the factors influencing its persistence. Vegetation surveys were conducted at 293 sites, of which 93 supported wild rice in 2005. At each site, aquatic plants were sampled using a rake, identified, and related to lake depth. Logistic regression analyses revealed that wild rice survival is significantly influenced by proximity to EWM ($P < 0.01$), historical bed strength ($P = 0.03$), and distance from the main channel ($P < 0.01$). These results provide evidence to suggest wild rice decline is not sustainable and is affected by these factors. Future management should prioritize their efforts on invasive species management and habitat preservation. Continued monitoring and collaboration with the Leech Lake DRM will be essential for protecting wild rice beds, not only as ecological assets but also to protect the Indigenous cultural heritage of the land and water.**

I. Introduction

Wild rice (*mannoomin; Zizania palustris; ZIP*) is an aquatic grain that grows in slow moving rivers and shallow bays of many lakes in northern Minnesota. Wild rice holds sediment in place on shorelines and riverbanks protects those areas from erosion (Drewes 2020). For fish, rice fields provide habitat, nutrients, and organic material necessary for seasonal spawning and feeding (Freed et al. 2020). On a nutritional basis, this native plant has many health benefits in comparison to other grains. As a complete protein, wild rice replaced bread for the early indigenous people for centuries. Many tribes of the Dakota, Menominee, and Ojibwe people use manoomin in their everyday diets, and as a useful medicine. Wild rice is crucial to native culture and many native peoples' incomes. Anishinaabe believe whatever happens to the mannoomin happens to them. As a result, many tribes of the north would fight each other over control of wild rice rivers (Vennum 1988). These tribes to this day try their best to protect ricing beds from all the threats that occur in lakes and rivers.

Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) is an invasive species that comes from Asia. The EWM invasion of North America is hypothesized to have originated along the U.S. Eastern Seaboard. The earliest North American herbarium collections of EWM are from Washington, D.C., in 1942 (Moody et al. 2016). EWM was first found in Leech Lake in 2005 on the south shoreline. This plant reproduces very rapidly by vegetative propagules such as stolons and fragments. Stolons, or runners, reproduce by branching off the main plant. Once a runner touches the ground, roots can develop and grow into an identical copy of the plant prior (Madsen et al. 1998). EWM can also fragment, meaning when a piece of the plant breaks off, it can begin to grow into a new plant. This invasive is especially harmful because it can out-compete many of the native plants by creating dense mats that can seal off the competing plants from sunlight, resulting in an environment that is less biodiverse. As a result of the dense mats at the end of the growing season, a high biomass decomposition leaves high concentrations of nitrogen and phosphorus in the water collum (Grace and Wetzel 1978).

Lakes in Minnesota and many other states are dealing with affected fisheries because of many invasive species. Leech Lake, located in northern Minnesota, is one of the most revered muskellunge and walleye fishing lakes in the world, even being crowned the muskellunge capital in 1955. EWM affects these fish communities by changing the macrophyte community of a waterbody, leading to a cascade of abiotic and biotic alterations that can change fish species diversity and reduce fish abundance (Kusnierz et al. 2024). EWM does this by obstructing swimming space of pelagic fishes while sheltering juvenile fishes and disrupting foraging movements of piscivores. With EWM outcompeting native plants that support a diverse array of invertebrates and macrophytes, food shortages for fish may occur due to reduced sunlight penetration and limited water movement (Engel 1995). The objective of this study is to assess the factors contributing to the decline of wild rice since 2005, using vegetation survey data collected by the Leech Lake Department of Resource Management and the Minnesota Department of Natural Resources.

II. Methods

The sample site for this project was Headquarters Bay, Leech Lake. The lake itself is primarily shallow water with a mean depth of 18 ft and 50% of the lake is less than 15 ft deep and 80% is less than 25 ft deep. It is characterized as a hard water, mesotrophic lake. Headquarters Bay where the study was conducted is more fertile than the main lake. The bay is entirely shallow, with maximum depths of less than 15 ft. This bay is relatively protected from wind and most boaters. Bottom substrates are sand and muck making it the desired environment for wild rice growth (Perleberg et al. 2010).

Surveys were conducted by boat with two to three surveyors over 293 sites. Surveyors navigated to each site using a handheld Global Positioning (GPS) unit. Surveyors attempted to navigate within 15 feet of the point and precision varied due to wind and boater experience. One side of the boat was pre-selected as the sampling side, and at each sample site surveyors recorded water depth in one-foot increments using an electronic depth finder, or a measured stick in water depths less than eight feet. At each survey site, surveyors approximated a 3 foot squared area and recorded any plant taxa visible from the boat surface. A double-headed long-handled rake with a rope attached to the end of it was lowered vertically to the bottom and dragged over a defined sampled area (Johnson et al. 2011). To survey vegetation not visible from the surface, taxa were identified recorded to the lowest level possible typically to the species level (Perleberg et al. 2010). Wild rice is a protected plant with boating being prohibited within the rice beds. Some of the points unable to be reached/surveyed due to the wild rice being abundant in those sample sites. Those points were then labeled as either “wild rice only present and was not surveyed” or “other emergent plants present.”

Data Analysis

To assess the relationship between the presence of wild rice and EWM and depth, a logistic regression was performed. For this analysis wild rice and EWM were either 1 (presence) or 0 (absence), and depth was a continuous predictor. All data points from both years were used in this analysis.

To evaluate how the strength of rice beds affected wild rice survival, data was collected from the 93 locations that had wild rice present in 2005. Wild rice bed strength was measured at each site in 2005 and assigned a value from 0 to 7, representing the number of neighboring sites with wild rice present in 2005. In 2024, wild rice presence or absence was recorded at each site to assess changes over time. A logistic regression model was applied to examine the effect of bed strength on the probability of wild rice survival.

To examine the relationship between wild rice survival and EWM proximity, data was collected from the 93 locations that had wild rice present in 2005. EWM proximity was calculated by measuring the distance to nearest EWM in 2024 using a GIS map of the points. Wild rice presence or absence was recorded at each site in 2024. A logistic regression model was applied to examine the effect of EWM proximity on the probability of wild rice survival.

To determine if there was a relationship between wild rice survival and proximity to the main channel, data was again used from the 93 locations where wild rice was present in 2005. Proximity to the channel was calculated by measuring the distance to the nearest point in the channel using a GIS map. In 2024, each site was assessed for wild rice presence or absence. A logistic regression model was applied to examine the effect of the main channel on the probability of wild rice survival.

III. Results

Presence or absence of Wild rice and Eurasian watermilfoil was determined at 293 sites in both 2005 and 2024. There was a significant negative relationship between depth and wild rice presence with ($P < 0.01$; Figure 1) indicating wild rice is more likely to occur in shallower waters.

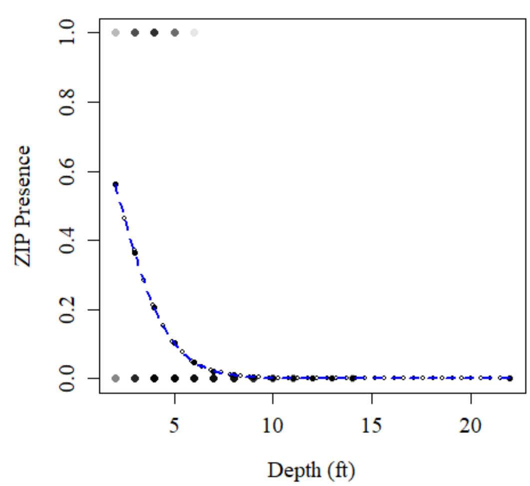

Fig. 1. Probability of wild rice (*mannoomin; Zizania palustris; ZIP)* presence in relation to depth in Headquarters Bay, Leech Lake, MN. Presence absence data was recorded at 293 sites sampled in both 2005 and 2024.

While the fitted model suggests a negative trend. That decreasing probability of EWM presence with depth, the effect is not statistically significant (*P* = 0.61; Figure 2). The large p-value suggests that depth alone does not explain much of the variation in EWM presence.

There was a significant negative relationship between bed strength and wild rice survival with a (*P* = 0.03; Figure 3). Wild rice was more likely to persist in areas with lower bed strength and where fewer neighboring wild rice were present, suggesting that both clustering and possible disease can influence survival.

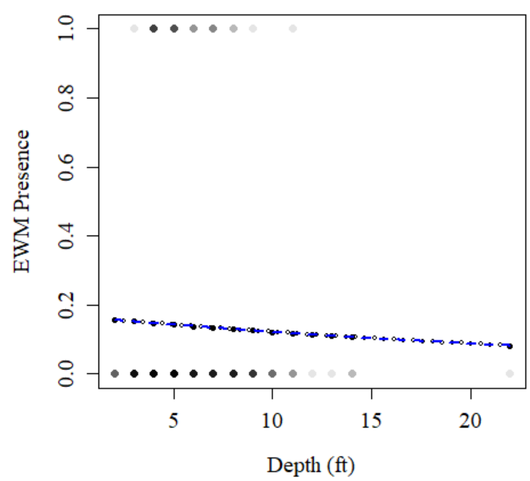

Fig. 2. Probability of Eurasian watermilfoil (*Myriophyllum spicatum*; EWM) presence in relation to depth in Headquarters Bay, Leech Lake, MN. Presence–absence data were recorded at 293 sites sampled in both 2005 and 2024.

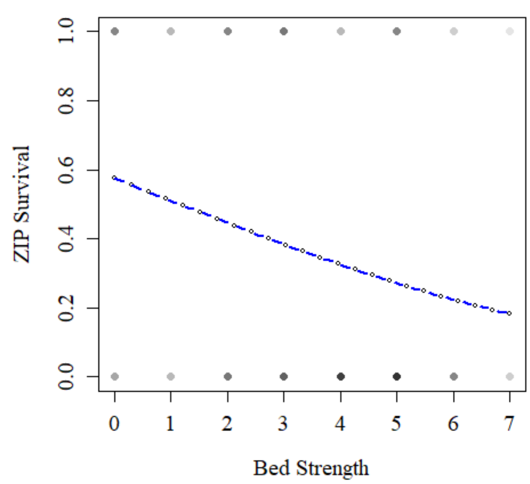

Fig. 3. Probability of wild rice (manoomin; *Zizania palustris*; ZIP) survival in relation to bed strength in Headquarters Bay, Leech Lake, MN. Presence–absence data indicating wild rice survival in 2024 were recorded at 93 sites where wild rice was present in 2005.

There was a significant positive relationship between distance from EWM and wild rice survival (*P* < 0.01; Figure 4). The fitted curve indicates that wild rice is more likely to persist in areas farther from EWM patches, suggesting that proximity to EWM may negatively influence wild rice through competition, habitat alteration, or other ecological factors.

There was a statistically significant positive relationship between distance from the main channel and wild rice survival (*P* < 0.01; Figure 5). The fitted regression curve indicates that wild rice is more likely to persist farther from the main channel, possibly due to reduced boat traffic and less flow disturbance.

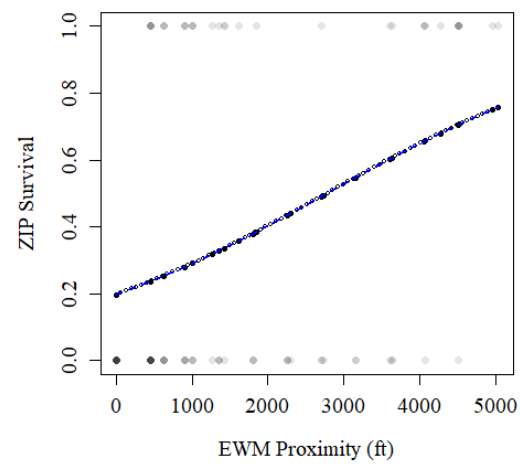

Fig. 4. Probability of wild rice (manoomin; *Zizania palustris*; ZIP) survival in relation to EWM proximity in Headquarters Bay, Leech Lake, MN. Presence–absence data indicating wild rice survival in 2024 were recorded at 93 sites where wild rice was present in 2005.

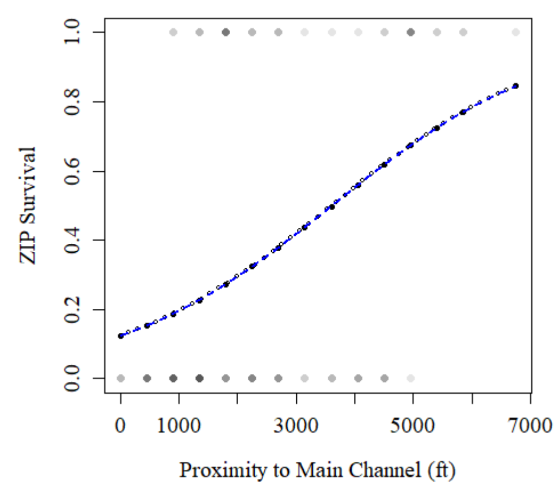

Fig 5. Probability of wild rice (manoomin; *Zizania palustris*; ZIP) survival in relation to proximity to main channel in Headquarters Bay, Leech Lake, MN. Presence–absence data indicating wild rice survival in 2024 were recorded at 93 sites where wild rice was present in 2005.

IV. Discussion

The primary finding of this study is that wild rice has declined significantly since 2005, with 46 sites lost and only 5 new sites established. While 34 sites remained stable, this trend points to an unsustainable rate of loss. This decline appears to be associated with multiple environmental factors, including proximity to the main channel (where boat traffic is highest), proximity to existing EWM populations, and the strength/size of existing wild rice beds. These factors appear to influence whether wild rice sites persist, decline, or are displaced by other species. Sites surrounded by other wild rice locations in 2005 were more likely to be lost by 2024, suggesting that disease might have taken place in this large portion of the wild rice beds. For example, when paddies are flooded in the spring, infested crop debris floats to the surface and conidia of *B. oryzae* and *B. sorokiniana* infect leaves and stems of wild rice as they emerge from the water (Johnson et al. 1992).

Proximity to EWM sites was significantly associated with wild rice decline. Between 2005 and 2024, thirteen wild rice sites were replaced by EWM, suggesting possible competitive displacement. This finding supports the hypothesis that EWM can outcompete wild rice for space, light, and nutrients, altering the native plant communities of Leech Lake over time. These results are consistent with previous research showing that dense EWM growth can significantly reduce the abundance and diversity of native aquatic vegetation (Getsinger et al. 1997).

Proximity to the main channel was significantly associated with wild rice persistence. Sites located closer to the main channel where boat traffic and wave energy are typically higher were less likely to support surviving wild rice populations. This finding points to the negative effects of disturbance on wild rice sustainability. In Jefferson County, Wisconsin macrophyte abundance has declined in areas with high motorboat activity. The decline in macrophytes appears to result mainly from sediment disruption and direct mechanical damage (Asplund et al. 1997).

This study also examined whether wild rice and EWM occupy similar depth ranges, which could indicate competition for habitat. Wild rice is more likely to occur in shallower depths, with occurrence decreasing as depth increases. These results were significant and consistent with other studies showing wild rice requires the presence of shallow, relatively clear water, where water depth is 0.33 to 3.28 ft (Biesboer et al. 2009).

EWM had an equal distribution among different depths and was insignificant. More than likely other factors influenced distribution in depth. In a study conducted on lake Wingra, WI. distribution of Eurasian watermilfoil (*Myriophyllum spicatum L.*) was researched. The linear regression model for predicting milfoil biomass was weak, but optimal ranges of water depth were able to be identified (Nichols 1994).

This study provides clear evidence that wild rice in Headquarters Bay has experienced significant declines since 2005, driven by the factors above. These findings suggest the need for management to prioritize both invasive species control and habitat preservation. Continued monitoring and collaboration with the Leech Lake DRM will be essential for protecting wild rice beds, not only as ecological assets but also to protect the Indigenous cultural heritage of the land and water.

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APPENDIX A. ADDITIONAL FIGURES

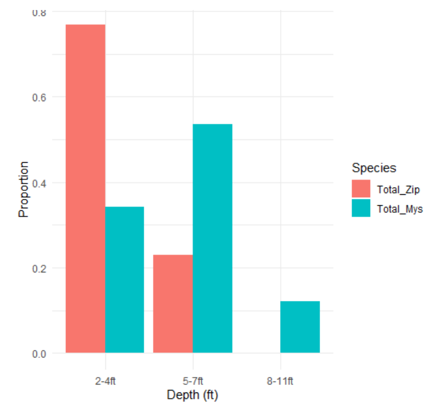

Fig A1. Depth distribution of wild rice (manoomin; *Zizania palustris*; ZIP) and Eurasian Watermilfoil (*Myriophyllum spicatum*; EWM) in Leech Lake MN.

DOES DEPTH AFFECT ZEBRA MUSSEL LENGTH IN SCALP LAKE?

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***Abstract*—Since its first sighting in 1996, zebra mussels *Dreissena polymorpha* have been confirmed in hundreds of Minnesota lakes. Not only were they found in lakes, but they are also found in rivers too. With lack of effective treatments to impede their spread, zebra mussels continue to colonize new areas causing ecological as well as economic harm. The objective of this study was to see if there was a relationship between a zebra mussel length and the depth, they are found using shell length (mm) and depth (cm). The comparison of zebra mussel length and depth was done by performing a regression analysis. On 18 October 2024, 150 zebra mussels were sampled at 10 different depths from the north, east, south, and west parts of Scalp Lake. Scalp Lake was chosen for the lake to sample because of the high-water clarity and the high number of zebra mussels present. Average zebra mussel length was 12.00 mm (SD = 1.20) on the north shore; 12.64 mm (SD = 1.64) on the east shore; 16.58 mm (SD = 1.57) on the south shore; and 12.65 mm (SD = 1.06) on the west shore. There was no significant relationship between zebra mussel length and depth in any quadrant around the lake (north shore P = 0.85; east shore P = 0.42; south shore P = 0.35; west shore P = 0.29). The results show that zebra mussel size is more related to lake location and less depth. This could be due to the fact that vegetation densities are different around each quadrant of the lake.**

I. INTRODUCTION

Originally from Eastern Europe, the zebra mussel *Dreissena polymorpha* is one of the most alarming invasive species in North America. It has drastically changed in recent decades, altered natural processes and resulted in billions of damaged objects. Every time a boat or other aquatic vessel goes to a different body of lake or river, there is a chance that zebra mussels get introduced to that water system. Since its first appearance in 1986 in Lake St. Clair (Roberts 1990), zebra mussels have rapidly expanded throughout the United States.

Zebra mussels are extremely efficient suspension filter feeders that take nutrients from the water column's phytoplankton while interfering with the normal flow and circulation of organic material. They have a significant impact on the water clarity of lakes and rivers due to their feeding on phytoplankton, bacteria, and other organic compounds (Cohen and Weinstein 1998). The more they eat, the clearer the water becomes. The invasion of shallow lakes and ponds by zebra mussels can potentially lead to a redirection of production and biomass from pelagic to benthic food webs (MacIsaac 1996). This shift in ecosystems can result in the transition to an alternative state.

Determining the length of a zebra mussel under water, at a certain depth, is difficult without the physical collection of the specimen. The objective of this study is to see if there is a relationship between the length of zebra mussels and how deep they are when they are collected in the north, east, south, and west parts of Scalp Lake.

II. METHODS

Study Area

Scalp Lake is a 103-hectare lake in the city of Frazee, within Ottertail County. The lake has a maximum depth of 27 meters. The recent zebra mussel infestation and the paucity of information regarding the population's condition are the reasons Scalp Lake was selected for this investigation. Furthermore, it is crucial to comprehend the potential effects of this invasive species on Scalp Lake and the surrounding lakes, since it is a component of numerous other bodies of water.

Sample Collection

Sampling occurred on 18 October 2024. Ten to fifteen zebra mussels were taken from 10 different depths (10 – 100 cm) from the north, east, south, and west quadrants of Scalp Lake. Waders were used to walk in the water and to locate the zebra mussels at the appropriate depth. Once the zebra mussels were taken from the water ground, they were put in plastic bags which were labeled with the appropriate quadrant and depth that they were collected. The zebra mussels were put in a cooler and then transported to the lab for measurements.

Data Analysis

The zebra mussels were measured to a hundredth of a millimeter (mm) using a micrometer. The measurement was taken along the longest axis of each individual zebra mussel. To test for a relationship between the zebra mussel length and sample depth, regression analysis was used on each quadrant of the lake separately.

III. Results

A total of 600 zebra mussels were collected and measured. Average zebra mussel lengths were taken from the north, east, south, and west shores. North shore: average zebra mussel length 12 mm (SD = 1.20). East shore: average zebra mussel length 12.64 mm (SD = 1.64). South shore: average zebra mussel length 16.58 mm (SD = 1.57). West shore: average zebra mussel length 12.65 mm (SD = 1.06). Regression analysis was used for each quadrant, (north shore P = 0.85); (east shore P = 0.42); (south shore P = 0.35); and (west shore P = 0.29).

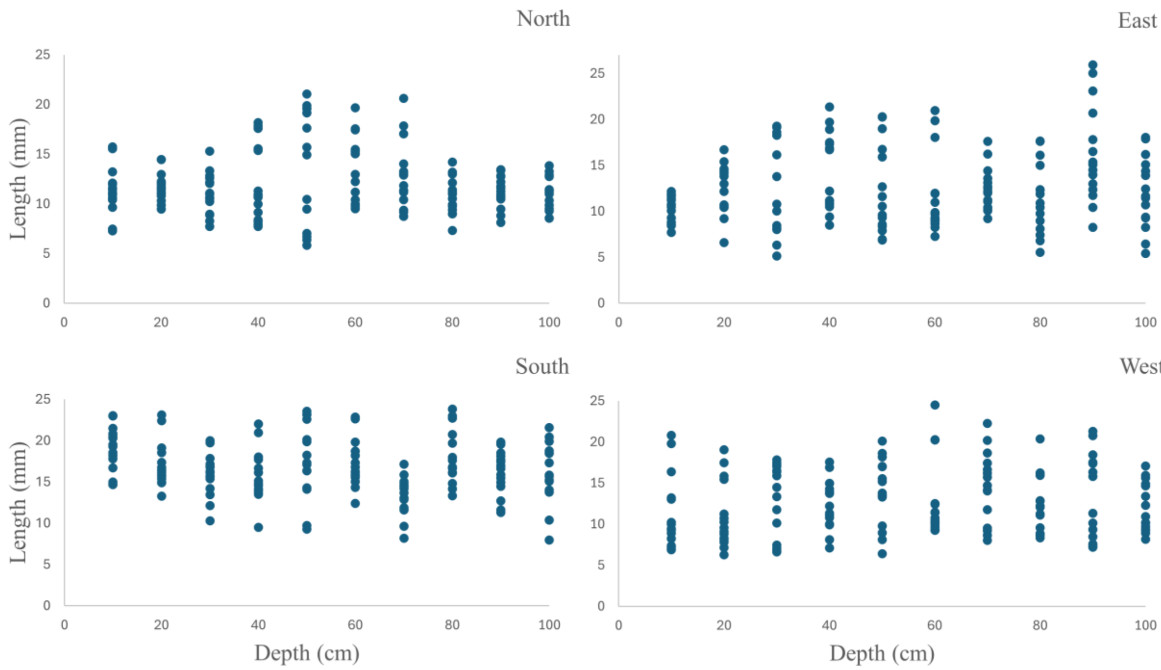

Fig. 5. The relationships between zebra mussel length (cm) and depth (cm) at the north, east, south, and west shore at Scalp Lake.

IV. Discussion

On the north, south, and west shores of Scalp Lake, there was not a significant relationship between zebra mussel length and depth. This result could be because of different limnological variables in Scalp Lake like Chlorophyll. Chlorophyll is important in determining zebra mussel growth because of the number of zooplankton and algae present at that location (Chakraborti et al. 2008). Zebra mussels thrive in nutrient rich environments which leads to rapid growth. The lack of zooplankton and other algae organisms is possibly why there is not a significant relationship with zebra mussel length and depth.

The growth rates of zebra mussels may have been influenced by many factors such as water temperature, food availability, and competition with other species (Garton and Johnson 2000). According to a study done in Lake Wawasee, scientists were doing a study on measuring the response of shell growth of zebra mussels in different environment gradients. Shell growth in mussels decreased with initial shell length and depth, with shallow water mussels having growth rates nearly twice that of deeper water mussels. In Lake Wawasee, growth took place early in the spring and differed greatly between locations. Additionally, the study discovered that mussel shell growth was not significantly impacted by the cage design or the spacing between growth cages.

By establishing a correlation between zebra mussel length, sample depth, and the areas of the lake from which they were gathered, this study developed a framework for comprehending Scalp Lakes zebra mussel populations. To support these conclusions, more research should be done on population patterns and the ensuing effects on the nearby water systems.

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SIZE OF AGE-0 *SANDER VITREUS* PRE AND POST *DREISSENA POLYMORPHA* INFESTATION ON BRAINERD AND BEMIDJI AREA LAKES

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***Abstract*—Walleye *Sander vitreus* is the Minnesota state fish and a vital predator fish in lake ecology. Zebra mussels, *Dreissena polymorpha* were first found in Minnesota inland lakes in the 1980's. These mussels have had huge impacts on water clarity as well as phytoplankton and zooplankton population levels which affect how age-0 fish feed, especially a low light feeding predator fish like walleye. A study took place on age-0 walleye size and it supported the conclusion that age-0 walleye were negatively impacted by zebra mussels in Minnesota's large lakes. The goals of this study were to determine how the size of age-0 walleye are impacted by changing water clarity in two Minnesota lake regions (northwest and central). Fall electrofishing data was collected on age-0 walleye, zebra mussel invasion dates and water clarity measurements were collected by the Minnesota Department of Natural Resources and Minnesota Pollution Control Agency. The factors of lake ($P = 0.00008$), year ($P = 0.00273$), and zebra mussel status ($P = 0.00069$) all had statistically significant relationships with age-0 walleye size. The effect of average June Secchi ($P = 0.13310$) did not have a statistically significant difference on age-0 size. Age-0 walleye size increased post-invasion (11 mm) as well as water clarity depths (1.16 m). Water clarity measurements varied from lake to lake, but all lakes consistently increased in the first five years post invasion.**

I. INTRODUCTION

The zebra mussel, *Dreissena polymorpha* have been a highly debated and researched invertebrate since their introduction into Minnesota's inland waters. These small inhabitants can alter ecosystems dramatically as has been seen in many lakes as a result of their invasion in the mid 1980s (Depew 2021). Zebra mussels are very efficient aquatic invaders in freshwater lakes and are highly specialized to filter feed on pelagic food, then returning their feces to the benthic zone. Through secreting this food out of the pelagic zone it increases the water clarity in lakes, increasing the depth surface light can reach into the water column. For example, in Oneida lake in New York, the average depth receiving 1% surface light was increased from 6.7 m pre zebra mussel invasion to 7.8 m after the invasion of zebra mussels. This represented a 23% aerial expansion (Zhu et al. 2006). The water clarity change not only affects phytoplankton abundance, but it also affects zooplankton abundance in the initial years following invasion of an ecosystem (Pace 2010). This is not only impactful on micro and macroinvertebrates, but it also will affect the whole lake ecology. Meaning it affects how age-0 fish feed and what they can feed on.

The state fish of Minnesota is the walleye *Sander vitreus.* This one species of fish plays a vital role in most of Minnesota's lakes ecology and has a significant role in Minnesota's economy. The Minnesota Department of Natural Resources (MN DNR) invests a lot of money, time, and effort into stocking, surveying, and researching walleye. It is logical to put in effort to gain understanding of how effective the stocking and management plans are working. One prime example was Mille Lacs Lake in central Minnesota, which was infested with zebra mussels in 2006. The Mille Lacs walleye population, which was more stable than any other walleye population in the larger lakes in Minnesota, dropped to historically low levels in the five-year timespan from 2007-2012 (Kumar 2015). This lake has been highly altered by the zebra mussel colonization and the walleye population has been in fluctuation throughout the past decade.

A previous study conducted in the year 2020 hoped to find the effects of zebra mussels on age-0 walleye. Data from nine lakes, including a majority of Minnesota's large lakes, was used. This data included near shore seins collected in years ranging from 1983-2018 from mid-July to mid-August each year (Hansen et al. 2020). This study found age-0 walleye were 9.2% smaller in zebra mussel invaded systems than uninvaded systems (Hansen et al. 2020). The 2020 study differs from this study in lakes, data collection, and classification of invaded versus uninvaded.

Through the analysis of age-0 walleye electrofishing data of five central Minnesota lakes and six northern Minnesota lakes, this study looks to find how zebra mussel invasion has affected age-0 walleye size and growth. Water clarity measurements are used as evidence of zebra mussel invasion on all analyzed lakes. Monitoring of age-0 walleye occurs on many Minnesota lakes through fall electrofishing to see how well recruitment was for the age-0 and age-1 year classes. Year class physical size and mortality is heavily affected by abiotic conditions in the spring of the year as well as the previous winter's environmental effects. Fall electrofishing is meant to measure the age-0 and age-1 cohorts in order to adjust possible management plans or stocking rates. This study uses all fish caught and measured electrofishing to analyze the size of fish in a certain timeframe.

II. Methods

Using Secchi disk measurements as evidence of zebra mussel infestation, this study looked at the total lengths of age-0 walleye pre and post zebra mussel invasion to see what effects zebra mussels and water clarity have had on the fish. Five of the eleven lakes used in this study are located in central Minnesota, near the Brainerd lakes area. The lakes are as follows: Gull Lake, Pelican Lake, Thunder Lake, Edward Lake, and Washburn Lake. Washburn Lake has yet to be labeled infested with zebra mussels. The other six lakes in this study were from the Bemidji area and are: Bemidji, Big, Blackduck, Plantagenet, Julia, and Itasca. Julia, Itasca, and Blackduck are lakes not yet infested with zebra mussels. Bemidji, Big, and Plantagenet are lakes that contain zebra mussels. The lakes not infested with zebra mussels were used as comparative lakes to see if other abiotic factors like weather could have played a role in age-0 walleye size in a specific year.

June Secchi data was collected from the Minnesota PCA data set (MN PCA 2025). This data was collected in the field from a variety of agencies or lake monitoring groups. The month of June was chosen due to inconsistent data collection in the month of May over time. Later in the summer such as July-September warmer water temperatures support spontaneous algal blooms creating irregular data. Algal concentration in a lake is a dynamic variable that often changes drastically within a short period of time (Kislik 2018). Most years there were two to four Secchi depths taken in the month of June. To better asses the Secchi depth of that month, each Secchi measurement was then averaged.

All the lakes in this study were smaller than Minnesota's designated large lakes. The lakes in this study ranged in size from 1 to 13 km^2, while many of the large lakes are over 40 km^2. Data for the year that each lake was labeled infested with zebra mussels was collected from the Minnesota Department of Natural Resources infested waters list (MN DNR 2024).

For many Minnesota lakes routine fall electrofishing occurs within the months of August, September, and October. The data collected by these electrofishing surveys was the data used in this study. Total lengths were recorded during the surveys and some specimens were kept and aged to ensure where the size break was between age-0 and age-1 fish. In this study all the fish lengths were then averaged per year to get the mean age-0 size per year. The average fish size per year was then plotted according to lake. Lakes are monitored for zebra mussels through water surveys, survey plates, and most commonly, equipment inspection during end of year removal. The Minnesota DNR keeps a record of what year lakes have been infested. This study analyzed data from 2000-2024. This timeframe includes years pre and post zebra mussel infestation of the lakes.

To test if there was a significant difference between the continuous variable of June Secchi depth and age-0 size, a regression test was used. To test if there was a significant relationship between the categorical factor of lake and age-0 size, an ANOVA test was used. To find if there was a significant relationship between the categorical factor of year and age-0 size, an ANOVA test was used. To analyze if there was a significant relationship between the categorical fact of zebra mussel status, an ANOVA test was used. Program R was used to run these analyses. Through this testing, the main goal was to isolate the age-0 walleye size variable as much as possible to get the most accurate measurement of the effect that zebra mussels and water clarity have had on the fish.

III. Results

The average size of age-0 walleye varied significantly among the lakes analyzed ($P < 0.001$; Figure 1). Both Blackduck Lake (μ = 170 mm, σ = 13.3) and Edward Lake (μ = 170 mm, σ = 20.2) had the largest average age-0 size, while Big Lake had the smallest age-0 size (μ = 147 mm, σ = 18.0) including two years of 0 fish electro-fished over the 24 year timespan. Washburn lake had the most consistent walleye size (σ = 9.68), while Plantagenet had the most inconsistent walleye size (σ = 26.0) over the timespan analyzed.

Most all lakes included in this survey had an average Secchi of around two to six meters in depth. Including all years of data in the 24 year timespan, whether or not zebra mussels were present or absent, the lake with the deepest Secchi measurements was Pelican Lake (μ = 6.14 m, σ = 1.41 m). The lake with the shallowest Secchi measurements was Blackduck Lake (μ = 3.51 m, σ = 0.85 m). Including data from all lakes within the study's timeframe the average Secchi depth increased post invasion (1.16 m).

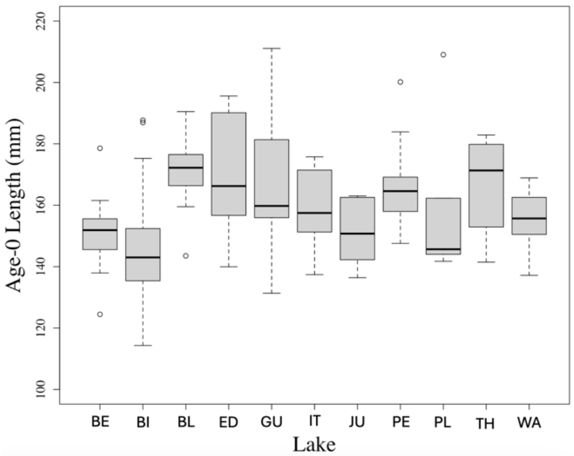

Figure 1. Age-0 walleye lengths on Bemidji and Brainerd Minnesota area lakes (P = 0.00008). All data was from the year 2000 to 2024. BE is Bemidji, BI is Big, BL is Blackduck, ED is Edward, GU is Gull, IT is Itasca JU is Julia, PE is Pelican, PL is Plantagenet, TH is Thunder, and WA is Washburn.

Average June Secchi depths in the years pre-zebra mussel infestation were relatively consistent within a meter or two of their ten-year average (Figure 2). Once infested with zebra mussels, the lakes water clarity increased drastically within the first five years of infestation. Through producing graphs of Secchi depth measurements over the last twenty-four years of data, visual confirmation supports the conclusion that water clarity increases in the years following a lakes zebra mussel status change.

Water clarity and age-0 size data appears more clustered with lakes grouping together instead of a negative correlation (Figure 3). This supports the insignificance that water clarity has on the age-0 size. Using Secchi depth measurements pre and post zebra mussel invasion a regression test was done. The water clarity variable affecting age-0 size was not statistically significant (*P* = 0.13310). Water clarity has many factors such as chlorophyll, turbidity, sediment levels, and nutrient levels. These factors can vary dramatically year to year, sometimes even day to day on certain water bodies. Age-0 size according to lake tended to group together in this graph as well suggesting the statical significance of the lake-to-lake variability.

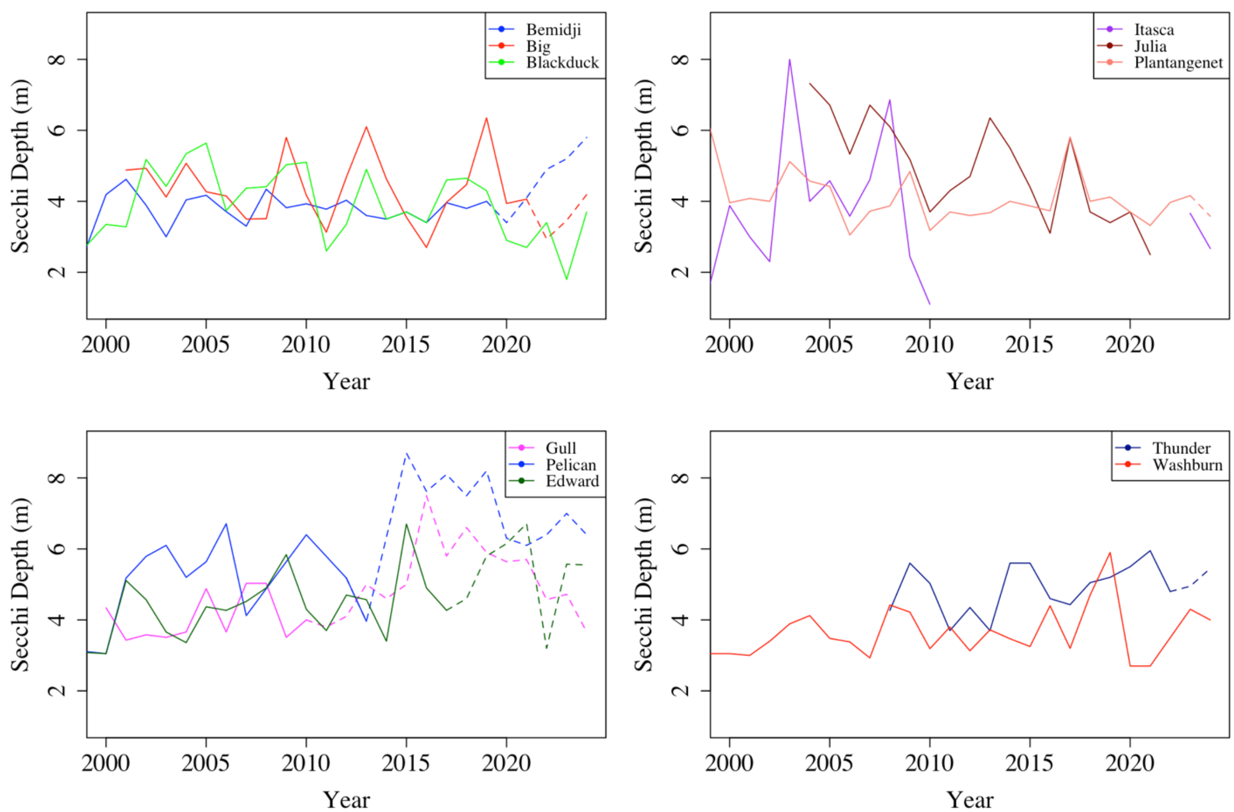

Fig. 2. Average June Secchi depths on all eleven lakes analyzed in both the Bemidji and Brainerd, Minnesota areas. Lakes are labeled by color, and the lake name appears in the legend of each graph. The dotted part of lines is data from years' post-zebra mussel invasion.

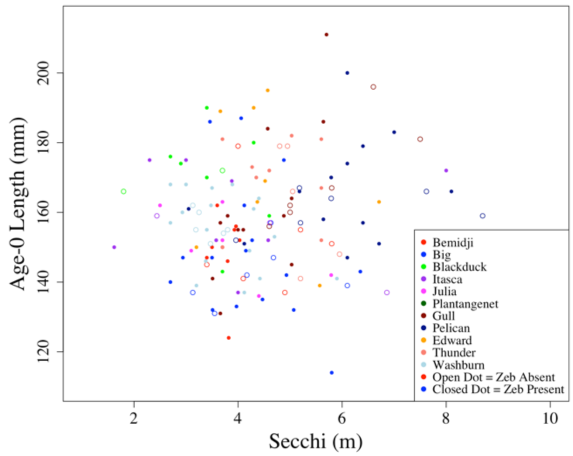

Fig. 3. Mean age-0 walleye length (y-axis) compared to average June Secchi measurements (x-axis; P = 0.13310). Each dot represents the average age-0 size each year. Open dots are years zebra mussels are absent in the lake, while closed dots are years when zebra mussels are present.

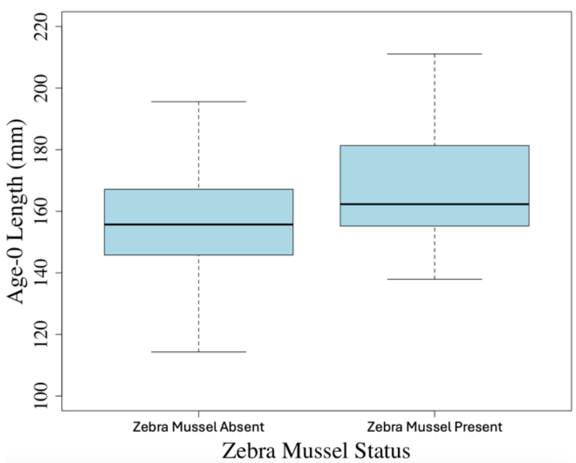

Fig. 4. Average age-0 walleye size for all lakes in this study. Each year pre and post zebra mussel infestation was analyzed in this graph. Average pre infestation age-0 size was 156 mm and average post infestation size was 167 mm (P = 0.00069).

Including every lake in this study and all age-0 size measurements pre and post zebra mussel invasion, it was found that age-0 walleye size pre and post zebra mussel invasion had a statistically significant relationship ($P = 0.00069$). On average, age-0 walleye size was shown to increase (11 mm) in the years post zebra mussel invasion (Figure 4). Age-0 size was also significantly different among years ($P = 0.0273$).

IV. Discussion

The primary finding of this study was that age-0 walleye size was positively affected by zebra mussel presence on a low level (11 mm). This finding was similar to a Lake Erie study in 1999, where Trometer and Busch (1999) found no significant differences in age-0 growth for yellow perch *Perca flavescens* and walleye *Sander vitreous* pre and post zebra mussel invasion. These findings, however, are in contrast to another study of similar foundation. Hansen et al. (2020) found that walleye were smaller at mid-summer and grew more slowly throughout the growing season, resulting in mid-August lengths that were 15 and 18 mm (12 and 14%) smaller in lakes containing *Bythotrephes* and zebra mussels, respectively, compared to uninvaded systems. A major factor that differs from this study is lake size. Each of the large lakes that was analyzed have varying stocking rates, which could possibly vary size of individual age-0 fish throughout the year by increasing CPUE. As found across the Great Lakes region, study to study differences suggest differing results in the impact of zebra mussel on age-0 fish size. To verify the increase in size through this study, more lakes of similar size would need to be analyzed as well as possibly expanding the research region to Minnesota's southern regions. Abiotic conditions such as global warming or other unnatural occurrences such as decreased average wind over a time period could cause a shift of age-0 size on a lake.

Another large factor that was not analyzed in this study was CPUE pre and post zebra mussel invasion. This study's aim was to determine if water clarity increase, due to zebra mussel presence, has affected age-0 size. Adding analysis of a factor such as CPUE would have exceeded this studies time frame. In Oneida Lake in New York for both yellow perch and walleye, trawl CPUE was lower during the post zebra mussel period; however, recent catches often were not below projected catches (Rudstam et al. 2016). Analysis of electrofishing CPUE would need to be conducted to analyze Minnesota's age-0 catch rate. Through analysis, some of the lakes in this study have seen a decrease in number of fish caught while fall electrofishing, more specifically in lakes that have had a longer period of time post invasion. These lakes are located on the southern end of this studies range around Brainerd, Minnesota. There are also many factors that play a role in CPUE analysis. Some of the critical factors that can have a lot of variability could be the amount of time spent electrofishing as well as abiotic weather conditions experienced on the night that electrofishing occurred.

Through this study, another finding was that lake to lake variability between age-0 walleye size was significant. In a previous study, it found that inability to account for variability in Walleye abundance among lakes of the same size can lead to a mismanaged fishery (Hansen et al. 2015). This supports how crucial it is to recognize that there can be significant differences in quantity of walleyes in lakes with similar size and even within the same region. Another potential underlying difference between lakes (including the presence of other invasive species) could be driving variation in growth rates among walleye populations (Nienhuis et al. 2014). Most lakes in this study are only infested with zebra mussels. However, other non-bivalve species like starry stonewort *Nitellopsis obtusa* is found in Lake Bemidji, Thunder Lake, and Blackduck Lake. An aquatic invasive plant, Eurasion watermilfoil *Myriophyllum spicatum,* is found in Washburn Lake. Blackduck Lake has also acquired the faucet snail *Bithynia tentaculata* recently. Other aquatic invasive species, such as an aquatic plant or the faucet snail, would have to be studied further to see if it is suspect that they could affect fish growth. Growing degree days, stream or ground flow levels varying among lakes, or even base phytoplankton and zooplankton forage levels without zebra mussel effects are major factors in lake to lake variability. Some lakes are naturally more apt to growing age-0 fish more efficiently than others.

The factor of year was analyzed to be statistically significant through this study. Year factor has a lot of variables that would need to be included in its analysis. The main variable being growing degree days which can be influenced by abiotic factors such as wind, water levels, and water temperature of a specific year. Another variable that can play into year factor is interaction among other populations of fish through interspecific competition and even predation (Deangelis et al. 1993). These biotic factors can change yearly, which makes this near impossible to track or predict without further research on the specific ecosystem in question. Since many variables must be considered when comparing multi-year data sets for lake ecosystems, it is hard to analyze what the exact cause of age-0 size difference is on a year-to-year basis.

Another major finding through data analysis was the noticeable increase in water clarity in the first five years following the year of zebra mussel invasion. Around year five post invasion the clarity seemed to stable off, then in some cases even begins to decrease in years to follow. On Lake Erie's western and central basins, in the two earliest post-invasion years for which there is data (1989, 1991) did not appear to differ from pre-invasion years, but by 1996 chlorophyll a had decreased substantially (Barbiero et al. 2004). On Lake Ontario Secchi depths increased from an average of 3.9 to 6.5 m between the periods 1985-1991 and 1996- 2004, respectively (Barbiero et al. 2009). Both examples support a similar lag in peak clarity increase or turbidity decrease after the year of invasion (1989). Lake Erie and Lake Ontario are drastically different aquatic systems than the lakes in this study, with lake fetch alone being substantially different. However, possibly through more analysis of inland lakes and areas around the great lakes this pattern may be something to note.

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EVALUATING THE CONDITION OF AGE-0 BURBOT IN RELATION TO DIET

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***Abstract*—Burbot are becoming more and more popular for angling and aquaculture. However, populations have been declining across the world. Very little information is available about their early life and their interactions in the ecosystem due to challenges in sampling. Condition estimates and diet metrics were used to better understand the status of burbot in Lake Bemidji. Using backpack electrofishing, 45 young of the year burbot were collected. The lengths ranged from 86 to 146 mm and the wet weights ranged from 4.29 to 24.36 g. The stomach contents were removed and stored in ethanol for later analysis. The burbot were then dried in an oven at 60 °C and weighed to calculate percent dry weight. R was used to construct an NMDS plot and to calculate both percent dry weight and prey-specific abundance. Amphipods and yellow perch *Perca flavescens* were the most frequently consumed prey. Prey type was not significantly correlated to condition (*P* = 0.61). Juvenile burbot had an average percent dry weight of 18.1% (SD = 0.87) which appears to be consistent with observations of condition from other systems.**

I. Introduction

Burbot *Lota lota* is the only member of the cod family in freshwater ecosystems. Their range spans the Northern Hemisphere across North America, Asia, and Europe. They inhabit systems with cool water, such as lakes and streams that support trout or deep-water lakes. In North America, burbot spawn under ice from about February to March in water temps from 1 to 4 °C (Stapanian and Madenjian 2013).

Burbot is gaining popularity for angling and aquaculture. Many populations worldwide are in decline, thus, learning the factors that will affect future management and protection is important (Stapanian et al. 2010). Some problems affecting burbot populations are development, overfishing, and lack of monitoring (Stapanian et al. 2010). Many populations in the United States have been affected by dam construction and agriculture, affecting spawning habitats and changing food webs. Burbot have not been considered game fish in many regions, and thus have had limited protections, leading to overfishing. They also do not sample well in standard gill nets, meaning government agencies cannot track trends in their populations.

Few studies have looked at the diet of young of the year or juvenile burbot in the wild. Most past studies looked at adults who consume primarily other fish species and invertebrates such as crawfish and insects. (Rudstam et al. 1995; Schram et al. 2006). Furthermore, the subject of condition and diet has not been explored. The few studies that evaluated the diet of juveniles found they consume primarily the rotifer *Asplanchna* early on and then switch to different copepod species as they age, with prey length being the limiting factor (Ghan and Sprules 1993). Burbot preference for copepods could be concerning due to the risk of their population decreasing in response to increasing pollutants (Di Marzio et al. 2013; Di Lorenzo et al. 2014). To better understand the life history and health of the burbot, this study aims to evaluate the diet of juvenile burbot in Lake Bemidji and compare it to the condition of the fish.

II. Methods

In October 2024 age-0 burbot were sampled using a backpack electrofisher. Rocky habitat was targeted along the western shore of Lake Bemidji. The fish were taken back to the lab, where length and weight measurements were taken. An incision was made down their ventral side, and their stomachs were then removed using scissors and forceps. The stomach contents were flushed into a 7.62 cm PVC pipe with an 80 mm filter on one end using ethanol. The ethanol was allowed to dry, and the filter was weighed. The stomach contents were then preserved in ethanol for later analysis. The fish and empty stomachs were placed in a bag and frozen for later. They were then dried in an oven at 60 °C until the weight remained constant. The invertebrates were identified to the lowest taxonomic unit possible, while fish were identified to species. They were then sorted by species, counted, and dried. Frequency of occurrence and prey specific abundance was calculated for each prey type then plotted against each other. Percent-dry weight (%DW) was used to estimate the condition of the sampled burbot. Percent-dry weight and total length were plotted against each other. To find the frequency of prey occurrence (O_i), the number of fish that ate a specific prey (J_i) was divided by the number of fish with stomach contents (P).

$$O_i = \frac{J_i}{P}$$

To calculate prey abundance (P_i), the total count for a single prey type (S_i) was divided by the abundance of all prey in stomachs that contained that particular prey (S_{ti}).

$$P_i = \frac{S_i}{S_{ti}}$$

A NMDS plot was built by using the metaMDS function in the Vegan package (Oksanen et al. 2020). It was used to organize data to understand the relationships between the prey types and their effect on the condition estimates. An envfit analysis was performed using the NMDS, %DW, and total length data to determine if prey species was affecting the condition estimates.

III. Results

The average length of the burbot sampled was 118 mm (SD = 15.97). The average wet weight was 11.91 g (SD = 5.07), and average percent dry weight was 18.10% (SD = 0.87; Figure 1). Empty stomachs were present in 15 of the 45 burbot sampled. A total of 387 prey items were identified (Table 1) the most abundant prey type were amphipods (27%) and perch (2%). Six of the burbot consumed sand or gravel. Amphipods were consumed most often across all the sampled burbot followed by perch and chironomids (Figure 2). The *envfit* analysis returned P-values of 0.61 for percent dry weight and 0.84 for total length.

TABLE 1. TOTAL COUNTS, FREQUENCY OF OCCURRENCE (O_i), AND PREY SPECIFIC ABUNDANCE (P_i) OF PREY TAXA. PREY WERE COLLECTED FROM STOMACH CONTENTS OF LAKE BEMIDJI BURBOT SAMPLED IN OCTOBER 2024.

Prey Species	Count	(O_i)	(P_i)
Amphipods	102	56.25	69.86
Hirudinea	4	9.38	21.05
Annelids	4	12.50	9.76
Chironomidae	10	21.88	22.73
Unidentifiable Fish	2	6.25	66.67
Perca flavescens	9	21.88	6.16
Copepod	2	6.25	10.53
Decapoda	1	3.13	3.45
Gastropod	3	9.38	14.29
Bivalvia	1	3.13	50.00
Ephemeridae	1	3.13	11.11
Ephemeroptera	3	3.13	30.00
Rocks/sand	39	15.63	61.90
Ostracoda	1	3.13	50.00
Unidentified Invert	5	15.63	17.24
Tipulidae	1	3.13	4.55
Dreissena polymorpha	1	3.13	4.55

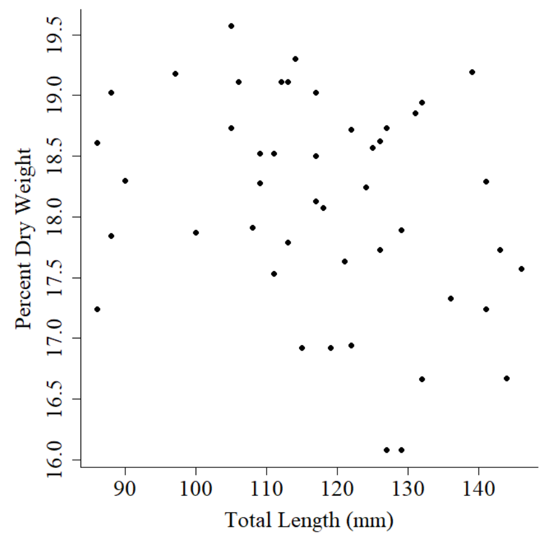

Fig. 1. Relationship between percent dry weight and total length (mm) of burbot *Lota lota* sampled from Lake Bemidji in October 2024.

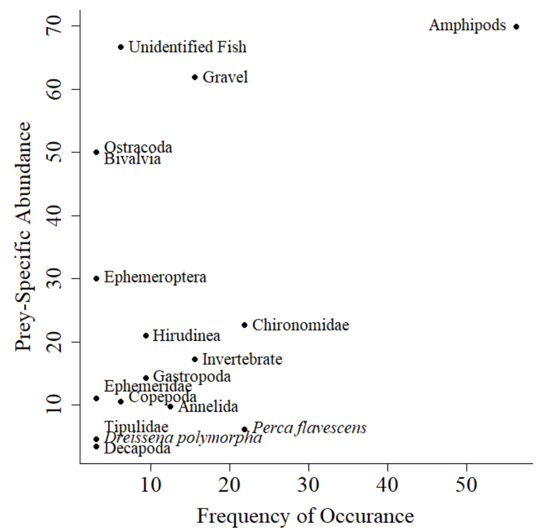

Fig. 2. The relationship between frequency of occurrence and prey-specific abundance of identified burbot prey items. Prey items are sorted to the lowest taxonomic unit that was identified. Prey was collected from burbot sampled from Lake Bemidji in October 2024.

IV. Discussion

There was not a significant relationship between prey type and %DW or total length of age-0 burbot in Lake Bemidji. Studies indicate that prey and forage habitat is dependent on the size of the individual (Ghan and Sprules 1993; Fischer 2004). Where the larger individuals control better forage sites and can consume larger prey. With better forage and a wider range of available prey, growth and condition would likely be affected. However, that is not what this study observed with the p-values of the envit analysis being insignificant. It is known that juvenile fish increase in length more than weight (Bacon et al. 2005). This combined with lethal studies providing a limited temporal view into the diet of fishes could help explain these results.

The %DW estimates of the burbot were consistent with those of a typical population. Previous studies have found that burbot have energy densities between 3350 and 5000 J/g (Rudstam et al. 1995; Schram et al. 2006). Energy densities are correlated to %DW in fish (Hartman and Brandt 1995). Using that relationship burbot %DW can range between 18% and 23%. The burbot in this study were between 16.0% and 19.5%, slightly below the expected range. It has also been shown that juvenile fish have a lower lipid content than adult fish (Martin et al. 2017). More research needs to be done on the bioenergetics of burbot, however, these results seem to be consistent with what others have found.

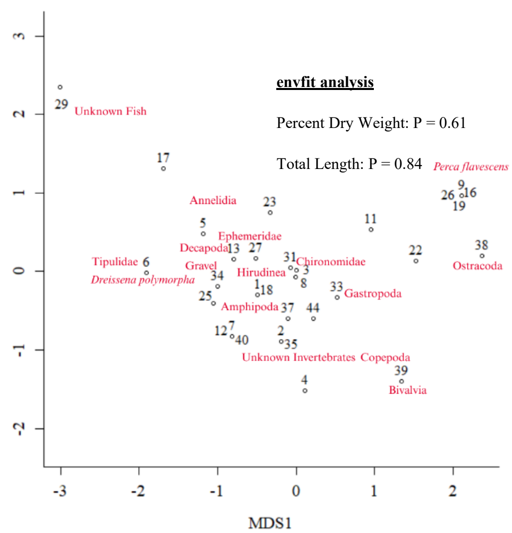

Fig. 3. NMDS (nonparametric multi-dimensional scaling) of sampled burbot and prey taxa. Individual fish are represented by their fish id number. Fish and prey located closely together are correlated to each other. Burbot were sampled from Lake Bemidji in October 2024.

Amphipods and yellow perch (*Perca flavescens*) are important prey species for juvenile burbot in Lake Bemidji. While both taxa were commonly consumed, individual fish typically consumed only one of the two. The abundance of amphipods is consistent with other studies (Ryder and Pesendorfer 1992; Blabolil et al. 2018). Blabolil et al. (2018) also found that fish were not frequent prey of juvenile burbot. Yellow perch and amphipods are both abundant food sources in Lake Bemidji and that is reflected in the stomach contents. The isolation between these two prey species can be explained by the size differences. The yellow perch that were consumed were smaller than the burbot, but occupied most of the available stomach volume. Implying that individuals were consuming perch or amphipods when the other was not available.

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APPENDIX A

TABLE 2. TOTAL LENGTH (TL), WET WEIGHT (WW), AND PERCENT DRY WIGHT (%DW) FOR BURBOT SAMPLED FROM LAKE BEMIDJI IN OCTOBER 2024.

Fish_ID	TL (mm)	WW (g)	%DW
1	146	24.36	17.57
2	144	21.13	16.67
3	121	13.00	17.63
4	143	21.60	17.73
5	124	14.50	18.24
6	132	16.55	16.66
7	126	15.41	17.73
8	108	9.29	17.91
9	139	20.63	19.19
10	129	13.77	17.89
11	113	9.38	17.79
12	114	8.97	19.30
13	111	8.32	18.52
14	122	11.61	18.72
15	117	10.66	18.13
16	119	12.16	16.92
17	141	18.91	18.29
18	115	10.83	16.92
19	127	15.87	16.08
20	117	10.72	19.02
21	132	16.65	18.94
22	141	20.67	17.24
23	111	9.05	17.53
24	109	8.52	18.28
25	122	11.84	16.94
26	113	9.17	19.11
27	105	9.11	18.73
28	117	9.79	18.50
29	109	8.29	18.52
30	100	6.34	17.87
31	86	4.29	17.24
32	88	5.18	19.02
33	86	4.57	18.61
34	88	5.09	17.84
35	125	13.48	18.57
36	136	18.94	17.33
37	131	13.67	18.85
38	129	15.92	16.08
39	118	9.83	18.07
40	126	12.68	18.62
41	105	7.33	19.57
42	106	8.37	19.11
43	112	8.65	19.11
44	97	5.64	19.18
45	127	12.37	18.73
46	90	4.67	18.30

THE EFFECT OF ZEBRA MUSSELS ON MINNESOTA FISH COMMUNITIES

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***Abstract*— Zebra mussels *Dreissena polymorpha* are an aquatic invasive species that has been spreading quickly throughout Minnesota water bodies since 1989. Zebra mussels are known to change certain morphological conditions in lakes such as turbidity, phytoplankton abundance, and aquatic invertebrate populations. However, studies showing impacts of the infestation of zebra mussels on the health of a fish community have been hard to prove due to a lack of repeated surveys and a suite of other confounding variables such as natural and seasonal variability, plant community, and lake morphometry. Therefore, the objective of this study is to determine whether zebra mussels have an impact on fish communities in Minnesota lakes. The Minnesota Department of Natural Resources (MN DNR) has been collecting Fish Index of Biological Integrity (FIBI) scores since the early 2000's to measure the health of the lakes fish community throughout Minnesota. Lakes in Minnesota that had an FIBI survey taken both before and after the introduction of zebra mussels were included in this study. The scores from these lakes were then graphed and analyzed by a paired t-test to determine changes in fish community health. Additionally, years since infestation and the change in the FIBI score was graphed and analyzed using regression statistics. This regression model determined the predicted change in score for every year that the lake has been infested. Variables included in FIBI calculation for each lake type were then ranked and plotted using NMDS plots as well to determine if one variable significantly influenced scores more than others. A significant difference in FIBI was found in scores before, versus after infestation ($P < 0.01$), however, no significant relationship was found between the years since infestation and change in FIBI score ($P = 0.68$). No single metric was influencing scores more than another.**

I. INTRODUCTION

Zebra mussels *Dreissena polymorpha* are an invasive freshwater mussel to North America and have been spreading in Minnesota since 1989. When this bivalve first invades a system, populations will increase rapidly in the first one to two years (Strayer 2019). They will spawn multiple times a year, producing and releasing over a million eggs per individual in one year. Zebra mussels are primarily filter feeders and can filter up to one quart of water a day that feed primarily on algae, macroinvertebrates, bacteria, and other organic compounds (Vanderbush et al. 2021). They will often outcompete native mussels for food and space, along with changing the biology of aquatic systems (Borchedring 1991).

While fish community changes in at least one Minnesota lake have been hard to track due to lack of repeated community surveys (Jones 2020), evidence of the impacts of zebra mussels has been looked at across different lakes in other regions and shown mixed results. In Lake Oneida in New York, gillnet catches of pelagic fishes were reduced while catches of littoral and benthic fishes were not (Irwin et al. 2011). In Lake Erie, walleye *Sander vitreus*, white bass *Morone chrusops*, yellow perch *Perca flavescencs*, and freshwater drum *Aplodinotus grunniens* populations did not change after the introduction of zebra mussels (Gopalan 2014). However, the populations of gizzard shad *Dorsoma cepedianum* in Lake Erie might have been affected (Gopalan 2014).

Indices of biological integrity are used to help monitor the overall composition of a water body by utilizing multiple community attributes from the water body. They have been used for a wide variety of ecological systems and communities using many different indicators (Karr 1981). FIBIs have been used in Minnesota since the early 2000s and allow agencies to better understand fish communities and their health. Four different FIBIs are currently being used in Minnesota to describe ecological condition in the diverse lakes across Minnesota (Bacigalupi et al. 2021). IBIs can also be used by system managers to identify different stressors potentially harming the lake (MN DNR Fish IBI Program 2018).

New infestation of zebra mussels in freshwater systems can lead to many changes, especially in fish communities and their dynamics (Vanderbush et al. 2021). No published studies have used an FIBI score to look at the impact of zebra mussels on fish communities. The objective of this study is to compare how the infestation of zebra mussels changes fish-based IBI scores and the respective fish communities in certain Minnesota lakes.

I. METHODS

Four different types of traditional fisheries gear were used to obtain FIBI scores for each of the surveyed lakes. Wadeable, nearshore stations were surveyed with seines measuring 15.2 or 4.6 m long by 1.5 m deep with 3 mm bar mesh and backpack electrofishers for the 30.5 m station. Backpack electrofishing was done in two passes, the first pass being completed as close to the shoreline as possible and the second pass being completed in water ~1 m deep. In the case of steep shorelines or heavy aquatic vegetation, boat assisted seines and/or boat assisted backpack electrofishing were completed instead. The number of sites from lake to lake ranged from 10-24 based on the lake size and morphological conditions. Fish captured in nearshore gears were identified to species and counted, with a subset of each specimen being vouchered to be independently verified in a lab setting (Bacigalupi et al. 2021). To sample the littoral area of the lake, double frame 19 mm mesh trap nets were used, with 9-15 locations selected according MNDNR lake survey methods (MNDNR 2017). Standard graduated mesh gill nets (15.2 m long with 1.8 m deep panels of 19, 25, 32, 38, and 51 mm bar mesh) were set to sample the limnetic areas. Gill nets were completed in sets of 6-15, and sites were chosen to represent available habitat in each lake (Bacigalupi et al. 2021; MNDNR 2017). Fish from gill and trap nets were identified to species, weighed to the nearest gram, and measured to the nearest mm.

Lakes were then fit into one of the four fish-based IBIs used by the MN DNR; applied to four different lake groups, and scored appropriately (Drake 2007; Bacigalupi et al. 2021). Group 2 lakes are the deepest of the lakes, spanning a wide range of sizes and locations in Minnesota, having the highest average fish species diversity, and most will thermally stratify. Lakes in group 4 are also deep and often thermally stratify, although they are smaller in size (>200 ha), and are located in the central and northern portions of the state. Group 5 lakes are found most often in central and northern Minnesota and are typically shallow, with a range of sizes and few experiencing partial and infrequent winterkill events. Group 7 lakes are the shallowest, with some experiencing a partial winterkill event at some point (Bacigalupi et al. 2021).

Lakes with completed FIBI surveys were then cross-referenced with the MN DNR infested waters database to find lakes that had both FIBI surveys completed before and after the infestation of zebra mussels (MN DNR 2025). In the case of multiple surveys before a lake was infested, the survey closest to the date was taken to give the most accurate representation of the lake beforehand. For the case of multiple surveys after, the last survey taken was used, to measure the effects of the infestation over the longest possible period. This method resulted in 87 surveyed lakes.

To test for a difference in FIBI score before and after infestation, a paired t-test was used. To test for relationship between change in FIBI score and years since observed infestation, a regression test was used. Variables used in each FIBI model across the four lake groups for score calculation were then plotted using non-metric multidimensional scaling (NMDS) to look for similarities between variables in before and after surveys of the lakes.

III. Results

Survey scores from the 87 lakes increased an average of 5 points (SD = 10.14) after an observed infestation of zebra mussels (Figure 1; $P < 0.01$). FIBI survey scores ranged from 11.00 to 89.00 before infestation and 18.00 to 81.00 after infestation. Statewide, lakes had an average score of 49.19 before infestation and 53.70 after.

Regression analysis showed no significant relationship was observed in the change in last FIBI score compared to the time since infestation of the zebra mussels ($P = 0.68$; Figure 2).

Trends in NMDS modeling based off 95% confidence ellipses were used for each lake group and identified that lake group 2 did not have any specific variable from FIBI calculation that impacted scores more significantly than another. Lake groups 4, 5, and 7 also showed to this same trend, however more lakes would need to be sampled to confirm this (Figure 3).

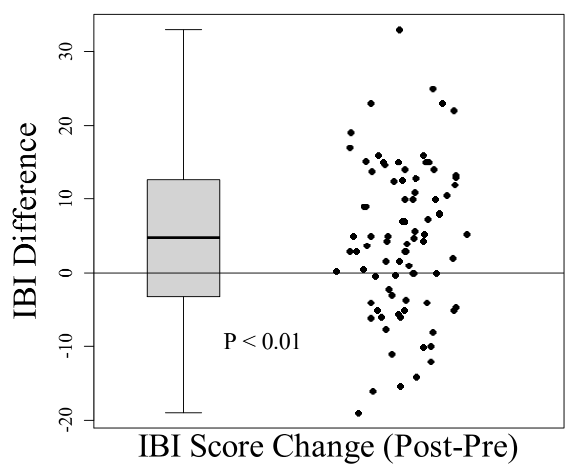

Fig. 1. The change in FIBI score (Score after-score before) for each lake with corresponding box and whisker plot. The rectangle of the box and whisker plot represent the 25th and 75th percentiles, bars are 5th and 95th percentiles and horizontal midline is median.

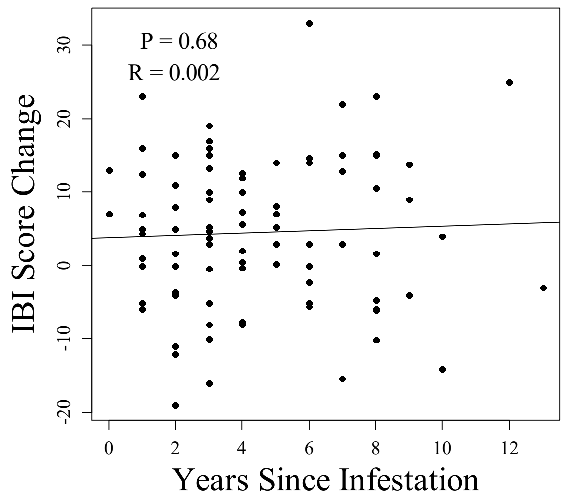

Fig. 2. Relationship of change in IBI score and years since infestation.

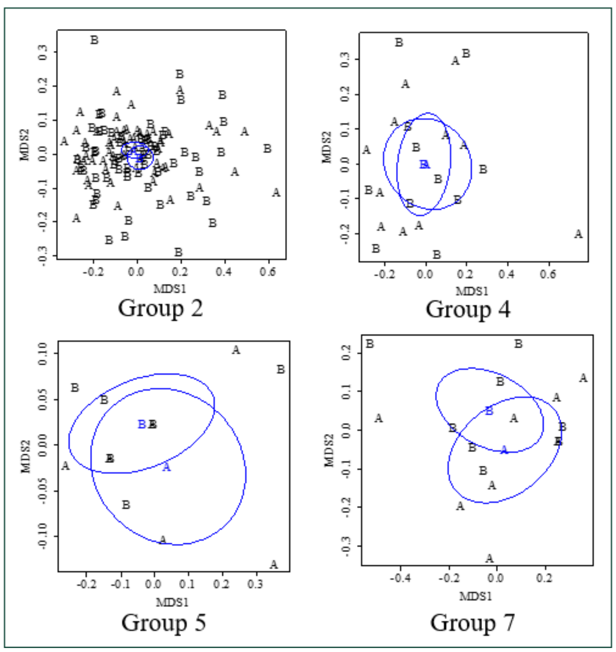

Fig. 3. Total number metrics compared against each other to see if one metric is affecting the FIBI score more. B represents lakes score before and A represents a score after. Ellipses represent 95% confidence.

IV. Discussion

Overall, when zebra mussels were introduced, a change in FIBI score was seen, yet we do not know why this change happened

Besides zebra mussels, there are quite possibly other variables that also contribute to the rise in FIBI scores that are not included in the calculation of scores. No stressor variables such as lake eutrophication, riparian shoreline development, aquatic plant removal, etc. are included in the calculation of an FIBI score (Bacigalupi et al. 2021). However, the premise of an FIBI is that it responds predictively to a stressor gradient. Changes in FIBI score over time could be explained from stressors not associated with the impacts of zebra mussel infestation.

Water quality across the state of Minnesota has generally stabilized in the past 20 years along with over 75% of lakes meeting water quality standards (Minnesota Pollution Control Agency, unpublished data). This trend of stability can decrease the stress on fish and allow them to thrive better (Adams 2003). Additionally, lake groups 2 and 4 make up most of our survey lakes (73 of our 87 lakes). These lakes are much deeper than lakes in group 5 and 7, leading to less nutrient accumulation and higher oxygen levels (Kramer 1987), leading to better habitat conditions for most fish (insectivores, plant dwellers, small benthic dwellers; Kramer 1987).

Although no increase was seen in any specific metric in this study, other studies have found specific populations of fish to benefit from or be damaged from the presence of zebra mussels. Populations of muskellunge *Esox masquinongy*, smallmouth bass *Micropterus dolomieu*, other centrarchids, and yellow perch have benefited from the presence of zebra mussels (Vanderploeg et al. 2002). Yellow perch and centrarchids are classified as insectivores (Bacigalupi et al. 2021), potentially benefiting from the increase of different invertebrate populations that also increase when zebra mussels are present (Vanderbush et al. 2021). Additionally, muskellunge and smallmouth bass are both classified as intolerant species according to FIBI classifications (Bacigalupi et al. 2021), potentially increasing FIBI scores when populations increase. Fish species such as walleye were shown to decrease with the presence of zebra mussels, potentially altering FIBI scores as well.

The ecological response of fish communities across Minnesota lakes to zebra mussels is largely site and species specific. Lakes did show a general increase after an infestation of zebra mussels, although it was widely variable (SD = 10.14). Besides zebra mussels, it is quite possible that other factors are influencing the health of fish communities more (MN DNR Fish IBI Program 2018; Adams et al. 2003).

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NAVIGATING WASTEWATER: MINNESOTA MINING WASTEWATER PERMIT CHALLENGES AND EVOLVING COMPLIANCE STANDARDS

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I. INTRODUCTION

February of 2021 marked the beginning of Minnesota clearly establishing that groundwater qualifies as a Class 1 water in a legal battle involving United States Steel, the Minnesota Pollution Control Agency, WaterLegacy, Fond du Lac Band of Lake Superior Chippewa, the Minnesota Court of Appeals, and eventually the Minnesota Supreme Court (MNSC). Minnesota has a water classification system, as required by the federal Clean Water Act, with 7 classes of use. Class 1 waters are waters used for domestic consumption (MPCA 4, n.d.). According to Minnesota Statute § 7050.0220, the acceptable level for sulfate is 250 mg/L in Class 1 waters, the acceptable level for sulfate in wild rice waters is 10 mg/L, and for comparison the acceptable level for mercury, another common mining byproduct pollutant, is 0.2 ng/L in Class 1 waters (Minn. Stat. § 7050.0220, Subp. 3a). The MNSC decision on groundwater being a Class 1 water is significant as it can serve as a precedent for future cases (Minnesota Judicial Branch, n.d.).

Northern Minnesota is home to several iron mines as well as advanced projects for copper, nickel, and platinum that have yet to begin full production (Minnesota Department of Natural Resources, 2016). A major component of mines getting the green light to operate is permitting. Without proper permitting from the state, production can be delayed indefinitely as seen with NewRange Copper Nickel, formerly NorthMet and PolyMet, over the past two decades (State of Minnesota, 2018). Stricter standards for groundwater help to better protect the environment but also cause snags in industry production and jobs for Minnesotans. This leads to questions surrounding the future of pollution permits in Minnesota and their impact on local economies within the state. The purpose of this research is to delve into the MPCA permitting process and history for Minntac mine to attempt to answer the following: Is the current pollutant permit system, managed by the MPCA, effective? Will efficacy or enforceability of future permits be impacted by new stricter groundwater standards?

II. HISTORY

Minnesota's permitting process for water pollutant discharge involves 2 types of permits. The first type is the National Pollutant Discharge Elimination System (NPDES) permit which was established under the Clean Water Act as a federal program. This permit's purpose is to better regulate disposal systems and water treatments that discharge pollutants into surface waters. The State Disposal System (SDS) permit is a Minnesota permit program. This permit's purpose is to regulate discharges to protect groundwater. The table below describes the steps to issuing SDS and NPDES permits in Minnesota (Minnesota Pollution Control Agency 2, n.d.).

One specific pollutant type the permits regulate are sulfates. Sulfates can occur naturally in water and are often found in discharged waters of mines. The Minnesota Department of Health (MDH) advises sulfate levels below 500mg/L for infant formula and warns that folks not acclimated or tolerant to higher levels of sulfates in water may experience diarrhea and dehydration for a few days until tolerance is reached. Animals, particularly young animals, are also susceptible to diarrhea and dehydration leading to death from too much sulfate in drinking water. The MDH states that most of the state has naturally occurring levels of sulfate at or below 250 mg/L, and the southwestern areas of Minnesota typically see higher levels (Minnesota Department of Health, n.d.). Wild rice, which is a cultural, biological, and economical component of Indigenous people's tribes within Minnesota, is also impacted by sulfates. Research done by the University of Minnesota Duluth found that sulfate levels over 50 mg/L significantly declines productivity of plants. Most wild rice studied

TABLE 1. STEPS TO ISSUANCE OF NPDES/SDS PERMITS

Steps	Description
Application	Application completed and submitted to MPCA by permittee with fees.
Review	MPCA conducts technical and compliance reviews, impaired waters and total maximum daily allowed reviews, and effluent limits reviews and recommendations.
Permit and Document Development	All requirements are established for limits, monitoring, and reporting. Compliance schedules and plans to minimize pollutants are established. Standard permit conditions and fact sheets/statement of basis sheets are completed.
Permit Review	Applicant receives a draft of the permit after application review and development by the MPCA for review prior to public notice.
Public Notice	Interested parties may comment, request a meeting, or petition for a contested case hearing. Comments received may lead to permit draft revisions.
Final Issue	Permit is finalized, issued for 5 years (NPDES) or 10 years (SDS), and made effective. Permittees must apply for reissuance prior to the permit's expiration date.

in sulfate levels above 250 mg/L did not survive (Pastor et al., 2016). Minnesota adopted a standard of 10 mg/L for sulfates in wild rice waters in 1973. There are currently around 2400 waters listed in Minnesota for use of wild rice production. The wild rice aspect is an important piece of the Minntac permitting issue as waters from the mine's site impact groundwater that reaches streams, rivers, and lakes designated as wild rice waters (MPCA 3, n.d.).

In 1964 Minntac hit the northern Minnesota mining scene with their plant location in Mountain Iron. Production at the plant subsequently began in October of 1967. They became the largest producer of taconite in North America producing 15 million tons of taconite pellets annually. The plant features an 8700-acre pond for waste materials called a tailings basin (Minnesota Pollution Control Agency, (n.d.). The first NPDES/SDS permit covering the tailings basin was issued in 1987 initially without limits for sulfates but monitoring for the pollutant instead. In 1992 the permits were due for renewal, and the MPCA called for United States Steel (USS), Minntac's parent company, to resolve upcoming non-compliance of sulfate levels in the basin to adhere to new permit regulations. Between 1992 and 2001 USS worked on a variance application for sulfate levels at 3000 mg/L. The Minnesota Pollution Control Agency would only allow a variance if USS reduced current sulfate levels to prove forward moving progress in reduction. This led to a schedule of compliance being put into place in December of 2001 after nearly 10 years of back and forth regarding non-compliance. The schedule of compliance ended in 2007 with USS needing to submit a permit application within 90 days with new evaluations of seepage, groundwater impact modeling, and a revised water management plan. By 2009 USS submitted a permit application with propositions of a new water treatment system that would reduce pollutant concentration in the basin by half within the following 5 years. In 2012/2013 USS had several meetings surrounding permit reissuing and pollutant levels with the MPCA, EPA, and Minnesota DNR. By 2016 the permits still had not been reissued, only a public draft had been created, with the MPCA denying USS a variance in January of 2017. The final permit from the 2016 draft became effective at the end of 2018 marking 25 years of not having an active permit and working toward renewing the original 1987 permit (Minnesota Pollution Control Agency 6, 2018).

The new permit had 4 short months of use before facing a lawsuit brought forth by Fond du Lac Band of Lake Superior Chippewa and WaterLegacy, a non-profit organization working to maintain wetland health and tribal rights (WaterLegacy, 2023). This appeal filed at the end of 2018 challenged the MPCA for the failure to set pollution limits for the Minntac tailings basin's direct discharge and failure to control seepage affecting downstream waters. In 2019 the Minnesota Court of Appeals issued a decision affirming that groundwater seepage wasn't covered by the clean Water Act. The court also ruled that the direct discharge of the tailings basin did violate the Clean Water Act. WaterLegacy and Fond du Lac Band then made the decision to petition for review of the decision by the Minnesota Supreme Court in 2020 (WaterLegacy, 2023). In February of 2021, the Minnesota Supreme Court agreed to hear the case and decided unanimously that groundwater is a Class 1 water by interpreting the meanings of Minn. R. 7050.0220 subp. 2 and Minn. R. 7050.0221 subp. 1, which are rules describing water classes and groundwater in Minnesota, interpretive history of MPCA previously categorizing groundwater as a Class 1 water in Statements of Need and Reasonableness (SONARs), legislative intent of the MPCA applying Class 1 standards to groundwater through evolution of water quality rules, and deferring to the MPCA for expertise in interpreting technical language (Minnesota Supreme Court, 2021). This means the MPCA correctly applied secondary drinking water standards to USS's Minntac permit in 2018. This standard makes groundwater impacted by the tailings basin subject to the sulfate standard of 250 mg/L. The ruling reverses the Appellate Court's decision that seepage through groundwater to surface water isn't regulated by the Clean Water Act. This case also decided that NPDES permits need to not only comply with the CWA, but they must also comply with regulatory and statutory standards of the state. (Minnesota Supreme Court, 2021)

Part of this decision referred to the 2020 U.S. Supreme Court case of *County of Maui v. Hawai'i Wildlife Fund.* The Supreme Court's decision holds that a permit is required for pollutants through groundwater if they are "the functional equivalent of direct discharge from the point source into navigable waters." (*County of Hawaii v. Hawaii Wildlife Fund*, 2020). Part of the case got into specifics relating to what constitutes "traceable" pollutants and "proximate cause" for groundwater pollution (*County of Hawaii v. Hawaii Wildlife Fund*, 2020). Essentially this ruling establishes that the CWA prohibits polluting into groundwater without a permit. This has bearing in the USS MPCA case as groundwater seepage is covered by the CWA, which was initially ruled otherwise by the Minnesota Court of Appeals.

III. Analysis

Several MPCA monitoring stations are set up throughout the state to collect data and assess discharge in waters. The chart below with data from the MPCA station data access tool shows a few of Minnntac's stations and their pollutant assessments (MPCA 5, n.d.).

TABLE 2. MPCA MONITORING STATIONS AND THEIR POLLUTION ASSESSMENTS

Area and Station ID	Average Sulfate Level in 2020 (mg/L)	Average Mercury Level in 2020 (ng/L)
Tailings Basin Dark River (MN0057207-SD-001)	1092.5	0.607
Tailings Basin Near Sand Lake (MN0057207-SD-006)	943.73	N/A
Mining Area West Two River (MN0052493-SD-004)	464	<0.5-1.3
Tailings Basin Timber Creek (MN0057207-SD-005)	697	N/A
Mining Area Main Pit (MN0052493-SD-001)	419.5	<0.5-0.709

The three stations nearest the tailings basin on the west and northeast sides have higher concentrations of sulfates than stations nearer to the plant itself such as the West Two River station and the Main Pit station (MPCA 5, n.d.). This is consistent with findings and evaluations during the permitting process where the MPCA requested that Minntac reduce current sulfate levels coming from the tailings basin specifically to obtain a variance for further operating. As of 2020 when all 5 stations listed above were last assessed within the same calendar year, none of the stations are in compliance with sulfate levels below 250 mg/L for groundwater or 10 mg/L for wild rice waters.

The NPDES/SDS permit issued in 2018, prior to the MN Court of Appeals ruling and later Minnesota Supreme Court ruling, set the sulfate limit to 250 mg/L for groundwater surrounding the plant by 2025 and a reduction to 357 mg/L in the tailings basin itself by 2028 (Minnesota Court of Appeals, 2019). With the MNSC ruling, Minntac will be held to the sulfate limits upon reissuing of the corrected permit from the MPCA. It is unclear what the timeline will look like at this point as 2024 is presently coming to a close. The final permit is awaiting a contested case hearing regarding whether USS will be granted a groundwater standard variance at the Court of Appeals level. The MPCA will also have to perform analyses of groundwater discharges at Minntac, to comply with standards for groundwater discharge set by the Supreme Court in the Maui decision, prior to final permit issuance (Minnesota Supreme Court, 2021).

IV. Discussion

The last permit issued for the plant in 1987 didn't consider the possibility of seepage discharges to groundwater later impacting surface water. The 2014 draft of a new permit neither provided a date regarding when compliance for standards would be met nor a plan for achieving compliance, as stated by the EPA (Pierard & US EPA, 2014). In a comment from the EPA regarding the revised permit draft in 2016, the organization noted that the CWA requires authorization from an NPDES permit for discharges to surface waters coming from the tailings basin. At the time of this document, the tailings basin was considered point source discharge to both Sand and Dark River watersheds (Pierard & US EPA, 2016). US Steel was aware of these potential bumps in the road for the permit before its approval in 2018 and following court cases. The company continued to kick the can down the road on compliance until they were backed into a corner forcing legal action. The standards weren't being met in the 1990's when the original permit expired, and current standards for the permit awaiting reissue aren't being met today (MPCA 5, n.d.). The classification of groundwater as a Class 1 water does increase the difficulty of meeting standards, but this isn't a new issue. This is the same issue from 30 years ago eventually making its way to Minnesota's highest court due to somewhat ineffective permitting practices. If Minntac hasn't been able to meet compliance with looser guidelines, I'm inclined to believe it is unlikely to change with more stringent limits.

The MPCA encountered another permitting blunder in 2023 with the Minnesota Supreme Court ruling that they issued a permit to the NewRange Copper Nickel mine, formerly PolyMet/ NorthMet, arbitrarily with knowledge of potential pollution dangers. Permitting was remanded by the courts back to the MPCA to decide if a variance for groundwater pollution would be appropriate given the situation. The mine was proposing a wastewater treatment system that would treat the water from the tailings basin then allow that water to discharge to surface waters, a situation similar to the USS case. The 2021 U.S. Steel permit reissuance decision by the MNSC was cited to explain that while NPDES permits must comply with the CWA, they must also comply with regulatory and statutory standards of the state (Minnesota Supreme Court, 2021).

V. Conclusion

The CWA has strict regulations that the EPA works to enforce, but when regulations are particularly strict with steep penalties for violations, we see defunct permits and variances running the show. Minntac is an incredible economic asset to northern Minnesota and the state with over 1400 employees, annual payroll around $153 million, and more than $45 million paid annually in taxes on production alone (U. S. Steel, n.d.). However, this economic asset is impacting the environment, and neither the state nor federal agencies involved have come up with a legitimate solution to ensure pollution compliance and pollutant reduction for the future. Allowing a business this large to operate without proper permits on variances for 30 years seems lax. The company was allowed to begin operation and production under specific environmental regulations, but there hasn't been active continued enforcement on said regulations. 1967 was a different era of environmental concerns, understandably. As the plant continued production, and environmental concerns continued to evolve, there were opportunities to address and enforce appropriate regulations. 1984 was the first year of regularly testing sulfate levels at the tailings basin, yet here we are in 2024 with no clear plan for remediation (MPCA 6, 2018).

Minnesota's own House of Representatives and Senate have had significant forward moving environmental progress, especially with the Clean Water Fund. 33% of tax revenue from sales and use tax goes into the fund. The fund contributes to our ability to have water monitoring, scientific studies, and restoration and protection activities. The Amendment was initially passed in 2008 and will continue through 2034. $1.8 billion has been appropriated to the fund from 2010-2025. For the fiscal year of 2024-2025, the MPCA received $48.375 million to do state and federally required water quality protection. Water monitoring itself received $18.1 million, and TMDL development received $12.7 million (MPCA 7, 2024). In the 2024 performance report, the MPCA states that there are more requests for water quality projects than there are funds available for said projects. Lack of funds is also stated as the reason the numbers of previously impaired waters now meeting standards is considered not meeting targets as determined by the agency (MPCA 7, 2024).

We have existing projects still not meeting targets with a continued influx of new projects ever arising. If we don't have funding to remediate existing water issues, we need to do a better job of ensuring compatibility with compliance for any new industries looking to be granted NPDES/SDS permits. Groundwater being a Class 1 water for permitting purposes requires additional reviewing of future permits for compliance's sake. This could mean fewer industries are granted permits, more industries are granted permits but deal with steep penalties, or more industries could operate on 30-year variances as seen with Minntac. Minntac's scenario of operating on a lengthy variance isn't unheard of. Keetac, an iron mine located in Keewatin also owned and operated by USS, last submitted a permit application to the MPCA in 2009 for air quality that reached the public comment phase in May of 2024 (MPCA 8, n.d.). Northshore Mining located in Babbitt is another example of operating without an active permit as they applied for a permit in 2009 that reached the public comment phase in 2024 as well (MPCA 9, n.d.). Mesabi Nugget mine located in Aurora eventually faced shutdown due to noncompliance with water quality. In 2012 the MPCA proposed issuing Mesabi Nugget a water pollution permit along with variances. The EPA then issued a comment of concern regarding the variances and requested a study for sulfate with monitoring. The MPCA approved the application and variances in December of 2012 despite public opposition and EPA concern. This resulted in a lawsuit where the courts remanded the permit variances back to the EPA in 2014. The variances were then disapproved by the EPA contributing to Mesabi Nugget's eventual closure in 2015 (WaterLegacy, 2020). These examples point to a likelihood of Minntac's lengthy permit process and variances not being outliers for the MPCA's permitting practices.

We need some level of accountability within this system to make forward progress on environmental quality. If the penalties are too steep to implement resulting in many industries being granted variances, we need to address and change that. If it's a matter of legitimate financial hardship for existing industries to adopt technologies to meet compliance, we need reachable targets for pollutants with moderate enough penalties to not entirely discourage economic growth in areas relying on said industries. If the scenario is that new industries must wait years to begin production to ensure proper care of the environment, that might be a hard decision Minnesota has to make. We need industries that promote responsible use of our natural resources along with policies and procedures that guide us in that direction. Industry within Minnesota shouldn't be an economic versus environment battle. We need to find better ways to build symbiotic relationships between the environment and economy of our state.

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THE RELATIONSHIP BETWEEN PLANT DENSITY AND MICROPLASTIC CONCENTRATION IN THE WATER IN LAKE BEMIDJI, MN

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***Abstract*—Microplastics, plastic particles smaller than 5 mm, are an increasing environmental threat, particularly due to their widespread distribution and toxicity. While much research has focused on larger freshwater and marine ecosystems, the presence and impacts of microplastics in smaller inland lakes remain less understood. This study investigates the relationship between plant density and microplastic concentrations in Lake Bemidji, a smaller freshwater lake. A total of 30 water samples were collected across varying vegetation conditions, ranging from open water (0%) to 90% plant coverage. Microplastics were identified and categorized into six morphological types: fibers, fragments, films, spheres/beads, and foam. The samples contained a total of 2,790 microplastic particles, with an average of 93 particles per sample and an average concentration of 8.16 microplastics per liter (SD = 3.06). The most abundant colors were blue (2,204 particles) and black (460 particles), while red and green were the least common. A regression analysis was conducted to directly test the relationship between the total count of microplastics and plant density, and there was a significant negative relationship between microplastic count and plant density ($P < 0.01$). The findings suggest that plant density does influence the distribution and deposition of microplastics in freshwater systems, with higher concentrations observed in areas with lower plant coverage.**

I. INTRODUCTION

Microplastics, plastic particles smaller than 5 mm, are becoming a looming environmental crisis, with their pervasive spread and toxicity posing a growing threat to ecosystems around the world. As Conowall et al. (2023) emphasize, microplastics are a significant concern because of their alarming global presence and the dangerous toxicity they introduce into the environment, furthering this growing environmental crisis. This problem has been worsened by the surge in plastic production, which surpassed 350 million tons as of 2019 (Negrete Velasco et al. 2020). The uncontrolled manufacturing and consumption of plastics, combined with their resistance to degradation, have resulted in their accumulation within the environment (Negrete Velasco et al. 2020).

While much of the research on microplastic pollution has focused on marine environments and large freshwater systems, there is a growing need to examine smaller, inland lakes like Lake Bemidji. One factor that may influence these microplastic concentrations in these ecosystems is plant density. Vegetation in aquatic systems can impact water flow, sedimentation, and particle retention, which may affect how microplastics are transported and deposited within lakes. This shift in research focus reflects the broader trend in science, where initial studies on marine plastic debris dating back to the around the 1970s eventually expanded as the ecological impact of plastic became more apparent. Once unnoticed, the accumulation of microplastics is now recognized as a major environmental concern across marine and freshwater systems (Andrady 2011).

Despite the importance of plant density in shaping the dynamics of microplastic pollution, limited research exists on how this factor influences microplastic concentrations in smaller freshwater lakes. This study aims to address this gap by investigating the relationship between plant density and the concentration of microplastics in the water of Lake Bemidji. By examining this relationship, the study seeks to enhance our understanding of the environmental factors that contribute to microplastic pollution and to inform future efforts to mitigate its impacts in freshwater ecosystems.

II. METHODS

All water samples were collected from Lake Bemidji on the west side, just north of Cameron Park (47°30.2231'N 94°52.1179'W), on 8 October 2024. A total of 30 samples were taken across varying vegetation conditions to assess the impact of plant density on microplastic concentrations. Plant density was recorded put into categories: these plant densities were 0%, 5%, 10%, 25%, 50%, and 90%. Plant density was measured using a 0.5 m^2 quadrat, with the percentage of emergent and floating vegetation within the quadrat being visually estimated. Plant density values for the samples ranged from 0–90% coverage.

Water samples were collected using a 11.4 L stainless-steel stock pot, submerged to a depth of approximately 0.5 m. To avoid contamination, the stock pot was rinsed with distilled water before each use. After collection, the contents of the pot were poured through a 106 µm stainless-steel testing sieve to filter out larger debris. The material collected in the sieve was back washed with distilled water into pre-decontaminated glass mason jars. An approximate 12% hydrogen peroxide solution was added to each jar to break down organic material, and the jars were left to sit for at least one week.

After the organic material had been allowed to be broken down, the contents of each jar were examined under a dissecting microscope, in a glass dish, at 1-4x magnification. White paper was placed under the dish to help identify colored microplastics. Suspected microplastics underwent a stress test with a sterilized dissecting needle (with a wooden handle) to confirm their identity. Plastic particles were confirmed if they did not crumble or break under the pressure of the needle. Identified microplastics were separated into six morphological categories: fiber, fragment, film, sphere/bead, and foam. In addition, the color of each microplastic particle was recorded. A regression analysis was run to directly test for a relationship between the log-transformed total count of microplastics and plant density.

III. Results

Data was collected from Lake Bemidji across six varying plant density levels: 0%, 5%, 10%, 25%, 50%, and 90% plant coverage. The total microplastic count for each density level is summarized in Table 1, along with the average number of microplastics (ANMP) per sample and the microplastic concentration (MPC) per liter.

TABLE 1: TOTAL MICROPLASTIC COUNT, AVERAGE NUMBER OF MICROPLASTICS (ANMP), AND MICROPLASTIC CONCENTRATION (MPC), FOR EACH PLANT DENSITY LEVEL. COLLECTED ON 8 OCTOBER 2024 ON WEST SHORE LAKE BEMIDJI.

Plant Density (%)	Total # of plastics	ANMP (particles/per sample)	MPC (particles/ per liter)
0	1362	136.20	11.95
5	629	104.83	9.20
10	472	98.25	8.62
25	231	77.00	6.75
50	99	33.00	2.89
90	76	19.00	1.67

TABLE 2. MICROPLASTIC MORPHOLOGY DISTRIBUTION IN LAKE BEMIDJI SAMPLES. PERCENTAGE AND TOTAL COUNT OF EACH SHAPE ARE SHOWN. COLLECTED ON 8 OCTOBER ON WEST SHORE LAKE BEMIDJI.

Microplastic Morphology	Total # of Plastics	Morphology Distribution (%)
Fiber	289	10.36
Fragment	2420	86.77
Film	32	1.15
Sphere/Bead	47	1.68
Foam	1	0.04

TABLE 3. MICROPLASTIC COLOR DISTRIBUTION IN LAKE BEMIDJI SAMPLES. PERCENTAGE AND TOTAL COUNT OF EACH COLOR TYPE ARE SHOWN. COLLECTED ON 8 OCTOBER ON WEST SHORE LAKE BEMIDJI.

Color	Total # of Plastics	Color Distribution (%)
Blue	2204	79.00
Black	460	16.49
Clear/White	88	3.15
Magenta	34	1.22
Red	2	0.07
Green	2	0.07

As plant density increased, both the total number and the concentration of microplastics in the samples decreased. At 0% plant coverage, the average microplastic count per sample was 136.20 particles, and the microplastic concentration was 11.95 particles per liter (Table 1). In contrast, at 90% plant coverage, the average count per sample dropped to 19.00, with a concentration of only 1.67 particles per liter. The breakdown of microplastic types demonstrated that most particles were fragments, making up 86.77% of the total microplastics (Table 2). Fibers accounted for 10.36%, followed by films (1.15%), spheres/beads (1.68%), and foams (0.04%). In terms of color distribution, blue microplastics dominated the sample, comprising 79.00% of the total microplastic particles (Table 3). Black microplastics were the second most abundant at 16.49%, while clear/white particles made up 3.15%. Magenta, red, and green particles were found in trace amounts, representing 1.22%, 0.07%, and 0.07%, respectively.

A regression analysis was conducted on the log-transformed total count of microplastics to directly test the relationship with plant density, and there was a significant negative relationship between microplastic count and plant density with a p-value < 0.01, suggesting a strong inverse correlation between plant density and microplastic concentration in the lake (Figure 1).

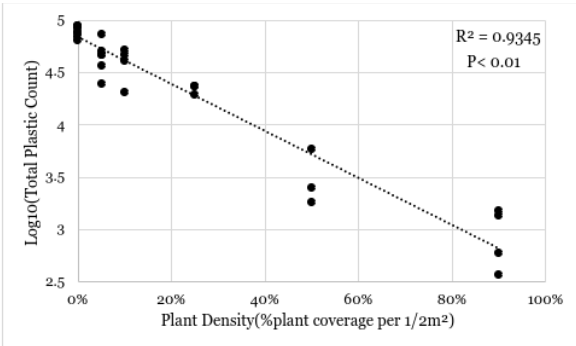

Fig. 1. Log-transformed total plastic count data from the regression analysis for all of the samples in Lake Bemidji, MN (8 October 2024).

IV. Discussion

The primary finding of this study is the strong inverse relationship between plant density and microplastic concentrations in Lake Bemidji, as higher vegetation coverage was associated with lower concentrations of microplastics. This result aligns with findings by Helcoski et al. (2020), who reported higher vegetation cover and stem density in wetlands reduced microplastic retention, in an urban tidal freshwater wetland in Washington DC, USA. A potential explanation for this pattern is that dense aquatic vegetation acts as a natural buffer, reducing water flow and trapping microplastics in sediments or plant structures before they can remain suspended in the water column. Inversely, in open water with little or no vegetation, microplastics are likely to remain suspended due to higher water flow and turbulence, leading to higher concentrations.

An additional finding is that microplastic fragments dominate the samples from Lake Bemidji, accounting for 86.77% of the total particles, followed by fibers at 10.36%. This distribution contrasts with global trends, where Yang et al. (2022) report fibers are the most common morphology in Asian lake waters (64.5%) and sediments (93.5%), while fragments dominate European lakes (68.8% in water and 29.9% in sediments). In the Americas, fibers are the primary morphology in water (56.2%), but sediments show a more balanced distribution between fibers (37.0%), fragments (31.8%), and foams (29.9%). African lakes, in contrast, are dominated by pellets in both water (75.7%) and sediments (70.3%), likely reflecting specific industrial inputs. Lake Bemidji's predominance of fragments, alongside the limited presence of films, foams, and pellets, highlights regional differences in plastic use, degradation, and hydrodynamic conditions offering valuable insights into the localized sources and pathways of microplastics into freshwater systems.

An important note in this study is the appearance of blue, black, and clear/white microplastics as the most common colors in the samples. This color distribution mirrors findings by Conowall et al. (2023), who observed a similar prevalence of these colors in microplastic samples from four inland lakes in Minnesota. The dominance of blue and black microplastics may be linked to their frequent use in consumer products, such as textiles and packaging materials, which are common sources of microplastic pollution. Clear/white microplastics may represent degraded or weathered plastics that have lost pigmentation over time. The trace presence of other colors, like magenta, red, and green, suggests limited contributions from specific or localized sources of these hues, such as fishing lines or plastic toys.

In summary, the study highlights the critical role of plant density in reducing microplastic concentrations, underscores the dominance of fragments as the primary microplastic type, and reveals a distinct color distribution pattern with blue and black microplastics as the most common. These findings enhance our understanding of the environmental dynamics of microplastics in smaller freshwater systems and provide valuable insights for targeted mitigation efforts in Lake Bemidji and similar ecosystems.

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RELATIONSHIP OF UTRICLE AND TRAP DOOR SIZE ON COMMON BLADDERWORTS *UTRICULARIA MACRORHIZA*

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***Abstract*—The common bladderwort *Utricularia macrorhiza* is a carnivorous aquatic plant that captures organisms with its bladders. This study was aimed at setting the stage for future research which might determine if *U. macrorhiza* could be a biological method of control for invasive species. The objective of this study was to determine the relationship between utricle and trap door sizes and to gather data on the size ranges of these two morphological features. A regression analysis was used to obtain results which showed there was enough evidence to suggest that the size of bladderwort trap doors have a significant, positive relationship with the size of its utricles ($P < 0.01$). Additionally, the size of utricles ranged from 2.00 - 3.87 mm, and the size of utricle trap doors ranged from 0.53 - 1.47 mm. When looking at future samples of *U. macrorhiza*, the relationship between the two morphological features can be used to easily estimate trap door size which is a time-consuming measurement to obtain. Knowing trap door size is important when determining if the common bladderwort might consume an invasive species, such as the spiny water flea *Bythotrephes longimanus*, or if the trap is limited by the size of the invasive organism.**

I. Introduction

The carnivorous aquatic plant *Utricularia macrorhiza*, also known as the common bladderwort, belongs to the lentibulariaceae family. The common bladderwort is one of 250 carnivorous plants belonging to the *Utricularia* genus. Only about 20% of *Utricularia* species are aquatic. (Miranda et at. 2021). The bladders, or traps, on these plants are often referred to as utricles and are what make this species distinct as the traps are how the bladderwort obtains its prey. To catch its food, the utricles first actively pump water out of themselves. Then, trap doors on the utricles are activated when trigger hairs on the outside are stimulated by prey. This sets off a suctioning mechanism used to acquire the bladderwort's food (Castaldi et al. 2023). Once prey is trapped inside the utricle, hydrolytic enzymes are used to digest the organism. The utricles of *Utricularia* species have also been shown to spontaneously be set off which incidentally can result in the apprehension of prey. The diet of *Utricularia* species has been shown to consist of micro-crustaceans, nematodes, rotifers, and insect larva along with many other organisms (Miranda et at. 2021).

The goal of this study is to set the stage for further research on common bladderworts which may help determine whether they are a plausible method of control for invasive species. A study by Couret et al. (2020) was conducted to test whether or not *U. macrorhiza* could be a potential solution to regulate mosquitos. This was done by evaluating the proficiency of the plant's consumption of mosquito larva from two different species, *Aedes aegypti* and *Aedes albopictus*. The study showed that the common bladderwort had a high predation efficiency on the larva, and thus should be explored as an option for biological control of mosquitos. This study introduces the idea that the common bladderwort might be used as a means to control unwanted species. Something to consider is whether the desired species to be controlled would be a potential choice of prey. A study done by Harms & Johannson (2000) demonstrated that the common bladderwort exhibited a preference for cyclopoid copepod *Eucyclops serrulatus* over cladocerans *Polyphemus pediculus*, suggesting that *U. macrorhiza* actively selects for its prey. Another study (Ceschin et al. 2021), highlights that the feeding strategy of *U. australis* is highly dependent upon prey availability and size. An invasive species will more than likely be in abundance; therefore, an important factor to look at in future studies would be size.

This being said, the size of utricles and trap door openings on *U. macrorhiza* should further be studied to help rule out whether an invasive species that scientists desire to control would be actively selected for. This study can be used as a basis for future research which might look at the characteristics of invasive species to determine if the common bladderwort would be able to consume them.

Furthermore, the relationship between utricle and trap door size may be useful when looking at bladderworts in other water systems or when they are at different stages in their life cycle. These factors may have an influence on trap door size which is a difficult measurement to obtain. Knowing the relationship between the utricle and the trap door would make it simple to estimate trap door size since utricles can easily be measured. The objective of this study is to determine the relationship between utricle size and trap door size and to gather data on the size ranges of these two morphological features.

II. Methods

Samples of *U. macrorhiza* were pulled from two locations on Lake Bemidji using a rake with a rope attached to the end. One area of sampling was conducted on the lake side of the bridge near the inlet where the Mississippi flows into Lake Bemidji. The other area of sampling was conducted in the outlet where Lake Bemidji flows into Lake Irvine near the wastewater treatment plant. Three plants were selected from the samples taken at each location, and five utricles were carefully pulled off the plant at random. The traps from each location were placed into a plastic sample container to which 95% ethanol was added to preserve the bladders.

The bladderwort samples were processed by placing them under a compound microscope. Each of the 30 utricles were observed under the lowest power objective lens of 4x. A microscope with lenses containing a scale was chosen so measurements could be made. Using a calibration slide, a conversion factor was created to convert all measurements into millimeters. The size of the utricle while laying on its side was recorded to the hundredth place using the scale within the microscope, and the same was done for the trap door when the utricle was held upright in order to see the opening. To find out if there was a significant relationship between utricle and trap door size, a regression analysis was used.

III. Results

The size of utricles ranged from 2.00 - 3.87 mm, and the size of utricle trap doors ranged from 0.53 - 1.47 mm. There is enough evidence to suggest that the size of common bladderwort trap doors have a significant, positive relationship with the size of the utricles (Figure 1; $P < 0.01$).

IV. Discussion

In this study, it was found that the size of the utricles on *U. macrorhiza* were directly correlated to the size of the openings on the traps. The two features had a positive, linear relationship, meaning as utricle size increased, trap door size increased. Numerous other studies of organisms have shown similar findings when looking at relationships between morphological structures. At times, certain physical characteristics are loosely, yet not significantly, correlated. Nevertheless, there are many studies that have found significant relationships between the sizes of morphological traits. The relationship between a plant's features can be useful for scientists in many ways. For example, several taxonomic keys use relationships between important characteristics, like petiole and leaf length, as ways to identify plants.

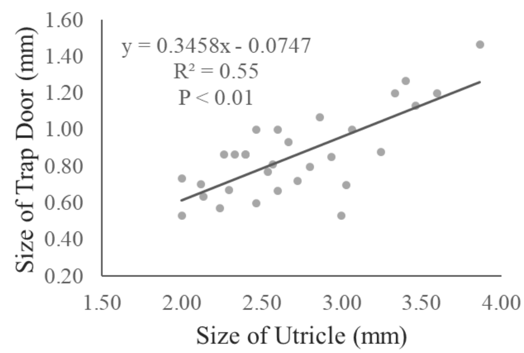

Fig. 1. Measurements of 30 utricles and their trap doors from collected *U. macrorhiza* samples represented in a scatterplot with a positive, linear trendline ($P < 0.01$).

The findings of this study are significant because in future studies, the size of trap door openings on the common bladderwort can easily be estimated. This would be important to know when determining if certain invasive species might be subject to consumption by the carnivorous plant. The feeding of *U. macrorhiza* is largely influenced by the size of its prey (Ceschin et al. 2021). This limits the selection of organisms that bladderworts may feed upon which becomes of interest when considering if the common bladderwort could be a method of control for spiny water fleas, *Bythotrephes longimanus*.

The spiny water flea is a particularly large planktonic crustacean that creates detrimental effects on zooplankton communities. It is a sight predator and surfaces at night to feed on zooplankton smaller than itself. The disruption of native zooplankton communities can then negatively affect native fish populations. Spiny water fleas are characterized by the barbs on their singular long tail. This tail may have one to four barbs, and they make it difficult for small fish that feed on zooplankton to consume the water fleas (Noreen et al. 2013). Spiny water flea eggs are resistant to drying and freezing, and they may pass through the digestive tract of fish (Kerfoot et al. 2011).

In future studies, the size of spiny water fleas may be looked at to determine whether they would be able to enter and fit into the utricles of *U. macrorhiza*. Similar to the study conducted to test if common bladderworts could be a biological method of control for mosquito species, the larva of spiny water flea would most likely be looked at. This is due to the fact that adults may reach up to 12 mm (Noreen et al. 2013), which is much larger than the size of *U. macrorhiza* utricles. Since this study has already obtained data on utricle and trap door opening size, it should be easy to determine if size is or isn't a limiting factor when considering if bladderworts could be a method of control for spiny water fleas.

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EFFECTS OF ROUND-UP ON THE ENVIRONMENT

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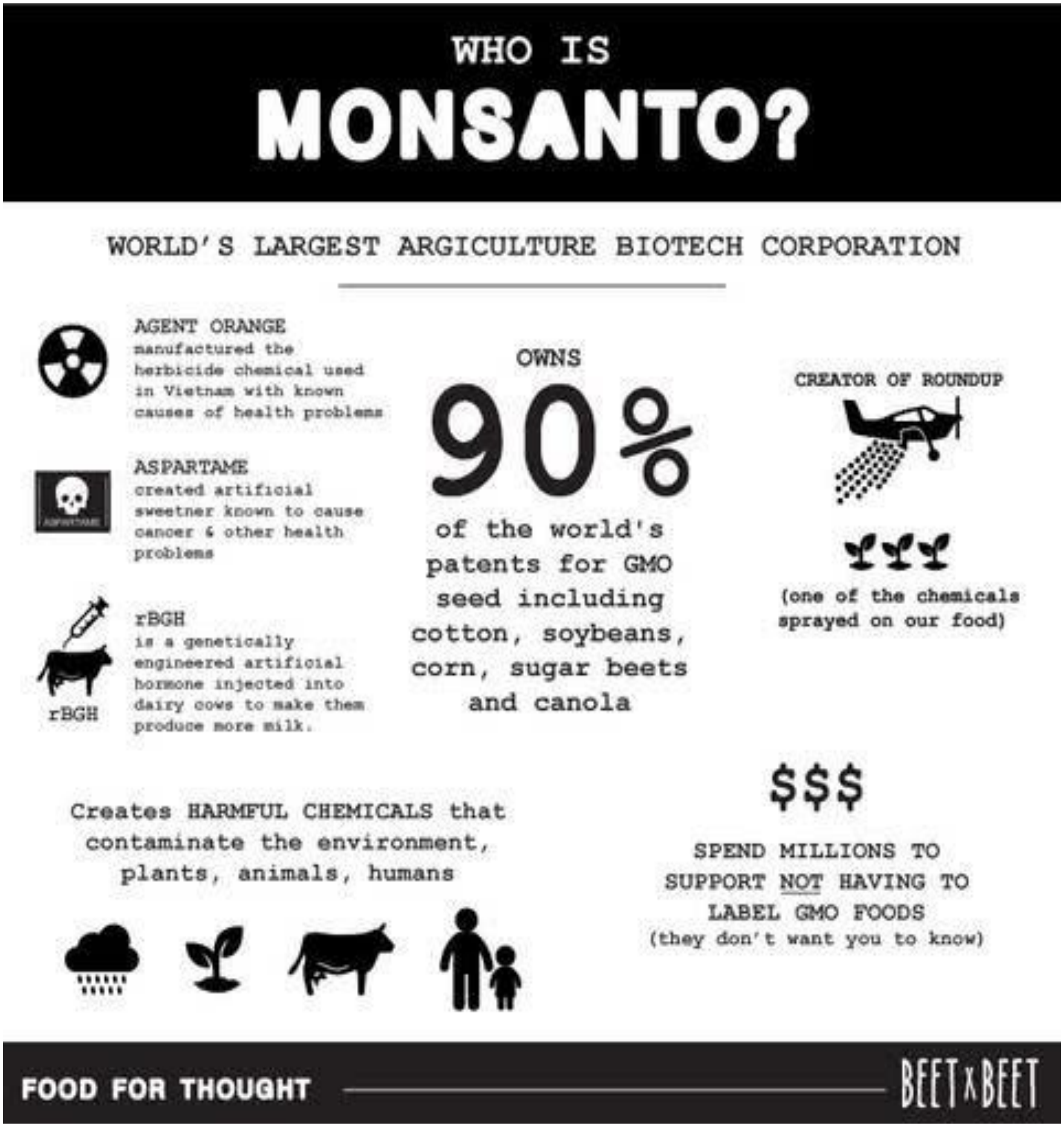
Pulse, S. (2018)

I. Introduction

Many people around the world have used and still currently use Roundup but are unaware of the effects it has on the environment. Roundup is a spray on application weedkiller that is widely used around the world today both residentially and commercially. It enables its user to grow a garden or a field of crops with a no-tilling approach to eliminate weeds. It is a well-known and popular choice for killing weeds that has been around since the mid 1970's (Oca, 2017). John Franz, a Monsanto scientist discovered that glyphosate (main ingredient in roundup) was an herbicide or weedkiller, and it began selling on the shelves to the public in 1974 (Oca, 2017). The U.S. Environmental Protection Agency, at that time, classified glyphosate as a Group C chemical (aka a possible human carcinogen), based on the presence of kidney tumors in male mice (Staff, 2020). Glyphosate has applications in agriculture, forestry, industrial weed control, lawn, garden, and aquatic environments (Jennings & Li, 2017). It is used on corn, wheat, sorghum, citrus, stone fruits, potatoes, onions, asparagus, coffee, peanuts, and pineapple (Jennings & Li, 2017). Nonagricultural uses include ornamental plants, turf, forests, rights-of-way, and weed control in ponds, reservoirs, waterfowl sanctuaries, and waterways (Jennings & Li, 2017).

Upon spraying the weeds with Roundup, glyphosate enters the plants through their leaves and travels around the plants inner system until it reaches the crown and the roots (Roundup, n.d.). Once Roundup penetrates the leaves it prevents the plant from creating the much-needed protein for cell division. One downside to Roundup is that it is non-selective and will kill a wide range of plants that it encounters including crops in agricultural settings (RoundUp, n.d.). For this reason, a higher emphasis was placed on creating genetically modified organisms (GMO) seeds that were glyphosate resistant, also known as Roundup ready crops. The introduction of these Roundup Ready crops created easy application for the farmers in the agricultural industry, simply spray and plant. Farmers spray Roundup usually before seeding, after harvesting; and sometimes other crops require in between spraying such as canola or soybeans (Jake, 2016).

The Monsanto Corporation is the brains behind Roundup and the above visual marks important chemicals (along with the side effects of them) that Monsanto developed aside from Roundup such as: Agent Orange (herbicide used in Vietnam), Aspartame (artificial sweetener), and rBGH (artificial hormone). In the center, it highlights that Monsanto owns 90% of the world's patents for GMO seeds including cotton, soybeans, corn, sugar beets, and canola which means it has a heavy influence in controlling the crops around the world. In addition, it states that it is the world's largest agricultural biotech corporation. The visuals to the bottom left illustrate that all these chemicals are harmful to the environment, plants, animals, and humans. Lastly, another important statement on the bottom right corner points out that Monsanto would rather pay millions of dollars to avoid labeling all the foods that are truly made from GMO products rather than simply paying less just to include them on all food products that contain them. This visual was included to show how much the Monsanto corporation controls many important products that are sold daily around the world and the consequences of their chemical products.



(Classaction.com, 2016)

There has been much controversy about the Roundup product and its true effects on the environment and humans because of misleading advertising and warnings being left out on the labeling of its products. From the very beginning the Monsanto company advertised Roundup as completely safe with no effects to the environment or humans (Roundup Legal Center, n.d.). However, years later many studies were done on animals, soil, and even humans that provided legitimate reasons to question Monsanto's claims about Roundup (Roundup Legal Center, n.d.). What's more, the Monsanto company had a prior history of creating toxic products since they started operations (Roundup Legal Center, n.d.).

Saccharin, their first chemical product, is an artificial sweetener that was used in the early days of Coca-Cola (Roundup Legal Center, n.d.). Monsanto was sued by the government to halt production of saccharin due to it being poisonous (Roundup Legal Center, n.d.). Saccharin was found to negatively affect the balance of bacteria in the gut, which may lead to type 2 diabetes, obesity, and cancer (Meeks, 2021). It is still used in products that are sold on the shelf today such as:

- bakery products
- candy
- chewing gum
- deserts
- jelly
- salad dressings
- sweet & low

(Meeks, 2021)

However, this lawsuit failed and allowed the Monsanto company to continue producing toxic chemicals (Roundup Legal Center, n.d.).

In the 1920s through the 1940s the company worked on industrial chemicals and drugs, working with the U.S government and producing seeds for agricultural use. Monsanto also began producing many household cleaners, plastics, and began developing pesticides (Roundup Legal Center, n.d.). However, these newly developed agricultural pesticides contained dioxins (Roundup Legal Center, n.d.). Dioxins are chemical compounds that are toxic pollutants caused by industrial processes. The wide distribution of dioxins in products like pesticides has caused them to become present in food-chains and ecosystems around the globe (Roundup Legal Center, n.d.).

Furthermore, in the 1960's Monsanto helped produce Agent Orange, an herbicidal weapon. This chemical was deployed during the Vietnam war and was known to be deadly and this chemical also produced dioxins (Roundup Legal Center, n.d.). As a result, The Red Cross in Vietnam estimates that over 1 million people suffer from health problems due to exposure to the herbicide. Additionally, U.S veterans who were exposed have higher cases of leukemia, Hodgkin's lymphoma, and other cancers according to the U.S government (Roundup Legal Center, n.d.). Aspartame is yet another chemical produced by Monsanto and is still widely used as a sugar substitute today (Roundup Legal Center, n.d.). Dozens of studies have linked aspartame to serious health problems such as: cancer, cardiovascular disease, Alzheimer's, seizures, strokes, dementia, intestinal dysbiosis, and even mood disorders (Malkan, 2023). The Monsanto Corporation was eventually purchased by the Bayer company in 2016. However, now Bayer has had to deal with the 30,000 plus left-over lawsuits due to harmfulness to humans by means of non-Hodgkin lymphoma (NHL) from prolonged exposure to Roundup, which has taken a toll on Bayers overall stock market value dropping it by 44% as of May 2019 (Roundup Legal Center, n.d.). Now Bayer is suggesting it will discontinue the use of glyphosate in Roundup beginning this year in 2023 but only in its residential products and only in the U.S., it will still sell Roundup (glyphosate) to other countries (*Bayer to Pull Roundup From Shelves in 2023 to Avoid Further Lawsuits*, n.d.). Bayer's main goal with this move is to stop the financial bleeding and they have set aside millions of dollars for the remaining pending lawsuits and any new potential ones.

Now that it is understood what Roundup is, what it does, and the history of the company that stood behind it, let's dig deeper about the effects on the environment, the effects on humans and discuss what alternatives there might be to Roundup.

II. Environmental Effects

There have been many studies done on the effects of Roundup and the harm it has caused to our environment. According to the Ecology Center some of these effects include:

(1) Acute toxicity to fish and birds.
(2) Can kill beneficial insects and soil organisms that maintain ecological balance.
(3) Leaves residues in the soil that can last over one year.
(4) Residues of Roundup chemicals have been found in strawberries, wild blueberries and raspberries, lettuce, carrots, and barley.
(5) Glyphosate (main chemical in Roundup) has been measured 1,300 – 2,600 feet away from its application site.

The biochemical pathway that glyphosate takes to kill a plant itself is not the same biochemical pathway needed to kill an animal and should be nearly harmless to animals for those reasons (Biochemical Toxicology, n.d.). However, there are adhesive chemicals needed in Roundup to aid in glyphosate sticking to a plant called surfactants. The most common surfactants found in glyphosate-based products are called POEA (polyethoxylated amine) and it is known to be more toxic to animals than glyphosate itself (Biochemical Toxicology, n.d.).

	Tissue Damages	DNA damages	Enzyme inhibition	Endocrine disruption	Development disruption causing malformations	Carcinogenesis caused by glyphosate
Animals		X	X	X		X
Fish	X	X	X	X		X
Amphibians	X	X	X	X	X	X
Mammals		X	X	X		X
Humans		X	X	X		X
Invertebrates		X	X	X	X	X
Reptiles	X	X	X	X	X	X

(Ecology Center, n.d.)

There have been 233 published papers from 1979 to the present day that evaluated the toxicology effects of glyphosate in aquatic environments (Biochemical Toxicology, n.d.). Many studies have reported: The toxic effects from glyphosate were studied in various types of organisms such as invertebrates, fish, amphibians, plants, and the aquatic environment (Biochemical Toxicology, n.d.). Acute toxicity tests measure the effects of toxic agents on aquatic species over a short period of time over the life span of the organism while chronic toxicity tests measure the effects of chemicals on species which may cover some or all the life cycle of the organism (Biochemical Toxicology, n.d.). These toxicology tests determine the maximum concentration of herbicide (like Roundup) that can be tolerated in an environment without causing significant damage (Biochemical Toxicology, n.d.). Herbicides are included in a wide range of micro-pollutants that have ecological impacts. For instance, water can be contaminated by runoff from herbicides, but contamination can also occur directly through application or indirectly through exposure of residues to the environment (Biochemical Toxicology, n.d.).

Robert Kremer, a retired research microbiologist with the USDA-Agricultural Research Service and adjunct professor at the University of Missouri, Dobberstein wrote that glyphosate quietly lingers in soil years after it's been sprayed, damaging non-target crops, and suppressing beneficial mycorrhizal fungi, which help plants obtain nutrients from the soil while offering protection against disease (EcoWatch, 2021).

In addition, the herbicide also harms beneficial soil organisms such as small insects and earthworms, while leaving behind chemical residues that wind up in our waterways (EcoWatch, 2021).

Further evidence suggests that species richness was reduced by 22% with Roundup. Roundup completely eliminated two species of tadpoles and nearly exterminated a third species, resulting in a 70% decline in the species richness of tadpoles (Relyea, 2005). Furthermore, most glyphosate-based herbicides are not approved for use in the aquatic environment, but measurable quantities of the active ingredient and surfactants are detected in surface waters, giving them the potential to alter the physiology of aquatic organisms (Annett et al., 2014).

Reports show that glyphosate imposes diverse effects on the biology and ecology of rhizosphere microorganisms and on their interactions with plant roots when released into the rhizosphere (Kremer & Means, 2009). The rhizosphere is a dynamic region governed by complex interactions between plants and organisms that are in close association with the root (Broeckling et al., 2008). Beneficial or harmful relationships exist between rhizosphere organisms and plants, which ultimately affect root function and plant growth (Broeckling et al., 2008). In addition, the rhizosphere may include organisms that do not directly benefit or harm plants but clearly influence plant growth and productivity (Broeckling et al., 2008).

The potential for developing critical pathogen levels in soils that affect crop health, altering rhizosphere microbial communities involved in nutrient transformations, and shifting the balance of beneficial and detrimental plant-associated microorganisms are legitimate concerns regarding the impact of glyphosate on crop productivity and environmental sustainability. This is especially significant with consideration to the current widespread use of glyphosate in glyphosate-resistant seeds (Kremer & Means, 2009).

III. Effects on Humans

There have been five countries that have officially banned the use of the herbicide glyphosate: Malta, Sri Lanka, The Netherlands, Argentina, and Belgium (Simmons, 2017). Malta was the first European country to ban the use of the weedkiller. There were 30,000 doctors in Argentina that demanded the prohibition of glyphosate in their country because those doctors found it to be associated with cancer,

Alternatives w/Essential Oils	**Alternatives w/Soap Salts**
Avenger Organic Weed Killer	Avenger
Bioganic Broadleaf Killer	Certis
Dr. Earth Final Stop	M-Pede
EcoSmart Weed & Grass Killer	
SaferGro	**Alternatives w/Acetic Acid**
Weed Zap	
	AllDown
Alternatives w/Iron	Apple Cider Vinegar
EcoSense	Green Goblin
Elements Lawn Weed Killer	Weed Pharm
Fiesta	White Vinegar
Iron X	
Whitney Farms	

spontaneous abortions, birth defects, skin diseases, respiratory illness, and neurological disease (Simmons, 2017). The president of Sri Lanka banned the use of glyphosate nationwide to protect the health of the citizens. In northern Sri Lanka the herbicide had been linked to a quintuple increase in chronic kidney disease in the nation resulting in around 20,000 deaths (Simmons, 2017).

In 2015 the World Health Organization's International Agency for Research on Caner (IARC) found a strong correlation between glyphosate and non-Hodgkin lymphoma, which is a type of blood cancer that affects the white blood cells called lymphocytes. After this report was announced by the IARC the lawsuits began flooding in from the United States for Monsanto's famous Roundup product (Simmons, 2017).

In addition, a study done by the Munich Environmental Institute revealed that the 14 most popular brands of beer were all contaminated with glyphosate (Bell, 2016). German brewers reacted defensively, claiming they monitored beer ingredients regularly, and had never found excess glyphosate, although conceding that the pesticide was "found virtually everywhere." German people pride themselves on the purity of their beer dating back to April 23, 1516, which was when the Purity Law by the Bavarian Duke Wilhelm IV was enacted to only include pure ingredients in their beer (German Beer Purity Law, n.d.). So, for nearly 500 years, barley, hops, and water have been the only permissible ingredients until the slippage of glyphosate decided to add itself to the ingredient list (Bell, 2016).

Research on Roundup and cancer has found that cancer risk increased by 41% with high long-term exposure to glyphosate (Ecology Center, n.d.). Currently, the warning label on Roundup products does not indicate this risk, but the U.S. Environmental Protection Agency is re-evaluating whether glyphosate is an environmental contaminant and, as part of this review, will re-evaluate its cancer risk (Drugwatch, 2023).

Further research has linked glyphosate to non-Hodgkin lymphoma and oxidative stress in the body, which can be a precursor to cancer and other diseases. Additionally, a 2023 study shows that glyphosate may increase estrogenic activity in breast tissue cancer cells, causing abnormal cell growth (Ecology Center, n.d.). Moreover, results from studies in healthy humans have shown that more than one-half of the human microbiome (often used to describe the microorganisms that live in or on the body such as skin or gastrointestinal tract) are intrinsically sensitive to glyphosate (Puigbò et al., 2022).

The bottom line is that traces of Roundup have been found in crops around the world and leaked into the soil, which not only leads to water contamination, but it also leads to human health consequences upon ingesting glyphosate through food (Cox, 1998). Glyphosate-containing products are acutely toxic to humans and animals alike. Common symptoms can include eye and skin irritation, headache, nausea, numbness, elevated blood pressure, and heart palpitations (Cox, 1998). The surfactant used in a common glyphosate product like Roundup is more acutely toxic than glyphosate itself; the combination of the two is yet more toxic (Cox, 1998).

If these findings are not enough to blow your mind, "scientists also reported that glyphosate-based herbicides, including Roundup, contain toxic levels of heavy metals, including arsenic" (EcoWatch, 2021). Researchers such as Professor Gilles-Eric Seralini from the University of Caen Normandy, France and his colleagues ran several studies and found that 11 glyphosate-based herbicides (including Roundup) contained heavy metals: arsenic, chromium, cobalt, lead, and nickel which are all known to be toxic endocrine disruptors (Wisner Baum, 2023).

IV. Possible Alternatives

These links between Roundup and cancer leave people searching for alternatives to use such as soaps, oil, or acid to kill weeds, but some other options use salt, vinegar, or boiling water (Drugwatch, 2023). These safe alternatives to Roundup can be effective if used properly. There is always good old-fashioned manual weed control methods, such as mulching, which are very effective at controlling young weeds.

The cost of mulching on average is between \$15-\$65 per cubic yard for a bulk truck load delivery (Grupa, 2023). Below are several different types of products that do not contain glyphosate and can be alternatives to Roundup:

(Drugwatch, 2023)

Roundup is a popular herbicide because it's very effective and kills the entire weed, including the root. Chemical alternatives to Roundup do come with limitations. Some products aren't as effective during hot weather, and many only kill small broadleaf weeds but not large plants (Drugwatch, 2023). As with Roundup, these herbicides should not encounter surrounding plants because they are not selective and can cause cosmetic damage or kill nearby plants (Drugwatch, 2023). Although a few chemical alternatives are as effective as Roundup, these products are not better for weed control because many contain chemicals that pose health risks to humans and animals (Drugwatch, 2023).

They can also cause environmental contamination (Drugwatch, 2023). Manual weed control methods like mulching or using cover crops can block emerging weeds without chemicals. However, the downside to this method is if done incorrectly and applied too thickly or too often it can cause soil compaction which can lead to waterlogging, poor drainage, and suffocation of the roots (Rivera, 2023). This compaction can also deter earthworms and other insects that contribute nutrients and help break up the soil (Rivera, 2023). On the other hand, if the mulch is applied too thinly then it would promote weed growth instead of preventing it (Rivera, 2023). Therefore, it is important to note proper quantities and when re-application may be necessary.

V. Conclusion

Monsanto ended up pleading guilty to over 32 environmental crimes in 2021, of these 30 environmental crimes were related to the use of a pesticide on cornfields in Hawaii, and the company further agreed to plead guilty to two other charges related to the storage of a banned pesticide that were the subject of a 2019 Deferred Prosecution Agreement (Hunter, 2021). Additionally, Monsanto was charged with 30 misdemeanor crimes related to the use of a glufosinate ammonium-based product sold under the brand name Forfeit 280 and ended up having to pay over \$22 million dollars in fines (Hunter, 2021). Monsanto also pleaded guilty to two felony charges filed in 2019 that the government would have dismissed if the company had complied with federal law (Hunter, 2021). Monsanto has also reached close to 100,000 settlement agreements in Roundup lawsuits and paid around \$11 billion since it began in 2015 after the report from the World Health Organization's International Research on Cancer published a report linking cancer in humans with glyphosate (Miller, 2023).

However, keep in mind the company brought in \$14.6 billion dollars in revenue per year (Zippia, 2023). Despite the alleged link between glyphosate and certain types of cancer, the new Bayer company that merged with Monsanto continues to assert that the ingredient is safe (*Bayer to Pull Roundup From Shelves in 2023 to Avoid Further Lawsuits*, n.d.).

According to Bayer pulling glyphosate Roundup off the shelves in the United States for consumers only is not being done to address the health or environmental concerns, "this move is being made exclusively to manage litigation risk and not because of any safety concerns." (*Bayer to Pull Roundup From Shelves in 2023 to Avoid Further Lawsuits*, n.d.)

When sales of the product cease, it will be replaced with a new formula that will not include glyphosate. However, this change will only apply to residential customers in the American market. Commercial farmers in the United States will still have access to the original formula, and it will continue to be sold for both residential and commercial use overseas (*Bayer to Pull Roundup from Shelves in 2023 to Avoid Further Lawsuits*, n.d.).

According to the company's website, this move is part of a five-point plan to reduce the risk of further lawsuits and put "uncertainty" behind them, so the company and its shareholders can "move on." Since most claims come from residential users in the United States, Bayer believes this change will curb most future claims. (*Bayer to Pull Roundup From Shelves in 2023 to Avoid Further Lawsuits*, n.d.)

After all the findings linked to environmental and health problems around the world regarding Roundup and its toxicity, it has left both the old Monsanto company and the new Bayer company without many consequences for their actions.

The Bayer company will continue to produce and sell Roundup with glyphosate everywhere except in the U.S. consumer market only to avoid paying out extensive lawsuits that will cut into their profit margins. There is no discussion of future changes or prevention methods, nor a mention of all the people that surround the location of manufacturing because the company's focus is not the people or the environment, it is simply just to mitigate the aftermath.

All of this makes a person wonder if environmental fines or even lawsuits are truly enough to stop large companies from polluting our environment. It seems the Bayer company is simply trying to avoid any future accountability by discontinuing the main ingredient (glyphosate) that caused the uproar in the first place but only with the United States consumer population that threw the most lawsuits at them, only if to keep them quiet. Lessons are generally learned, however, when it comes to the environmental consequences and the overall health of our population these lessons are simply being avoided regarding Roundup. New ways to navigate around problems and avoid them is the oldest innovation for companies like Monsanto and Bayer when the only true goal in mind is their bottom line.

Perhaps it is time to implement harsher consequences for companies such as Monsanto and Bayer, so the environment and humans do not have to incur such heavy consequences because of one company's decisions. Clearly, millions of dollars in fines and lawsuits are not enough to affect them to change their practices, the company simply finds alternate routes to continue bringing in billions every year. For a first offense of an environmental crime the fines should be assessed based on the company's revenue, the more revenue the corporation or company produces the higher the environmental fines should be.

For example, in this situation Monsanto could have been fined around $5 billion dollars because this would take 35.7% of their profits, placing a significant dent in their pockets. Monsanto would not have turned their head the other way once those high fines had been imposed, it would have forced the company to make changes so they could continue making profits instead of giving money away. Monetary fines are only beneficial if they make the company think twice before continuing bad practices. Furthermore, it can be suggested that after a said number of violations, say three to five environmental crime violations, the company should be proposed (by the EPA) to be shut down unless proven implemented changes have been put into practice. Millions of dollars in fines cannot replace the damage done to the environment that will take many years to fix, nor will it stop the death of hundreds of people that have been diagnosed with the last stage of cancer. However, harsher consequences can be put in place to avoid further environmental damage and human despair.

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