UNIVERSITY OF MICHIGAN, SCHOOL FOR ENVIRONMENT AND SUSTAINABILITY

Restoring the Shiawassee Flats:

The Second Year of Ecosystem Monitoring During Hydrologic Restoration at Shiawassee National Wildlife Refuge

A project submitted in partial fulfillment
of the requirements for the degree of Master of Science
at the University of Michigan.

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Prepared by Julie Dellick, Jon Gorter, Anna Greenberg, Xinmiao Liu, and Maria Salem

Clients:
Eric Dunton and Pamela Repp, USFWS
Dr. Kurt Kowalski and Alexandra Bozimowski, USGS

Faculty Advisors:
Dr. Karen Alofs and Dr. Paul Seelbach

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EXECUTIVE SUMMARY

Established in 1953, Shiawassee National Wildlife Refuge (SNWR) serves as vital habitat for migratory and resident birds, and numerous fish species. Since designation, US Fish and Wildlife staff at SNWR have been restoring the area's historic wetland, marsh, and flooded forest ecosystems, which had been drained for agricultural and timber development. The refuge is split into separately managed units that can be connected or disconnected to the Shiawassee River to mimic the area's natural floodplain hydrology and ecology.

Refuge staff recruited a group of students from the University of Michigan School for Environment and Sustainability in 2019 to perform post-restoration ecological monitoring of Maankiki South (MS), Maankiki North (MN), and Pool 1A (P1A). P1A was established in 1958 and has been connected to the Shiawassee River ever since. The two other units, MS and MN, were first flooded in 2018 and 2017 respectively. Our team continued this post-restoration field research and followed the same sampling procedures utilized by the 2019 team, which were modeled after the Great Lakes Wetland Monitoring Program (Uzarski et al. 2016). Our research adds to a log of annual data on biological communities within the floodplain units, helps inform habitat management strategies, and further refines data collection and analysis methods. This report describes our research of the water quality variables, vegetation, macroinvertebrates, and fishes in MS, MN, and P1A, and contextualizes it with the findings from 2019 sampling.

Water Quality: We describe each water quality variable sampled (average temperature, dissolved oxygen, pH, conductivity, turbidity, and phosphorus and nitrogen levels) and provide statistical analyses of each variable across units, months, and in comparison to 2019 data.

- Average DO in P1A was lower than both MS and MN. We suspect this may have influenced our fish sampling, as fish likely moved to deeper, more oxygenated, waters.
- *Phalaris* zones had high DO levels through the whole summer due to the zone's high surface cover and root destiny, which results in increased dissolved oxygen release.
- There was some notable monthly variation in DO and pH which we attribute either to increased riverine water inputs from the May 2020 flooding event or sampling location.
- Even though MN and MS are both much younger than P1A and have followed almost the same restoration schedule since their completion in 2016, we found MN and MS to be two distinct units from each other, with MS being most similar to P1A.

Vegetation: We describe the plant species located and identified throughout the refuge units, and the statistical analyses and indices used to characterize the quality of habitat sampled.

- Quality assessments show all three units are of "Low" quality, though particularly in MN. We attribute this to the abundance of invasive species and lack of sensitive species.
- Though all units had the same FQA score, P1A had the greatest number of plant species (37), followed by MN (30) and MS (17). Much of the diversity in MN was attributed to a small strip of dry earth which hosted an array of terrestrial plant species.

- SAV and *Typha* vegetation zones were quite similar across units.
- The most dissimilar zones were the *Nymphaea* zone in P1A*,* Mudflat zone in P1A, and *Phalaris* zone in MN, and they were each dominated by vegetation unique to each zone.
- The dissimilarity index and PCA indicate that vegetation composition of floodplain units is affected by water depth, land use history, invasive species, and canopy coverage.

Macroinvertebrates: We describe the abundance and composition of macroinvertebrate communities across the three study units in 2020 and compare our findings to those from 2019. We also consider these data in relation to water quality, vegetation, season, and refuge location.

- Similar abundances and families were captured in both the 2019 and 2020 field seasons, though we recorded a higher CPUE in 2020 despite a lower overall sampling effort.
- We did not find month or vegetation to be statistically significant in determining community composition. However, July and the SAV vegetation structure had the highest diversity and evenness, while August and the flooded forest had the lowest diversity.
- P1A, the refuge's oldest unit, which serves as a reference site, had the greatest richness and evenness. MN had low diversity and a more distinct community composition than the other units, which we attribute to the relative abundance of Scuds captured.
- IBI scores for all three units classify them as "Mildly Impacted", so no unit shows drastic anthropogenic degradation, despite the very recent restoration of MN and MS.

Fish: We describe the abundance, composition, and size structure of 2020 fish communities across the three study units, and compare these findings to those from the 2019 sampling season. We also consider these data in relation to water quality, vegetation, season, and refuge location.

- We performed fish sampling using multiple frame- and mesh-size fyke nets.
- We found fewer fish species than were recorded in 2019, though unique species were found during both sampling years. We recorded significant changes in overall and per-unit species abundances between 2019 and 2020, which we attribute to either the May 2020 flooding or to standard annual variation in ecosystem composition.
- Assemblages of the five most abundant species were not identical across study units. However, there were several dominant species consistent across years, including Black Bullhead *Ameiurus mela*s and Pumpkinseed *Lepomis gibbosus*.
- Future recommendations include incorporating water depth analyses for the study units and tracking the relationship between conductivity in the Shiawassee River and the units.

Our research has implications for ecosystem development, as we found factors like water depth and flood history to potentially be more influential on annual ecosystem variation than temporal length of floodplain connectivity and restoration. However, in this second year of monitoring, we also recorded increases in habitat quality in some units, which provides evidence of the progress of SNWR's habitat restoration efforts.

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REGIONAL AND REFUGE HISTORY

The Shiawassee National Wildlife Refuge (SNWR) is a large floodplain area where four tributaries merge to form the Saginaw River. (Figure 1.1) Though located 20 miles inland, SNWR is a coastal wetland influenced by the water levels and seiche dynamics of Lake Huron, and is a central feature of the large Saginaw Bay watershed, which drains 15% of Michigan's land area (USFWS 2018). This significant wetland resource provides several ecosystem functions, including flood attenuation, chemical processing, and a diversity of habitats supporting productive regional and migratory wildlife. Most notably, SNWR is a crucial stopover point for thousands of migratory birds and is globally recognized as an Important Bird Area by the National Audubon Society. In addition, SNWR is home to a productive, summer resident fish community

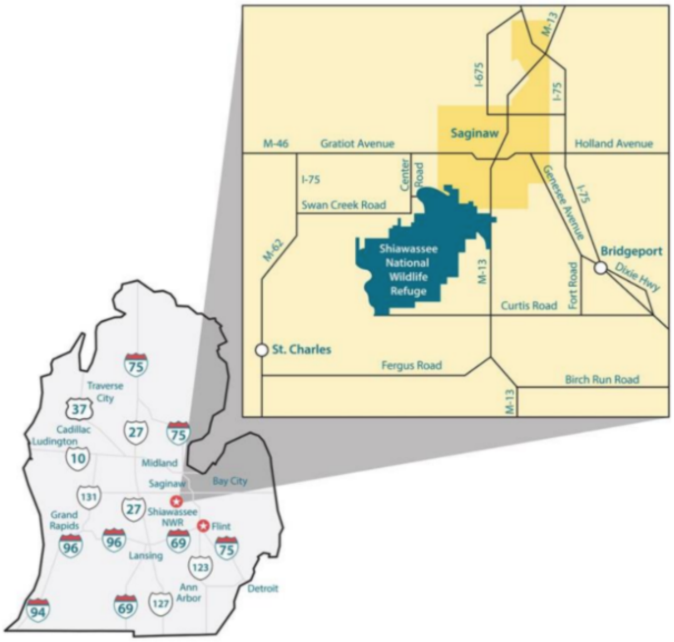


Figure 1.1: Inset map of SNWR's location near Saginaw, Michigan.

In the early 1800s, European colonizers settled the area surrounding what is now SNWR for timber harvest, coal mining, and farmland. These economic pursuits required heavy alteration of the land, severely altering vegetation across the landscape and rerouting and channelizating the area's rivers with miles of dikes (Heitmeyer et al. 2013). This landscape alteration caused dominant land cover to shift from lowland forests and wetlands to agricultural land and developed urban space. Though timber harvests were highly profitable and land that was previously flooded became arable, this land cover shift disrupted crucial ecosystem services of the area and degraded habitat quality. Most notably, it disconnected the Saginaw River from its floodplain, which led to greatly decreased treatment of nutrients, floodwater storage, and native habitat productivity.

SIGNIFICANCE OF RESTORATION

Globally, it is estimated that upwards of 50% of all wetlands have been destroyed in just over a century and many are still disappearing (Davidson 2014). The situation is equally dire in the United States, and especially in Saginaw County, Michigan, where 50-90% of all wetlands have been destroyed since 1830 (Comer 1996; Buchanan et al. 2013).

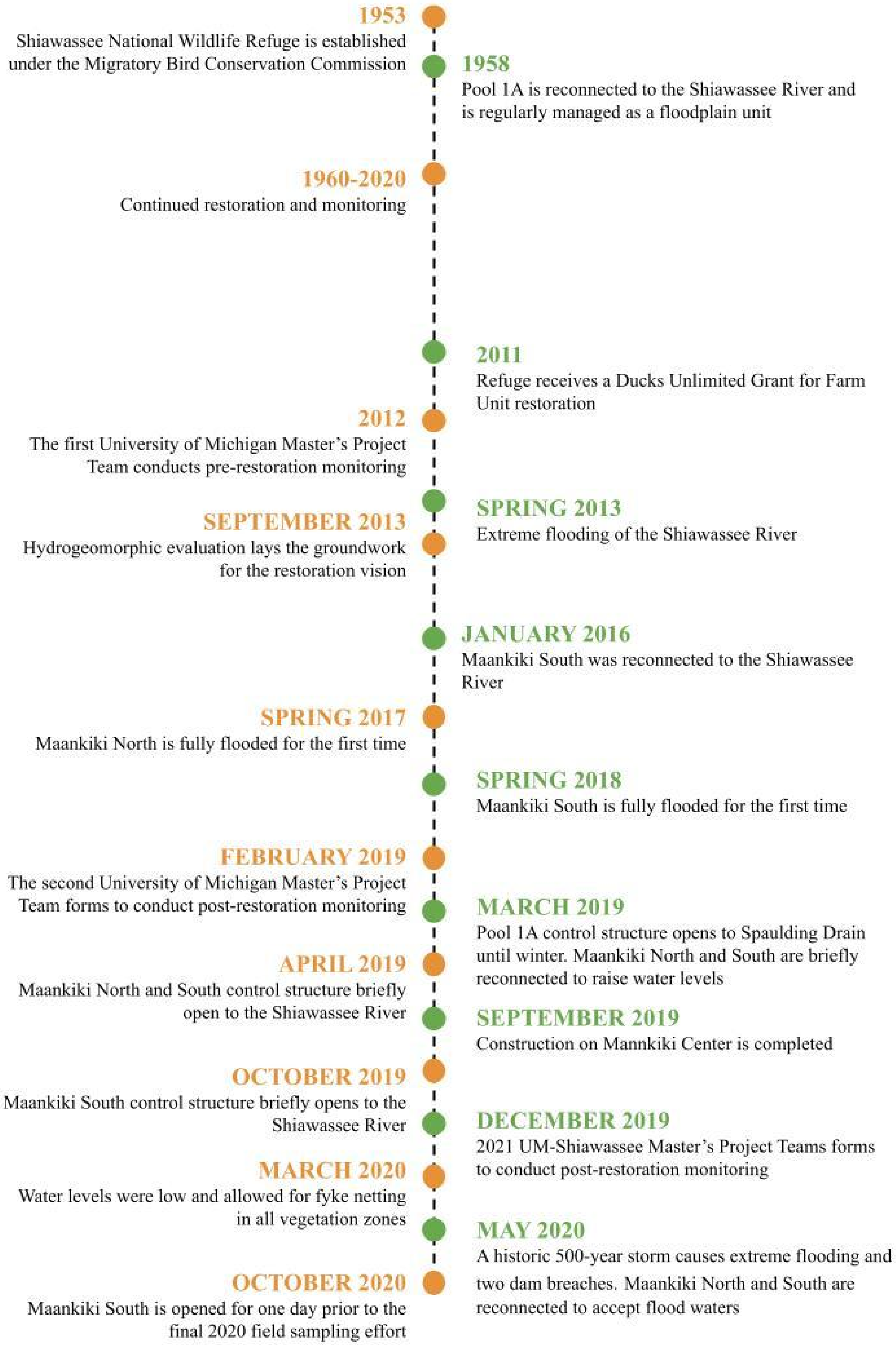
To recover and sustain the valuable ecosystem processes provided by refuge habitats, SNWR was established in 1953 as a sanctuary for migratory birds under the Migratory Bird Commission (USFWS 2001). Since then, SNWR has focused heavily on ecosystem restoration and management for the benefit of waterfowl and fish through the provision of high-quality habitats. SNWR is managed under the jurisdiction of the U.S. Fish and Wildlife Service (USFWS), a branch of the Department of the Interior. Within the last decade, SNWR has received millions of dollars through the federal Great Lakes Restoration Initiative (GLRI). This funding has supported the ongoing hydrologic restoration work of reconnecting floodplain wetlands to river channels through strategically placed and monitored gates. Post-restoration monitoring began in 2019 and its continuation will allow USFWS to determine the ecological impacts of ecosystem restoration and reconnection. Ultimately, SNWR hopes to increase the quality of vital habitat, improve ecosystem services for local and regional communities, and create an environment ecologically similar to that which existed prior to major anthropogenic disturbance. This will allow the refuge to meet its mission of conserving, protecting, and enhancing wildlife for the continued benefit of the American public.

HISTORY OF RESTORATION MONITORING

Throughout the Refuge history, key events have shaped the trajectory of restoration. After the establishment of the refuge in 1953, the formation of its largest floodplain unit, Pool 1A (P1A), helped re-establish a critical stopping point for migratory waterfowl. This unit became the focal point for most of the refuge's restoration efforts until 2011, when a grant from Ducks Unlimited helped fund new research and a comprehensive Hydrogeomorphic (HGM) evaluation of ecosystem restoration and management recommendations for SNWR (Heitmeyer et al. 2013). The HGM evaluation helped define past ecological conditions existing throughout the Shiawassee Flats (SF) and helped guide the refuge's restoration strategy for the Maankiki units, as well as for P1A. Further funding from the Great Lakes Restoration Initiative (GLRI) helped the refuge acquire additional acreage for restoration, which eventually became the floodplain units Maankiki North (MN), Maankiki South (MS), and Maankiki Center. In the past, these locations were disconnected from the SF floodplain and regularly drained for agriculture. However, through current restoration efforts, the Maankiki complex and other moist soil units are being reconnected to the larger floodplain of the SF region (USFWS 2018). Throughout the creation and maintenance of the refuge's floodplain units, various flooding events have had large impacts, though the extent of those impacts is not fully known. The two most severe flooding events occurred during the spring of 2013 and in May of 2020 (Figure 1.2).

In order to quantify and understand the restoration impacts of reconnecting the floodplain units of the Maankiki complex, the refuge has supported multiple research projects by students at the University of Michigan, School for Environment and Sustainability. The most recent of these was conducted by a group of Master's students referred to in this report as the 2020 UM-Shiawassee Master's Project team, or the 2020 team. The 2020 team monitored fish, macroinvertebrates, water quality, and vegetation throughout the Maankiki units and in P1A during 2019 and established protocols for continued monitoring into the future. These student monitoring efforts are projected to continue annually, until at least 2024.

We, the 2021 UM-Shiawassee Master's Project team, conducted the second consecutive year of monitoring. We followed the protocols established by the 2020 team and sampled water quality, vegetation, macroinvertebrates and fish during the 2020 field season. As described in each of the following sections, some of our sampling was limited due to restrictions related to the COVID-19 pandemic. However, we still gathered enough data (Appendix I) to add to the refuge's growing monitoring database and draw comparisons to findings from the 2019 season.



1953
Shiawassee National Wildlife Refuge is established under the Migratory Bird Conservation Commission

1958
Pool 1A is reconnected to the Shiawassee River and is regularly managed as a floodplain unit

1960-2020
Continued restoration and monitoring

2011
Refuge receives a Ducks Unlimited Grant for Farm Unit restoration

2012
The first University of Michigan Master's Project Team conducts pre-restoration monitoring

SPRING 2013
Extreme flooding of the Shiawassee River

SEPTEMBER 2013
Hydrogeomorphic evaluation lays the groundwork for the restoration vision

JANUARY 2016
Maankiki South was reconnected to the Shiawassee River

SPRING 2017
Maankiki North is fully flooded for the first time

SPRING 2018
Maankiki South is fully flooded for the first time

FEBRUARY 2019
The second University of Michigan Master's Project Team forms to conduct post-restoration monitoring

MARCH 2019
Pool 1A control structure opens to Spaulding Drain until winter. Maankiki North and South are briefly reconnected to raise water levels

APRIL 2019
Maankiki North and South control structure briefly open to the Shiawassee River

SEPTEMBER 2019
Construction on Mannkiki Center is completed

OCTOBER 2019
Maankiki South control structure briefly opens to the Shiawassee River

DECEMBER 2019
2021 UM-Shiawassee Master's Project Teams forms to conduct post-restoration monitoring

MARCH 2020
Water levels were low and allowed for fyke netting in all vegetation zones

MAY 2020
A historic 500-year storm causes extreme flooding and two dam breaches. Maankiki North and South are reconnected to accept flood waters

OCTOBER 2020
Maankiki South is opened for one day prior to the final 2020 field sampling effort

Figure 1.2: A timeline of significant events refuge restoration.

STUDY AREAS

As the Shiawassee River passes through SNWR, it joins with the Tittabawassee, Cass, and Flint Rivers to form the Saginaw River, which eventually drains into Saginaw Bay and Lake Huron. Additionally, the Spaulding Drain, a remnant of agricultural modifications to the landscape and hydrology, flows through the refuge and joins with the Shiawassee River. These systems form the hydrologic context of SNWR.

The refuge is split into multiple floodplain units that allow staff to control the hydrologic connectivity of each separate ecosystem to its surrounding units, and to the Shiawassee River or Spaulding Drain (Figure 1.3). Units are separated by earthen dikes that include connecting control structures, though some units are “unconnected” and do not have water control structures. Units with control structures can be either connected, allowing inter-unit or unit-river water passage; or unconnected, preventing water passage and maintaining water within a unit.

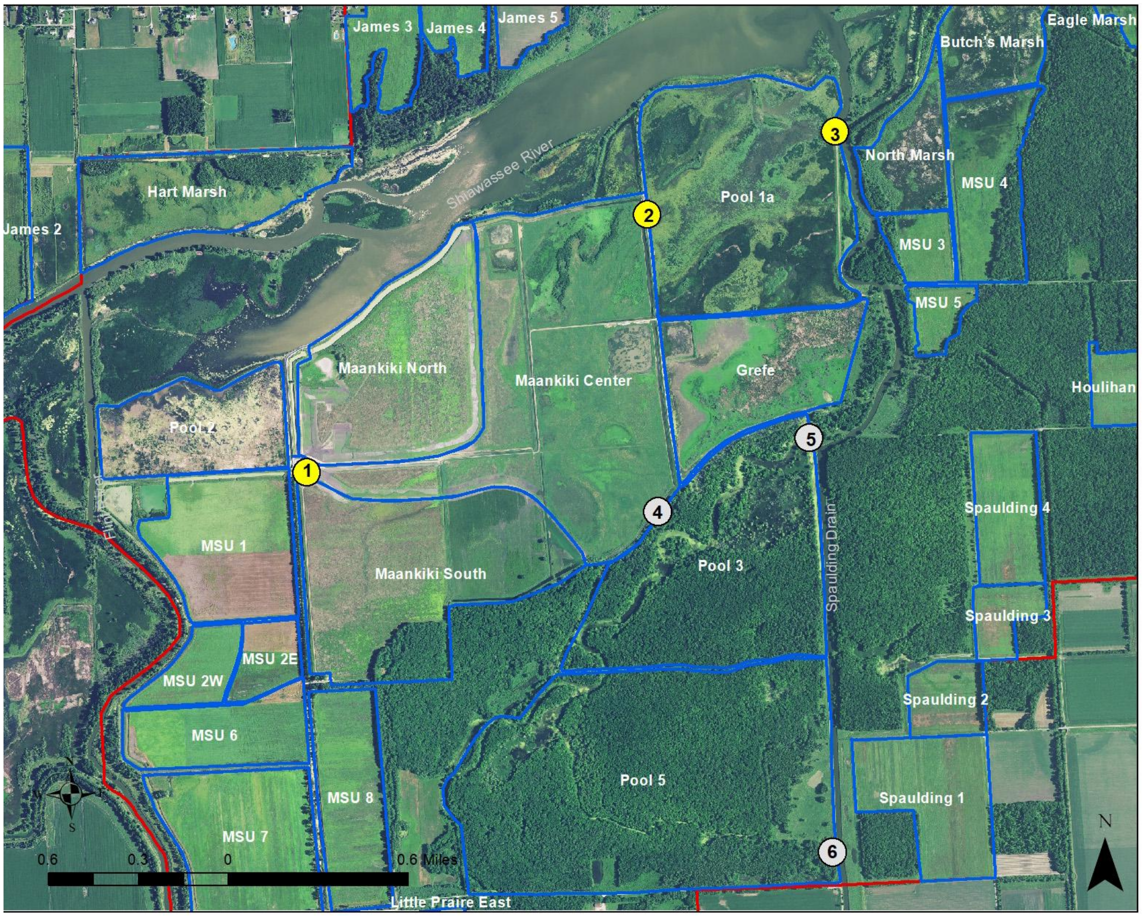


Figure 1.3: Aerial photo of SNWR. Blue lines indicate unit dike boundaries. Yellow points indicate connective control structures, gray points indicate proposed future connective control structures. Image pulled from 2020 UM-Shiawassee Masters Project team final report (Lugten et al. 2020).

In addition to the ecosystem variability induced by a unit's connectivity status, differences in local topography, and consequently hydrology, diversifies the water quality, vegetative, and biological character of each unit. Refuge staff must account for the numerous factors that dictate the biological community in each unit in order to support the management and conservation goals described in the Habitat Management Plan of 2018 (USFWS 2018).

Though the annual connectivity plan for most refuge units tends to vary, P1A has remained connected to the Spaulding Drain and Shiawassee River every year from ice melt to ice freeze since its establishment in 1958. P1A provides an experimental control, or reference, unit within SNWR, and it demonstrates what long-term connectivity can look like for Shiawassee floodplain ecosystems. Three emergent marsh units were added in 2016: MN, MS, and MC. Both our 2020 study and the 2019 study focused on P1A, MS, and MN for ecological monitoring. MS and MN are connected to the same water distribution basin, which runs between dikes and connects to the Shiawassee River by one common control structure. Consequently, any fish entering the distribution basin can either remain in the basin, move into MS, move into MN, or return to the river. P1A is not directly connected to MS nor MN.

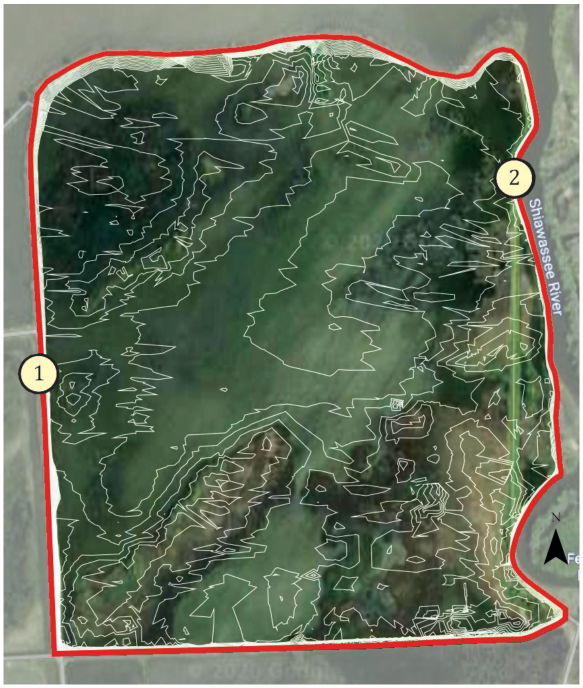


Figure 1.4: Aerial photo of the Pool 1A unit. Gray lines indicate water level topography. Point #1 indicates location of control structure connecting P1A to Maankiki Center; point #2 indicates location of control structure connecting P1A to the Shiawassee River. Image pulled from Lugten et al. (2020).

Pool 1A

P1A serves as a reference floodplain unit within SNWR. Since its reconnection in 1958, the unit has been open to the Spaulding Drain and Shiawassee River every year during the spring, summer, and fall months (Figure 1.4). In order to maintain water levels and provide adequate refuge habitat for overwintering species, the P1A control structure is closed each winter. The unit hosts a variety of vegetation zones including *Nymphaea* (water lilies), *Typha* (cattails), *Salix* (willow), and submerged aquatic vegetation (SAV). Though relatively flat, local topographical variation within the unit has resulted from nest formation by Canada Geese and Muskrats.

Maankiki South

MS, reconnected to the Shiawassee River in 2016, was first flooded in spring 2018 (Figure 1.5). Its connectivity to the river has varied seasonally. MS has primarily been a closed unit, though it was reconnected in March 2019 to raise water levels, in May 2020 to accept flood waters from a heavy precipitation and double dam breach event upstream on the Tittabawassee River, and for one day in October 2020. The interior perimeter of the unit is channelized and consequently deeper than the areas of the unit more towards the center. Topographically, MS is higher in elevation than its neighboring units and water levels are typically lower as a result. The unit is characterized by three principle vegetation zones: SAV, *Typha*, and flooded forest.

Maankiki North

Like MS, MN was reconnected to the Shiawassee River in 2016 but was first flooded in spring 2017 and again in March 2019 to raise water levels (Figure 1.6). Though locally variable in topography, most of this variation is attributed to the deep channels that constitute the interior perimeter of the unit. The unit primarily hosts *Phalaris, Typha*, and SAV vegetation zones, though a row of dead trees in the northwestern quadrant serve as a highly utilized roosting site for multiple water bird species. MN has typically been closed to the river, but its lower elevation results in deeper water levels throughout the unit. The unit was too deep for 2019 fish fyke net sampling in the SAV zone, but water levels during the 2020 season were notably low and allowed for fyke netting in all vegetation zones. Like MS, MN was opened in May 2020 to accept flood waters.

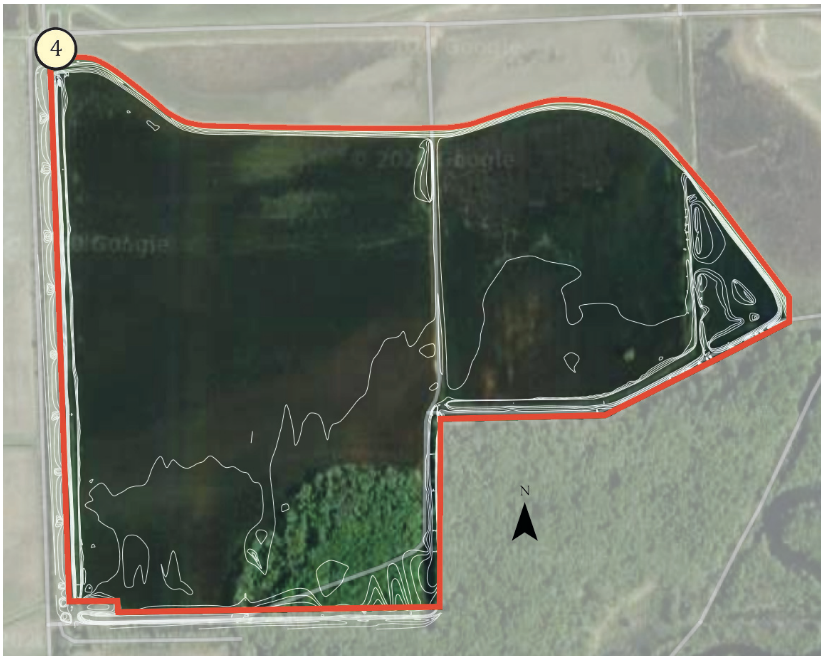


Figure 1.5: Aerial photo of MS. Gray lines indicate water level topography. Point #4 indicates the location of the control structure connecting MS to the Shiawassee River. Image from Lugten et al. (2020).

Figure 1.6: Aerial photo of MN. Gray lines represent water level topography. Point #3 indicates the water control structure that connects MN to the Shiawassee River. Image from Lugten et al. (2020).

RELEVANCE OF OUR PROJECT WORK

Ecological monitoring at SNWR is focused on enhancing habitats and communities of fish and vegetation through quality field monitoring. Our work was a direct continuation of monitoring efforts that began in 2019 by a previous group of student researchers from UM (Lugten et al. (2020). Our guiding questions for ecological monitoring were two-fold:

1. *How has the ecological community composition and water quality changed across floodplain units, throughout the restoration process?*
2. *How does this information help guide SNWR management?*

More specific research questions guided each particular section of ecological monitoring; they are listed in their respective sections. Following our project work, an additional group of Master's students from UM will continue monitoring in 2021.

OUTLINE AND OBJECTIVES OF THIS REPORT

The following report serves to document our 2020 UM-Shiawassee Master's Project team ecological Monitoring work. Our report objectives were to:

1. *Outline the ecological research questions we used to guide our field data analysis.*
2. *Describe the field monitoring procedures followed by our team for the 2020 season for sampling Water Quality, Vegetation, Macroinvertebrates, and Fishes.*
3. *Present analysis and discussion of ecological field data for Water Quality, Vegetation, Macroinvertebrates, and Fishes for the 2020 season, and compare these findings to results from the 2019 field season. (See Appendix I for how to access our raw data).*
4. *Provide recommendations for future ecological monitoring work.*

WATER QUALITY MONITORING

INTRODUCTION

In accordance with the 2020 UM-Shiawassee Master's Project team's protocols, we took water quality samples throughout SNWR within the floodplain units, at control structures between units, and in the Shiawassee River. We carried out water quality sampling in conjunction with fish fyke net sets and macroinvertebrate sampling throughout the summer. We also carried out separate sampling events in the months of May, June, and August 2020.

We determined water quality metrics to contribute to our understanding of the overall health of the wetland ecosystem at SNWR; and to better understand habitat conditions for biodiversity and health of various flora and fauna populations at the refuge, particularly fish, waterfowl, macroinvertebrates, and aquatic vegetation, all of which can be affected by water quality (Weaver & Fuller 2007). The parameters commonly studied in analyses of water quality include temperature (ºC), pH, conductivity (µS/cm), turbidity (FNU), and dissolved oxygen (DO) (mg/L). In combination and over time, these measurements can be used to characterize this aspect of overall habitat health.

Other useful measures of water quality are nutrient composition and prevalence, which can suggest the level of agricultural or runoff pollution in a system. Two nutrients, phosphorus and nitrogen, can be particularly telling of wetland health and pollution levels, as they are typically limiting nutrients (Baustian et al. 2018). Wetlands have the capacity to uptake nutrients and chemicals, so they have the potential to reduce loading rates to connected rivers and increase overall system health (Baustian et al. 2018). Two of the refuge floodplain units, MS and MN, have been recently hydrologically reconnected to the Shiawassee River, while the third study unit, P1A, has been connected since its completion in 1958. Due to this connection to the Shiawassee River, we would expect nutrient levels in the floodplain units to fluctuate throughout the year with changing conditions and flows depending on the connectivity status of their control structures. To characterize these levels, water quality samples were collected throughout the summer and sent to the Heidelberg University, National Center for Water Quality Research (NCWQR) for nutrient analysis, with a focus on determining phosphorus and nitrogen levels.

RESEARCH QUESTIONS

We developed the following research questions to guide our water quality data analysis:

- *What is the average temperature, dissolved oxygen, pH, conductivity, and turbidity in each unit throughout the sampling period? How do these values compare to data from 2019?*
    - *How do these indicators vary by month in 2020, as well as compared to 2019?*
    - *How do they vary within the units, based on vegetation types in 2020, and again compared to 2019?*

- *Are temperature, dissolved oxygen, pH, conductivity, and turbidity significantly different among units?*
- *How do high turbidity events affect phosphorus and nitrogen levels in the floodplain units, control structures, and surrounding rivers?*

METHODS

Multi-Parameter Sonde Sampling

We measured water quality using a YSI EXO III hand-held, multi-parameter sonde in refuge floodplain units, in accordance with protocols established by Lugten et al. (2020), with some minor modifications in how we analyzed the data. We deployed the sonde in conjunction with fish and macroinvertebrate sampling and as independent sampling events (Lugten et al. 2020). Sampling occurred in the months of June, July, August, September, and October for the wetland study units, MS, MN, and P1A. Sonde measures of DO, pH, temperature, conductivity, and turbidity were recorded in triplicates from the middle of the water column at every sampling site for fish and macroinvertebrates throughout the entire field season.

In addition to water quality measures and location information, depth and vegetation zone of the sampled area were also recorded for each sonde reading. Potential vegetation types included: submerged aquatic vegetation (SAV), flooded forest, *Typha*, *Salix*, *Nymphaea*, and *Phalaris*.

The 2020 UM-Shiawassee Master's Project team recorded distance from water control structures in their analysis of water quality for the 2019 sampling season in an attempt to determine if this had any effect on results (Lugten et al. 2020). This measure did not appear to have any significant effect on water quality, so our team did not include this information in our analysis for the 2020 sampling season.

Nutrient Sampling

We collected water samples and sent these to the Heidelberg University, NCWQR for nutrient analysis. Turbidity events can be associated with marked increases in concentrations of both phosphorus and nitrogen, the primary limiting nutrients in aquatic systems (Baustian et al. 2018). Typically, increases in turbidity can stem from high flows or storm events that increase water and sediment inputs into the floodplain units from surrounding rivers (Baustian et al. 2018). We made efforts to collect water samples at both high and low turbidity time periods and locations throughout our field season. If possible, samples were collected the day following storm events to get as many high turbidity data points as possible. This was a challenge, however, as our team was unable to stay at the sleeping quarters at SNWR due to COVID-19 restrictions. Consequently, we were only able to make the trip to the refuge on preplanned days. Additionally, the majority of water samples needed to be shipped to the NCWQR immediately to maintain the temperature integrity of the sampling, so our collection schedule was constrained by the availability of laboratory staff to run analyses.

We collected samples according to NCWQR protocols. Our samples were either full-panel nutrient samples or total phosphorus (TP) samples. Full-panel testing included analyses for TP, suspended sediments, dissolved nutrients and ions, and total Kjeldahl nitrogen (TKN). TKN is a measure of both organic nitrogen in a sample as well as inorganic ammonia-nitrogen (EPA 2001). Full-panel samples were collected once each month in July, August, and September in each of the three floodplain units of study, as well as at a water control structure and from Spaulding Drain. Sonde readings were also recorded at the time and location of sampling. Samples were collected by rinsing and emptying 500mL polystyrene bottles with water from the location of collection. The bottle was filled from the middle of the water column and immediately sealed and placed in a cooler with ice to maintain the temperature integrity of the sample. Samples were shipped overnight on ice to NCWQR for immediate analysis.

We performed additional sampling focused solely on TP. TP samples were collected using the same methodology as full-panel samples, but using a 250mL polystyrene bottle. Samples were collected periodically throughout the summer (July – September), either in the days following a major storm or from areas with noticeably turbid flow. Such areas included the three floodplain units of study, water control structures, and the Shiawassee River. Phosphorus samples can be frozen for up to one month and still yield accurate results, so these samples were stored in a freezer at the refuge for several weeks before being sent to NCWQR in bulk. TP testing only required a 125mL sample, so samples were divided into two 125mL samples: one to be sent to NCWQR and one to be retained frozen at the refuge as a backup.

DATA ANALYSES

Multi-Parameter Sonde

We organized the sonde data by month and averaged the three values collected during each sampling effort for DO, temperature, conductivity, turbidity, and pH. These values were visualized, assessed for normality and equal variance, and used to run ANOVA tests in R Studio, utilizing the packages ‘car’ and ‘dplyr.’ Code from the 2020 UM-Shiawassee Master’s Project team was used to carry out analyses (Lugten et al. 2019). The ANOVA tests analyzed the relationship between each water quality parameter and month, unit, and vegetation type. Data were analyzed by monthly variation (June, July, August, September, October), vegetation (forest, *Nymphaea*, *Phalaris*, *Salix*, SAV, Shore, and *Typha*), and unit (MN, MS, and P1A). Data was gathered from 120 different sites and water quality parameters were averaged for each site for analysis. While the 2020 UM-Shiawassee Master’s Project team took sonde measurements during macroinvertebrate and fish sampling events, they did not include these data in their statistical analyses, and only those data that were collected during independent water quality sampling events were included in their analysis. We included all sonde measurements in our data analysis to get a more detailed overview of water quality across time and space.

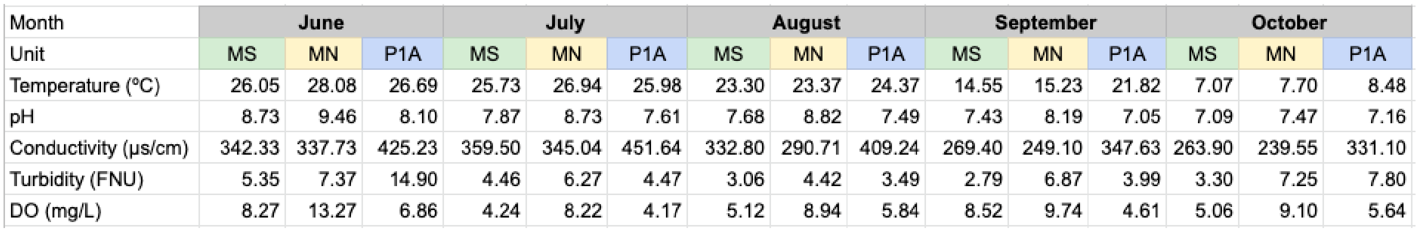
Nutrient Samples

Full-panel nutrient samples were analyzed and combined with data from the 2019 season to create a turbidity-nitrogen curve. Full-panel and TP data from the 2019 and 2020 seasons were used to create a turbidity-phosphorous curve. Such curves can be used to estimate total phosphorus and nitrogen retention in wetlands that have been hydrologically reconnected to floodplains (Baustian et al. 2018). This can suggest the extent to which a wetland may act as a sink for nutrients with fluctuating water levels, thus reducing loading rate for phosphorus and nitrogen in local waterways (Baustian et al. 2018). Such impacts can improve overall local water quality and even reduce issues of algal blooms.

RESULTS

Summary of Averages

We found that water quality varied significantly by month, unit, and vegetation zone in 2020. An alpha of 0.05 (p-value < 0.05) for statistical significance was used across all analyses. In 2019, the project team collected data on temperature, pH, conductivity, turbidity, and DO in MN , MS, and P1A in May, June, and August (Lugten et al. 2020). Our team did the same in 2020 from June through October (Table 2.1).



Month	June			July			August			September			October		
Unit	MS	MN	P1A	MS	MN	P1A	MS	MN	P1A	MS	MN	P1A	MS	MN	P1A
Temperature (°C)	26.05	28.08	26.69	25.73	26.94	25.98	23.30	23.37	24.37	14.55	15.23	21.82	7.07	7.70	8.48
pH	8.73	9.46	8.10	7.87	8.73	7.61	7.68	8.82	7.49	7.43	8.19	7.05	7.09	7.47	7.16
Conductivity (µs/cm)	342.33	337.73	425.23	359.50	345.04	451.64	332.80	290.71	409.24	269.40	249.10	347.63	263.90	239.55	331.10
Turbidity (FNU)	5.35	7.37	14.90	4.46	6.27	4.47	3.06	4.42	3.49	2.79	6.87	3.99	3.30	7.25	7.80
DO (mg/L)	8.27	13.27	6.86	4.24	8.22	4.17	5.12	8.94	5.84	8.52	9.74	4.61	5.06	9.10	5.64

Table 2.1: Average daytime water quality (temperature, pH, conductivity, turbidity, and DO) in June, July, August, September, and October for MN, MS, and P1A in 2020.

Variation by Month

ANOVA results showed significant differences between month and all water quality parameters (Table 2.2). This was also the case during the 2019 field season (Lugten et al. 2020).

Water Quality Parameter	Monthly Variation (June, July, August, September, October)	Vegetation	Unit (MS, MN, P1A)
Temperature	**<0.001**	0.736	0.813
DO(mg/L)	**0.005**	**<0.001**	**<0.001**
PH	**<0.001**	**<0.001**	**<0.001**
Conductivity	**<0.001**	**<0.001**	**<0.001**
Turbidity(FNU)	**0.004**	0.071	**0.039**

Table 2.2: A summary of p-values for tests of significant relationships (p-value < 0.05, bold) between selected physical variables and wetland water quality parameters in 2020.

Variation in Temperature by Month

Average temperature decreased monthly throughout the season from June to October. In total, temperatures decreased by 22ºC. June had the highest average temperature of 26.94ºC and October had the lowest average temperature of 7.75ºC. All months differed from one another significantly (p-value < 0.01 - 0.001) with the exception of June and July (Figure 2.1). Average temperatures were similar in both 2019 and 2020, though they were slightly higher in 2020 during the period from June through August (Figure 2.2). In June 2020, the average temperature was 3.96ºC higher on average than in June 2019. In August 2020, the temperature was on average 3.42ºC higher than August 2019.

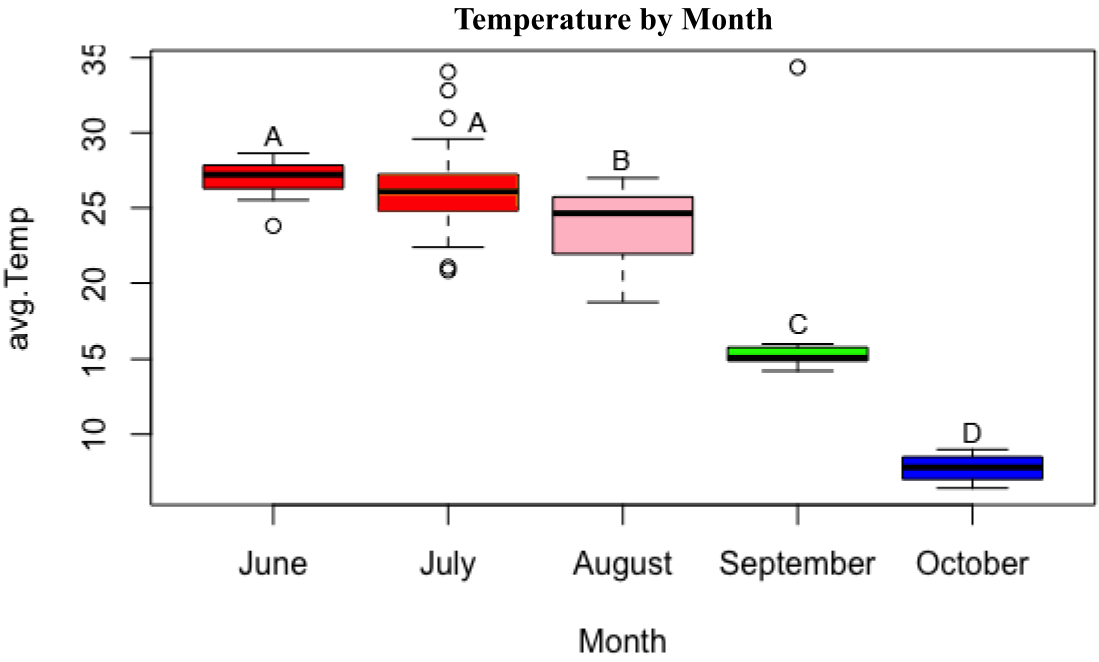


Figure 2.1: Temperature (ºC) levels by month for the 2020 sampling seasons. The different letters and colors each represent significant different temperature levels (June>July>August>September>October)

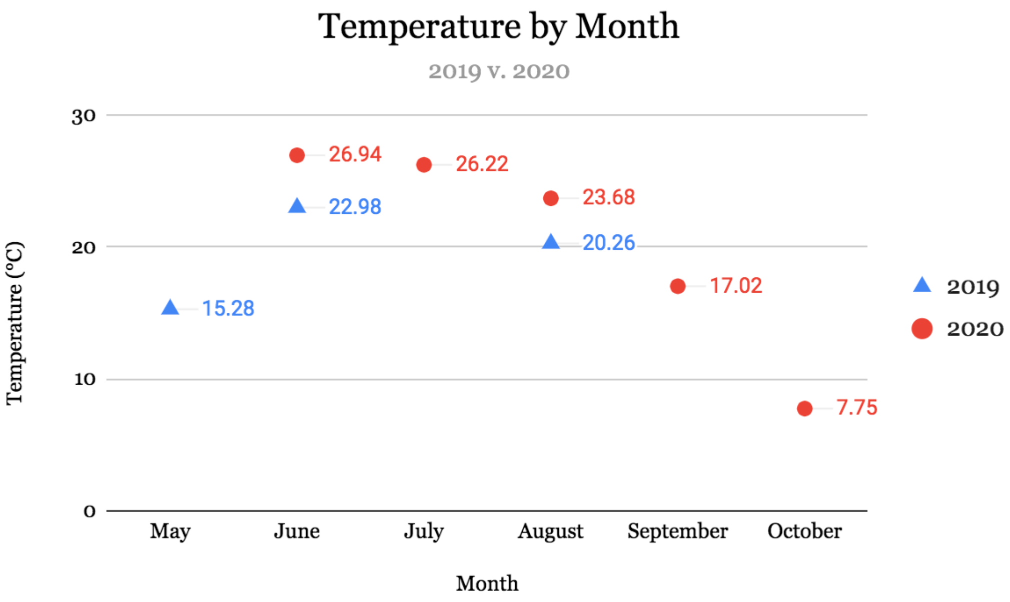


Figure 2.2: Temperature (ºC) levels by month compared between the 2019 and 2020 sampling seasons.

Variation in Dissolved Oxygen by Month

We found little difference between DO and month, though there was a significant difference between June and July. June's DO was found to be significantly higher than July, which had the lowest DO level of our entire 2020 field season. Overall, we saw a trend of heightened average DO in comparison to 2019 (Figure 2.3). Overall, DO seemed to vary more erratically by month in 2020 than it did in 2019, during which time values apparently declined steadily throughout the season (Figure 2.3). It is important to note, however, that DO was not recorded in July in 2019, so it is possible that a decline in DO occurred but was not captured in sampling.

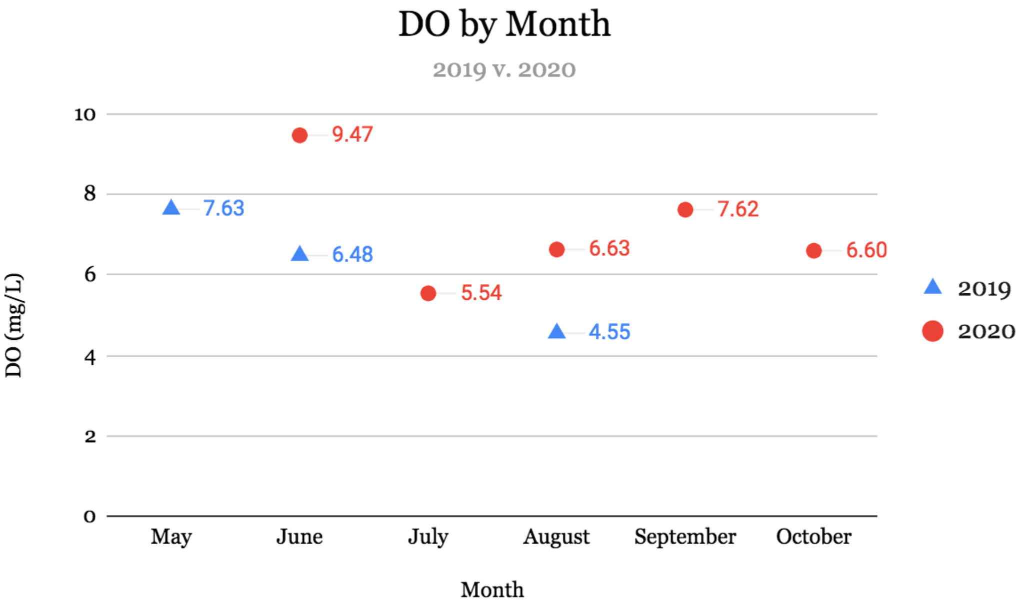


Figure 2.3: DO (mg/L) levels by month compared between the 2019 and 2020 sampling seasons.

Variation in pH by Month

Average pH in June 2020 was significantly higher than in all other subsequent months (Figure 2.4). The average pH in June was 8.76, and values slowly declined until October when the average pH was 7.24 (Table 2.1). Between 2019 and 2020, we found that the trends of pH were similar, with very slow declines in pH throughout the 2019 season as well (Figure 2.5). The average 2020 pH was slightly higher in all months than in 2019 (Figure 2.5).

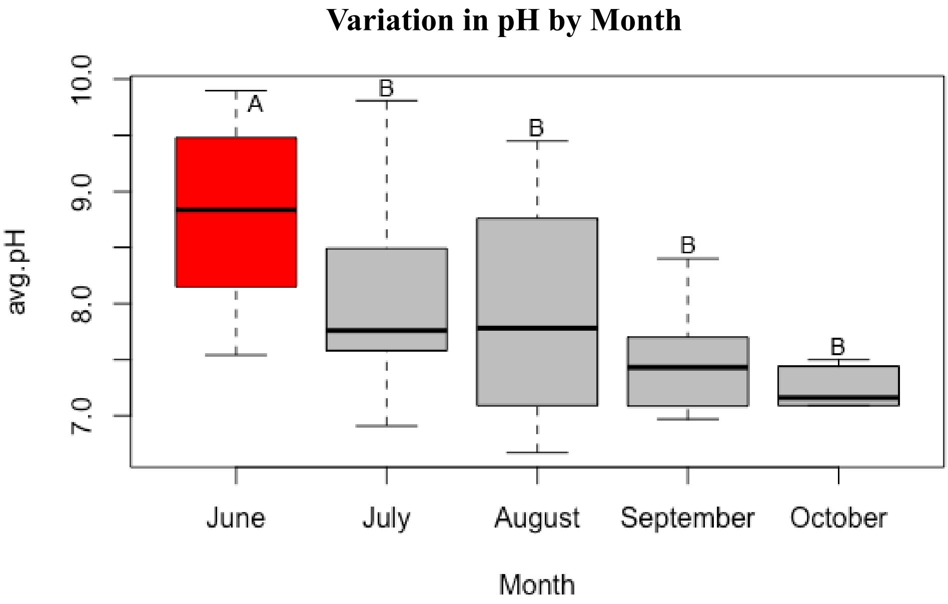


Figure 2.4: Average pH levels by month for the 2020 sampling season. The months that share a color and letter means that they are not significantly different from each other.

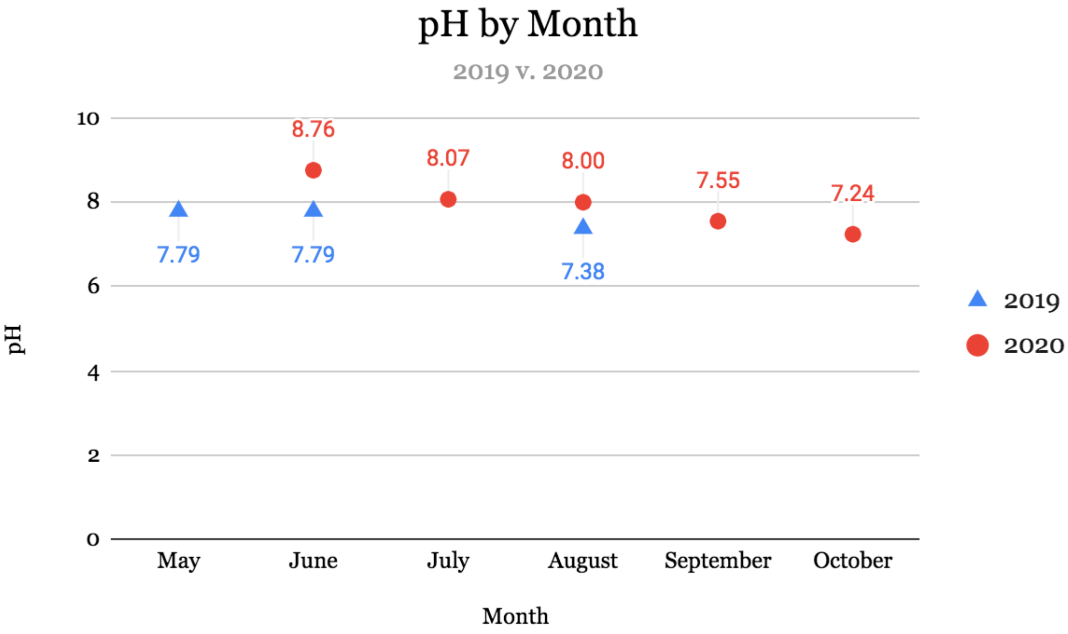


Figure 2.5: pH levels by month compared between 2019 and 2020.

Variation in Conductivity by Month

No month was significantly different in overall conductivity throughout the 2020 season, though July was higher than all subsequent months and June was higher than October 2019 (Table 2.1) and 2020 had similar trends in conductivity in June and July, though 2019 had slightly higher values in August (Figure 2.6).

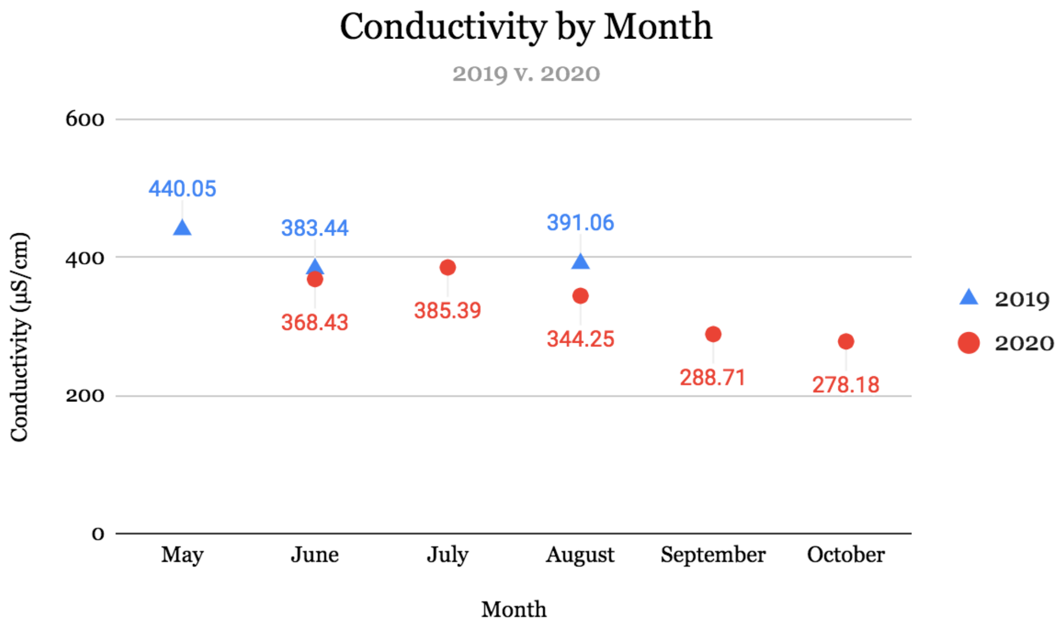


Figure 2.6: Conductivity (µS/cm) levels by month compared between the 2019 and 2020 sampling seasons.

Variation in Turbidity by Month

There was little difference in turbidity by month, though June was significantly higher than August (Table 2.1). Average 2020 turbidity decreased slightly from June to August and then increased again from August to October. The average turbidity was highest in June, at 9.21FNU, and the lowest in August, at 3.66FNU.

Trends in turbidity were very different between 2019 and 2020. In 2019, May had significantly higher turbidity than any other month, with an average of nearly 70FNU (Figure 2.7). Values dropped steeply to their lowest average in June followed by minor increases throughout the season (Figure 2.7). Every single month's average turbidity was higher in 2019 than 2020. This could be attributed to incorrect usage of the sonde, as noted by Lugten et al. (2020) or genuine variations in this water quality parameter.

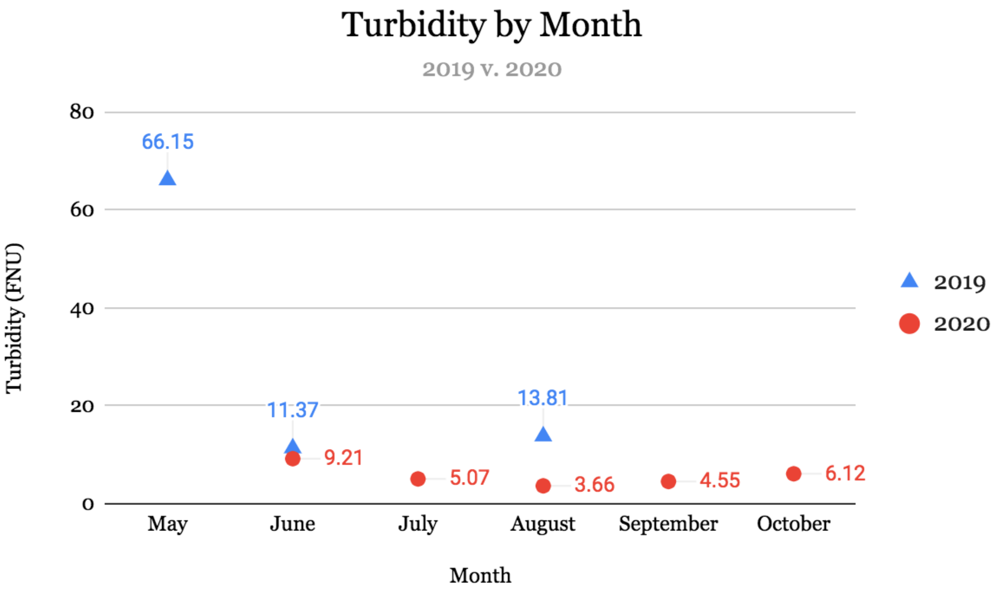


Figure 2.7: Turbidity (FNU) levels by month compared between the 2019 and 2020 sampling season.

Variation by Unit

Significant differences were found between units and in all water quality parameters except for temperature (Table 2.2). Similar trends were observed in 2019.

Variation in Temperature by Unit

There were no significant relationships between temperature and unit in 2020 (Figure 2.8). Average temperatures were very tightly clustered, though MN had the highest average temperature and greatest variation, while MS had the lowest average temperature. Temperature also did not significantly differ between units in 2019.

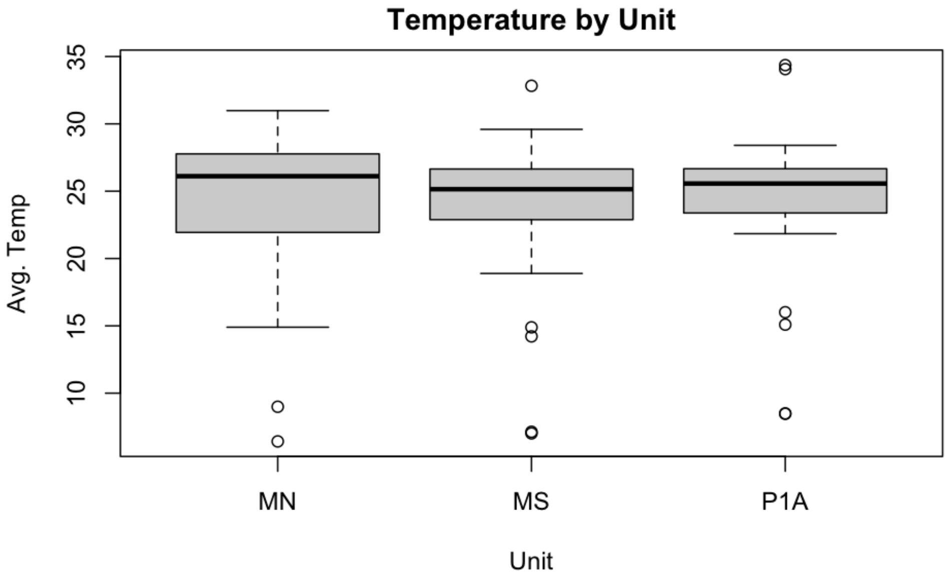


Figure 2.8: Average values of temperature (ºC) by unit. Note that there were no vegetation types that were statistically significant from each other.

Variation in Dissolved Oxygen by Unit

Average DO in MN (9.85mg/L) was significantly higher than MS (6.27mg/L) and P1A (5.42mg/L) in 2020 (Figure 2.9). There was no significant difference between MS and P1A. This result was similar to data in 2019, which revealed DO to be higher in MN than P1A(1.54mg/L) and MS (2.19mg/L).

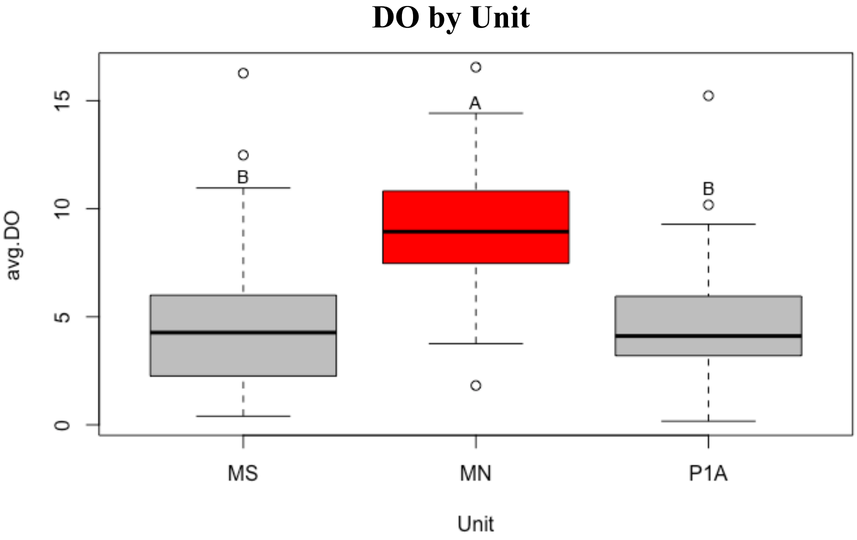


Figure 2.9: DO (mg/L) levels by unit from the 2020 sampling season. The units that share a color and letter are not significantly different from each other.

Variation in pH by Unit

MN had a significantly higher average pH than MS and P1A (Figure 2.10). There was no significant difference in pH between MS and P1A (p-value > 0.01). MN had the highest average pH at 8.58, while MS and P1A had lower, similar pH values. Our findings varied from the 2019 data, which showed MS having a lower average pH than both MN and P1A.

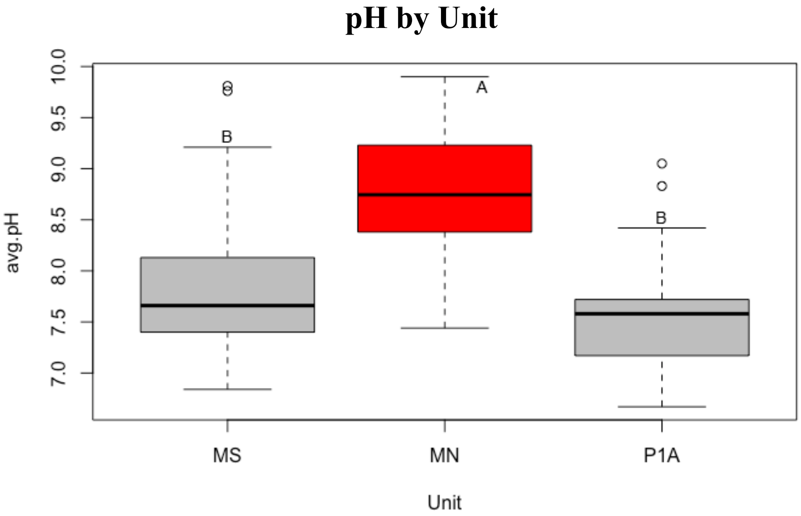


Figure 2.10: Average pH levels by unit for the 2020 sampling season. The units that share a color and letter are not significantly different from each other.

Variation in Conductivity by Unit

All three units had significantly different conductivity levels, with P1A having the highest and MN having the lowest (Figure 2.11). These findings are similar to those from 2019, in that all three units were significantly different from one another and that P1A had a significantly higher conductivity than either of the other units. While MN and MS had similar conductivity levels, MS was the lowest in 2019. In both 2019 and 2020, values for average conductivity in MN and MS were quite similar (300-400μS/cm). P1A had a slightly lower conductivity in 2020 than in 2019 (~425μS/cm as compared to ~475μS/cm), though in both years P1A was substantially higher than either other unit.

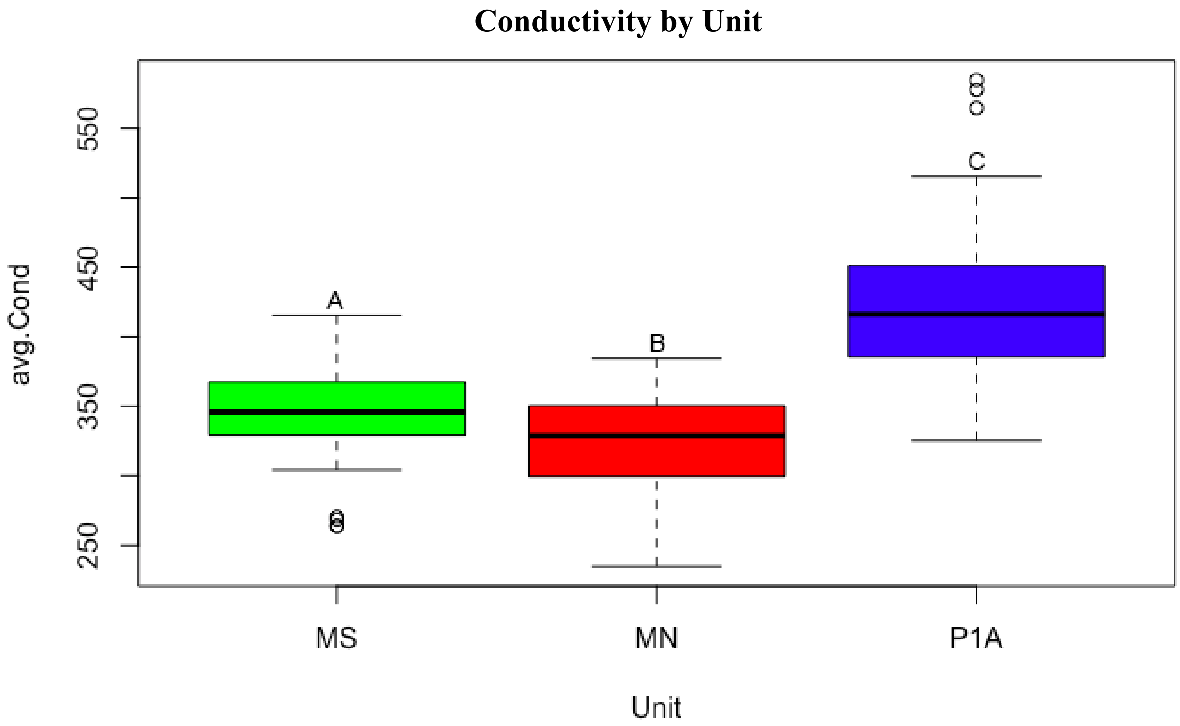


Figure 2.11: Conductivity (μS/cm) levels by unit for the 2020 sampling season. The different letters and colors each represent significantly different conductivity levels.

Variation in Turbidity by Unit

MN had a significantly higher average turbidity than MS (Figure 2.12). MN had the highest average turbidity, followed by P1A, then MS. The average turbidity across all three units was tightly clustered between 3FNU and 5FNU with nearly all values falling between 1FNU and 10FNU. In 2019, P1A was significantly more turbid than MS (by 21FNU, p-value < 0.009). While P1A was marginally higher than MS in 2020, none of our average turbidity values varied nearly this much. This could be due to errors in sonde usage in 2019, as described by Lugten et al. (2020), or to annual variations in turbidity in this particular unit.

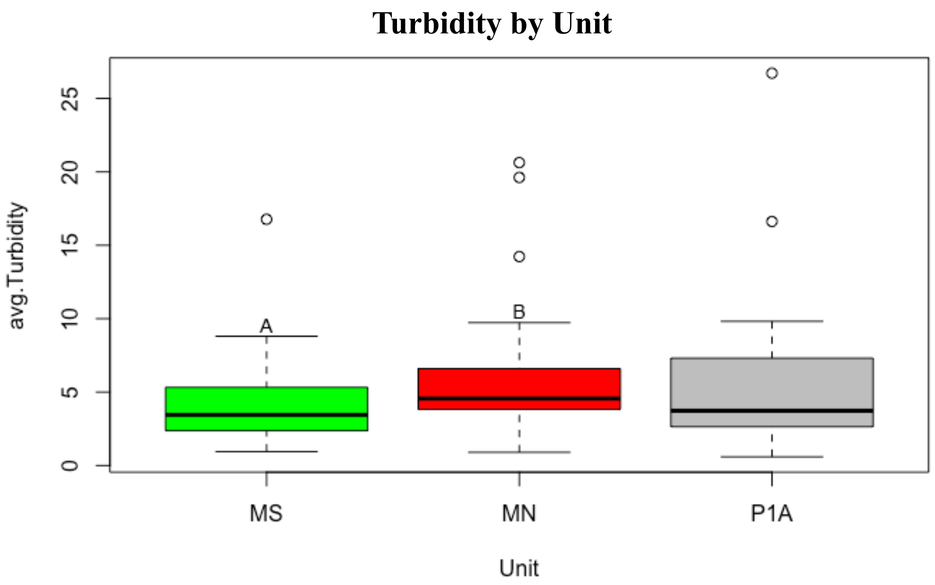


Figure 2.12: Turbidity (FNU) levels by unit. Different letters and colors represent significantly different turbidity levels.

Variation by Vegetation Type

Variation in Temperature by Vegetation Type

There were no significant differences in average temperature across different vegetation types for the 2020 field season (Figure 2.13, Table 2.2). This differed from the 2019 season, for which data showed *Salix* to have significantly lower temperatures (p-value < 0.001) than *Typha*, flooded forest, SAV, and *Nymphaea* (Lugten et al. 2020). Our data show that, while there were no statistically significant differences in temperature across vegetation types, the flooded forest had, on average, the lowest temperature, with *Salix* being the next lowest, but only marginally so.

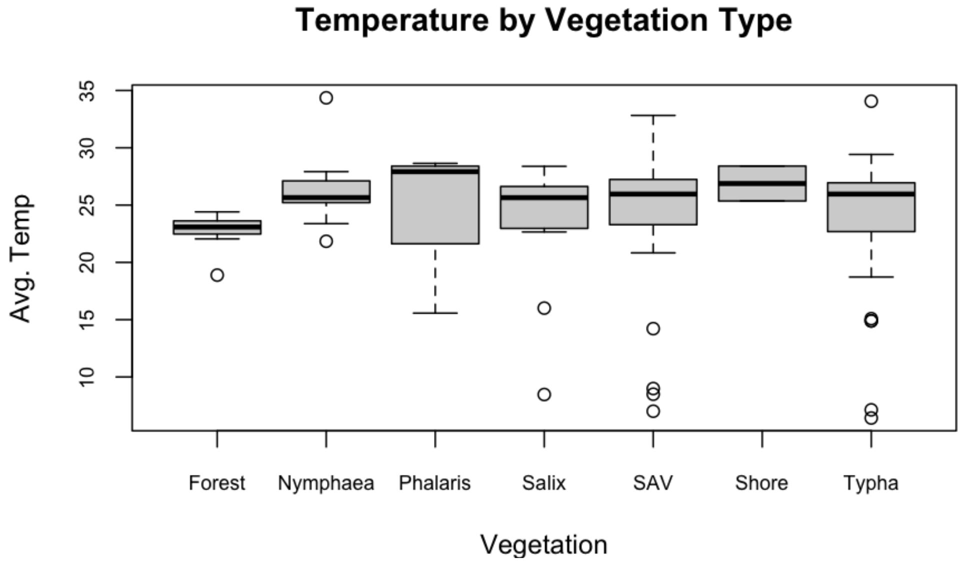


Figure 2.13: Temperature (ºC) by vegetation type. Note that no vegetation zone was statistically considered an outlier compared to all other zones, though there was significant variation between zones.

Variation in Dissolved Oxygen by Vegetation Type

There were several differences in DO between individual vegetation types in 2020 (Figure 2.14). Overall, *Phalaris* had the highest DO and was significantly different from all vegetations, except for the shore. This is likely because *Phalaris* was always found very near the shore when sampling this vegetation type. Based upon average DO levels for *Phalaris* and shore, it seems unusual that these vegetation types are not statistically different (Figure 2.14). We attribute this to the fact that the shore vegetation class was a rare sampling event, making this vegetation type difficult to compare statistically to all others.

It is important to note that *Phalaris* was sampled in different locations for water quality sampling than in vegetation sampling. *Phalaris* was found in shallow water during water quality sampling, while during vegetation sampling *Phalari*s was only found on shore. There was no significant difference between DO and vegetation type in 2019, which could be attributed to simple yearly variations in water quality parameters. Forest had the lowest average DO and was significantly different from SAV and *Typha*, in addition to *Phalaris*.

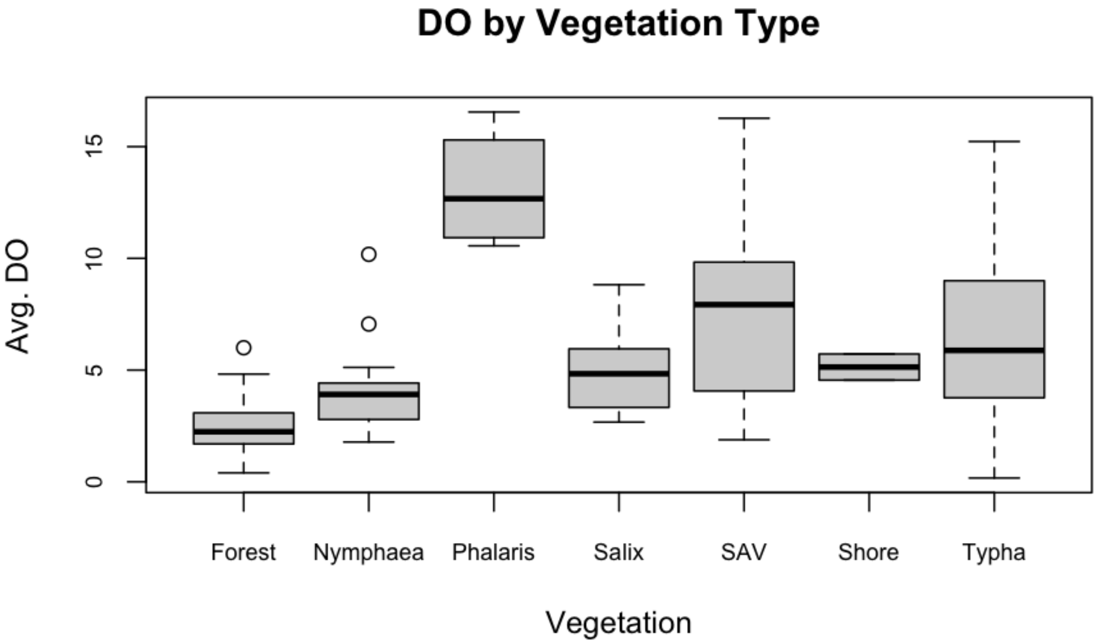


Figure 2.14: Average values of dissolved oxygen (mg/L) by vegetation type. Note that no vegetation zone was statistically considered an outlier compared to all other zones, though there was significant variation between zones.

Variation in pH by Vegetation Type

There were no outlying vegetation types in average pH, in that no single type was significantly different from all other types (Figure 2.15). However, there were many individual differences amongst vegetation types. As was the case for DO, *Phalaris* had the highest pH and forest had the lowest. *Phalaris* had a significantly higher pH than forest, *Nymphaea*, *Salix*, and *Typha*. Once again, based upon results it seems unusual that shore is not classified as significantly lower than

Phalaris (Figure 2.15). We attribute this to the fact that the shore vegetation class was a rare sampling event, making this vegetation type difficult to compare statistically to all others. The forest had a significantly lower pH than *Phalaris* and SAV. SAV, which had the second highest pH, was significantly higher than both *Nymphaea* and *Salix*. *Typha* did not significantly differ from any vegetation type in 2020, but was on average lower than SAV, as was the case in 2019. *Typha* had a higher average pH than *Salix*, which is very different from 2019.

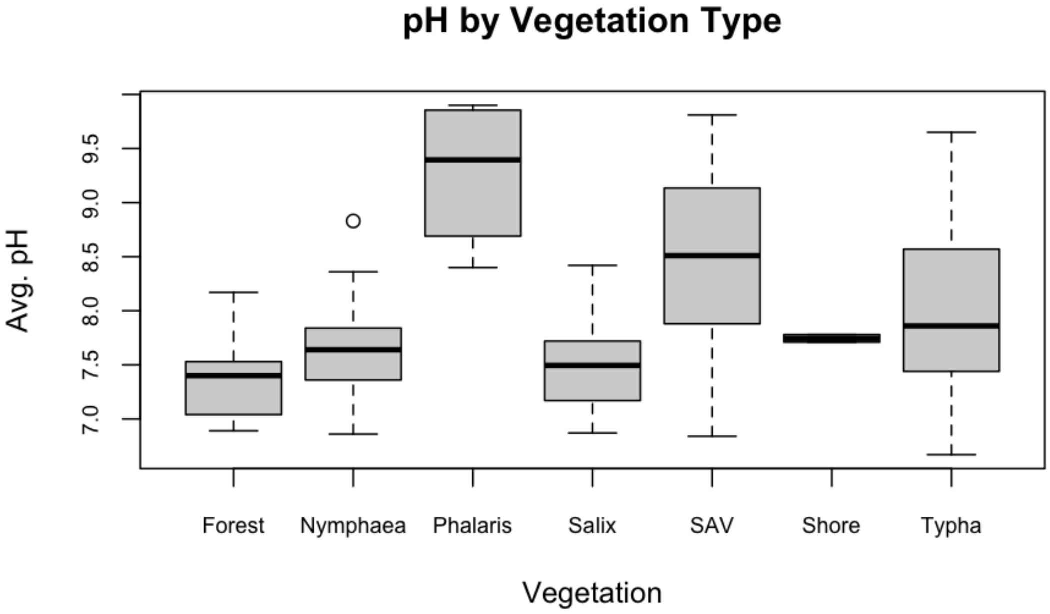


Figure 2.15: Average values of pH by vegetation type. Note that no vegetation zone was statistically considered an outlier compared to all other zones, though there was significant variation between zones.

Variation in Conductivity by Vegetation Type

There was no single vegetation type that was significantly different from all others in conductivity and there were no outlying vegetation types (Figure 2.16). SAV had a significantly lower conductivity than *Salix*, *Typha*, and *Nymphaea*, and *Salix* was significantly higher than forest. In 2019, *Salix* was also significantly higher in conductivity than forest, though it was also higher than *Nymphaea*, SAV, and *Typha*.

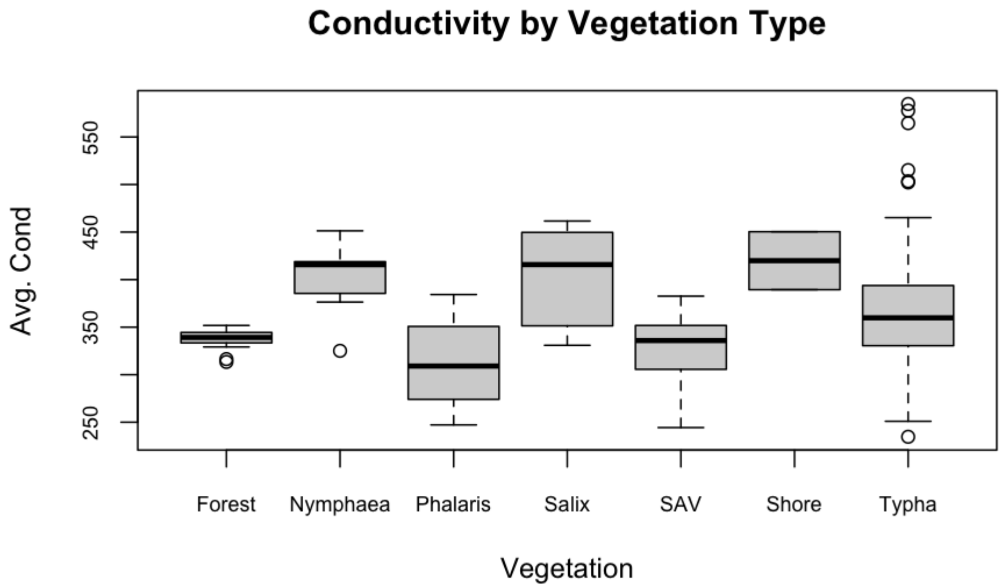


Figure 2.16: Average values of conductivity (µS/cm) by vegetation type. Note that no vegetation zone was statistically considered an outlier compared to all other zones, though there was significant variation between zones.

Variation in Turbidity by Vegetation Type

There were no significant differences in turbidity across different vegetation types for the 2020 field season (Figure 2.17, Table 2.2). This differs from the 2019 season, during which data showed that turbidity was significantly higher in *Salix* than in forest, *Nymphaea*, SAV, and *Typha* zones. Our data shows that turbidity was, on average, highest in *Salix*, though marginally so. This repeating trend across years is likely due to the difficulty of taking sonde readings in the very shallow and sediment-rich stands of *Salix* and not a genuine difference in turbidity across vegetation types.

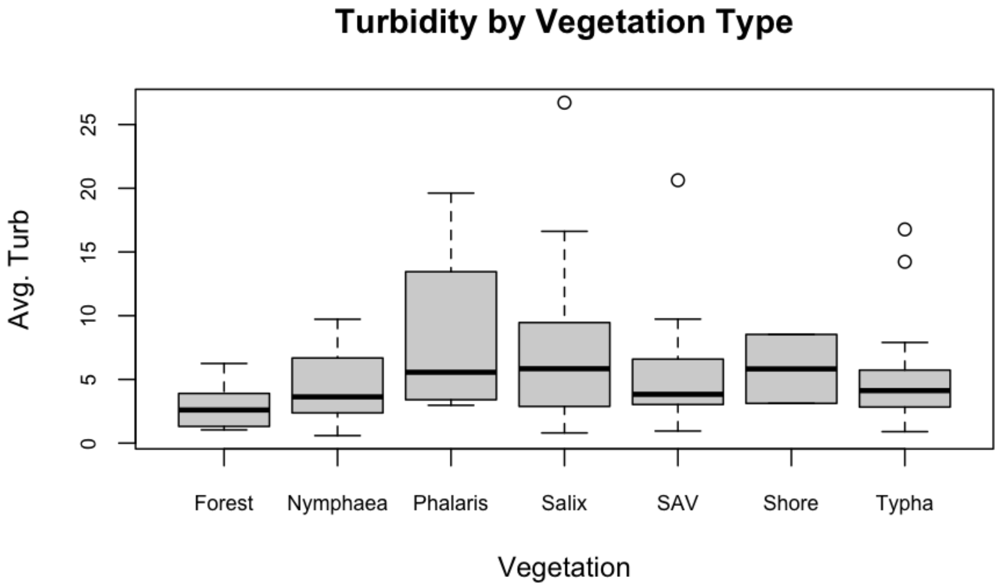


Figure 2.17: Average values of turbidity (FNU) by vegetation type. Note that no vegetation zone was statistically considered an outlier compared to all other zones, though there was significant variation between zones.

Nutrient Samples

Between 2019 and 2020, 43 total water quality samples were sent to the Heidelberg University NCWQR for TP and TKN analysis. All 43 samples were analysed for TP and 28 were further analyzed for TKN. Data from these analyses indicate a minor positive relationship between turbidity (FNU), TP (mg/L), and TKN (mg/L) (Figure 2.18 and Figure 2.19). Of the samples collected during 2019 and 2020, very few were marked by high turbidity.Current results have very low R^2 values, suggesting a poor trendline fit and low correlation between variables.

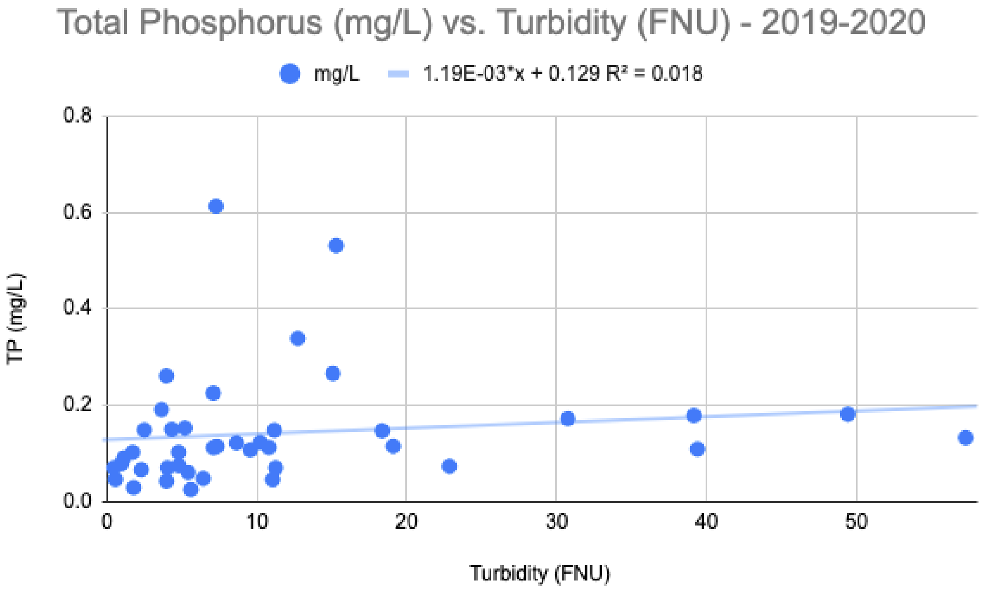


Figure 2.18: Relationship between total turbidity and total phosphorus. As turbidity increased, total phosphorus (mg/L) and total nitrogen (mg/L) slightly increased (R2 = 0.01879, 0.061135 respectively).

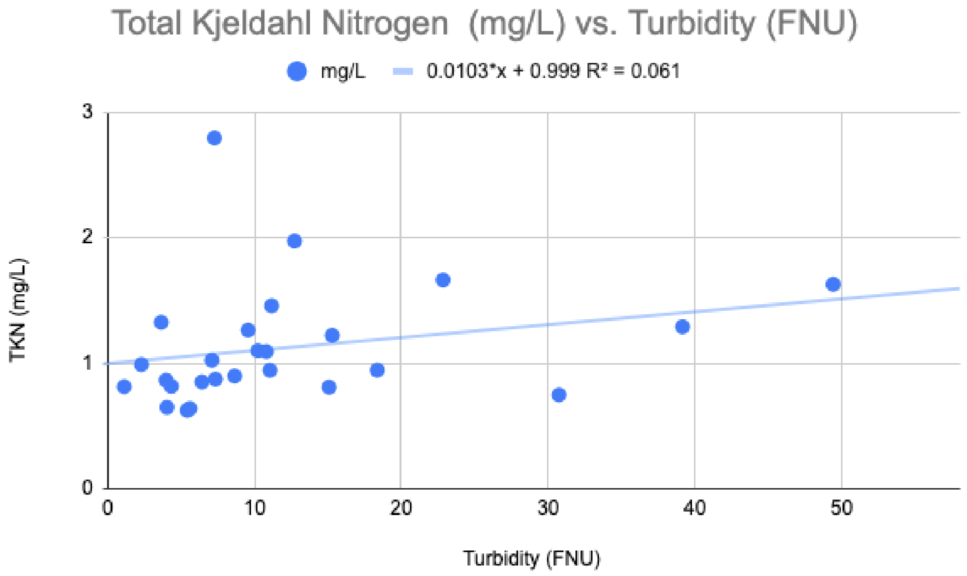


Figure 2.19: Relationship between total phosphorus and total nitrogen. Total phosphorus ranged from 0.025 to 0.612 mg/L. Total nitrogen ranged from 0.639 to 2.795 mg/L.

DISCUSSION

Main Findings Relative to Literature

Dissolved Oxygen (DO)

For wetland ecosystems to support fish and wildlife, they must contain sufficient DO (Kowalski et al. 2014). Certain threshold levels of DO are required to sustain particular species of fish. In Michigan, 6mg/L is considered an ideal level for cold water fisheries. For warm water systems, such as those in SNWR, 5mg/L is considered ideal for intolerant and sensitive species and 4mg/L is adequate for tolerant species (Environmental Protection Agency 1972). The hypoxic threshold for Great Lakes coastal wetlands is considered 3mg/L, and prolonged levels of DO beneath this threshold can result in fish die off events or exclusion of fish from an environment (Kowalski et al. 2014).

Generally, as temperature increases, DO concentration decreases (EPA 2021). We found this trend to be supported by our data, with the exception of the months of June and October. In June, the average DO was 9.47mg/L, whereas in July, the average DO levels had dropped to 5.54mg/L across units before gradually increasing from August through September. This heightened DO in June was unexpected, especially compared to July, since the average temperature in June was slightly higher than in July, at 26.94ºC and 26.22ºC respectively. We suspect that this unexpected finding is due to the Edenville Dam (on the Tittabawassee River) failure on May 19, 2020, which caused massive flooding at SNWR and the surrounding areas. In some areas, the refuge's floodplain units took on upwards of ten additional vertical feet of water. Floodwaters entering the refuge in May likely had increased levels of DO due to aeration from the river and velocity of the flood (Munn et al. 2018). This influx of aerated water resulted in higher DO levels in the floodplain units in June (Lyman 1944; Cherry 2011), even though average temperatures were high, which we would expect to correspond with lower DO. The flood would likely also have introduced a large volume of dead organic matter, the decomposition of which uses DO from the floodplain units (Liu et al. 2016). We hypothesize that the decrease in DO in July was due to stabilization of the refuge ecosystem, as decomposition of flood-introduced organic matter increased from May to July, with a lag in June. We believe that a combination of the necessarily high water temperatures in July and the rates of decomposition at that time likely accounts for the large and sudden decline in DO between June and July.

As temperature decreased between July and October, the concentration of DO increased, as was expected (EPA 2021). DO levels declined again marginally in October, which was the coldest month of sampling. This was unexpected, but could be due to random seasonal variations within floodplain units. As monitoring efforts continue into the future, patterns of variation for this system should become apparent.

Trends in DO seemed different between 2019 and 2020. However, due to differences in sampling timelines and methods, the two years are not easily compared. June and August are the only overlapping months with recorded DO values and are therefore the only months we can directly compare across years. In both cases, 2020 DO values were 2-3mg/L higher than in 2019, and water temperatures were about 3℃ higher than in 2019. This is also unusual, as it would be expected that 2019 DO levels would be higher than in 2020 given the lower temperature (EPA 2021). This could potentially be explained by water levels in 2019, which were on average shallower than in 2020. The higher levels in 2020 were likely a result of flooding in May. P1A was particularly shallow in 2019, thus allowing for warming of waters. Biological activity in combination with warm, shallow water likely depleted DO.

Water Quality Across Months

As we discussed above, we observed an unusual relationship between temperature and DO between June and July. Again, we believe the May flooding to be the most likely significant factor in DO levels between June and July, as the flood likely introduced DO-rich waters (Lyman 1944; Cherry 2011; Liu et al. 2016). The unusual trends in temperature and DO between June and July likely reflect the ecosystem stabilizing after the flood. Additionally, in June, the average DO was 3mg/L higher than 2019, and it was 2.08mg/L higher in August 2020 than in 2019. As described in the above DO section, this is unexpected, since average water temperatures in these months were higher in 2020 than in 2019. Since there was no flooding event in 2019, we attribute the difference between years to increased DO input from floodwaters (Lyman 1944; Cherry 2011).We believe higher water temperatures in 2020 were likely a direct result of increased monthly air temperatures between 2019 and 2020. Average air temperatures for 2019 were 18.8℃ and 20.8 ℃ for June and August respectively, while in 2020 temperatures were 20.9℃ and 22.1℃ for June and August ("Climate" *National Weather Service* 2021).

Freshwater lakes and ponds usually have a pH of 6-8, depending on the underlying soil and bedrock, and pH in deeper water is typically lower (6.5-7.5) than in shallower water (Fondriest Environmental 2013). In our study units the average pH from June to October was between 8.76 and 7.24. While ideal pH levels for fish are 7-8, most fish can adapt to the pH level for their environment (6.0-9-0) (Fondriest Environmental 2013). We recorded our highest average pH levels in June, with a steady decline as the season progressed. pH in June was significantly higher than in all other months, which may be explained by an increase in primary productivity resulting from the May 2020 flooding, since pH tends to increase as primary productivity increases (Rowe et al. 2020). The floodwaters would have introduced nutrients, like nitrogen, from surface and agricultural runoff to the floodplain units, increasing primary productivity and driving the average pH level up in June, the month immediately following the flood. As flood-introduced nutrients were used up and the ecosystem stabilized after flooding, pH steadily declined throughout the season, as reflected in our findings.

The highest turbidity values for all units were measured in the month of June, for which turbidity was found to be significantly different from August. We suspect that June had the highest values because of May flooding, which likely agitated the bottom sediments and introduced sediments from upstream and surrounding area runoff, increasing turbidity. The significance between June and August may be attributed to the sediments finally settling in the units. However, this would not explain why September and October were not significantly different than June.

Vegetation and Water Quality

Overall, *Phalaris* had the highest average DO levels throughout the whole summer and was significantly different from all vegetation types, except for the shore. We suspect that the high DO levels in *Phalaris* zones can be explained by the high surface cover and high density roots of *Phalaris* vegetation, which release oxygen into the water (Rehman et al. 2017). The forest zone had the lowest average DO and was significantly different from SAV, *Typha,* and *Phalaris*. We suspect this is because the forest grows in areas with shallower water and high inputs of allochthonous carbon, the decomposition of which uses up DO and decreases the overall oxygen levels in the water (Spieles & Mitsch 2003).

Phalaris had a significantly higher pH than the flooded forest, *Nymphaea*, *Salix*, and *Typha*. Water depth in the *Phalaris* zones sampled was notably low, and the area of *Phalaris*-dominated habitat shrank substantially between 2019 and 2020 (Personal Communication with Eliza Lugten 2020). We were unable to find any feasible explanation in the existing literature for why there may be this difference in pH between vegetation zones in 2020. It may be that this difference is due simply to annual variation, however, we recommend that future teams look for any trends in *Phalaris* water quality data. In MS, the flooded forest vegetation zone had a significantly lower pH than *Phalaris* and SAV. This difference is most likely due to the soil composition in forest zones, which can have higher acidity due to leaf material detritus from trees (Ovington 1953).

SAV areas sampled had significantly lower conductivity averages than *Salix*, *Typha*, and *Nymphaea*. Conductivity in water reflects presence of inorganic dissolved solids and increases as temperature increases (EPA 2012). Given that SAV was typically a dense vegetation type in deeper water than others, it makes sense that temperature, and therefore conductivity, would be lower than in vegetation types that are less dense or that dominate shallow waters. Additionally, *Salix* had a significantly higher conductivity than the flooded forest. Once again, this is to be expected, as *Salix* bushes were dominant in very shallow and relatively warm waters, while the forest has slightly deeper water that is almost entirely shaded by the dense canopy cover of mature trees. This is supported by the fact that the forest had the lowest average temperature of all vegetation types, despite there being no statistically significant differences in temperature across vegetation types.

Unit Comparisons

While average temperatures were not significantly different across units throughout the 2020 season, MN had higher levels of DO than both MS and P1A (Figure 2.9). These findings were consistent with those of the 2020 UM-Shiawassee Master's Project team, who attributed these differences to variations in depth (Lugten et al. 2020). However, our data showed MN and MS to have similar depths; both of which were deeper than P1A. For this reason, we believe that depth may not be the most reasonable explanation for DO levels. A possible explanation for lower DO in MS may be the high presence of algae in this unit, as a higher level of bacteria may lead to decreased DO (USGS 2021). Algal presence alone cannot explain such low values, however, as MS had the lowest values of DO in 2019 as well, when there was significantly less algae present (Personal Communication with Eliza Lugten 2020). Another likely reason for lowered DO in MS is the presence of the flooded forest. In the forest, tall trees create large subsidies of allochthonous carbon in the form of leaf litter. The decomposition of these leaves and detritus greatly reduces diel DO (Spieles & Mitsch 2003). Since the flooded forest is one of only three sampling locations in MS, and because this vegetation type had the lowest average DO of any vegetation type across units, it is logical that the unit may have a reduced DO overall.

P1A had significantly higher conductivity levels than MS and MN (Figure 2.7). Conductivity is a measure of ions in the water, so higher levels of conductivity in P1A are likely explained because P1A has been consistently connected to the Shiawassee River since 1958 (Lugten et al. 2020). This connection makes P1A more responsive to fluctuations in the Shiawassee River, which experienced conductivity levels between 550 to 750µS/cm during our sampling period (USGS 2021). Previous studies at SNWR found conductivity levels in the Shiawassee Flats area ranging from 153 to 977µS/cm (Buchanan et al. 2013), with similar results found at the restored wetlands at Crane Creek, where conductivity ranged from 106 to 1004µS/cm (Kowalski et al. 2014). The values we found were consistent with these and are considered typical of the region (Lugten et al. 2020). Additionally, the conductivity in MS was significantly higher than that of MN. This differs from 2019, when MN was significantly higher than MS. This difference may be due to annual variations or residual consequences from the May 2020 flooding. Continued research and monitoring are needed to determine what may be causing differences between these two similarly aged units.

The only significant difference in turbidity between units was between MN and MS; with MN being significantly higher than MS throughout the sampling season. These two units had similar control measures throughout the season and both were closed to the Shiawassee River for almost the entirety of our sampling season. The difference could simply be due to human error in sampling technique (taking sonde measurements in a plume of sediments generated by movement of the researcher), as was the case during the 2019 season (Lugten et al. 2020). However, we made an effort to take water quality measures out of the boat in MN in order to reduce a plume. For this reason, we think the higher turbidity in MN is not likely due to human error in 2020. This increased MN turbidity could also be due to the greater presence of bottom-dwelling Common Carp *Cyprinus carpio*, which we noticed more frequently in MN during casual observation and recorded as having a slightly higher abundance in MN than in MS. Though Common Carp are known to increase the turbidity of their habitats (Chumchal 2002), we did not record a significant presence of this species in our fish fyke net sampling efforts in either MS or MN. Therefore, the significance of the effect of Common Carp on turbidity in these units is inconclusive.

MN and MS are both much younger than P1A and have followed almost the same restoration and reconnection schedule since their completion in 2016. For this reason, we expected these two units to be the most similar. However, our data suggests that MN and MS are two very distinct units. This finding is in line with that of Lugten et al. (2020). Given that both units have similar vegetation structures and depth, additional research is needed to determine what makes these two units so distinct. MN was first connected to the Shiawassee River in 2017, while MS was not connected and flooded until one year later. It is possible that this one-year age difference is responsible for large differences in water quality, given how early these units are in the restoration process. Additionally, currently unstudied characteristics, such as the underlying soil structure of these units, could be contributing to these differences.

Turbidity-Phosphorus and Turbidity-Nitrogen Analysis

In previous studies utilizing turbidity-phosphorus analysis in hydrologically reconnected floodplain units at Crane Creek in Ohio, samples ranged from 1FNU to nearly 800FNU (Baustian et al. 2018). The average sample was less than 200FNU, but values between 400-600FNU were common in this study. By contrast, our highest turbidity sample was just under 60FNU, and we had a far smaller sample size than that of Crane Creek. While our samples display a slight positive trend of increasing TP as turbidy goes up, further study is needed to determine if any positive relationship exists between these variables at SNWR.

Future turbidity-phosphorus and turbidity-nitrogen analysis will require that more samples be sent to an outside laboratory for analysis. We recommend that teams in the future make special efforts to send as many high turbidity samples for testing as is possible. We were unable to do this in 2020 as a result of limitations due to COVID-19. We recommend that future teams make an effort to sample immediately after any major storm events and from areas with high flow, such as rivers or control structures. Storm events typically increase surface runoff into aquatic systems, which often includes dissolved nutrients like phosphorus and nitrogen. Turbidity, which can also increase after storm events due to sediment input from runoff, is therefore often associated with increases in phosphorus and nitrogen concentrations (Baustian et al. 2018). Consequently, consistently recording turbidity after storm events will help researchers determine the extent to which SNWR acts as a sink for limiting nutrients in the system. This can be useful in assessing the role of SNWR in improving water quality in the region by reducing nutrient loading into local waterways, since the vegetation and topography of the refuge helps pull dissolved nutrients from the water column (Baustian et al. 2018). Additionally, it is possible that SNWR plays a role in intercepting nutrients that could otherwise cause harmful algal blooms in the Saginaw Bay region of Lake Huron (Baustian et al. 2018).

Study Limitations

Water quality analysis during the 2020 field season was primarily limited by the COVID-19 pandemic and severe flooding in May 2020. Though sampling would ideally have started in May, we could not begin until July. Additionally, we were unable to stay on the refuge premises overnight during the field season due to USFWS pandemic restrictions. For this reason, the sampling schedule for all aspects of field monitoring were greatly truncated. Heidelberg University's NCWQR was also greatly affected by the pandemic, and this at times delayed scheduling of sample shipments to the laboratory.

We acknowledge that our movements through sampling sites disturbed sediments and likely impacted turbidity measurements by the sonde on occasion. Though we made an effort to reduce disturbance, it was sometimes impossible to escape the sediment clouds created by our movements. Consequently, we had some turbidity readings that likely far exceeded the true level, resulting in some inaccurate data. We avoided sediment clouds by sampling from a boat whenever possible.

Finally, use of the data recording platform Survey123 in the field sometimes resulted in numerical errors, though we reviewed all data entries to minimize these inaccuracies (Esri Inc. 2021). Survey123 sometimes malfunctioned and moved the decimal point of a numerical input, for example changing "7.43" to "743000.00". To our knowledge, these glitches were infrequent. We tried to correct these errors in the field whenever possible and also looked for them while organizing our data prior to statistical analysis. However, we acknowledge that we may have missed some of these errors or corrected them based on incorrect assumptions (for example, we may have changed the above error to "74.3" instead of the truly correct "7.43").

Management Implications

It is critical that SNWR closely monitor DO levels throughout future field seasons, as DO can have a significant impact on fish communities throughout the floodplain units (Kowalski et al. 2014). Compared to 2019, DO levels in 2020 were higher on average, though we suspect that these levels still dropped significantly during the diurnal cycle (Kowalski 2014). This is somewhat surprising, given that water temperatures were on average higher during 2020, and higher water temperatures are inversely associated with DO levels (Lugten et al. 2020). Logically, the most dominant species of fish at the refuge were those able to acclimate to fluctuating DO levels like Black Bullhead and several tolerant species of sunfish *Lepomis* (Rose

2006; Stuber et al. 1982; Jordan et al. 2009). It is still unclear whether species that are not able to survive the shifting DO levels throughout the study units are killed off by the DO levels, outcompeted by fish that can acclimate to the DO levels, or if (in the case of connected units) they evacuate the floodplain units, possibly to the Shiawassee River. In order to verify fish movements, particularly in and out of SNWR floodplain units, greater use of the ARIS sonar camera could help SNWR create a more detailed picture of how various fish species at SNWR respond to DO throughout the year as well as over a 24-hour period.

Understanding how flooding impacts water quality and community structure within floodplain units will also prove to be highly beneficial to refuge staff in the future. Given the newly increased connectivity of SNWR's wetlands to each other and the local river system, flood water will likely inundate units more frequently. It is expected that historic flooding events will occur at more frequent intervals as climate change intensifies (Byun et al. 2019). Our data from 2020, immediately following a historic flood and water infrastructure failure, will provide context to how such an event may influence water quality parameters and community structure. This year of data will act as a benchmark for comparison of water quality between flood years and non-flood years. In future flood years, SNWR will ideally be able to establish trends in how water quality and community composition may shift during a flood and will allow refuge staff to develop more specialized management plans for such an event.

Recommendations

We recommend that water quality measures continue to be sampled using the same methods as our team did during our field season, with minor modifications. We suggest that sonde readings continue to be taken with every fish fyke net set and macroinvertebrate sampling event to populate data on water quality, as was done in 2019 and 2020. For nutrient sampling, we recommend that samples are sent to an outside laboratory, preferably the Heidelberg University, NCWQR for interannual consistency, at more frequent intervals than were possible in 2020. In 2020, we were only able to send 25 total samples due to COVID-19 restrictions. Of these, all 25 were analyzed for TP, while only 10 samples were analysed for TKN and other nutrient analysis. We recommend that at least one full-panel sample per month (May through November) is collected from each unit and sent for analysis. This can help to inform general health and water quality of floodplain units throughout the year.

For total phosphorus testing, because these data are particularly important in establishing a more accurate turbidity-phosphorus analysis, we recommend that at least 25 additional samples be collected exclusively for phosphorus analysis. These samples should be taken in high turbidity areas throughout the refuge, such as near control structures, high flow channels, and throughout the floodplain units immediately after storm events. Ensuring that these samples are taken from turbid areas should result in a more accurate analysis of phosphorus loading, which can inform the extent to which SNWR may be acting as a phosphorus sink and the strategies for improving the water quality of connected waterways.

In addition to sonde use and nutrient analysis of water samples, we recommend the deployment of DO Loggers and HOBO Depth Loggers throughout the entire field season. We did not thoroughly monitor changing water levels throughout the season, but believe that this could be useful for the analysis of both fish and macroinvertebrate community analysis and general analysis of water quality. In addition, we believe that DO loggers would be valuable to deploy overnight during fish fyke net sets, as they will capture diurnal DO fluctuations over a 24 hour period within floodplain units and over the entire season. Because DO levels are directly related to the temperature and time of day that a reading is taken, these loggers would allow for the most thorough understanding of diel fluctuations of DO levels across time as is possible.

We recommend that future teams keep both physical and virtual logs of water quality data. Given the loss and potential inaccuracies of some 2020 data due to the use of Survey123, it would be best to keep a written record of sonde readings. This may prove to be inconvenient in the field, but will ensure completely accurate water quality data in the future.

Finally, we recommend that future teams do not sample water quality in the vegetation structure that we recorded as ‘shore’. This was a rare vegetation structure, and the inclusion of these data, made statistical analysis of water quality across vegetation types more difficult.

VEGETATION MONITORING

INTRODUCTION

Ecological monitoring at SNWR includes characterization of the floodplain unit vegetation communities, the factors that shape plant community composition, and the influence of vegetation on the ecology of the floodplain units (Lugten et al. 2020). Throughout SNWR, variation in presence of submerged aquatic vegetation (SAV) and emergent coastal wetland vegetation is crucial for the development of spawning habitat and seasonal refuge for macroinvertebrate and fish species of the Great Lakes (De Szalay & Resh 2000; Jude & Pappas 1992). The status of macroinvertebrate and fish communities affects migratory bird communities that feed on various plants, insects, fishes, and other organisms within the refuge ecosystem (Wilcox 2002; Lugten et al. 2020). Since protection of resident and migratory bird species is SWNR's primary mission, understanding the status of these prey populations, and how vegetation affects them, is vital.

RESEARCH QUESTIONS

Our team developed the following research questions to guide our vegetation sampling and data analysis:

- *How does vegetation vary among the three units?*
    - *Which species are present?*
    - *Which exotic species are present, and which invasive species are present?*
- *What is the variation in structure, composition, and abundance of species between each vegetation zone within and among units?*
    - *What are the emergent, submergent/floating species, groundcover, understory, and overstory species?*
    - *How do the floodplain units differ in structural composition?*
- *How does the composition of vegetation change among units and vegetation zones from 2019 to 2020?*
- *What can vegetation tell us about the quality of habitat?*

METHODS

We followed the protocols and methods described by the 2020 U-M Master's Project team (Lutgen et al. 2020). These methods were based on wetland monitoring conducted at Crane Creek coastal wetland complex in northwestern Ohio, which in turn, were based on protocols established through the Great Lakes Coastal Wetlands Monitoring Program (CWMP) (Kowalski et al. 2014; Uzarski et al. 2016).

Field Sampling

From August 18 to August 28, 2020, our team sampled vegetation composition within the three floodplain units: MN, MS, and P1A. We utilized stratified random sampling to capture variation within vegetation zones, both within and among units. Within each unit, we gathered at least ten samples from each vegetation zone. For vegetation types that dominated a particular unit, we collected 15 samples to better capture the true structure of the unit. In the field, we identified plant species with the assistance of SNWR biological technician, Eliza Lugten. Unfamiliar plants, or those at a development stage that made them difficult to identify, were pressed, dried, and sent to Dr. Kurt Kowalski, Research Wetland Ecologist with USGS, for identification. In addition, we used the plant identification mobile application (Glority LLC 2020), though each species identified in this way was cross referenced with a Great Lakes wetland vegetation field guide (Chadde 2012).

Vegetation Zone Delineation

Our team delineated vegetation zones prior to conducting sampling using visual observation and Geographic Information Systems (GIS) maps created by the 2020 U-M Master's Project team. Due to a drone restriction on US Fish and Wildlife (USFWS) property, our team was unable to collect aerial imagery to assist in delineating vegetation zones for our 2020 sampling period. We described vegetation zones based on what our team determined to be the key defining characteristics (e.g., SAV), dominant plant species (e.g., *Typha*), or unique habitat types (e.g., Mudflat) that had the greatest influence on the vegetation present.

Unit	# of Vegetation Zones	Vegetation Zones	Vegetation Zone Area (acres)	Average Depth (cm)	Total Vegetation Zone Area	Total Unit Area (acres)
MS	4	Dead *Typha*	146.11	88	275.11	288
		Forest	26.43	50		
		SAV	86.31	92		
		Typha	16.26	53		
MN	3	*Phalaris*	11.19	0	194.9	222
		SAV	67.17	88		
		Typha	116.54	46		
P1A	5	Mudflat	0.39	54	306.7	322
		Nymphaea	190.94	84		
		Salix	13.98	6		
		SAV	2.72	64		
		Typha	98.67	43		

Table 3.1: Vegetation characteristics across three floodplain units at SNWR.

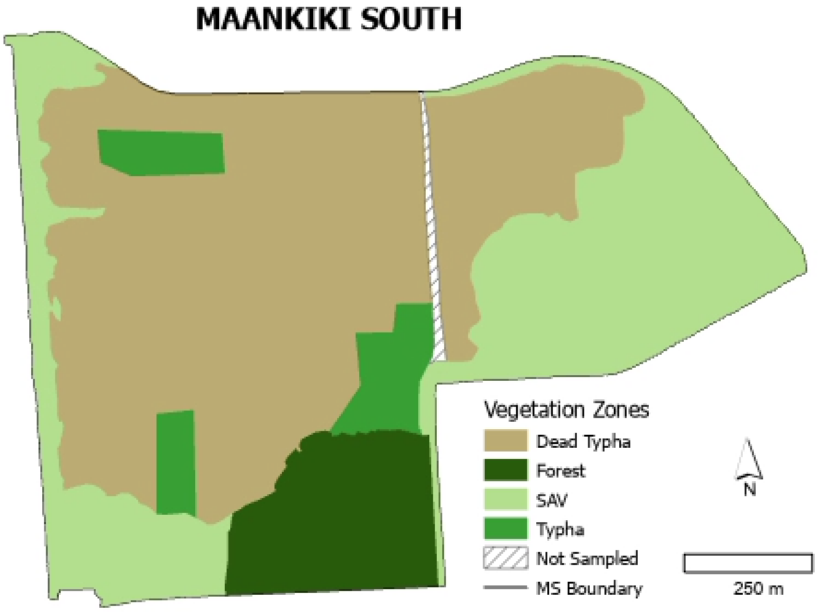


Image 3.1: Maankiki South vegetation zone map.

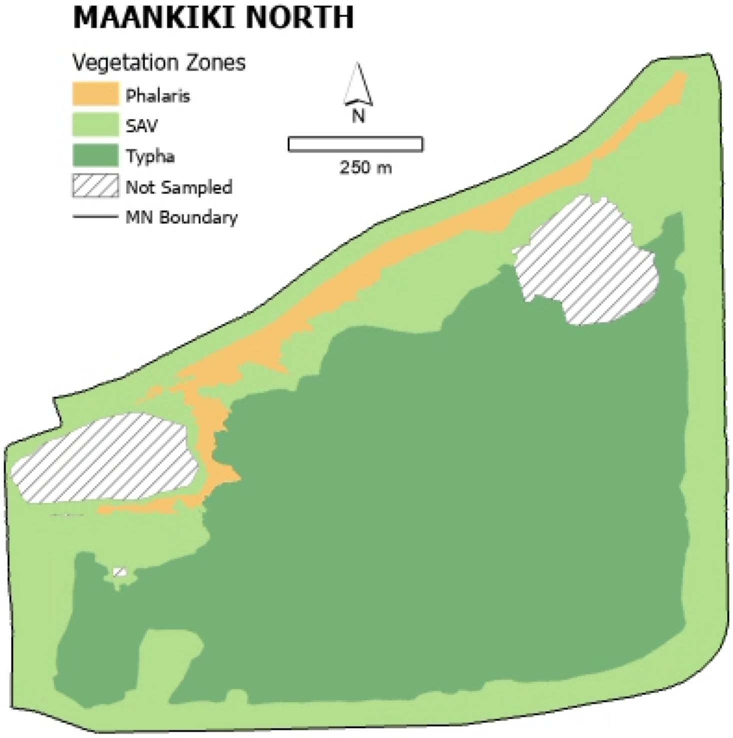


Image 3.2: Maankiki North vegetation zone.

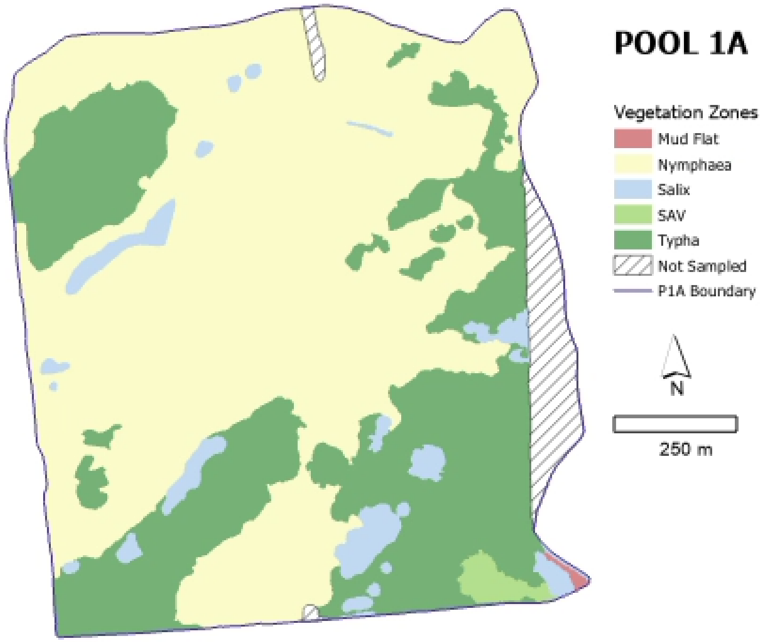


Image 3.3: Pool 1A vegetation zone.

Vegetation Survey

After each vegetation zone was defined, we created a map of each unit. Our team overlaid a grid across the map and assigned each grid cell a numerical identifier. Using a random number generator, we randomly selected 10-15 numbers from every vegetation type in each unit, identified the associated grid cell, and identified the precise on-the-ground area where we would survey within each vegetation zone. To further increase randomization, once arriving at the precise predetermined location, we walked a random number of steps into the area and haphazardly tossed a 1m^2 quadrat overhead to select the exact sampling site. We then sampled within the quadrat, recording the GPS location on an iPad using the Survey123 application (Esri Inc. 2021). We measured the depth of the water in centimeters to the nearest centimeter. If any area was not inundated with water, such as the *Phalaris* zone in MN, depth was recorded as 0cm.

Our team identified plants to the lowest taxonomic level possible in the field, and collectively assessed percent cover visually. To obtain the most accurate percent cover, each individual in our group silently estimated their own percent cover for a given species. We then announced our estimates and took the average of the group. Any unidentified plants were listed on the data sheet as “Unknown sp. #”, key characteristics were described, and then the species was collected, dried, and pressed to be identified later by the team or by Dr. Kurt Kowalski at USGS.
When determining percent cover of vegetation species present, we worked from the top down within the quadrat and water column. Percent cover referred to both vegetation surficial cover

and cover of the water column space within the quadrat's area. We determined and recorded the percent cover of large emergent species like *Typha* sp. first, then recorded floating species on the surface, like duckweeds, and finally assessed the percent cover of submerged species. We followed this sequential process so as not to disturb surface-dwelling species and to ensure we recorded as accurate a percent cover estimate as possible.

To determine the percent cover of shrub and tree species, particularly those encountered in the flooded forest vegetation zone in MS and the *Salix* vegetation zone in P1A, the quadrat was enlarged to either a 3m or 10m radius, depending on the relative size of the understory or overstory species (Figure 3.1). When understory species under 4.6m in height were present, we randomly placed the quadrat following the same protocol previously described and created a circle with a 3m radius, starting from the center of the quadrat. We measured understory species within this circle by taking the diameter at breast height (DBH) using a measuring tape and an arborist's tape. When we encountered an overstory species, we followed the same method, though we created a circle with a radius of 10m from the center of the quadrat.

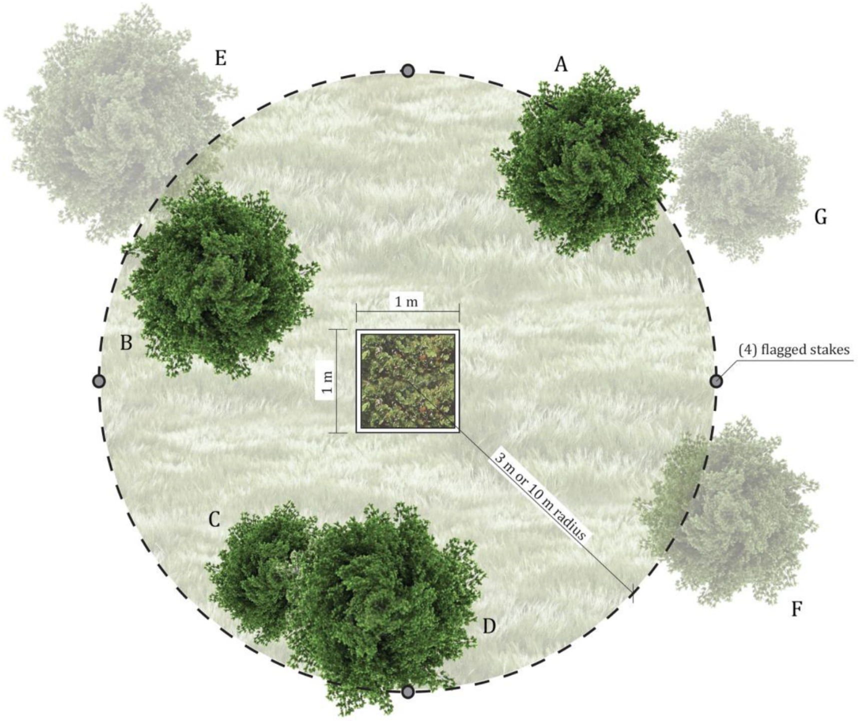


Figure 3.1: Plan diagram of an enlarged quadrat. We sampled the $1m^2$ quadrat in the center for any ground cover species, submerged/floating species, and emergent species. Then, we sampled the enlarged quadrat at 3m or 10m for understory or overstory species. Dead trees were not included. Trees located on or within the 10m boundary (A-D) were sampled. Trees with canopies in the boundary, but with trunks located outside of the boundary were excluded (E-F). Trees completely outside of the boundary were excluded (G). Diagram borrowed from Lugten et al. (2020).

DATA ANALYSES

To comparatively analyze our data and answer our research questions, we calculated Importance Value Indexes (IVI), Non-Metric Multidimensional Scaling ordinations (NMDS) of a Bray-Curtis dissimilarity index, Principal Component Analysis (PCA), Floristic Quality Assessments (FQA), and Indexes of Biotic Integrity (IBI). These tests and assessments address our research questions by indicating a metric of a plant species' importance (IVI) and using that metric to analyze the distribution of species and IVI throughout the refuge using the NMDS and the PCA. The FQA and the IBI highlight concerns of quality, as determined by wetland quality assessments conducted in Michigan in 2014, which are described in greater detail below.

Importance Value Index (IVI)

The Importance Value Index (IVI) is a numeric representation of a species' dominance, influence, or importance in a given measurement area. Our team utilized IVI to describe every species' importance across units and vegetation zones. The IVI is calculated using the following formula from Curtis and McIntosh (1951):

$$Frequency = \frac{\#\ of\ quadrats\ containing\ a\ given\ species}{Total\ \#\ of\ quadrats}$$

$$Relative\ Frequency = \frac{Frequency}{Sum\ of\ all\ species\ frequencies}$$

$$Abundance = \frac{Sum\ of\ percent\ cover\ for\ a\ given\ species}{Total\ \#\ of\ quadrats}$$

$$Relative\ Abundance = \frac{Abundance}{Sum\ of\ all\ species\ abundances}$$

$$Importance\ Value = (Relative\ Frequency + Relative\ Abundance) \times 100$$

After calculating each species' IVI, we used the IVI as the variable representing a given species for both the Bray-Curtis dissimilarity index and the PCA.

NMDS Ordination of a Bray-Curtis Dissimilarity Index

Our team conducted an NMDS ordination of a Bray-Curtis dissimilarity index to plot the relative differences between vegetation zones, based on the vegetation present and the respective IVI scores in a 2-dimensional space. This analysis was completed in R Studio, referencing the command code utilized by the 2020 UM-Shiawassee Team, using the 'vegan,' 'ggplot2,' and 'ape' packages (Lugten et al. 2020). Points plotted closer together indicate similarities, whereas points further apart on the plot show greater differences in plant IVI. Points are plotted against unconstrained axes which are not statistically determined, but are inferred through deductive reasoning, indicating gradients of biotic and abiotic factors that influence the wetland vegetation composition and structure.

Principal Component Analysis (PCA)

The PCA also plots similarities among units, but does so across new axes generated through a statistical analysis separate from the Bray-Curtis analysis. The PCA can be used to analyze large datasets by reducing the dimensionality in the original dataset into the key components, or eigenvalues, that explain the majority of the dataset's differences. The principle components created using this analysis are new variables and therefore do not have a direct correlation with the variables present in the original dataset. To visualize the PCA, we generated a variable correlation plot and a scree plot. The variable correlation plot places the vegetation zones of each study unit on a graph that illustrates the differences between unit vegetation zones by arrow direction and arrow length. The scree plot visualizes how much variance is explained by the eigenvalues in a scree plot format. For our analysis, data consisted of the IVI scores for each vegetation zone across the three study units at the refuge. Therefore, the principle components generated by the PCA analysis do not pertain to the difference caused by any particular vegetation zone or study unit, but are rather an account of a combination of biotic and abiotic factors influencing the distribution of vegetation throughout the study units.

Floristic Quality Assessment (FQA)

The FQA is a tool used to calculate a mean coefficient of conservatism (C), the score representing the deviation from an expected reference condition. The FQA also produces a floristic quality index (FQI), which is an adjusted form of C used for "better comparison between large sites with a high number of species and small sites with fewer species" (Lugten et al. 2020; Herman et al. 2001). The value of C calculated for a given study site ranges from 0-10, with 10 indicating a reference habitat and 0 indicating a highly degraded site.

The Universal Floristic Quality Assessment Calculator can be accessed online at universalfqa.org and is used to automatically calculate C and FQI when plant species for a site have been input (Freyman et al. 2016). We calculated an FQA for each floodplain unit and for the flooded emergent and dry emergent zones of each floodplain unit. Uzarski et al. defined dry emergent sites as quadrats with average water levels less than 1cm (2016). More detailed information on FQA use in Michigan and instructions on using the FQA Calculator are outlined by Herman et al. (2001).

Index of Biotic Integrity (IBI)

The IBI combines the FQA C value, the percent cover, and frequency of invasive species to measure a site's overall vegetation integrity (CWMP 2018). The IBI assigns scores for the total site, the flooded emergent zone (water level >1cm), and the dry emergent zone (water level <1cm). For each of these sites, the IBI takes into account the site's invasive cover, invasive frequency, and the mean conservatism index. The mean conservatism index is calculated by totaling the conservatism score for each species and dividing by the number of species. In addition to these nine metrics, a final metric is added that takes into account the relative cover and frequency of increased nutrient-, sediment-, and turbidity-tolerant SAV species. In sum, these 10 scores produce a ‘Combined Standardized Score’ which consists of a ‘Combined Numeric Score’ and ‘Combined Descriptive Score’. These standardized scores can be compared across sites within SNWR or across other coastal wetlands throughout the Great Lakes region (Lugten et al. 2020).

RESULTS

Species Characteristics Within and Among Units

We observed 67 species of plants throughout the units of study; 65 of which we identified and 2 which were unidentifiable because they were small, undeveloped, and generally lacked defining features (Appendix II). The greatest number of species were identified in P1A (49 species), followed by MN (36 species) and MS (25 species) (Figure 3.2). Each unit contained some unique species identified only in that unit: MS contained 7 unique species, MN contained 9 unique species, and P1A contained 23 unique species. Invasive species were located throughout each unit: 5 in MN, 3 in MS, and 4 in P1A. We did not identify any rare or endangered species based on lists provided by the Michigan Natural Features Inventory (MNFI) rare plant database (2009).

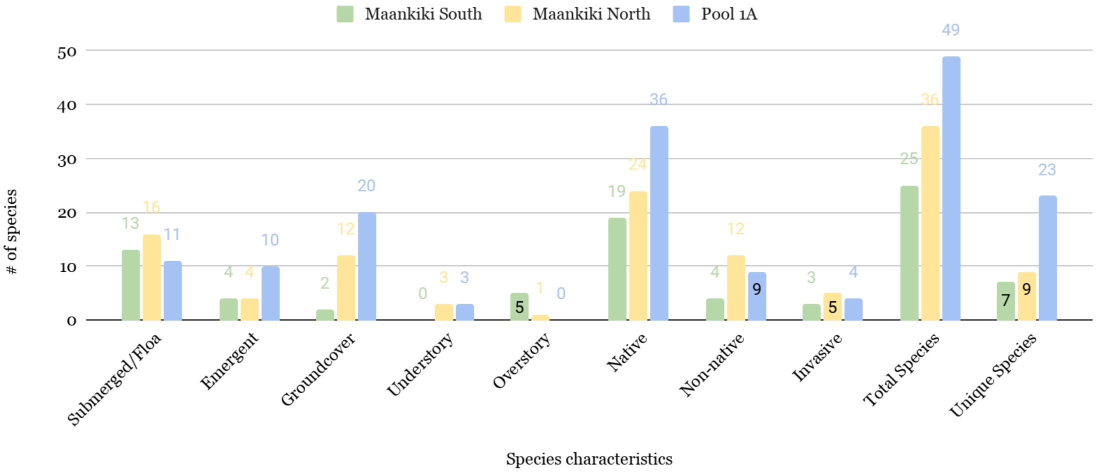


Figure 3.2: Total species counts across units and vegetation structure.

Importance Value Index (IVI)

The IVI for each floodplain unit and vegetation zone revealed the structural dominance of submerged and floating species across the study units (Tables 3.2 - 3.4). Various species of duckweed, like *Utricularia vulgaris* and *Lemna* spp., regularly ranked high in their importance value over other species in a given site. The *Salix* vegetation zone in P1A had the greatest structural complexity (three different structures represented in the top four zones), whereas the remaining zones only had two or a single structure present in the top IVI species. A comprehensive table of importance values can be found in Appendix III.

MAANKIKI SOUTH			
Vegetation Zone	**Dominant Species**	**IVI**	**Structure**
Dead *Typha*	*Utricularia vulgaris*	**53.4**	Submerged/Floating
	Dead *Typha* sp.	**25.3**	Emergent
	Elodea canadensis	**12.9**	Submerged/Floating
	Lemna trisulca	**12.4**	Submerged/Floating
Forest	*Lemna trisulca*	**56.2**	Submerged/Floating
	Lemna minor	**49.8**	Submerged/Floating
	Ceratophyllum demersum	**26.3**	Submerged/Floating
	Spirodela polyrhiza	**25.6**	Submerged/Floating
SAV	*Ceratophyllum demersum*	**39.2**	Submerged/Floating
	Elodea canadensis	**37.8**	Submerged/Floating
	Potamogeton nodosus	**31.3**	Submerged/Floating
	Algae spp.	**30.0**	Submerged/Floating
Typha	*Lemna trisulca*	**71.4**	Submerged/Floating
	Ceratophyllum demersum	**23.3**	Submerged/Floating
	Typha angustifolia	**21.7**	Emergent
	Wolffia spp.	**20.7**	Submerged/Floating

Table 3.2: Importance values for the four most dominant species in each vegetation zone of MS.

MAANKIKI NORTH			
Vegetation Zone	**Dominant Species**	**IVI**	**Structure**
Phalaris	*Phalaris arundinacea*	**103.7**	Groundcover
	Populus deltoides	**16.5**	Overstory
	Cyperus strigosus	**15.2**	Groundcover
	Abutilon theophrasti	**9.5**	Groundcover
SAV	*Ceratophyllum demersum*	**67.0**	Submerged/Floating
	Potamogeton nodosus	**26.5**	Submerged/Floating
	Elodea canadensis	**24.3**	Submerged/Floating
	Algae spp.	**22.8**	Submerged/Floating
Typha	*Typha angustifolia*	**24.8**	Emergent
	Ceratophyllum demersum	**14.4**	Submerged/Floating
	Dead *Typha* sp.	**7.0**	Emergent
	Stuckenia pectinata	**6.2**	Submerged/Floating

Table 3.3: Importance values for the four most dominant species in each vegetation zone of MN.

POOL 1A			
Vegetation Zone	**Dominant Species**	**IVI**	**Structure**
Mudflat	*Lemna minor*	**40.7**	Submerged/Floating
	Eleocharis palustris	**28.7**	Submerged/Floating
	Bidens cernua	**25.0**	Emergent
	Lemna trisulca	**22.2**	Submerged/Floating
Nymphaea	*Nymphaea odorata*	**38.7**	Emergent
	Najas minor	**19.0**	Submerged/Floating
	Ceratophyllum demersum	**13.9**	Submerged/Floating
	Potamogeton nodosus	**1.2**	Submerged/Floating
Salix	*Salix nigra*	**40.8**	Understory
	Spirodela polyrhiza	**17.9**	Submerged/Floating
	Pilea pumila	**17.6**	Groundcover
	Phalaris arundinacea	**16.5**	Groundcover
SAV	*Ceratophyllum demersum*	**42.0**	Submerged/Floating
	Algae spp.	**35.8**	Submerged/Floating
	Elodea canadensis	**34.9**	Submerged/Floating
	Najas minor	**25.0**	Submerged/Floating
Typha	*Typha angustifolia*	**57.4**	Emergent
	Spirodela polyrhiza	**43.8**	Submerged/Floating
	Dead *Typha* spp.	**24.1**	Emergent
	Typha latifolia	**18.2**	Emergent

Table 3.4: Importance values for the four most dominant species in each vegetation zone of P1A.

Invasive Species

Various invasive species were present throughout the study units (Table 3.5). Dominant non-native invasive species included *Typha angustifolia* and *Lythrum salicaria*. The dominant native invasive species was *Phalaris arundinacea.* MN had the greatest number of non-native species (12) and the greatest number of invasive species (5), followed by P1A and MS, respectively.

Invasive Species	Vegetation Zone	IVI	Unit
Butomus umbellatus	*Phalaris*	1.95	MN
Cirsium arvense	*Phalaris*	5.85	MN
Dead *Typha*	*Typha*; Dead *Typha* *Typha* *Typha*	16.35; 25.32 7.02 24.10	MS MN P1A
Lythrum salicaria	*Phalaris* *Nymphaea; Salix*	1.80 0.08; 1.77	MN P1A
Myriophyllum spicatum	SAV; *Typha*	15.01; 0.84	MN
Phalaris arundinacea	SAV; *Typha*; Dead *Typha* *Phalaris* Mudflat; *Salix*	7.48; 1.32; 0.97 103.72 7.30; 16.45	MS MN P1A
Typha angustifolia	*Typha*; Dead *Typha* *Typha; Phalaris* *Typha*	21.69; 0.97 24.84; 1.85 57.42	MS MN P1A

Table 3.5: Chart of invasive species found throughout the floodplain units. Along with each species is listed the vegetation zones and floodplain unit where they were located, and the importance value indexes (IVI) for each species in each corresponding vegetation zone.

NMDS Ordination of a Bray-Curtis Dissimilarity Index

The NMDS ordination of the Bray-Curtis dissimilarity index shows relative similarities and differences in specific vegetation zones among units (Figure 3.3). Vegetation zones plotted closer together are more similar in their plant community compositions than zones that are plotted farther apart. For example, all three units have a *Typha* zone and an SAV zone, and the composition of these six zones are more similar to each other than to any other zone within their respective units (Figure 3.3).

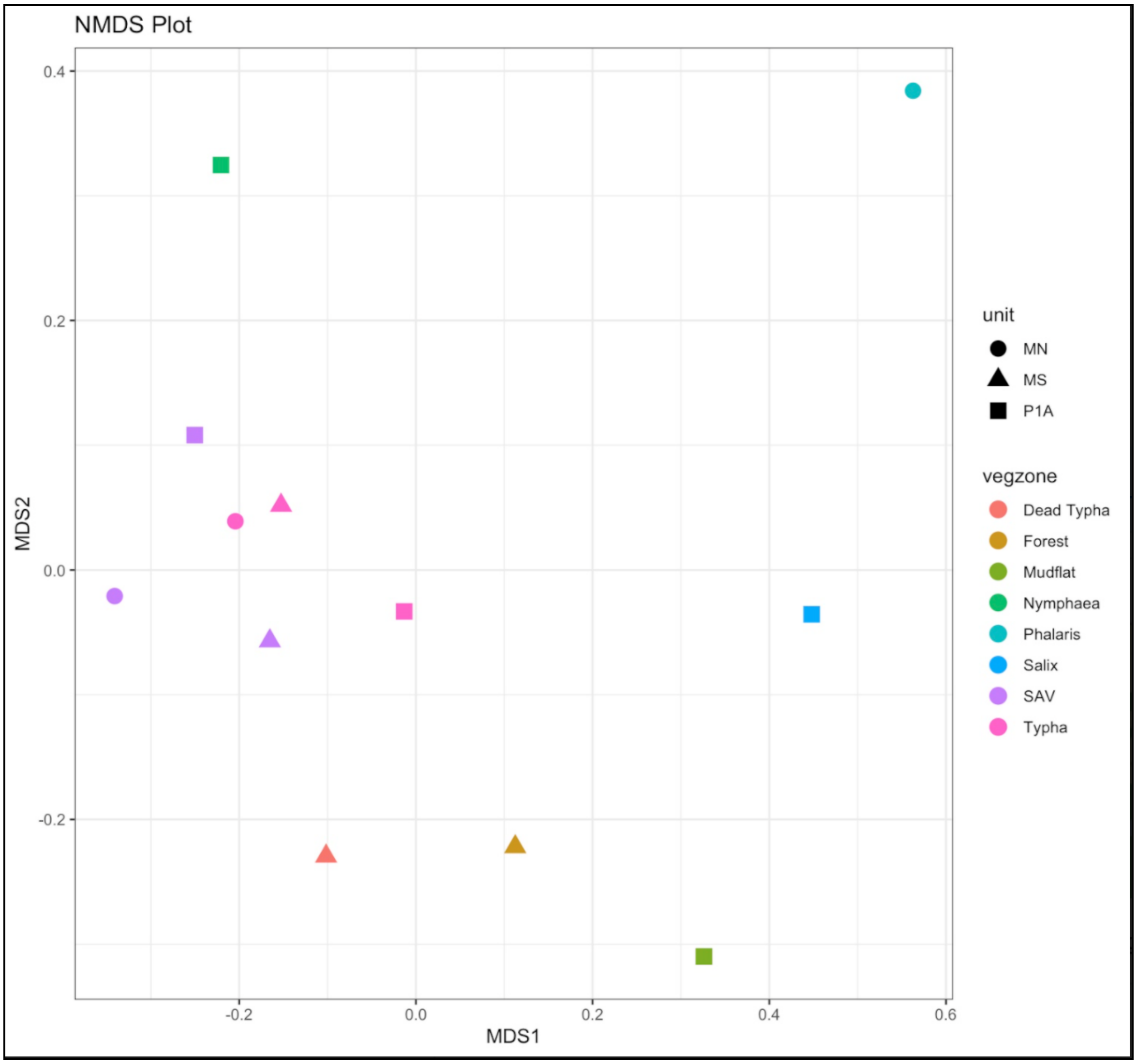


Figure 3.3: An NMDS ordination of a Bray-Curtis Dissimilarity that plots the differences between plant species' IVIs alongside one another. Vegetation zones that are more similar in composition are plotted more closely (i.e., all *Typha* zones and SAV zones). The axes of the plot are

We also plotted the data gathered in 2020 alongside data gathered during the previous sampling season in 2019 in order to compare vegetation similarities across years. Both *Typha* and SAV zones from all sites and years are clumped close together, indicating a high level of similarity (Figure 3.4). The most dissimilar site recorded was the 2019 MS Mudflat, which was defined by receding water levels and hosted a unique combination of plants, including *Populous deltoides* (Eastern Cottonwood), *Sagittaria latifolia* (Broadleaf Arrowhead), and non-native species like *Alisma plantago-aquatica* (Common Water-Plantain) and *Phalaris arundinacea.*

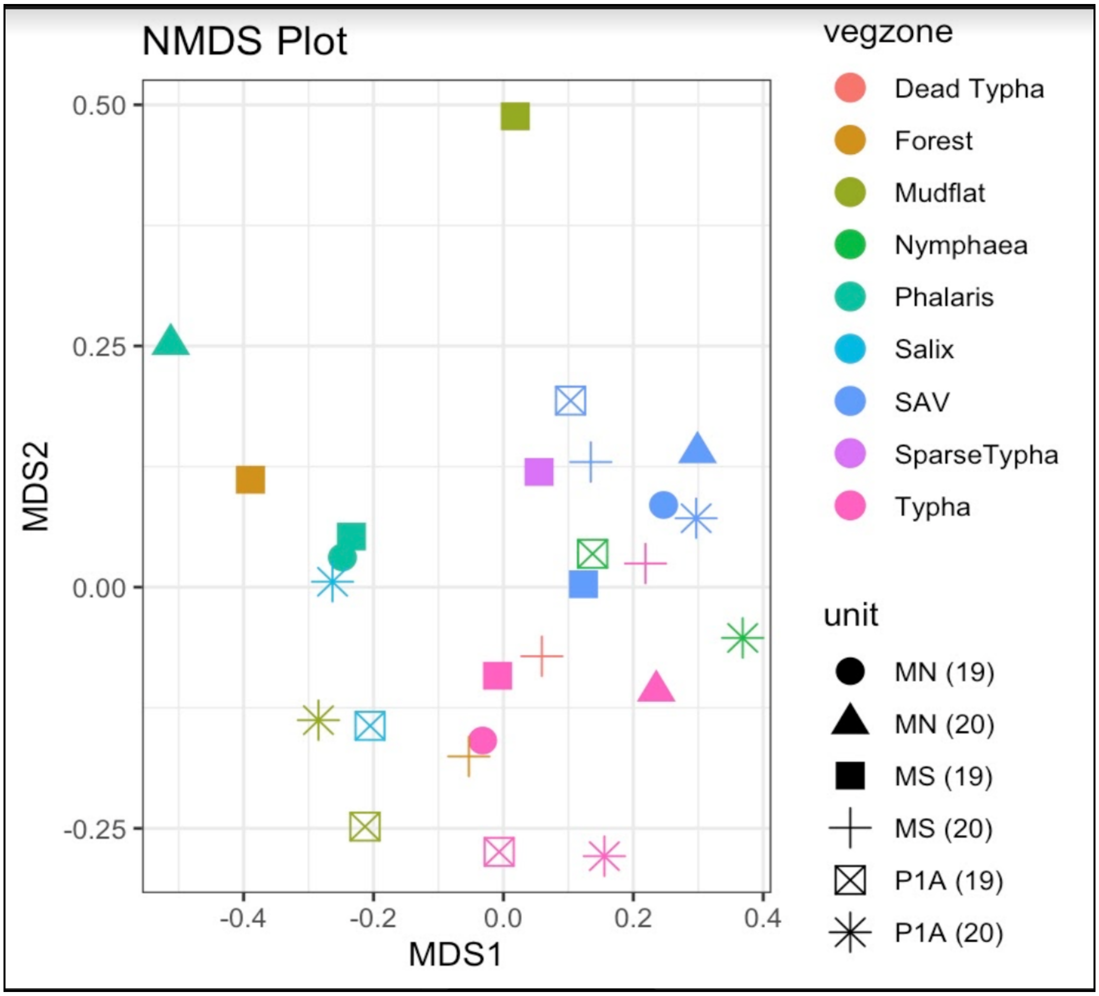


Figure 3.4: NMDS ordination of a Bray-Curtis Dissimilarity, using data from both 2019 and 2020.

Principal Component Analysis (PCA)

Our PCA analyses showed that the P1A *Nymphaea* vegetation zone and the MN *Phalaris* vegetation zone were highly different from one another, because their arrows point in opposing directions (Figure 3.5). In contrast, the SAV vegetation zones of all three study units were clustered close together, showing that each ranked with great similarity in the analysis. The length of the arrows conveys how strong the explanation of a particular vegetation zone and unit combination is, and how distinct that particular zone is from the average vegetation composition. In the plot below, the MS Forest zone is not only isolated from the other arrows, but the arrow is comparatively long (Figure 3.5). This indicates that MS Forest had a unique compilation of vegetation species. This result is consistent with what we might expect, since this was the only tract of the flooded forest habitat surveyed at SNWR and had greater influence from canopy species like *Acer saccharinum* (Silver Maple) and *Populus deltoides* (Eastern Cottonwood), even though these were not accounted for in the IVI due to the lack of percent cover estimates.

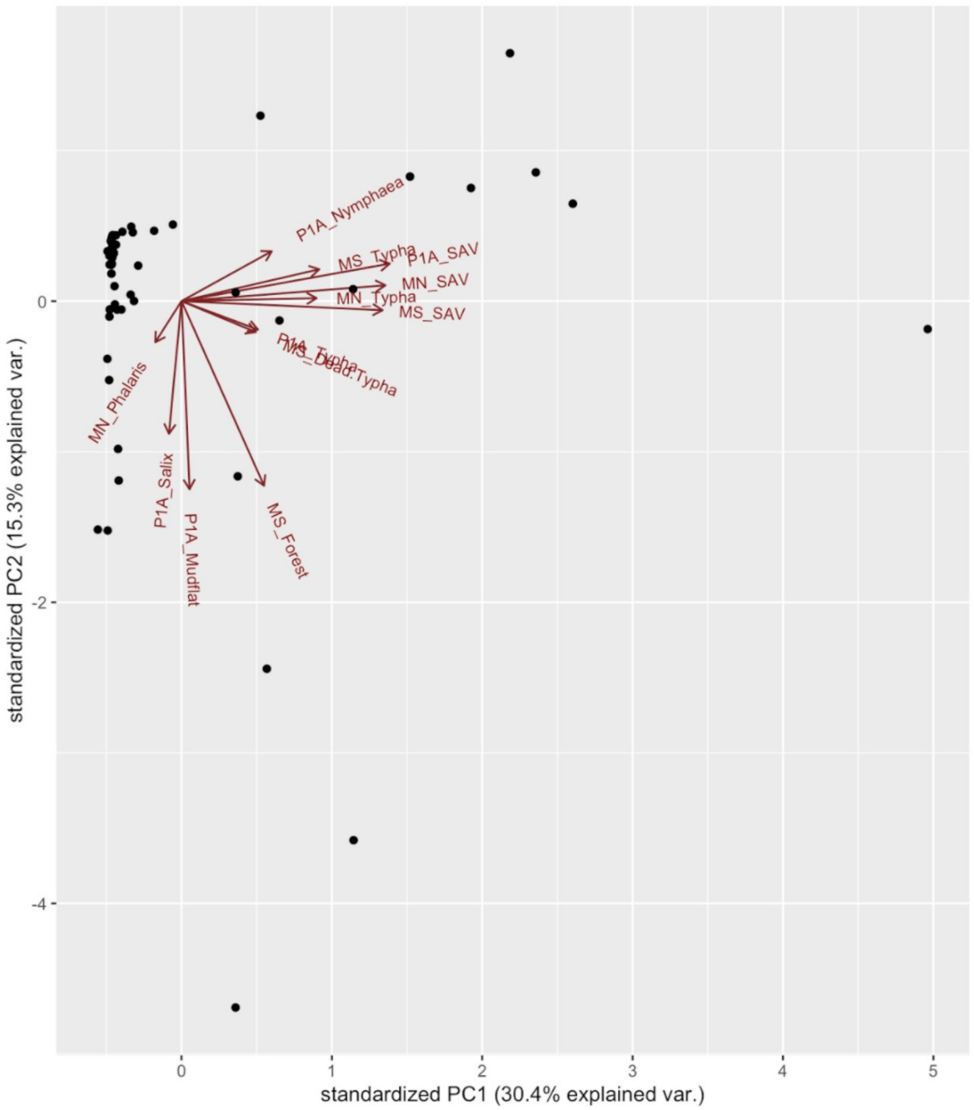


Figure 3.5: Principal Component Analysis of vegetation zones and units displayed in a variable correlation plot.

In addition to creating a variable correlation plot with the PCA, we can also plot the eigenvalues for the vegetation variability using a PCA scree plot. The scree plot illustrates how much variance each dimension explains within the data. Represented in the scree plot, the first eigenvalue captures nearly one-third (30.4%) of the total variance present in the data (Figure 3.6). The next eigenvalue captures 15.3%, and the remaining eigenvalues capture smaller portions of variance. These values reveal that one particular dimension has the greatest effect on the vegetation distribution throughout the study sites. All other eigenvalues are within 15 percentage points of each other, indicating that the remainder of explained variance is captured by many factors and is not easily captured in only a few statistical components. These data suggest that dimensionality within the vegetation data are complex, likely dependent and influenced by a multiplicity of factors that likely range from flooding events to year-round water levels, shade levels from canopy coverage, and land use history.

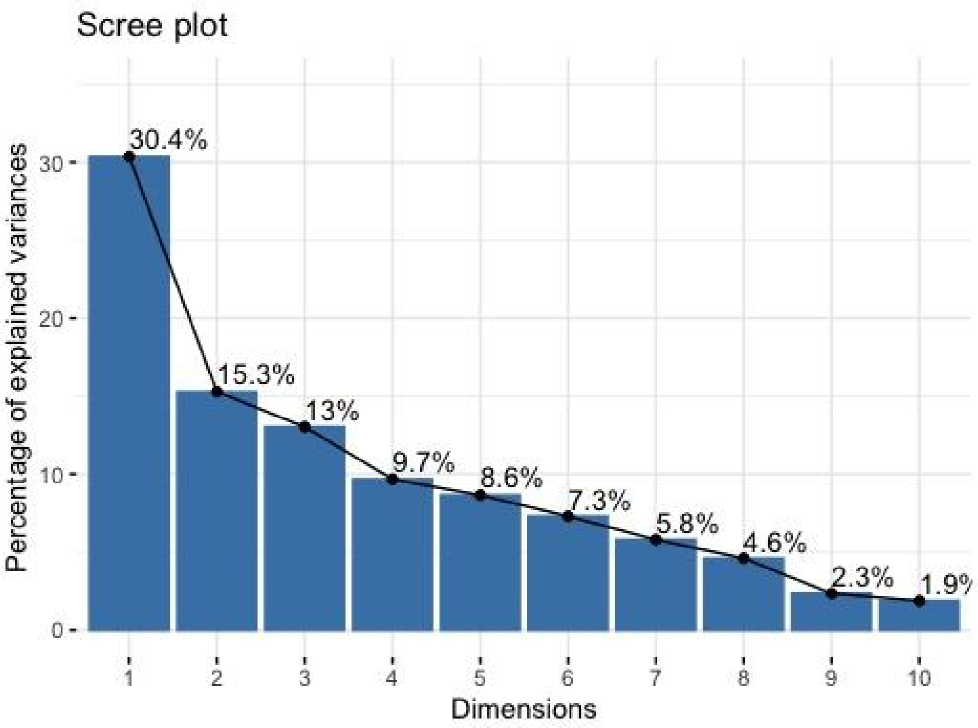


Figure 3.6: Scree plot of the top 10 eigenvalues explaining the variance in the data.

Floristic Quality Assessment (FQA)

The FQA compares the wetland species for a site against an ideal reference condition for a Michigan wetland, represented by a score of "10" for C. Each of our sample sites significantly diverged from the reference condition. MS scored the highest, with a mean C of 2.8, followed closely by P1A with a mean C of 2.7, and trailed by MN with a mean C of 2.5. The FQI for MS was 11.5, the lowest of the units. MN had a slightly higher FQI at 13.9, followed by P1A with an FQI of 16.4. It is important to note that the FQA does not account for any dead species or non-vascular species in a wetland; therefore, the total species numbers are lower than indicated above in Figure 3.2.

Unit	Forb	Grass	Sedge	Shrub	Vine	Tree	Native Species	Total Species*	Mean C with invasive species	FQI Value (Natives Only)
MS	12	1	0	0	0	4	14	17	2.8	11.5 (12.7)
MN	22	3	1	1	2	2	20	31	2.5	13.9 (17)
P1A	26	4	3	0	2	2	29	37	2.7	16.4 (18.8)

Table 3.6: Outputs of the FQA Calculator for each floodplain unit. Mean Cs show significant deviation from the reference score of 10 with MS having the highest Mean C of 2.8, followed by P1A with 2.7, and MN with 2.5. When scores are adjusted to FQI, P1A had the highest score at 16.4, trailed by MN with 13.7, followed by MS with 11.5.

*The FQA does not take into account dead or nonvascular species; therefore, total species across each unit are lower than counts displayed in Figure 3.2.

Index of Biotic Integrity (IBI)

The IBI calculation reveals a similar pattern to the FQA results in that scores reflected significant distance from reference condition. MS and P1A both scored 17 for Total IBI, compared to MN which scored only 8 for Total IBI. Each floodplain unit scored Low on the IBI scale (Table 3.7).

2020												
Unit	**Total Site**			**Dry Emergent Zone (water level <1 cm) ***			**Flooded Emergent Zone (water level >1 cm)**			**Submergent Coverage**		
	Invasive Cover	Invasive Frequency	*C*	Invasive Cover	Invasive Frequency	*C*	Invasive Cover	Invasive Frequency	*C*	Tolerant Submergents	Total IBI	Descriptive Score
MS	3	0	3				3	0	3	5	17	Low
MN	0	0	1	0	0	0	1	0	1	5	8	Low
P1A	3	3	1				3	1	1	5	17	Low

Table 3.7: IBI scores across the floodplain units. Total IBI scores take into account invasive cover, invasive frequency, and calculated C throughout the total site, the dry emergent zone, and the flooded emergent zone. These scores are combined with the overall tolerant submergent coverage for a given site to produce a "Total IBI" and "Descriptive Score" (Lugten et al. 2020).
*The Dry Emergent zone was not observed in MS or P1A.

For reference, the table displaying IBI results from 2019 is also documented here (Table 3.8).

2019												
Unit	**Total Site**			**Dry Emergent Zone (water level <1 cm) ***			**Flooded Emergent Zone (water level >1 cm)**			**Submergent Coverage**		
	Invasive Cover	Invasive Frequency	*C*	Invasive Cover	Invasive Frequency	*C*	Invasive Cover	Invasive Frequency	*C*	Tolerant Submergents	Total IBI	Descriptive Score
MS	1	0	3	3	1	5	1	0	3	5	22	Medium
MN	1	0	1	0	0	5	1	0	1	5	14	Low
P1A	1	1	3							5	20	Low/ Medium**

Table 3.8: 2019 IBI scores among wetland study units (Lugten et al. 2020).
*This zone was not observed in P1A.
**Although P1A's score is technically rated 'Low,' we modified its Descriptive Score to 'Low/Medium' as it is at the high end of the 'Low' score and to show it is a higher quality site than MN, but slightly more degraded than MS.

DISCUSSION

Invasive Species

Ecological management for wetland vegetation communities at SNWR has focused on establishing the diversity, composition, distribution, and regenerating mechanisms of native vegetation communities (Heitmeyer et al. 2013). To meet these management goals, vegetation across SNWR has been managed by adjusting hydrological flow through the floodplain units by strategically opening and closing gates, managing invasive species through the application of herbicide, and manipulating natural river drainage and floodplain topography (Heitmeyer et al. 2013). Despite these efforts to restore and propagate native vegetation, invasive species still dominate the refuge study units. Large portions of each unit contained entire vegetation zones defined by *Typha angustifolia*, an pervasive invasive species. Additionally, *Lythrum salicaria* (Purple Loosestrife) and *Phalaris arundinacea* also covered significant portions of study units.

These species can greatly reduce wetland vegetation diversity by outcompeting native vegetation through competitive exclusion, thereby negatively impacting overall habitat quality (Liu & Stiling 2006; Chen 2010). Though not captured in our sampling efforts, we also located *Hydrocharis morsus-ranae* (European Frogbit; EFB), an exotic species with highly invasive potential, in both P1A and MN. It is not fully understood how EFB entered these units at SNWR, but two possibilities are likely. EFB travels primarily by hydrochory, or dispersal by water currents, but has also been known to hitchhike on the legs of waterfowl like Great Blue Heron *Ardea herodias* (Nault & Mikulyuk 2009). Since Great Blue Herons have been present at the refuge for many years, it would seem unusual for EFB to only have entered the refuge units now, though it is possible it entered earlier, but conditions were not right for it to become established. Instead, we think it is more likely that EFB entered P1A and MN following the major flooding event of May 2020, when flood waters could have carried EFB disseminules from upstream. Also in 2020, Lake Huron water levels were at a record high , according to measurements dating back to 1918 (U.S. Army Corps of Engineers 2021). The refuge is only a few miles upstream along the Saginaw River from Lake Huron, and experiences backflows as a result of lake level rises (Buchanan et al. 2013). Because 2020 experienced both serious flooding and heavy backflow from Lake Huron, it is possible that EFB may have traveled upstream from the Saginaw River and Lake Huron, or downstream with flood water flows.

Further compounding their impact, particular invasive species may create more ideal conditions for other invasive species to thrive. This process of facilitation may pose a significant risk to ecosystem health because it can dramatically accelerate the impacts of invasive species (Simberloff & Von Holle 1999). For example, the presence of invasive *Typha* has been shown to have strong association with the spread of EFB (Monk 2019). This interaction likely occurs because EFB requires low wave disturbance, a condition which is created within large stands of invasive *Typha* (Monk 2019). In P1A, invasive *Typha* was particularly dominant, with an IVI of 57.4, forming dense stands. This may have made P1A even more suitable for EFB upon introduction. Though EFB was also located in MN, stands of invasive *Typha* in MN were less dense than in P1A, and only accounted for an IVI of 24.8 in the MN *Typha* vegetation zone. MS had the least invasive *Typha* throughout with an IVI of 21.7 in the MS *Typha* vegetation zone, and no EFB was located in this unit.

According to the FQA and the IBI, the vegetation assemblages across all three study units at SNWR had a descriptive quality score of Low (Table 3.7). These scores were heavily influenced by the presence of non-native and invasive species, particularly dominant *Phalaris* and *Typha*. Of the three study units, MN had the lowest qualitative score (8), which dropped from its score of 14 during the previous sampling year. One reason for this drop could be due to an increase of non-native and invasive species in the *Phalaris* vegetation zone. In 2019, only one invasive species, *Phalaris arundenacea,* was found in the *Phalaris* vegetation zone. In 2020, we recorded five invasive species: *Butomus umbellatus, Cirsium arvense, Lythrum salicaria, Phalaris arundinacea,* and *Typha angustifolia* in the *Phalaris* zone (Table 3.5). This increase in the number of prominent invasives from 2019 to 2020 in MN is likely due to a drop in water level in this vegetation zone.

Water Depth and Microtopography

In 2019, water levels in the *Phalaris* vegetation zone in MN averaged 12.4cm across ten sampling locations. In 2020, water was entirely absent from the *Phalaris* vegetation zone in MN for an average depth of 0.0cm. Without any inundation, aquatic species like *Lemna* spp. and *Ceratophyllum demersum* could not survive and the dry soil became dominated by *Phalaris arundinacea* (IVI 103.7) and other invasives. The domination of invasives like *Phalaris arundinacea* is a well-recognized successional process that frequently occurs on exposed ground within wetland restoration sites (Craft 2015). As water levels fluctuate in future years throughout the units, areas of ground where water recedes entirely will most likely have heavy colonizing pressure from invasives that can quickly capitalize on the altered conditions.

The wetland study units at SNWR differ from typical lacustrine and riverine wetland systems due to their lack of a riparian gradient of water levels (Lugten et al. 2020). Instead of a gradual increase in depth away from shore, as found in a typical coastal wetland (Uzarski 2017), the study units at SNWR have steep banks that form the dikes surrounding each unit. Additionally, each unit has deep troughs adjacent to the dike banks where substrate was extracted to build the dikes. Though not included in our sample, we observed that these steep banks often contained *Phalaris,* but also occasionally had *Vitis riparia* (Riverbank Grape), *Cornus sericea* (Red-Osier Dogwood), and *Salix* spp., suggesting that the dike embankments are suitable, to some degree, for native shrub species.

Study Limitations

Though stratified random sampling of vegetation was comprehensive across the study units, we still may have missed various species. For example, we observed EFB in P1A and MN, but this species was not accounted for through our subsampling efforts. In addition, there were some species we could only identify to the taxonomic rank genus, and we were unable to identify one plant specimen entirely.

Another limitation was that we neglected to calculate percent cover for overstory species in the flooded forest in MS. This would have required that we estimate the canopy coverage of each species within the quadrat; however, we did not take this measurement due to time constraints. Therefore, we lacked the data necessary to calculate the IVI for overstory species in MS.

Due to cybersecurity protocols established by the Department of the Interior, we were unable to operate drones to gather aerial imagery of the study units, as was conducted to develop the vegetation maps in 2019. Instead, we ground truthed maps from 2019 and estimated vegetation zones onto our own maps for 2020. However, these were rough adaptations and aerial imagery would have been more accurate.

Management Implications

A realistic time frame for restored wetlands to reach a high quality status is between 20 and 100 years (Zedler & Callaway 2002). We can easily understand the impact of age on floodplain unit diversity and health when we compare the newest floodplain unit, MS, to the oldest floodplain unit, P1A. Established in 1958, P1A is over 50 years older than MS, which was first fully flooded in 2018. We located 49 species in P1A and only 25 species in MS. For native vegetation species, P1A held 36 species, MN held 24 species, and MS held 19 species; however, it is worth noting that P1A is always open to the Shiawassee River and has a much greater chance of receiving native plant disseminules than MS, which is open to incoming waters much less frequently. Additionally, the soils in MS and MN have been much more recently disturbed than P1A; construction of that unit was only completed in 2016, making the conditions in MS more ideal for invasive species that can quickly colonize disturbed soils (Craft 2015). The most significant course of action SNWR can take in order to continue improving the vegetation quality within the study units may be to continue the current restoration effort, continue annual monitoring and progress evaluation, and allow time to pass for native plant communities to reestablish (Zedler 2003).

The floodplain units of study all received a descriptive score of Low on vegetation quality calculations. We suspect that the dominance of invasive species greatly contributed to these low scores. The refuge must continue their invasive species management practices, while also expanding their strategies to include the location and management of new species, particularly EFB.

Recommendations

We recommend continued vegetation monitoring following the protocols established by Puz et al. (2020). We believe this protocol for vegetation sampling is sufficient and needs no major modifications.

We recommend that, when allowable, future teams capture aerial imagery to develop accurate maps of the distribution of vegetation throughout the study units. Accurate maps will help research teams determine the major vegetation zones and help in the randomization process for determining where to sample. Additionally, it may be helpful to analyze topographic variation throughout the study units through a formal water depth analysis. Analyzing the microtopography of the study units will help future researchers identify any patterns between water depth, topography, and vegetation distribution.

Though *Typha* management can be time-intensive and difficult, we identify this as particularly important due to the likely effect *Typha* has on allowing EFB to spread. One effective management strategy for reducing *Typha* in Great Lakes wetlands has been to manually cut *Typha* below the water. Monk et al. found that *Typha* could be significantly reduced by first harvesting it above water, and then subsequently cutting all remaining severed invasive stems below the water surface using a specialized sickle-bar mower attachment (2019). Harvesting *Typha* in this way limits regrowth, and allows for greater water flow and air flow across the water, creating unfavorable conditions for EFB. We acknowledge that this is a labor-intensive recommendation. However, depending on refuge staff's vegetation conservation goals, it may be a worthy endeavor to prevent invasional meltdown (Simberloff & Von Holle 1999).

Another prominent invasive species throughout our study units was *Phalaris arundinacea.* Though the combination of persistent prescribed burns and applications of herbicides have helped reduce *Phalaris* in some managed wetlands across the Midwest, the impacts tend to be difficult to sustain if stable populations of *Phalaris* exist nearby (Adams 2006). Shade, however, has been shown to help reduce *Phalaris* vitality. In MN, where *Phalaris* was most dominant among the units studied, a greater presence of shrubby species that have been found at the refuge in the past, like *Salix* spp. or *Cornus sericea* (Red-osier Dogwood), could stunt *Phalaris* growth. We therefore recommend that the refuge consider planting or encouraging the growth of these native, shady species, especially in and among *Phalaris*-dominated vegetation zones.

In addition to controlling invasive species, the refuge should place a high priority on propagating native species, which will help reduce the recolonization by invasive species and expedite development of healthy native vegetation communities (Adams 2006). Two native species the refuge could look to propagate are Northern Wild Rice *Zizania palustris* and Southern Wild Rice *Zizania aquatica*. At present, neither species of Wild Rice, or Manoomin in the Anishinaabe language, were captured in our sampling at SNWR; however, these species were historically growing throughout the Shiawassee Flats (Heitmeyer et al. 2013). Manoomin can be propagated by dispersing seeds in water up to three feet deep, and supports macroinvertebrates, fishes, muskrats, water fowl, and other wetland species (NOAA 2020). This native plant species is central to the greater Anishinaabe Indigenous community and Great Lakes wetland ecosystems (NOAA 2020).

MACROINVERTEBRATE MONITORING

INTRODUCTION

Aquatic macroinvertebrates are an integral part of the wetland food web, providing a source of nutrients to other organisms including fish, birds, and other macroinvertebrate species. Additionally, macroinvertebrates serve as ecosystem bioindicators that provide information about overall water quality, including factors like pH, temperature, dissolved oxygen concentration, turbidity, nutrient loads, and pollutants (Kenney et al. 2008).

SNWR has prioritized macroinvertebrate monitoring due to their biological significance to fish and bird communities (Lugten et al. 2020). Macroinvertebrate monitoring began during the 2019 field season and continued through our 2020 season.

RESEARCH QUESTIONS

Our team developed the following research questions to guide our macroinvertebrate data analysis:

- *How does the abundance and diversity of macroinvertebrate communities vary across units and between years?*
- *How does vegetation structure influence the abundance and diversity of invertebrate communities?*
- *How does water quality influence the abundance and diversity of invertebrate communities?*
- *What does the invertebrate community imply about the success of restoration?*

METHODS

Macroinvertebrate collection procedures were adapted from the 2020 UM-Shiawassee Project team's sampling protocol (Lugten et al. 2020). Our team made modifications to this protocol to accommodate a reduced field season due to the COVID-19 pandemic. Instead of sampling once per month, macroinvertebrates were collected every other week from July to August for a total of four sampling events: once in June, twice in July, and once in August. During each sampling event, we collected specimens from each of three wetland study units: MN, MS, and P1A.

Within each study unit, sample sites were randomly stratified by vegetation type. Vegetation types included submerged aquatic vegetation (SAV), *Typha, Nymphaea, Salix*, *Phalaris,* and flooded forest. At each site, three 1m quadrats were placed along a transect 1m apart. Before sampling for macroinvertebrates, measurements of total percent cover of vegetation and water depth were taken. Triplicate water quality parameters were also recorded using a sonde.

A 0.5mm mesh D-net was used to sweep nine times within each quadrat. The full water column was sampled including substrate, vegetation, and any present open water. Invertebrates collected from all sweeps were combined into a gridded, white enamel tray. All large and highly visible individuals were collected regardless of location in the tray. Then, grid numbers were randomly selected and were thoroughly picked using forceps and pipettes before the next grid section was examined. Specimens were collected for a combined effort of 30 minutes across participants. Individuals were preserved in jars of 70% ethanol that were labeled with unit of collection, date, and vegetation zone. Individual macroinvertebrates were later sorted, identified to either genus or family, and counted with the use of a dissecting microscope. Genus and family identifications were made in accordance with protocols of the Great Lakes Coastal Wetland Monitoring Program (CWMP) and two identification guides (Hilsenhoff 1995; Thorp & Covich 2009) . It is worth noting that macroinvertebrates collected during the 2019 field season were identified only to the family level. This resulted in inaccurate Index of Biotic Integrity scores for 2019. We identified organisms to the genus level in 2020 wherever CWMP protocol required it. This also makes it easier for SNWR macroinvertebrate data to be compared to other coastal wetlands in the region.

DATA ANALYSES

Macroinvertebrate counts were standardized by unit effort, here classified as the total number of individual macroinvertebrates of a given genus captured at each of site sampled within a given unit, vegetation type, or sampling period. We visualized our data, assessed them for normality and equal variance, and ran ANOVA tests in R Studio, utilizing the packages 'car' and 'dplyr.' R code from the 2020 UM-Shiawassee Master's Project team was modified and used to carry out analyses.

These statistical tests were used to assess differences in community composition between each water quality parameter and month, unit, and vegetation type. We also generated Bray-Curtis dissimilarity indices to better visualize differences in community composition between units, months, and vegetation types.

In an effort to better characterize the overall health of floodplain units and track restoration success, we calculated Index of Biotic Integrity (IBI) scores for each unit. Results were calculated using parameters established by the Great Lakes CWMP (Burton et al. 1999; Uzarski 2004). These parameters require the use of specific vegetation criteria, so we used the closest vegetation structure for each unit, which was *Typha* throughout all three units.

RESULTS

Overall Abundance

In total, we collected and identified 7,763 individual macroinvertebrates from 45 sites within the 3 floodplain units of study. From these, we identified 14 orders, 50 families, and 100 genera of macroinvertebrates (Appendix IV). Not all taxonomic groups were found in each unit. MS had

37 families and 52 genera, MN had 34 families and 53 genera, and P1A had 39 families and 58 genera (Table 4.1). In 2019, 43 families were identified in total, with 41, 32, and 36 families found respectively in MS, MN, and P1A (Table 4.1). This shows that, at the gross level, community structure has remained fairly stable throughout the first two years of restoration.

Unit	Number of Families 2019	Number of Families 2020	Number of Genera 2020
MN	32	34	53
MS	41	37	52
P1A	36	39	58

Table 4.1: Total number of families and genera collected across each unit of study during the 2019 and 2020 field seasons.

Nearly 76% of all macroinvertebrates belonged to nine families: Hyalellidae, Coenagrionidae, Caenidae, Chironomidae, Hydrachnidae, Physidae, Libellulidae, Corixidae, and Pleidae (Table 4.2). This was consistent with 2019 data, which included eight of these nine families, though in a slightly different order of abundance (Table 4.2). Belostomatidae was the ninth most abundant family in 2019, and Libellulidae was not in the top nine families in 2020. It is worth noting that 2020 catch per unit effort (CPUE) was on average higher and had a greater range than 2019, despite a lower overall percentage of individuals belonging to the top nine families: 85% in 2019 compared to 76% in 2020 (Table 4.2). Two of the top taxonomic groups caught in 2020 were found to be in the top five most abundant groups in all three units (Table 4.3).

Family 2019	Total CPUE	Family 2020	Total CPUE
Caenidae	28.0	Hyalellidae	67.8
Hyalellidae	21.9	Coenagrionidae	18.0
Chironomidae	17.0	Caenidae	14.4
Coenagrionidae	10.8	Chironomidae	5.7
Corixidae	6.1	Hydrachnidae	5.7
Pleidae	3.1	Physidae	5.4
Physidae	2.8	Libellulidae	4.8
Hydrachnidae	2.8	Corixidae	4.7
Belostomatidae	2.4	Pleidae	3.8

Table 4.2: Top nine families for all sampling efforts across all units in 2019 and 2020 ranked by CPUE.

MS			
Family 2020	**Genus**	**Common Name**	**CPUE**
Caenidae	*Caenis*	Mayfly	14.8
Hyalellidae	*Hyalella*	Scud	14.5
Chironomidae		Chironomid	5.6
Physidae	*Physa*	Ram's Horn Snail	4.9
Hydrachnidae		Mite	4.1

MN			
Family 2020	**Genus**	**Common Name**	**CPUE**
Hyalellidae	*Hyalella*	Scud	154.1
Caenidae	*Caenis*	Mayfly	15.1
Hydrachnidae		Mite	11.6
Sphaeriidae		Pill Clam	9.1
Coenagrionidae	*Ischnura*	Damselfly	7.8

P1A			
Family 2020	**Genus**	**Common Name**	**CPUE**
Hyalellidae	*Hyalella*	Scud	46.1
Coenagrionidae	*Enallagma*	Damselfly	12
Caenidae	*Caenis*	Mayfly	11.9
Coenagrionidae	*Ischnura*	Mayfly	9.6
Physidae	*Physa*	Ram's Horn Snail	9.1

Table 4.3: The top five dominant taxonomic groups for each sampled unit ranked by CPUE. Taxonomic groups highlighted in gray were found in all units.

Monthly Variation

We found minor differences in community composition of floodplain units across time, given the high degree of overlap of ellipses in ordination space (Figure 4.1). Both sampling periods in July reveal the highest variability in community structure, as the ellipses span the greatest ordination space (Figure 4.1). Throughout both July sampling periods, 67 genera and 40 families of macroinvertebrate were identified. In June and August, 31 and 35 families respectively, were collected. August has the narrowest ellipse and most tightly clustered points in NMDS ordination space, suggesting a less diverse community composition than in earlier sampling periods (Figure 4.1). This is supported by the observation that August had relatively high abundances of the top nine families throughout units but very low numbers of other families and genera (Table 4.1).

There were no statistically significant differences between months for either CPUE or total number of genera identified (ANOVA, p-value = 0.269 and p-value = 0.488) (Table 4.4). With more extensive sampling, some trends in data could become significant, particularly for the months of July and August where the CPUE and total genera and families tended to be more different than other months. These findings are consistent with those of the 2019 field season.

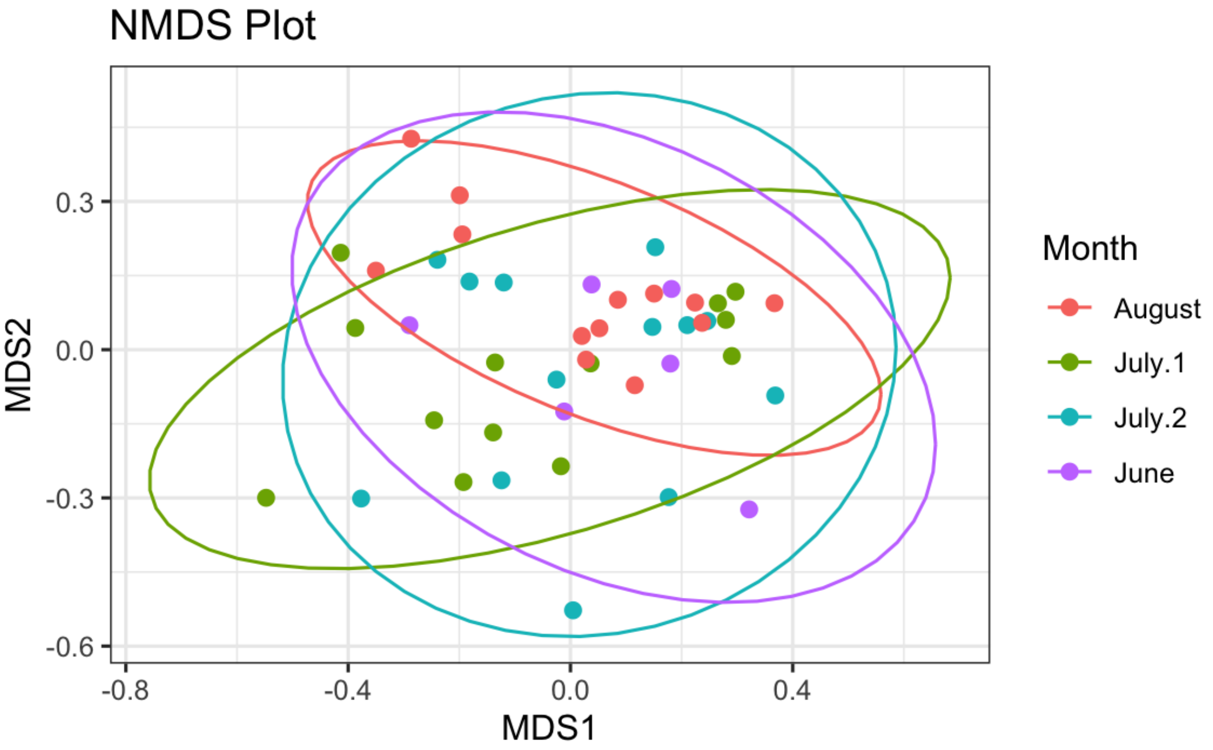


Figure 4.1: NMDS Bray-Curtis dissimilarity index for invertebrate sampling between sampling periods by month. Ellipses show sites sampled in the same sampling period by month. Ellipses show similarity of community composition by site in a given month.

	2019 Monthly Variation	**2020 Monthly Variation**	**2019 Vegetation Type**	**2020 Vegetation Type**	**2019 Water Quality**	**2020 Water Quality**
Site CPUE	0.562	0.269	**<0.001**	0.500	0.714	**<0.001**
Number of Genera	0.186	0.488	0.054	0.244	0.303	**0.002**

Table 4.4: P-Values for tests of significant relationships between selected physical variables and invertebrate CPUE and the number of genera at a site. Significant p-values (<0.05) are in bold.

Influence of Vegetation Type

We found little difference in macroinvertebrate community composition based on dominant vegetation structure. The NMDS Ordination plot shows that both SAV and emergent vegetation, such as *Nymphaea*, had similarly placed ellipses and clustered points (Figure 4.2). SAV had a slightly longer and wider ellipse than other vegetation zones, suggesting that this vegetation structure may have a marginally more diverse community composition (Figure 4.2). Given that the flooded forest was only sampled for macroinvertebrates on three separate occasions, there were not enough data points to create an ellipse. The position of the three forest points on the plot suggest that, had an ellipse been generated, it would have been significantly narrower, suggesting a decreased community composition as compared to the other two dominant vegetation types (Figure 4.2).

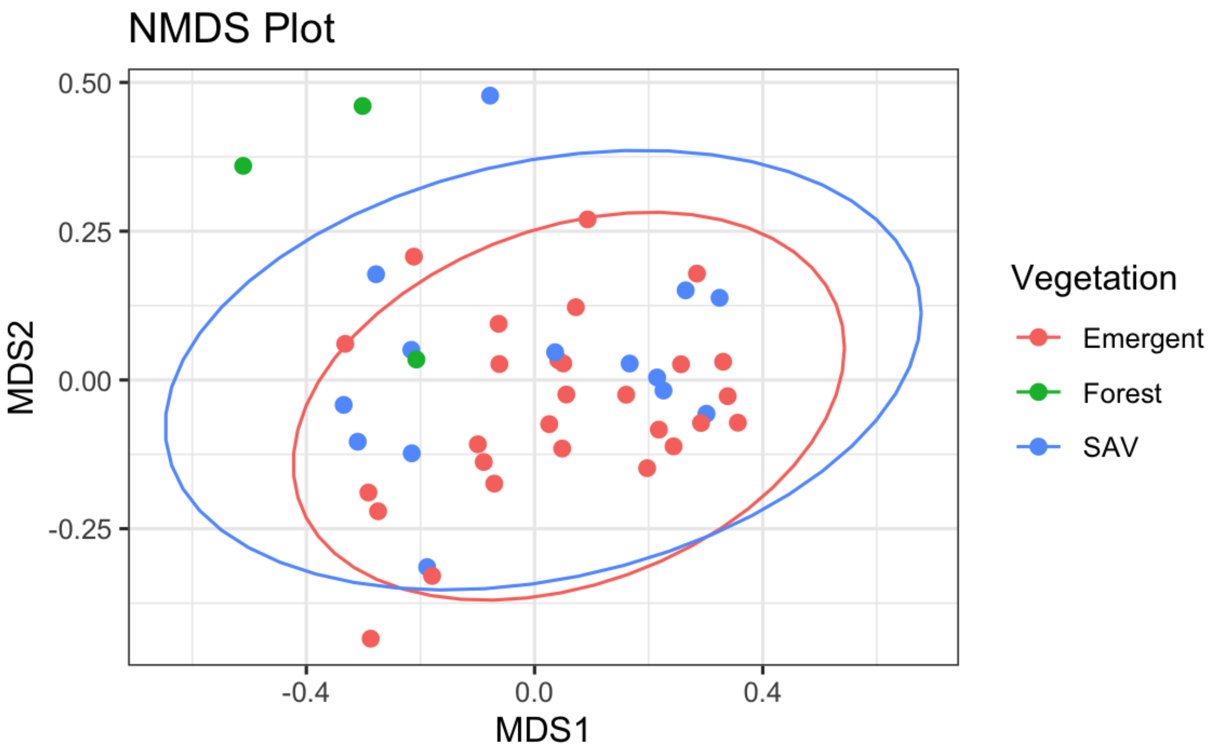


Figure 4.2: NMDS Bray-Curtis dissimilarity index for invertebrate sampling by dominant vegetation structure. Ellipses show sites sampled within the same dominant vegetation structure. Ellipses and points show similarity of community composition by vegetation structure. Too few samples were taken to generate an ellipse for the forest vegetation structure.

The lack of difference in community composition displayed by the NMDS plot is supported by insignificant statistical relationships for both CPUE and number of genera identified (ANOVA, p-value = 0.500 and p-value = 0.244) (Table 4.4). These results are inconsistent with the findings for the 2019 field season, where there was a highly significant difference in CPUE between vegetation types and a very nearly significant difference in number of families identified by vegetation type (Table 4.4). The significant p-value for 2019 resulted from the forest vegetation type having a far lower CPUE than any other vegetation type sampled (Table 4.5). While the forest still yielded a lower CPUE than other vegetation types in 2020, this difference was much less significant. The 2020 CPUE for the forest was 70.3 as compared to 28.0 in 2019 (Table 4.5). This value was similar enough to other vegetation types that a significant difference was not found.

Vegetation Type	MS 2019	MS 2020	MN 2019	MN 2020	P1A 2019	P1A 2020
Nymphaea	178.6	n/a	n/a	n/a	100.2	170.0
Typha	141.8	119.8	112.4	281.3	92.6	163.2
SAV	106.9	94.4	106.0	306.2	86.4	n/a
Phalaris	137.8	n/a	93.0	n/a	n/a	n/a
Forest	28.0	70.3	n/a	n/a	n/a	n/a
Salix	n/a	n/a	n/a	n/a	92.0	184.5
Shore	n/a	124.0	n/a	164	n/a	174.0
Open Water	287.0	n/a	130.0	n/a	n/a	n/a

Table 4.5: CPUE for each vegetation type across floodplain units for the 2019 and 2020 field seasons.

Influence of Water Quality

We found water quality to have statistically significant impacts on both CPUE and number of genera identified (Linear Regression, p-value < 0.001 and p-value = 0.002) (Table 4.4). Of the five water quality parameters tested, only temperature and conductivity had significant relationships with site CPUE (Table 4.6). All water quality parameters except dissolved oxygen (DO) had significant relationships with the number of genera identified in a sampling event (Table 4.6). These findings are inconsistent with the 2019 field season, where water quality was not found to affect site CPUE nor number of families found. It is worth noting that in 2019, an oversight was made in comparing macroinvertebrate composition and water quality parameters. An ANOVA test was run to compare macroinvertebrate composition against water quality as a single variable. Therefore, only a single p-value was recorded to compare the overall parameter of water quality to macroinvertebrate community composition, rather than exploring the relationship between macroinvertebrates and each individual water quality parameter measured.

	Temperature	Conductivity	Turbidity	Dissolved Oxygen	pH
Site CPUE	**0.021**	**0.049**	0.154	0.293	0.165
Number of Genera	**0.027**	**<0.001**	**0.035**	0.326	**0.036**

Table 4.6: P-values for significant relationships between water quality variables and invertebrate CPUE and the number of genera at a site. Significant p-values (<0.05) are in bold.

Influence of Unit

As was the case in 2019, MS and MN were dissimilar in macroinvertebrate community structure, despite being most recently reconnected to the Shiawassee River and managed under similar water control conditions. NMDS Ellipses showed that MS had a far larger spread over the ordination space than both of the other units, while MN had a much tighter clustering of points (Figure 4.3). While all units had a similar number of genera present, MS had the lowest overall richness and evenness of genera. This lack of evenness is likely the cause of the large ellipse for

MS. In contrast, despite a larger number of genera overall, MN had the highest evenness of genera and a low Simpson's index score 0.0891, a possible reason for the tightly clustered points on the NMDS plot (Figure 4.3). Additionally, MN had a CPUE for *Hyalella* more than ten times greater than that of MS and more than three times greater than P1A, possibly accounting for the rightmost positioning of the MN ellipse (Figure 4.3).

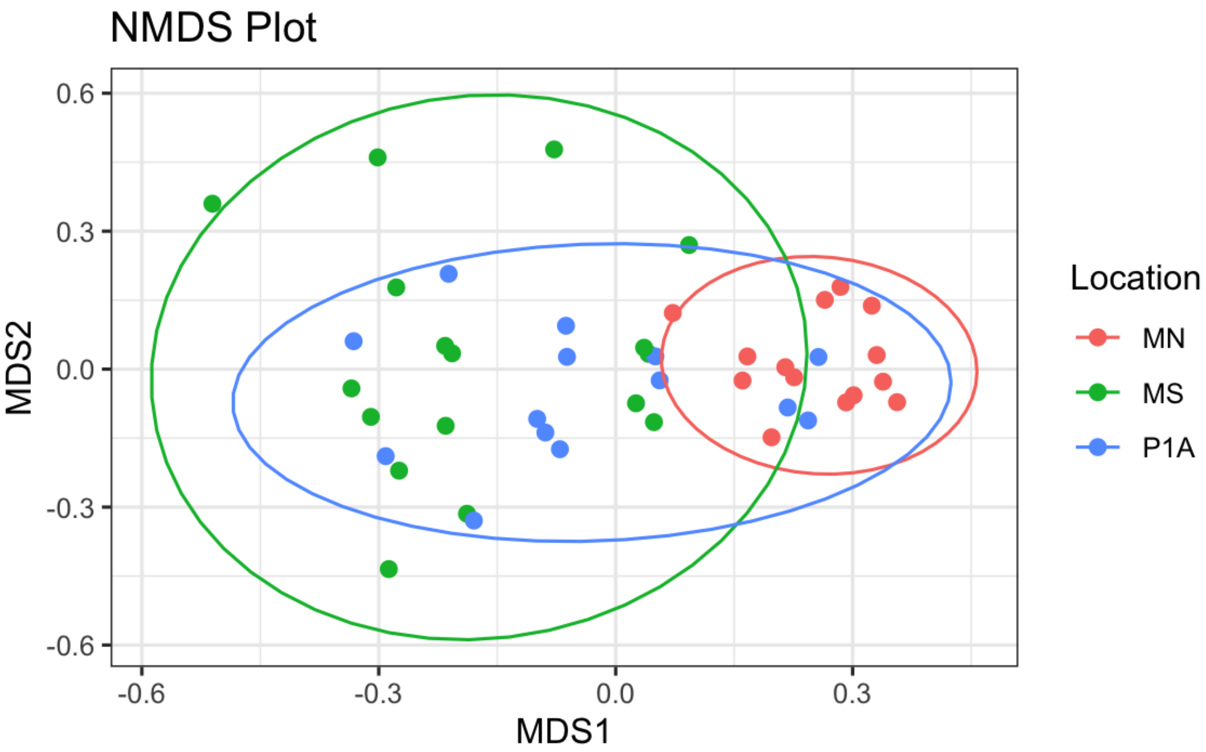
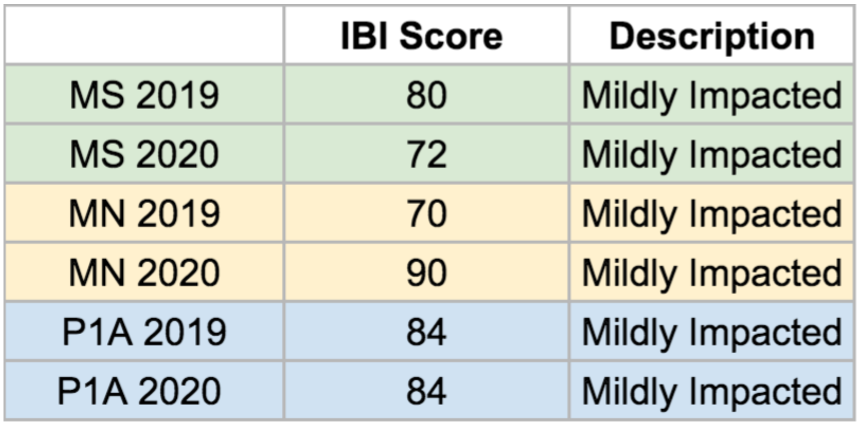


Figure 4.3: NMDS Bray-Curtis dissimilarity index for invertebrate sampling between units of study. Ellipses and points show sites sampled in the same unit. Ellipses show similarity of community composition by site in a given unit.

Restoration Success

We calculated IBI scores for each floodplain unit as a measure of overall health and level of anthropogenic disturbance for these units (Table 4.7). In 2019 and 2020, all three units were rated as Mildly Impacted for the dominant vegetation structure of *Typha*. We adjusted IBI scores from 2019 to correct for an error in calculations that resulted in MN and MS being classified as Moderately Impacted in 2019. The score for MS decreased slightly between 2019 and 2020, while MN increased by 20 points (Table 4.7). This large increase could be accounted for by the fact that scores in 2019 were calculated using only family level identifications, likely reducing overall abundance and richness scores. P1A remained constant between years, at a score of 84 (Table 4.7). These scores, which place all three units firmly in the Mildly Impacted condition, imply a low level of anthropogenic disturbance at SNWR and a reasonably healthy system. It is worth noting that in 2020 MN was one point shy of being classified as Reference Condition, an impressive classification for a floodplain unit so recently reconnected to the local river system.



	IBI Score	Description
MS 2019	80	Mildly Impacted
MS 2020	72	Mildly Impacted
MN 2019	70	Mildly Impacted
MN 2020	90	Mildly Impacted
P1A 2019	84	Mildly Impacted
P1A 2020	84	Mildly Impacted

Table 4.7: IBI scores for each floodplain unit in 2019 and 2020 with description of overall impact.

DISCUSSION

Major Findings Relative to Literature

Of all macroinvertebrates collected, 79.4% were from the nine families Chironomidae, Hyalellidae, Gammaridae, Coenagrionidae, Libellulidae, Physidae, Planorbidae, Corixidae, and Caenidae, all of which are considered typical of Great Lakes coastal wetlands (MacKenzie et al. 2004; Cooper et al. 2007). This compares to 86% belonging to families typical of coastal wetlands in 2019. The majority of these organisms are considered to be very tolerant of low oxygen levels and fluctuations in water quality, which are characteristic of wetlands (Hilsenhoff 1995). EPT taxa (members of the orders Ephemeroptera, Plecoptera, Trichoptera) are considered indicators of high quality water that is relatively free of pollutants and are thus reciprocally considered highly intolerant species and are not necessarily common in wetlands (USGS 2002). We caught 5 genera of Ephemeroptera and 7 genera of Trichoptera across all sampling. While these organisms are intolerant of chemical pollution, the majority that we caught are considered to be tolerant of high nutrient loads and fluctuating oxygen levels, as would be expected in a wetland system (Blinn & Ruiter 2013; Jacobus et al. 2019).

Similar patterns were observed in macroinvertebrate community composition between years. Eight of the nine top observed families remained the same between 2019 and 2020, though CPUE was higher in 2020 despite a lower overall sampling effort. We suspect that this could be a result of annual variations in the water quality and the community compositions of this ecosystem. Continued monitoring of macroinvertebrate, fish, and vegetation community structures, and changes in water quality will help SNWR to become familiar with patterns of variation for this dynamic system.

We found little difference in macroinvertebrate community composition across months in 2020. The most noticeable trend was that July had a more varied and even community structure, though a lower average CPUE, than June or August. We suspect this can be attributed to the emergence from the water of insect groups like Mayflies (order *Ephemeroptera*), which begin to transition to their terrestrial adult forms in early summer (Voshell 2011). We caught a substantially higher abundance of members of the Mayfly family, Caenidae, in June than we did in both July and August. Additionally, we began to catch more diverse samples of groups like Trichoptera, Diptera, and various snails in the month of July. It is likely that the reduction in CPUE of certain groups, like Caenidae, and increases in diversity across many other groups led to July having such a high level of evenness. Though August had a higher total CPUE than July, it seemed to have the lowest diversity of all months. This could be due to the complete emergence and dispersal of these same insects that began to emerge in the early and mid-summer (Voshell 2011). This dispersal would lead to a reduced overall richness of taxa caught in our sampling, though total CPUE remained high for a reduced number of taxa.

We also found no major difference in macroinvertebrate composition between vegetation types in 2020. This is in contrast to 2019, when flooded forest CPUE was significantly lower than in any other vegetation type. While forest was still the vegetation zone in which we recorded the lowest CPUE and a low diversity of macroinvertebrates in 2020, we found no statistically significant difference with other zones. We attribute this change to differences in DO levels between 2019 and 2020, which were on average lower (2 – 3mg/L) throughout the 2019 field season. Low water levels and DO in 2019 would have likely decreased macroinvertebrate abundance, even for the most tolerant species. Additionally, because of significant allochthonous carbon subsidies from the large maple trees of the flooded forest, diel DO is greatly reduced already in this vegetation type, thus providing suitable habitat only for highly tolerant organisms (Spieles & Mitsch 2003). The most common tolerant species we would expect to find in the harsh conditions of a seasonally flooded forest are members of the Chironomidae family (Brooks & Colburn 2012). Our data supports this expectation, as Chironomidae was the most abundant group we found in the flooded forest vegetation zone in 2020.

The SAV vegetation type had the highest average invertebrate CPUE, particularly in MN. This differs from 2019 findings, which showed the highest CPUE was found in the *Typha* vegetation type. Despite this difference between years, the overall implication remains the same: the majority of macroinvertebrates caught at SNWR are detritivores, which feed on the dense decaying plant matter abundant in vegetation types such as *Typha* and SAV (Voshell 2011). Additionally, macroinvertebrates have been found to prefer dense substrates for protection from predation, particularly in vegetation with high surface area, like SAV (Watkins et al. 1983). This reinforces our conclusion that the vegetation structure of SAV most closely matches the food resource and refuge habitat needs of macroinvertebrate communities.

We found water quality to be a significant correlate with macroinvertebrate CPUE for the 2020 field season, but it was not significant in 2019. While this result could be indicative of annual variability in overall water quality trends, it is more likely attributed to incomplete statistical analysis in 2019. In 2019, an ANOVA test was used to compare macroinvertebrate CPUE and number of families against water quality as a single variable and only a single p-value was recorded to compare the overall category of water quality to macroinvertebrate community structure. Individual comparisons for each water quality parameter (e.g., DO or pH) were not generated in 2019, as we did in 2020. This means that we are unable to draw comparisons between 2019 and 2020 on how each water quality parameter affects macroinvertebrate composition.

We found several differences in macroinvertebrate community composition among units. Most notably, P1A data showed a greater richness and evenness than either of the other units. We suspect that this can be attributed to P1A's connection to the Shiawassee River, which has been constant since 1958. Due to its age and relative lack of anthropogenic disturbance, which allows for more development of vegetation and overall ecological community, we suspect that this unit has a more stable community structure than either of the two more recently connected units. MN and MS, by contrast, were agricultural fields only five years ago. It is logical that P1A would have a more well-established and consistent vegetation structure that hosts a higher richness of macroinvertebrates.

MN displayed the most tightly clustered community composition. NMDS analyses suggested a low richness throughout the unit and a community structure that was different than either of the other two units due to the high number of Scuds (genera *Hyalella* and *Gammarus*) that were captured in our sampling. In MN, 65% of all macroinvertebrates captured in this unit were Scuds, nearly all of them belonging to the genus *Hyalella*. This could be explained by the dietary preference of *Hyalella*, which favor *Elodea* and *Myriophyllum* vegetation structures, common in the SAV vegetation zone (Scriber 2013). In MN, SAV is one of only three dominant vegetation structures and accounted for 43% of all macroinvertebrate sampling in this unit. *Elodea canadensis* was found to be one of the dominant species within SAV in this unit, and although *Myriophyllum* was not dominant here, MN was the only unit where we captured this genus in our vegetation survey. This makes MN an ideal habitat for *Hyalella* due to the increased availability of a primary food source (Scriber 2013). Further, SAV in MN produced our single highest invertebrate CPUE for all vegetation types across all units sampled. MS, which also had a high cover of SAV, including *Elodea canadensis*, would also be considered ideal for Scuds. However, this unit had a broader diversity of dominant vegetation types with varying macroinvertebrate communities, which we believe is the reason for the lower overall percentage of Scuds captured in our survey.

Study Limitations

Macroinvertebrate sampling in 2020 was primarily limited by COVID-19 restrictions, which prevented us from sampling in May and early June. These restrictions also slowed the macroinvertebrate identification process, as we were unable to access our laboratory or collaboratively identify specimens due to social distancing protocols. While we made every effort to maintain consistency in identifications across team members, it is entirely possible that a minority of macroinvertebrates were misidentified at the level of genus. Additionally, since we were unable to verify each other's identifications, it is possible that individual team members were individually consistent with identifications but inconsistent across group members. For example, one person may have always identified a given macroinvertebrate as *Hyalella* while another always identified that same macroinvertebrate as *Gammarus*. We believe misidentification to be limited in scope, especially because we each sent any specimens that were difficult to identify to one of our clients at USGS for verification.

Our ability to make comparisons to other locations using the CWMP macroinvertebrate sampling protocol was also slightly limited by our use of pipettes in our collection methods. Pipettes allowed us to collect more and smaller macroinvertebrates than would have been possible with forceps alone. Since CWMP protocols only allow for the use of forceps, our data may be skewed toward small organisms and consequently less comparable to other CWMP sampling efforts. We recommend that future groups not use pipettes in their sampling.

Management Implications

Invertebrates as Biological Indicators

The IBI we used, which was developed specifically for use in Great Lakes coastal wetlands, allows for an estimate of overall ecosystem health and integrity for a system that is typically characterized by more tolerant macroinvertebrate communities (Uzarski 2004). As such, we believe that the continued use of this Great Lakes CWMP IBI index will provide the most accurate characterization of the overall health of macroinvertebrate communities at SNWR. In addition, the use of this particular IBI allows for the comparison of SNWR macroinvertebrate communities to those of other coastal wetlands in the Great Lakes region.

It is worth noting that the vegetation structures for which the CWMP indices were created do not perfectly match those at SNWR. SNWR is characterized primarily by *Typha, Nymphaea,* and mixed SAV that primarily contains *Utricularia*, *Ceratophyllum*, *Elodea,* and *Lemna*. CWMP scoring protocols in Lake Huron coastal wetlands exist only for the vegetation types *Typha*, wet meadow, and *Schoenoplectus* (Burton et al. 1999). SNWR is not characterized by the presence of *Schoenoplectus,* nor were macroinvertebrates collected in wet meadows. All three units of study were characterized by dominant and dense *Typha*, though only a portion of our total macroinvertebrate sample was collected specifically from net dips within the *Typha* zone. Therefore, the use of only the *Typha* IBI category could lead to minor inaccuracies in IBI scores, thus suggesting the need for an IBI index that takes into account vegetation types such as *Nymphaea* or SAV.

With this in mind, all three units of study had IBI scores classified as Mildly Impacted using the *Typha* index, which is unchanged from 2019. MN had the highest score of 90 in 2020, followed by P1A and MS, with scores of 84 and 72 respectively. This differs from the 2019 season, when P1A had the highest score of 84 and MN was lowest with a score of 70. While it did not impact the overall classification of Mildly Impacted, the increase of 20 points between 2019 and 2020 for MN is of particular interest. This could indicate that a major change in community composition occurred and or that there were massive improvements in the success of restoration for this unit. It could also simply be a function of annual fluctuations in community composition that are not yet understood for this system due to lack of sufficient annual data. However, we suspect that the primary source of this change is that IBI scores in 2019 were calculated using macroinvertebrates that had only been identified to the level of family, rather than genus. Various components of the IBI score require the input of total genera richness and genera richness for specific groups of macroinvertebrates (Burton et al. 1999). Substituting genera for family totals would have resulted in lower overall richness and therefore a lower score. For MN, only 32 families were used for calculations in 2019, as compared to 53 genera from 34 families used in 2020. We suspect that these differences in methodology account for changes in IBI scores across years, particularly for MN.

The fact that MN and MS, which were so recently agricultural fields, are considered to be only Mildly Impacted is highly encouraging and poses key implications for restoration at SNWR. IBI scores indicate that these floodplain units have been restored to a reasonably high level of ecological quality and integrity in a very short timeframe. While the systems will continue to evolve over time, this rapid initial progress is highly indicative of a successful management and restoration strategy. This may help SNWR make additional strides in wetland restoration in the future or inform restoration efforts in similar systems in the region.

Invertebrates in the Food Web

Macroinvertebrates are integral links in wetland food webs, as they provide sources of energy to various fish and waterfowl species. Being that waterfowl protection is the primary goal of SNWR, it is important to understand how macroinvertebrate presence and abundance might impact management decisions for these birds. A wide variety of waterfowl, particularly ducks, utilize macroinvertebrates as a large portion of their diet either year-round or during the breeding season (Ducks Unlimited Canada 2016). Various species, such as the Blue-Winged Teal *Anas discors* and Ruddy Duck *Oxyura jamaicensis*, which typically consume vegetation as their primary food source, will shift to nearly 100% reliance on macroinvertebrates during the breeding season (Ducks Unlimited Canada 2016). Conservation of these species requires intact habitat that is suitable not only for the waterfowl, but also for macroinvertebrates of high nutritional value and abundance.

Many species of fish also consume macroinvertebrates as their primary means of nutrition. As a resource of concern at SNWR and an important game and fisheries resource in the Saginaw Bay region, Yellow Perch *Perca flavescens* are of particular interest to the refuge. Dietary studies of Yellow Perch populations in Saginaw Bay have shown that these fish, particularly young of year and juveniles, prey primarily on zooplankton such as *Daphnia*, Chironomids, Amphipods, *Trichoptera*, and other macroinvertebrates (Staton et al. 2014). While we did not capture a large number of Yellow Perch in our sampling, we did capture a large abundance of *Lepomis* Sunfishes, which have also been found to prefer *Trichoptera* and especially Chironomids in their diets (Kirby 1982). Each of these prey groups was present at high levels in 2020 sampling, with a group of Amphipods being our most abundant macroinvertebrate and Chironomids as our fourth most abundant (Figure 4.2). Though not analyzed statistically, various Zooplankton, particularly *Daphnia*, were observed to be abundant throughout the sampling season. Dietary analysis also suggests seasonal variability in the diet of Yellow Perch, with Chironomids being of peak importance to the fish during spawning in the spring and early summer, while Sunfishes utilize this food item year round (Kirby 1982; Staton et al. 2014). While we were unable to sample in the spring due to COVID-19, we found a significantly higher CPUE of Chironomids in June than in either July or August, suggesting that this macroinvertebrate group would be a highly available resource to Yellow Perch and Sunfishes utilizing the floodplain units in early summer.

Managing the health of macroinvertebrate communities should be a high priority for SNWR, as these organisms have significant bottom-up trophic effects on the system. To ensure an overall healthy system, the refuge might consider managing for vegetation types that yielded high CPUE of macroinvertebrates, like SAV and *Typha*, and particularly for those containing groups most preferably utilized by various fish and waterfowl species.

Recommendations

Our team recommends the continued use of the macroinvertebrate sampling protocol established by the 2020 UM-Shiawassee Master's Project team, with one modification. In accordance with CWMP macroinvertebrate sampling standard operating procedure, on which our own protocol was based, we recommend that the number of macroinvertebrates collected at each sampling site not exceed 150 individuals. Following this procedure, team members will collect macroinvertebrates for either 30 minutes of combined effort or until 150 individual organisms are collected. It is recommended that hand counters are used to tally total individuals and that only forceps are used to collect individuals, so as not to bias the type and size of organism collected (CWMP 2019). This will involve discontinuation of the use of pipettes for collection of smaller individuals, which our team employed during the 2020 field season. This change in sampling procedure should result in a yield that is representative of the overall site community composition with the added benefit of identifying macroinvertebrates under a more efficient timeframe. In addition, having all site counts standardized to 150 or fewer individuals should make sites more statistically comparable across time and space.

Our second recommendation is that all future Master's Project teams continue to identify macroinvertebrates to the level of genus. Identifications that stop at the level of family or higher should only be made if specified by CWMP protocols as appropriate. This will not only result in continued accuracy of IBI scores for refuge units, it will also allow for a more thorough comparison of community composition across years of study. Having a deeper understanding of annual variations in macroinvertebrates as well as how populations change in response to other biotic factors may help SNWR make management decisions. In addition, we recommend that statistical analyses compare individual water quality parameters to macroinvertebrate data so data between years may be compared across time.

FISH MONITORING

INTRODUCTION

Great Lakes coastal wetlands serve as spawning, seasonal refuge, and residence habitats for numerous fish species (Uzarski et al. 2016). The floodplain units of SNWR serve as seasonal habitat for Great Lakes, riverine, and resident wetland fish species. Refuge floodplain units are independently managed through flow control structures that either allow or prevent connectivity of the units to each other and the Shiawassee River. Consequently, fish communities within the units are heterogeneous. Fish communities can also indicate the health of each unit and serve as indicators of responses to various management strategies. By studying fish species richness and abundance within separate units, we generated quantifiable data related to the health of each unit and of particular species of concern, including Yellow Perch *Perca flavescens*.

We followed the monitoring protocols developed by the 2020 UM-Shiawassee Master's Project team (Sens & Mitchinson 2020), who collected field data during the 2019 field season, and sampled within the floodplain units MN, MS, and P1A. The sampling protocols were developed in accordance with those from the Great Lakes Coastal Wetland Monitoring Program (CWMP), led by a research group at Central Michigan University (Uzarski et al. 2016).

Due to the COVID-19 pandemic, our team was logistically limited in our sampling schedule and primarily utilized fyke netting to sample fish in shallow waters within the units. We excluded gill netting and electrofishing from our monitoring plan. We performed two sampling efforts using minnow traps but determined them ineffective for data collection. We did not include individuals caught in minnow traps in our statistical analyses. We sampled each study unit every two weeks from late June through August. We also sampled once per month during September and October.

RESEARCH QUESTIONS

The following research questions guided our fish data analysis:

- *What is the variation in composition, abundance, and size-structure of the community among and within the units, across months of our sampling season, and between matched months with 2019's sampling season?*
    - *What species were found in each unit? How does this compare to 2019 findings?*
    - *What is the abundance of each species in each unit? How does this compare to 2019 findings?*
    - *How does species richness and relative abundance compare among units?*
    - *How do Indices of Biotic Integrity compare among units? How does this compare to findings among units from 2019?*
    - *How does the species richness and abundance of each unit compare between months? How does this compare to the matched months from 2019?*

  - *How does the species richness and abundance of each unit compare between our 2020 season and the 2019 season?*
  - *How does size class vary among units? How does this compare to findings from 2019?*
- *What variables (DO, vegetation, HBI score, water depth, and temperature) influence species richness and species abundance among and within the units through time?*
- *How has the character of the fish community changed pre- and post-restoration?*

METHODS

Fyke Netting

Fyke netting was our team's primary fish collection method. Fyke nets are passive collection tools with four distinct sections: a lead, two wings, and a fyke trap. The lead and wing components are attached to the fyke trap frame, pulled taught, and staked into the sediment (Uzarski et al. 2016; Lugten et al. 2020). The transitional zone between open water and dense vegetation is an area of high fish movement, so we positioned the fyke net in this transitional zone. This positioning made it so fish swimming through the transitional zone would encounter the lead or a wing, become startled, and seek deeper water for refuge. We staked the fyke trap in this deeper water so that fish swam directly into the trap's funnels, or cods, and were unable to exit.

At each sampling site, our team evaluated water depth and vegetation type to determine the size and placement of each fyke net. Traps varied in both frame size (large or small) and netting mesh size (large or small). This gave us a total of four different net types. Frame size was selected based on water level. Nets were not set in areas where the water level exceeded the height of the fyke trap frame or the cods within the trap, as this would increase both the chances of fish swimming over or out of the net and of mortality of non-target species, like turtles. Previous studies have suggested there is no effect of mesh size on fish catches at SNWR, so mesh size was selected randomly (Lugten et al. 2020). Sites were chosen using a random number generator to determine step count from banks to the sampling location.

Nets were set at one site to gather fish data over two consecutive 24-hour sampling periods, or net sets. After the first 24-hour net set, data were collected, all sampled fish were released, and the net was reset in the same location. After collecting data from the second 24-hour net set, the net was disassembled and removed.

Minnow Traps

We tested the deployment of minnow traps in the study units during two weeks of the 2020 season. Traps were baited with approximately one quarter cup of dry dog kibble and submerged. Submerged traps were checked after one 24-hour set, any fish were counted, identified, and released, and then the traps were reset. Overall, minnow traps did not capture high-enough numbers of fish to contribute to the sampling dataset. For this reason, we discontinued the use of traps and excluded all individuals captured in this method from our dataset.

Study Areas

Our team monitored three floodplain wetland units during the open water seasons from June to October of 2020. Within each unit, we aimed to set at least two fyke nets in different vegetation types and depths to acquire an accurate and diverse representation of the fish community.

Maankiki South

Sampling in MS included three sampling locations in three different vegetation types: submerged aquatic vegetation (SAV), flooded forest, and *Typha* (Figure 5.1). These vegetation types represented the dominant habitats in the unit. Sampling locations were chosen based on their vegetation type, wading access, and depth.

Sources: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community

Figure 5.1: The map above shows the sampling locations in MS between the 2019 and 2020 field seasons. The 2019 field season sites are indicated by the red zones on the map. Our 2020 sampling sites are indicated by points and vegetation zones are differentiated by point color.

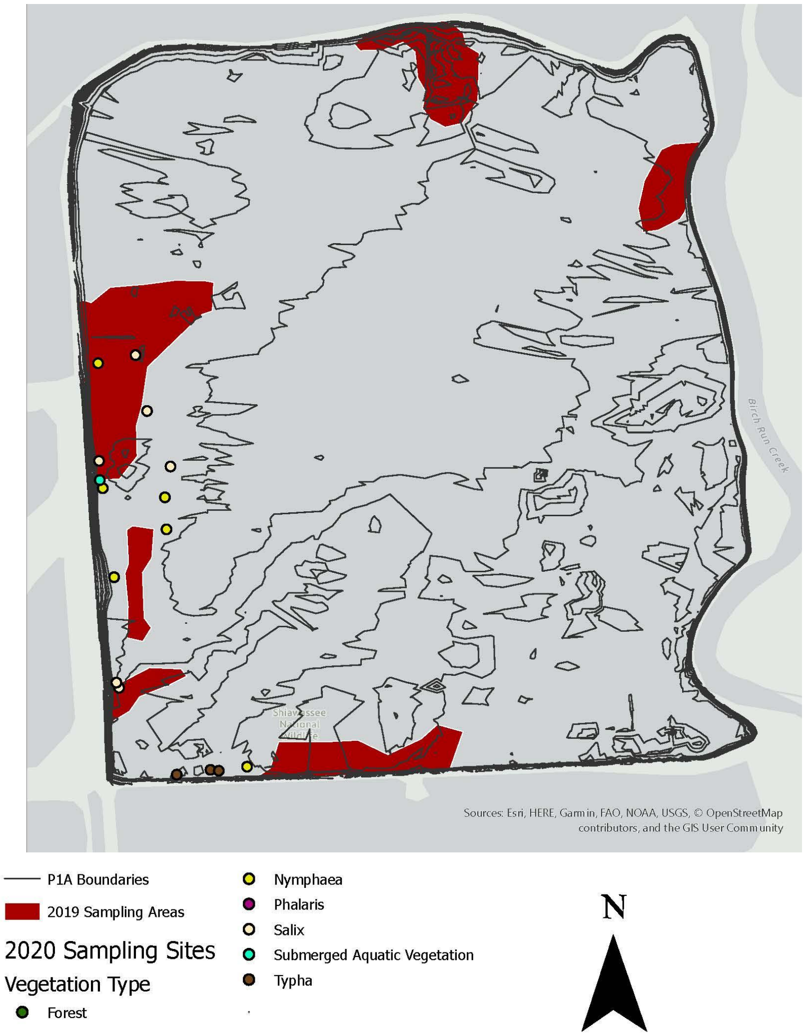
Maankiki North

MN is surrounded by unwadable channels, so each sampling site was accessed by boat. Our team set only two fyke nets in MN during each sampling period; these were set in *Typha* and SAV zones (Figure 5.2). Data from the *Typha* net set in MN were sometimes unreliable due to inconsistent submersion of the fyke net funnels from variation in water levels throughout the season. We excluded any compromised data from our analyses.

Figure 5.1: The map above shows the sampling locations in MN between the 2019 and 2020 field seasons. The 2019 field season sites are indicated by the red zones on the map. Our 2020 sampling sites are indicated by points and vegetation zones are differentiated by point color.

Pool 1A

Nets in P1A were set along the southern and western edges of P1A in a variety of vegetation types including *Nymphea*, *Typh*a, SAV, and *Salix* (Figure 5.3). As P1A is more responsive to changes in the Shiawassee River's water levels, depth in the unit varied greatly throughout the sampling period. During the summer months, large frame fyke nets were primarily used to accommodate the greater depth of the unit, while small frame fyke nets were used during the lower-depth, autumn sampling months. During mid- and late-summer, mortality of fish caught in P1A was notably high.



Sources: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community

Figure 5.3: The map above shows the sampling locations in P1A between the 2019 and 2020 field seasons. The 2019 field season sites are indicated by the red zones on the map. Our 2020 sampling sites are indicated by points and vegetation zones are differentiated by point color.

Sampling Schedule

Due to logistical limitations from flooding events in May 2020 and the COVID-19 pandemic, fish sampling did not begin until late June. We sampled every two weeks between late June and late August and once per month during September and October. During the summer months, each sampling period included two consecutive 24-hour net sets, while the fall sampling period included only one 24-hour net set. We decreased the number of fall net sets because we were unable to travel to the refuge three days in a row during the fall season.

Even though sampling efforts were greatly reduced in the fall, we wanted to gather data representative of potential fall fish migration to understand if unit fish assemblages were significantly different than during the spring and summer months. Seasonality influences the movement of many fish species, and the more stable character of the managed floodplain units serves as a refuge habitat for species over winter (Kowalski et al. 2014). Pre-winter sampling allowed us to gather data on which fish species, and in what abundances, may be using the floodplain units as over-winter refuge habitat.

Sampling Protocol

We followed the sampling protocol described for the preceding 2019 field season (Lugten et al. 2020). Fyke nets were positioned at the sampling site and water quality metrics were taken in triplicates using a YSI EXO III hand-held multiparameter sonde. This sonde recorded temperature (ºC), pH, conductivity (µS/cm), turbidity (FNU), and dissolved oxygen (DO) (mg/L). Frame size, mesh size, water depth, and vegetation type were also recorded (Lugten et al. 2020). Data recordings were made in Survey123 software using an iPad (Esri Inc. 2021).

Each net was set for two consecutive 24-hour sampling periods (except in the months of September and October, when we completed only one 24-hr net set per month). After the first net set, the same water quality metrics were collected using the sonde. Fish were collected in a bucket and brought to shore, and the fyke net was reset for the second 24-hour sampling period (Lugten et al. 2020). Fish were identified to species and the total count per species was recorded. Lengths were recorded in centimeters for the first 30 individuals of each species; after 30, individuals of that species were counted but not measured. Other observations of deformities, lesions, visible parasites, or mortality were noted. All fish were released away from the fyke net to prevent immediate recapture after data collection. This process was repeated for the second net set, though the net was disassembled and removed after the second fish collection.

Fyke nets could be compromised by animal tampering, weather conditions, or improper set up. Our team considered compromised nets to be those with sufficiently large holes below the water surface and beyond the first cod that could allow for fish to escape, or those with the cod end detached from its stake and open. Holes were repaired with a field kit on site. Compromised nets were recorded and data from these sites were still collected, but data from compromised nets were not included in any abundance calculations (Lugten et al. 2020). We recorded a total of seven compromised nets occurrences throughout the course of our sampling in 2020.

Fish Identification

We received guidance on fish identification from refuge biological technician and member of the 2020 UM-Shiawassee Master’s Project team, Eliza Lugten. We also used *The Royal Ontario Museum Field Guide to Freshwater Fishes of Ontario* for any challenging identifications (Holm et al. 2009). Identifications were primarily made based on body shape, fin count and location, coloration, number of dorsal spines, and tail shape.

DATA ANALYSES

Data were organized in accordance with 2019 datasheet formats. Many of the statistical tests necessary for our research questions were identical to those performed by the 2020 team (Lugten et al. 2020). Our team referenced the 2020 team's statistical analysis operations codes in R to perform analyses.

Species Accumulation Curve (SAC)

A species accumulation curve (SAC) was generated for each study unit to evaluate if our sampling effort was sufficient to characterize fish species richness. The SAC depicts the increase in the number of unique species caught as sampling effort increases. A curve that reaches an asymptote indicates that sampling effort was sufficient to characterize the fish community. Curves were generated in R Studio using the packages "vegan", "permute", and "lattice".

Catch Per Unit Effort (CPUE)

Catch per unit effort (CPUE) was calculated for each species at each sampling site and for each site for each sampling period. Species CPUE measures relative abundance as the ratio of total number of individuals of a species collected, divided by the total number of sampling efforts (Lugten et al. 2020). For example, if 100 individuals of the same species were caught across two net sets, the CPUE for that species would be 50 (100 divided by 2). Site CPUE was calculated by dividing the total number of all fish sampled at the site divided by the total number of sampling efforts.

The total number of sampling efforts was constrained by compromised nets. If one net set during a sampling period was compromised, fish from that net were not included in the total fish count and the sampling effort was excluded from CPUE calculation. Of the seven recorded compromised nets, three were from summer months, during which the sampling effort was two net sets, so fish totals were only included from the day with the uncompromised net. Four of the compromised nets were recorded in either September or October. Since these sampling efforts spanned only one net set, data from these sites were excluded entirely from analysis.

Testing for Differences in CPUE and Number of Species

T-tests were performed in R Studio to determine if total season CPUE and total unit CPUE for all species caught during our season were significantly different between the 2019 and 2020 sampling periods. We considered a significance threshold p-value of 0.07 for these analyses. Those significant relationships with p-values between 0.05 and 0.07 are indicated with an asterisk (*) in Table 5.2.

Linear regressions, F-tests, and T-tests were performed in R Studio to determine the relative influence of month, unit, vegetation zone, average pH, average turbidity, average temperature, average dissolved oxygen (DO), and average conductivity on site CPUE, number of species caught at a site, and the CPUE of eight species of interest. Linear regressions indicated significant differences in site CPUE and number of species within groups (for example, between *Typha* and *Salix* vegetation zones).

F-tests were performed on comparable linear models to determine the strength of one factor as a predictor of site CPUE, number of species, and the CPUE of select species. For example, to understand if "Unit" could be a predictor of Black Bullhead CPUE, we built two linear models differing only by presence of "Unit":

lm1 = (Black Bullhead CPUE ~ Month + Unit + Vegetation + Water Quality Factors)
lm2 = (Black Bullhead CPUE ~ Month + Vegetation + Water Quality Factors)

An ANOVA F-test was then performed to determine if the outputs of the two linear models were significantly different from one another. If significant, these results would indicate that "Unit" is a predictor of Black Bullhead *Ameiurus mela* CPUE. If the ANOVA F-test revealed a significant relationship between categorical variables with multiple sub-variables, an individual T-test was performed to determine which sub-variables were influential. For example, if ANOVA F-tests revealed that Unit was a significant predictor of Number of Species, a T-test was performed to determine which unit, MS, MN, or P1A, had a significantly higher or lower Number of Species recorded.

Index of Biotic Integrity (IBI)

Following the CWMP Index of Biotic Integrity (IBI) method, we calculated fish IBI scores per vegetation zone of each study unit. Our 2020 field season IBI scores are comparable to those from the 2019 field season and other Great Lakes coastal wetlands. Scores were calculated based on vegetation zone and weighted depending on fish species and classification within categories specific to the vegetation zone (Cooper 2018). For example, species in SAV zones were weighted differently depending on their trophic level, size, life duration, and pollution tolerance (Cooper 2018).

Not all of our vegetation types were represented by CWMP zones for IBI calculations. Consequently, we grouped our vegetation types into either the SAV or *Typha* IBI category based on habitat structure, following the same procedure used for the 2019 field data. Vegetation zones were combined into the following groups:

CWMP SAV IBI Category = SAV + *Nymphaea*
CWMP *Typha* IBI Category = *Typha* + *Phalaris* + *Salix* + Forest

RESULTS

We gathered data from a total of 43 uncompromised fyke net sets over the course of the sampling season. Net sets were not evenly divided between the three units due to variation in water depth and access (Table 5.1). We had 23 net sets in MS (excluding one net set from which data were lost), 19 net sets in MN, and 24 net sets in P1A. A total of 19 species was caught across the three units, fewer than the 26 caught during the 2019 season, with 14 caught in MS and 16 caught in both MN and P1A (Table 5.1). Note that young of year (YOY) sunfish (juveniles from species including Bluegill *Lepomis macrochirus*, Green Sunfish *Lepomis cyanellus*, and Pumpkinseed *Lepomis gibbosus*) were too immature to differentiate species and were consequently counted as a distinctive species (YOY sunfish were not counted as a separate species for 2019 season data).

We caught and identified 3,728 individuals to the species level, only 42% of the 8,855 individuals caught over 113 uncompromised net sets during the 2019 season. We attribute this difference entirely to the logistical constraints and safety protocols our team was required to follow due the COVID-19 pandemic. Notably, however, we caught 1,427 individuals in P1A, exceeding the 1,137 caught during the lengthier 2019 season (Table 5.1).

	MS		**MN**		**P1A**	
	2019	**2020**	**2019**	**2020**	**2019**	**2020**
Number of Net Sets	51	23	27	19	35	24
Number of Species	22	14	19	16	19	16
Number of Fish	4938	1525	2780	776	1137	1427
Species Unique to Unit	- Bluntnose Minnow - White Crappie - Central Mudminnow	None	None	- Brook Silverside - Johnny Darter	- Channel Catfish	- Northern Pike - Emerald Shiner

Table 5.1: Summary of fyke net results for both the 2019 and 2020 sampling seasons for each unit, including data on number of net sets, number of species caught, number of fish caught, and which species, if any, were unique to the unit. Note that data were lost from one net set in MS in 2020.

We generated species accumulation curves (SAC) to determine if our sampling efforts captured a representative picture of each units' fish communities. Our sampling efforts began to reach an asymptote at 20 sites in MS, 14 in MN, and 15 in P1A (Figure 5.4). In 2019, it took more net sets to reach an asymptote at a higher number of species caught (Figure 5.4). The SACs from 2019 clearly indicated that the sampling efforts adequately captured each unit's fish community, as all three curves in 2019 reach an asymptote. Our SACs were less clear (Figure 5.4). P1A reached a distinct asymptote and MS reached a less distinct, but still clear asymptote as well, indicating that sampling efforts in these units were adequate to capture the fish community. The SAC for MN did not clearly reach an asymptote, so sampling efforts in that unit were insufficient.

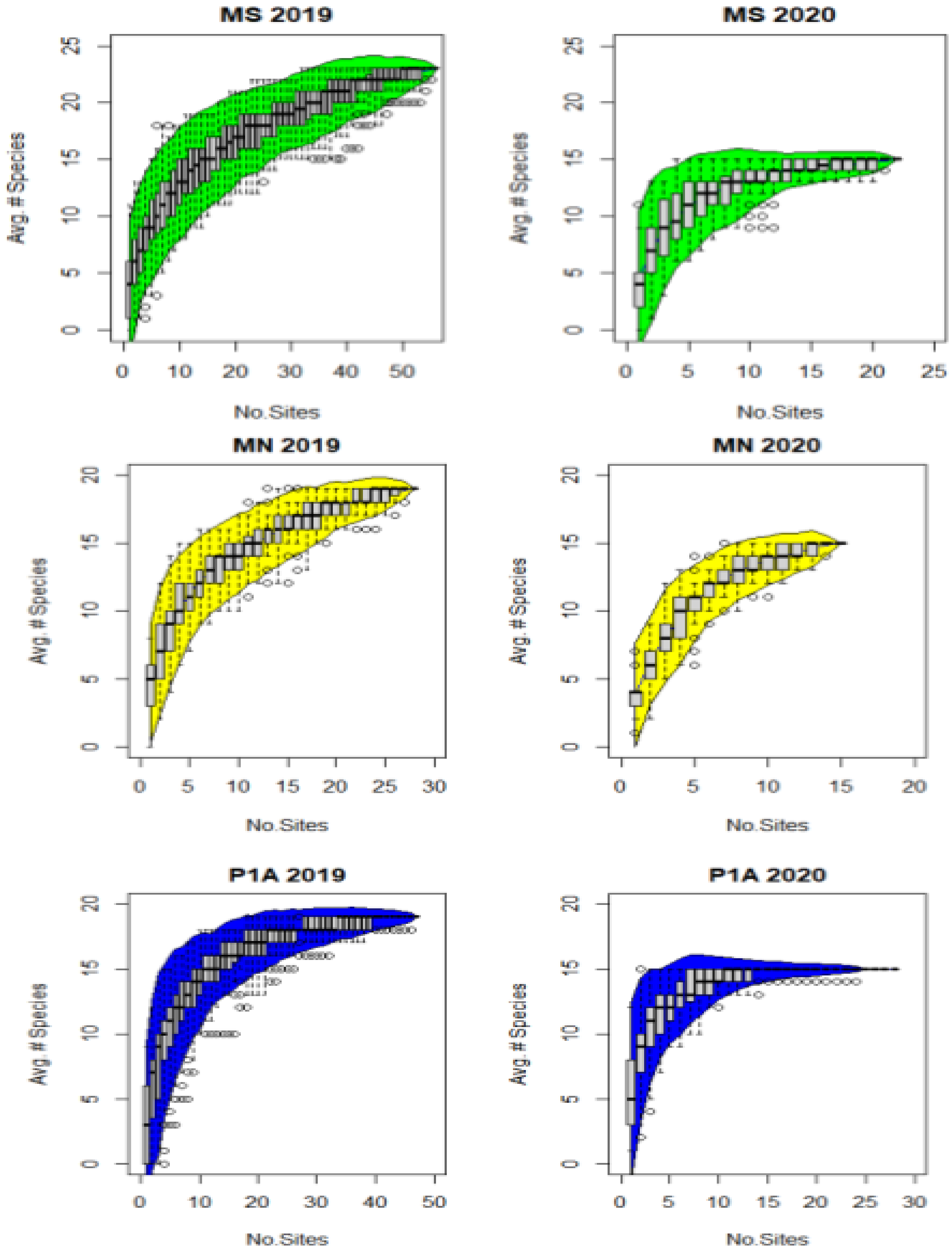


Figure 5.4: Species Accumulation Curves (SAC) for each unit for 2019 and 2020 field seasons. Panels on the left are from the 2019 sampling year and the right are from the 2020 sampling year. Red lines were added to the 2019 SAC to indicate the site count level at which the curve plateaued in 2020.

While three unique species were found in MS during the 2019 season (Bluntnose Minnow *Pimephales notatus*, White Crappie *Pomoxis annularis*, and Central Mudminnow *Umbra limi*), no unique species were found in MS during our 2020 sampling (Table 5.1). None of the 2019 species unique to MS were abundant in the unit, as indicated by the CPUE reported in Table 5.2 of Lugten et al. (2020). Additionally, they are all relatively common species in the region (Growe-Raney 2011; Parr 2013; Tingle 2015).

We identified two unique, non-wetland species in MN: Brook Silverside *Labidesthes sicculus*, which was not found in any unit during the 2019 season, and Johnny Darter *Etheostoma nigrum*, both of which are considered common throughout the region (Dewey 2012; Fuller et al. 2019). Neither species was abundant throughout MN based on our CPUE (Table 5.2). Though the 2020 team caught Johnny Darters in both MN and P1A, their CPUE was comparable to ours, indicating no change in MN abundance but some change in distribution (Table 5.2).

P1A had different unique species between both sampling seasons: Channel Catfish *Ictalurus punctatus* in 2019 and Northern Pike *Esox lucius* and Emerald Shiner *Notropis atherinoides* in 2020. Our team did not find any Channel Catfish during our 2020 sampling season. Northern Pike, which are a common species in the region, were identified in all three units during the 2019 season but only in P1A in 2020, indicating a shift of distribution between the two years. Emerald Shiners, another regionally common non-wetland species, were found in both P1A and MS in 2019 and significantly increased in abundance in P1A between 2019 and 2020 (Table 5.2).

Species Abundances Between Field Seasons

We calculated species CPUE between units and for the entire field season for both 2019 and 2020 (Table 5.2). The increase in total individuals caught in P1A between 2019 and 2020 (Table 5.1) can be attributed to increases in the number of Bluegill, Emerald Shiner, and Largemouth Bass *Micropterus salmoides* recorded during our season (Table 5.2). Bowfin *Amia calva* abundance decreased between 2019 and 2020 in P1A, but increased in MN. Total Bowfin CPUE between 2019 and 2020 was not notably different. Green Sunfish and Northern Pike were overall more abundant in 2019 due to counts in MS in particular (and in MN for Northern Pike). Pumpkinseed were more abundant in MS in 2019 but were more abundant in MN in 2020. The overall abundance of Largemouth Bass increased between seasons, likely due to higher counts in P1A in 2020. Both sampling years recorded the same two non-native species, Goldfish *Carassius auratus* and Common Carp *Cyprinus carpi*o, though no significant change in abundance was recorded (Table 5.2). Goldfish were identified in all three units during both sampling years and Common Carp were identified in all three units in 2019 and all but P1A in 2020.

Common Name	Scientific Name	GF	RF	WF	NN	Total Site CPUE		Total MS CPUE		Total MN CPUE		Total P1A CPUE	
						‘19	**‘20**	**‘19**	**‘20**	**‘19**	**‘20**	**‘19**	**‘20**
Black Bullhead	*Ameiurus melas*	Y	N	Y		51.43	26.65	56.58	57.22	91.37	21.84	12.86	1.17
Black Crappie	*Pomoxis nigromaculatus*	Y	Y	N		0.93	0.76	0.92	0.96	1.48	0.42	0.54	0.83
Bluegill	*Lepomis macrochirus*	Y	N	Y		5.45	10.42	7.88	1.70	4.59	2.74	**2.57***	**24.88***
Bowfin	*Amia calva*	N	N	Y		1	0.80	0.61	1.39	**0.7**	**0.11**	**1.8***	**0.79***
Brook Silverside	*Labidesthes sicculus*	N	Y	N		0	0.35	0	0.00	0	1.21	0	0.00
Central Mudminnow	*Umbra limi*	N	N	Y		0.008	0.06	0.02	0.13	0	0.00	0	0.04
Common Carp	*Cyprinus carpio*	Y	Y	N	Y	0.19	0.11	0.1	0.13	0.29	0.21	0.26	0.00
Emerald Shiner	*Notropis atherinoides*	N	Y	N		0.08	0.15	0.18	0.00	0	0.00	**0.03***	**0.42***
Gizzard Shad	*Dorosoma cepedianum*	N	N	Y		0.37	0.91	0	0.00	0.29	1.47	0.97	1.33
Golden Shiner	*Notemigonus crysoleucas*	N	N	Y		0.24	0.95	0.37	0.09	0.25	0.32	0.06	2.29
Goldfish	*Carassius auratus*	N	N	Y	Y	0.11	0.09	0.1	0.13	0.11	0.05	0.17	0.08
Green Sunfish	*Lepomis cyanellus*	Y	N	Y		**3.24**	**0.88**	**6.35**	**1.00**	0.7	0.58	0.86	1.00
Johnny Darter	*Etheostoma nigrum*	N	Y	N		0.01	0.03	0.02	0.00	0.03	0.11	0	0.00
Largemouth Bass	*Micropterus salmoides*	Y	N	Y		**0.32**	**3.05**	0.25	0.74	0.4	0.63	**0.37***	**7.17***
Northern Pike	*Esox lucius*	Y	Y	N		**0.27***	**0.12***	**0.16**	**0.00**	**0.4**	**0.00**	0.34	0.33
Pumpkinseed	*Lepomis gibbosus*	Y	N	Y		12.08	6.55	**21.51***	**2.39***	**1.51***	**7.84***	6.54	9.50
Warmouth	*Lepomis gulosus*	Y	Y	N		0.5	1.45	0.06	0.17	0.14	0.58	1.43	3.38
Yellow Perch	*Perca flavescens*	Y	Y	N		0.25	0.21	0	0.04	0.33	0.37	0.57	0.25
Young of Year Sunfish	*Lepomis*	Y	N	Y		N/A	2.95	N/A	0.26	N/A	2.37	N/A	6.00

Table 5.2: Species caught in fyke nets and CPUE calculations for both the 2019 and 2020 field seasons. Species are categorized as game (Y) or non-game (N) (GF column), riverine (Y) or non-riverine (N) (RF column), wetland (Y) or non-wetland (N) (WF column), and non-native (Y) (NN column). Differences in CPUE between 2019 and 2020 that were found statistically significant by T-test are indicated in bold. A significance p-value of 0.07 was used to determine statistical significance. Pairs with significance values between 0.05 and 0.07 denoted by an asterisk (*). Note only the 19 species found during the 2020 field season are listed here, fish that were unique to the 2019 are excluded. For the full list of the 26 species found in 2019, see Table 5.2 in the Lugten et al. report (2020).

We compared species CPUE per unit for the top five most abundant species in each unit (Figure 5.5). Black Bullhead was the most abundant species in MS and MN, but was not one of the top five most abundant species in P1A. Bluegill was the most abundant species in P1A and was found in the top five abundance lists of both MS and MN. Pumpkinseed was also one of the top five most abundant species in all three units.

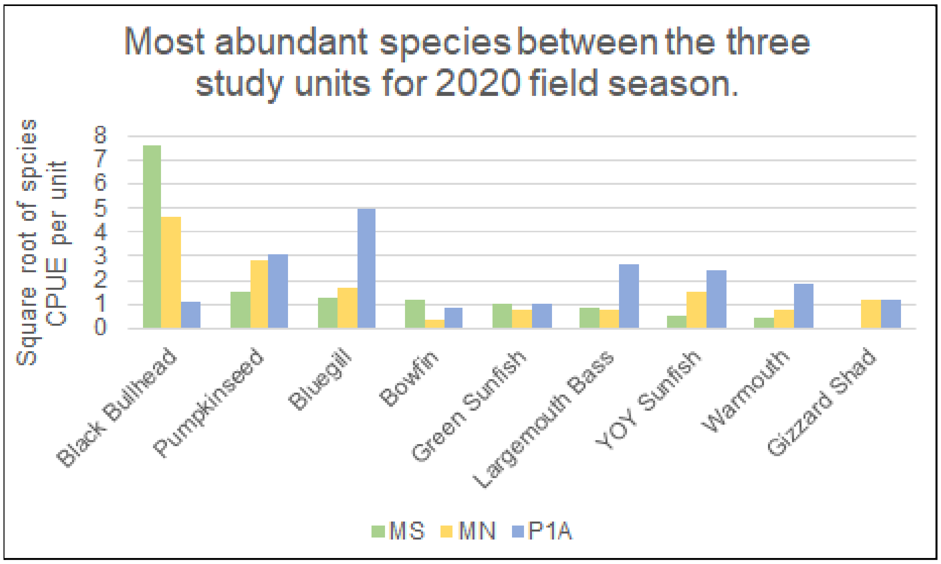


Figure 5.5: Bar graph showing the top 5 most abundant species in each unit. The y-axis shows the square root of species CPUE values to improve visualization of the relationships.

Influence of Month

ANOVA F-tests did not indicate that month was a statistically significant predictor of any of the variables tested (Table 5.3).

Month was not shown as having a significant predictor relationship with Gizzard Shad *Dorosoma cepedianum* CPUE by the F-test (Table 5.3). However, individual T-tests revealed significant relationships between certain months and Gizzard Shad CPUE, with September and October having significantly higher CPUE than other months. This discrepancy is likely due to errors in multiple testing. Consequently, we excluded these relationships from our results and from Table 5.4 because they were found to be significant only by individual T-tests and not *also* by the F-tests presented in Table 5.3.

Influence of Unit

ANOVA F-tests did not indicate that unit was a statistically significant predictor of any of the variables tested (Table 5.3).

Influence of Vegetation

From the ANOVA F-tests, we found vegetation to be a significant predictor in both site CPUE and Black Bullhead CPUE (Table 5.3) when controlling for the other variables considered. We determined that site CPUE was likely to be higher in SAV than in *Typha* and *Phalaris* (Table 5.4). Black Bullhead CPUE is likely to be higher in SAV than in Flooded Forest, *Typha*, and *Phalaris*.

Influence of Water Quality

We found average pH to be a statistically significant predictor of Yellow Perch CPUE, indicating a positive relationship. Average conductivity was found to be a predictor of Black Bullhead, Bluegill, and Pumpkinseed CPUE, and each indicated a negative relationship (as conductivity decreased, CPUE for each species increased) (Table 5.3).

We were unable to perform a statistical comparison of water quality findings between the 2019 and 2020 season due to errors in the analytical calculations in 2019 results. The 2020 team performed ANOVA analyses of the relationship between water quality, as one variable, with other variables like site CPUE and number of species. Consequently, these results were not specific to any water quality factors and do not allow for direct comparison between years.

	Month	**Unit**	**Veg.**	**Avg. Temp**	**Avg. pH**	**Avg. Cond.**	**Avg. Turb**	**Avg. DO**
Number of Species	0.501	0.132	0.1428	0.4673	0.719	0.057	0.2974	0.31
Site CPUE	0.3498	0.1489	**0.02339**	0.6756	0.4702	0.0905	0.2213	0.69
Black Bullhead CPUE	0.3951	0.2008	**0.00586**	0.8373	0.0813	**0.0482**	0.5362	0.5774
Bluegill CPUE	0.108	0.07948	0.4961	0.2722	0.899	**0.051**	0.3995	0.1734
Common Carp CPUE	0.3441	0.5909	0.5978	0.3999	0.2438	0.5355	0.2585	0.1817
Gizzard Shad CPUE	0.2013	0.2906	0.356	0.0687	0.9952	0.1466	0.4595	0.4596
Golden Shiner CPUE	0.8645	0.7116	0.1906	0.239	0.821	0.437	0.943	0.621
Green Sunfish CPUE	0.1585	0.2484	0.9749	0.565	0.918	0.129	0.326	0.429
Pumpkinseed CPUE	0.1217	0.06811	0.359	0.6213	0.2822	**0.0427**	0.2234	0.1403
Yellow Perch CPUE	0.4474	0.4287	0.3375	0.7732	**0.0101**	0.1818	0.2519	0.0638

Table 5.3: P-values from ANOVA F-test analyses showing strength of the variables Month, Unit, Vegetation type, average temperature, average pH, average turbidity, average conductivity, and average dissolved oxygen in predicting number of species, site CPUE, and the CPUE of eight species of interest. The p-values of significant variables are bolded. If a categorical variable (Month, Unit, Vegetation type) was found to be a significant predictor of a dependent variable, a separate T-test analysis was run to determine which group within the categorical variable was significant. Those results are presented in Table 5.4.

	Vegetation
Site.CPUE	**SAV** - *Typha* (0.00108) **SAV** - *Phalaris* (0.05558)
Black Bullhead	**SAV** - Flooded Forest (0.013021) **SAV** - *Phalaris* (0.045226) **SAV** - *Typha* (0.000143)

Table 5.4: Results from individual T-tests to determine which categorical sub-variables were significant predictors of the dependent variables in Table 5.3. Cells indicate the groups that were found significantly different from one another, with bolded groups indicating the higher value. P-values are shown in parentheses.

Index of Biotic Integrity (IBI)

We calculated IBI scores for each unit and compared our findings to scores from 2019. IBI scores range from 0 (high anthropogenic impact, low habitat quality) to 100 (low anthropogenic impact, high habitat quality). Scores are grouped and described in biotic integrity categories ranging from Degraded (<36) to Reference Quality (>60) (Cooper 2018).

In the SAV zone type, the MS scores decreased between 2019 and 2020, bringing the biotic integrity category down from Mildly Impacted to Degraded (Table 5.5). Scores for P1A SAV zone increased between 2019 and 2020 from Degraded to Moderately Degraded (Table 5.5). In the *Typha* vegetation zone type, we see the same pattern: the MS score decreased in both score and biotic integrity category, while the P1A score and category increased. MN remained the same in the *Typha* vegetation zone.

Unit	Vegetation Zone Type: SAV		Vegetation Zone Type: *Typha*	
	2019	2020	2019	2020
MS	54.54 Mildly Impacted	22.73 Degraded	55 Mildly Impacted	40 Moderately Degraded
MN	N/A*	45.45 Moderately Impacted	40 Moderately Degraded	40 Moderately Degraded
P1A	22.72 Degraded	40.91 Moderately Degraded	35 Degraded	40 Moderately Degraded

Table 5.5: IBI scores for each unit between 2019 and 2020. Note that fish sampling data from both SAV and *Nymphaea* were included in calculations for the SAV vegetation zone type and that fish sampling data from *Typha, Salix, Phalaris,* and Flooded Forest were included in calculations for the *Typha* vegetation zone type.
*The IBI score for 2019 MN was not calculated because water levels severely restricted fyke netting in that unit.

Length

Though we caught several notably large fish, the average size of fish caught was 10.3cm due to the high number of small fish caught. Large fish were commonly Bowfin species and one very large Common Carp (73cm). On average, Black Bullhead size increased over the sampling period. Largemouth Bass length steadily increased over the sampling period. Green Sunfish, Pumpkinseeds, and Bluegills were fairly constant in size throughout the season. Large numbers of YOY sunfish were captured in the month of July. The YOY sunfish were, on average, 2.9cm in length; any fish that were caught and measured under 2.0cm were not included in the dataset (Lutgen et al. 2020).

We found no significant differences in fish length across units (Figure 5.6). This was verified by running an ANOVA test of average length against the unit (Table 5.6). We also found no significant differences between months for any of the units (Figures 5.7 - 5.9).

Sites	P-value
MN - MS	0.9085488
P1A - MS	0.8377723
P1A - MN	0.9998433

Table 5.6: Table showing the p-values for an ANOVA of average fish length and month of net set.

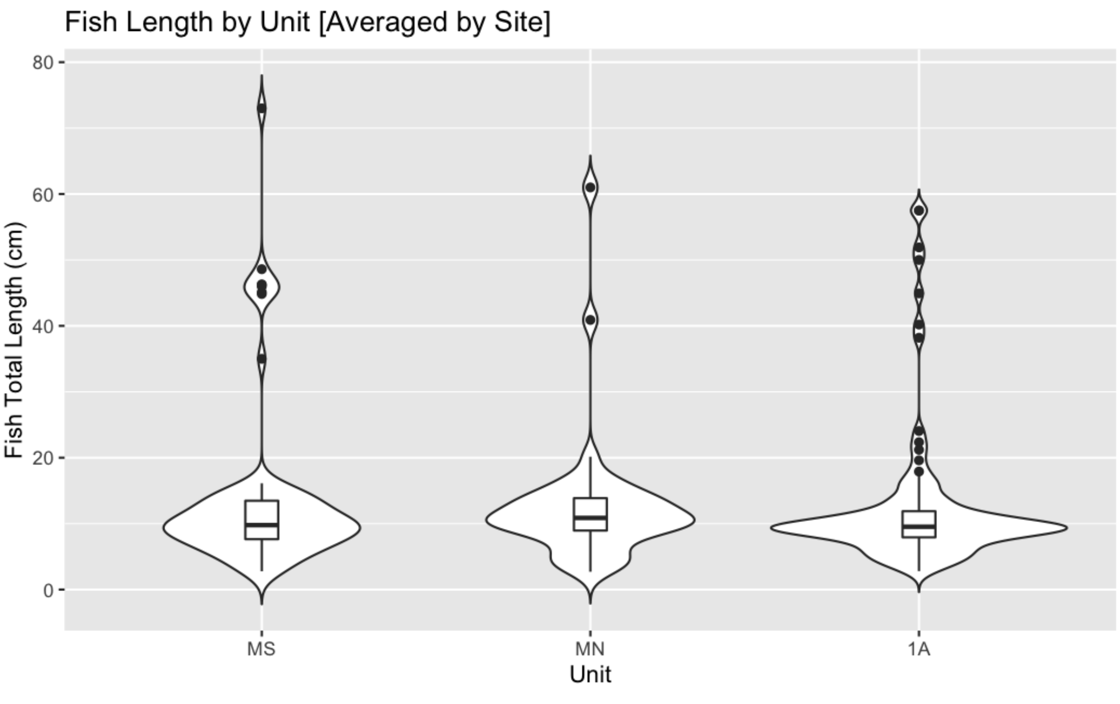


Figure 5.6: A violin plot of the distribution of fish lengths from the three sampled units. The width of the plot at a given length represents the frequency of occurrence for that size. Data were plotted using an average length of each species by site.

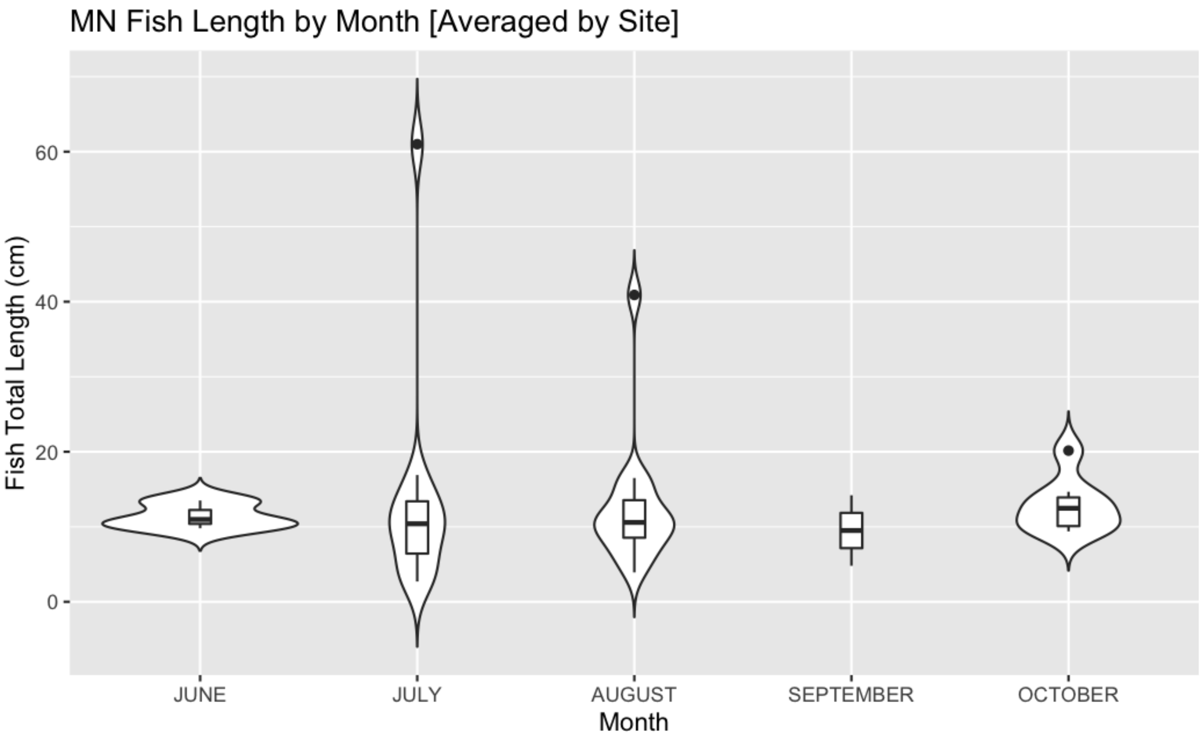


Figure 5.7: A violin plot of the distribution of fish lengths in MN. The width of the plot at a given length represents the frequency of occurrence for that size. Data were plotted using an average length of each species by site.

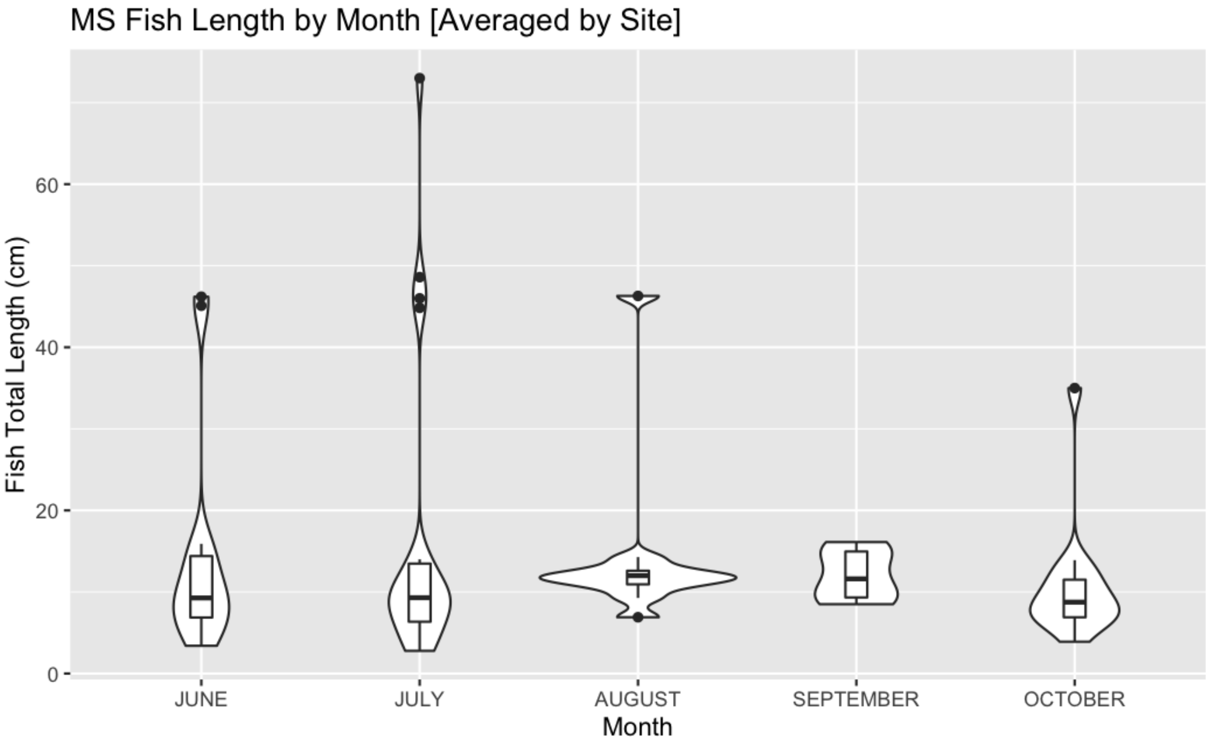


Figure 5.8: A violin plot of the distribution of fish lengths in MS. The width of the plot at a given length represents the frequency of occurrence for that size. Data were plotted using an average length of each species by site.

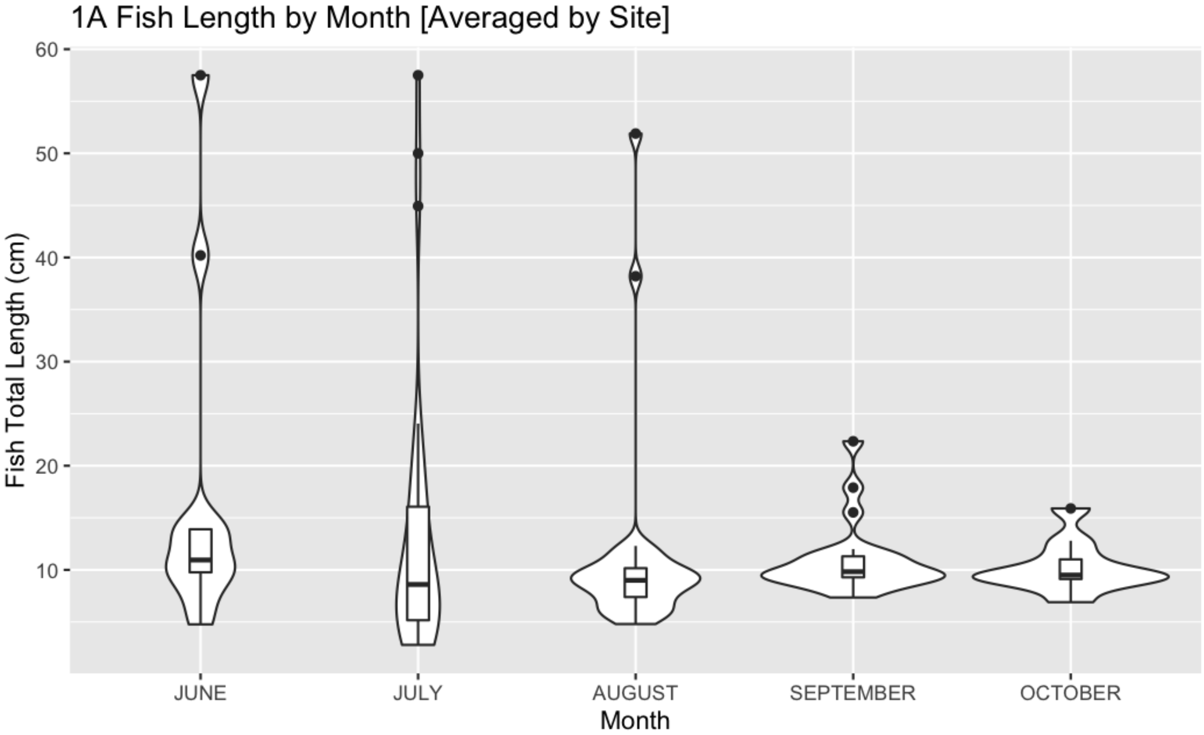


Figure 5.9: A violin plot of the distribution of fish lengths in P1A. The width of the plot at a given length represents the frequency of occurrence for that size. Data were plotted using an average length of each species by site.

DISCUSSION

Main Findings for Abundant Species

Black Bullhead

Black Bullheads were the most abundant fish caught during our sampling season and were in the top five abundant species for MN and MS. These fish are well-adapted to areas with low DO, high turbidity, high temperatures, and high levels of pollution that are not suitable for other fish (Rose 2006). This may make them less susceptible than other fish to diel fluctuations in DO and temperature in the units, allowing them to frequent the shallower waters where our nets were set.

Black Bullheads reach maturity at approximately 16cm (Rose 2006). MS had the smallest-sized Black Bullheads with an average length of 8.58cm, though MS also had the longest sampled individual, which was 26cm. The overall small size of this species in MS may be related to their high abundance. High population densities of these fish limit their growth and prevent them from reaching their full size capacity, and our data reflect the expected negative relationship for this species between length and abundance (Rose 2006). In P1A, Black Bullheads had an average length of 11.81cm, with 45% of sampled individuals being over 16cm in length. Despite MN being the unit with the highest average length, we did not catch any fish longer than 16cm, suggesting that this unit may not have many mature individuals.

Even though MN lacks mature individuals, swarms of juvenile Black Bullheads were seen in both MN and MS. The bulk of these fish (between 3–4cm) were found in the middle of July, which lines up with their breeding schedule, as Black Bullheads spawn in May to July and reach about 2.5cm two weeks after spawning (Rose 2006). Since both of these units were closed to the river and other units, this suggests that these habitats are suitable for Black Bullhead and indicates that the MS and MN populations are reproducing.

Bluegill and Pumpkinseed

The second and third most abundant species were Bluegill and Pumpkinseed, respectively. Both of these species were among the top five most abundant species in all three units. These sunfishes are found in warm, slow moving water with sand, mud, or gravel bottoms, and plenty of aquatic vegetation (Stuber 1982). They spawn in shallow water with gravel or rocky bottom sites for nest building (Brunson and Morris 2000). Between the two species, Pumpkinseeds are better able to withstand harsh conditions (Jordan et al. 2009).

Bluegills reach maturity when they are one to two years old and around 7.5cm in length (Paulson 2004; Parr 2013). The average length of Bluegills sampled across all units was 8.5cm. MN and MS had averages of 9.28cm and 9.25cm respectively, and P1A had an average of 8.25cm. From this, we estimate that 65% of all sampled Bluegills were likely mature.

Pumpkinseeds reach maturity after 2 years or after reaching 5cm in length (Danylchuk 1994). All Pumpkinseeds caught were greater than 5.4cm long, except for one outlier. The average length of Pumpkinseed caught was 10.84cm. Each unit had similar average lengths, of 10.84cm, 10.23cm, and 10.34cm for P1A, MN, and MS respectively. This shows that the populations in all units are of breeding age. Considering the size dynamics of these fish, it appears that they are growing well, so we can assume that the habitat is of suitable quality for this species.

Largemouth Bass

Largemouth Bass were the fourth most abundant species overall, but were only in the top five most abundant fish for P1A. The optimal riverine habitat for this fish is large, slow moving rivers, pools, or small streams with soft, but not silty, bottoms and relatively clear water (Stuber et al. 1982).

Male and female Largemouth Bass reach sexual maturity between three and twelve months of age (Laarman and Schneider 1985; Steed 2018). Largemouth Bass grow 10 to 15cm in their first year of life and 20 to 30cm within two years (Steed 2018). The average length of Largemouth Bass varied between units. P1A had an average length of 7.30cm, MN 16.04cm, and MS 9.43cm.

This indicates that the majority of sampled fish were yearlings and potentially of maturity. In addition, largemouth bass in all units displayed a positive trend in growth throughout the season.

The positive growth trends and high abundance of these fish may be due to the high presence of sunfish and Black Bullheads, which are known food sources for Largemouth Bass (Rose 2006; Parr 2013). However, this would not explain why Largemouth Bass were only in the top five species for P1A, as these prey fish were more abundant in MS. It is possible that, due to the connection between P1A and the Shiawassee River, Largemouth Bass preferred the habitat conditions of the river over those of P1A and were just wandering in. Another possible explanation for the higher abundance in P1A may be due to the pH value of the unit. Based on Stroud's (1967) criteria for freshwater fish, the optimal pH range for this fish 6.5–8.5. P1A was consistently within the optimal pH range, while the other two frequently exceeded the optimum value, particularly MN.

Young of Year Sunfish
YOY sunfish (2–4cm) were the fifth most abundant fish within the units and were in the top five fish for MN and P1A. All YOY sunfish were found in July, with the exception of one outlier in October. The most YOY sunfish were caught in P1A.

Since sunfish are known to nest in shallow waters typically between 0.33 to 0.66m, (Brunson and Morris 2000; Parr 2013), it is possible that water depth played a role in the higher presence of YOY sunfish in MN and P1A, as these two units had, on average, shallower waters than the sampled regions of MS. Another potential factor impacting YOY abundance could be turbidity and vegetation coverage (Stuber 1982). Increased turbidity would reduce predator visibility, making it easier for prey to escape. Similarly, increased aquatic vegetation provides more habitat in which prey can hide to avoid predation. Based on our data, it is more likely that turbidity impacted the population, as MN and P1A had higher turbidity averages than MS. Regardless, the presence of YOY shows that habitat in all units is suitable for sunfish populations to flourish as they are able to reproduce.

Other Species in Unit Top Five Abundances
In addition to the above species, Bowfin and Green Sunfish were in the top five most abundant fish in MS, Gizzard Shad was in the top five for MN, and Warmouth *Lepomis gulosus* was in the top five for P1A. All of these fishes are known to be able to survive harsh aquatic conditions. Bowfin are a sturdy species that are able to tolerate oxygen-poor waters, as they are able to breathe air (Univ of South Carolina Press 2009). Green Sunfish are able to tolerate environmental extremes and are usually the first sunfish to repopulate areas after disturbance (Brunson and Morris 2000; Clemons 2006). Gizzard Shad can live in highly turbid environments (Williamson & Nelson 1985; Fuller et al. 2021). Warmouth are able to survive in many different conditions and are often found in polluted and highly turbid waters (Larimore 1957).

Even though the majority of highly abundant fish can survive, and even thrive, in lower quality waterways, their presence may not be indicative of harsh habitat conditions. This is certainly indicated by the presence of Largemouth Bass and Bluegills, which are more sensitive to these harsh conditions. Additionally, we did not find substantial evidence of harsh conditions based on our water quality data. Overall, the floodplain units had low turbidity, midrange conductivity, neutral pH, and suitable temperatures for most warm water species. DO levels may be a bit harsher on Largemouth Bass and Bluegills.

Abundance Differences Between Field Seasons

Between the 2019 and 2020 field seasons, several statistically significant differences in species abundances were recorded between units and overall.

Sunfishes

Though Pumpkinseed and Green Sunfish were recorded in the top five species both years in MS, CPUE of both species decreased in the unit between 2019 and 2020. Pumpkinseed was recorded in the top five species in 2020 in both other study units as well, while Green Sunfish were only in the top five for MS. These two sunfishes require shallow waters to spawn. Water depth was not formally analyzed nor formally recorded in the field, however, we suspect water depth may explain this decline. Average water levels at our 2020 MS macroinvertebrate and vegetation sampling locations (water depths were not recorded at fish stations in 2019) were found to be higher than the averages from 2019 and potentially less suitable for spawning (Figure 5.10)

Additionally, the average length of Pumpkinseeds in MS increased from 7.4cm in 2019 to 10.34cm in 2020, and increased in MN from 9.8cm in 2019 to 10.2cm in 2020. Green Sunfish average lengths in MS increased from 7.9cm in 2019 to 11.6cm in 2020. Consequently, our team's samples likely represented both annual mortality and the development of surviving individuals from the 2019 cohort of both Pumpkinseed and Green Sunfish.

Pumpkinseeds reach maturity after two years or when they reach 5cm, which may occur earlier depending on resource availability (Danylchuk 1994). Since MS was first flooded in 2018, it is possible that we are tracking the development of the very first cohort in the unit. We would expect this cohort to be of mature size at this point, which is reflected in our findings. Further, MN was flooded one year prior to MS, in 2017, and 2019 data showed a higher average Pumpkinseed length in MN than MS. Again, this indicates that we may be tracking the first cohort of Pumpkinseed introduced to this unit as well.

We suspect the change in MS CPUE for both Green Sunfish and Pumpkinseed is more likely attributed to annual variation, rather than change in spawning habitat quality due to water depth. This is because Pumpkinseed CPUE also significantly decreased between 2019 and 2020 in MN where water levels decreased, which would make the habitat more suitable for Pumpkinseed spawning and would lead us to expect populations of juveniles there to increase (Figure 5.11).

We observed Bluegill abundance to increase significantly between 2019 and 2020 in P1A. Water depths decreased slightly in *Salix* and *Typha* vegetation zones, potentially facilitating increased spawning in those areas (Figure 5.12). We recorded only a negligible increase in average Bluegill length in P1A between 2019 and 2020 (from 7.997cm in 2019 to 8.213cm in 2020). We suspect that this increase in abundance is most likely due to the increased connectivity facilitated by the May 2020 flooding, which raised water levels in some areas of the refuge by up to 10ft over several days. Bluegill populations in aquatic systems with undisturbed flood patterns and connectivity have been found to have higher reproductive investments due to increased access to resource-rich floodplain and wetland habitats (Whitten et al. 2020). The May 2020 flooding may have directly carried individuals into the refuge wetlands during and in the days immediately following the flooding event and likely increased overall hydrologic connectivity in the region, allowing for easier movement of regional Bluegill into P1A. We would expect to see an increase in juvenile Bluegill in the 2021 season, following our abundance findings and Whitten et al.'s (2020) findings on reproductive investment.

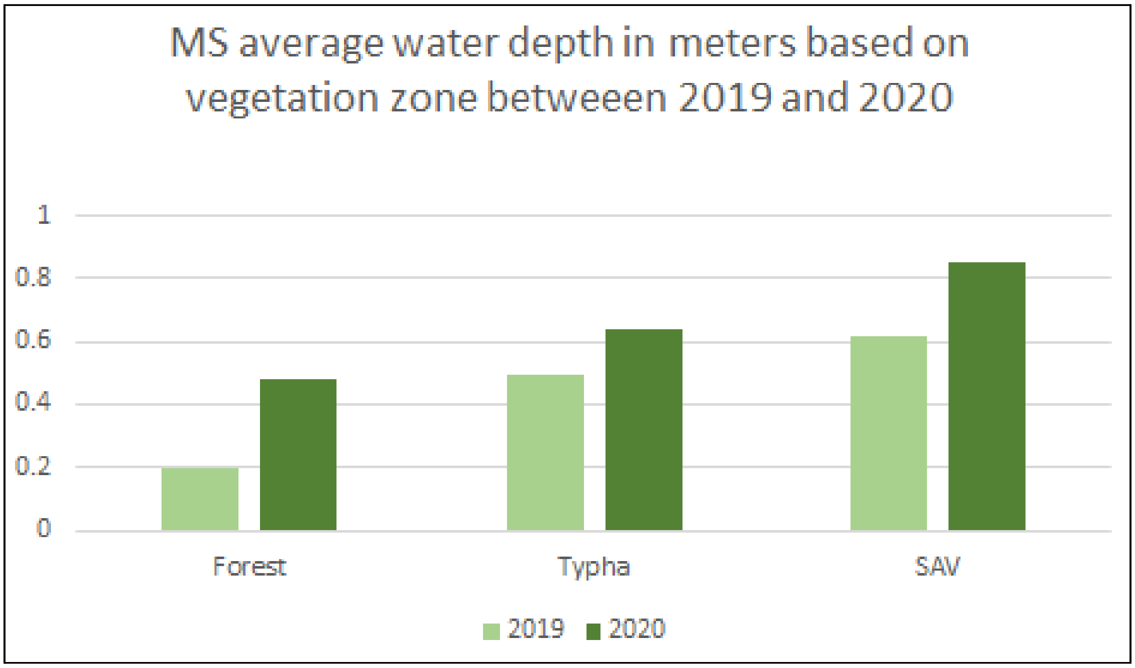


Figure 5.10: Average water depths at 2019 and 2020 MS macroinvertebrate and vegetation sampling sites. Water depth measurements from both macroinvertebrate and vegetation data were grouped by vegetation type and averaged across all months.

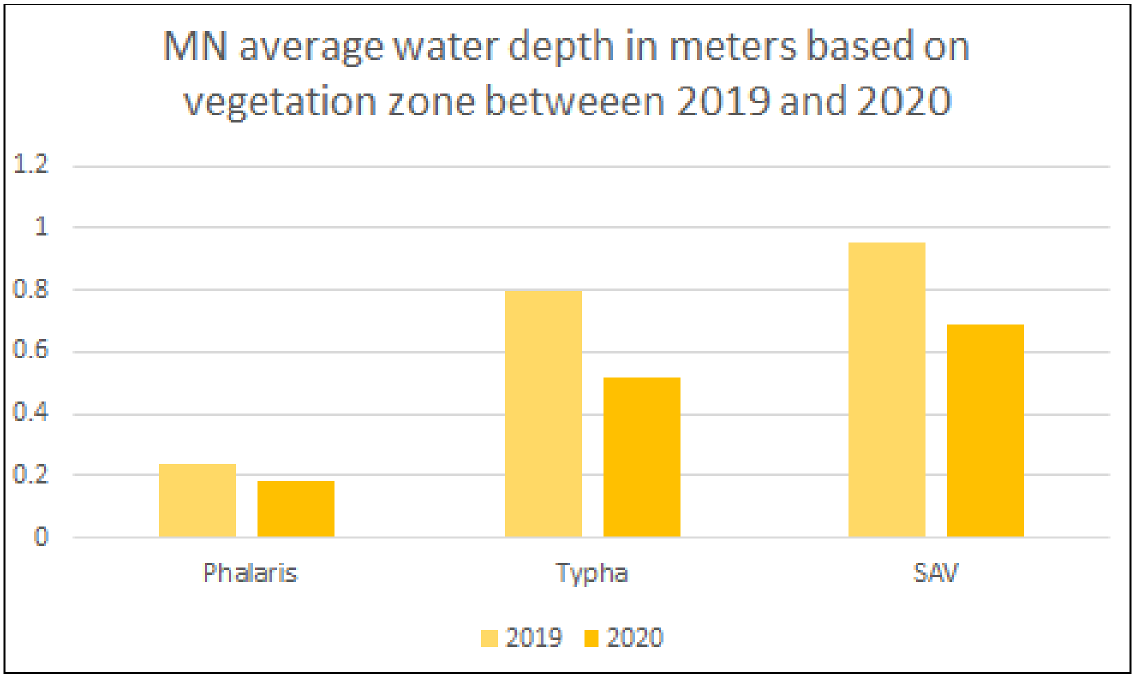


Figure 5.11: Average water depths at 2019 and 2020 MN macroinvertebrate and vegetation sampling sites. Water depth measurements from both macroinvertebrate and vegetation data were grouped by vegetation type and averaged across all months.

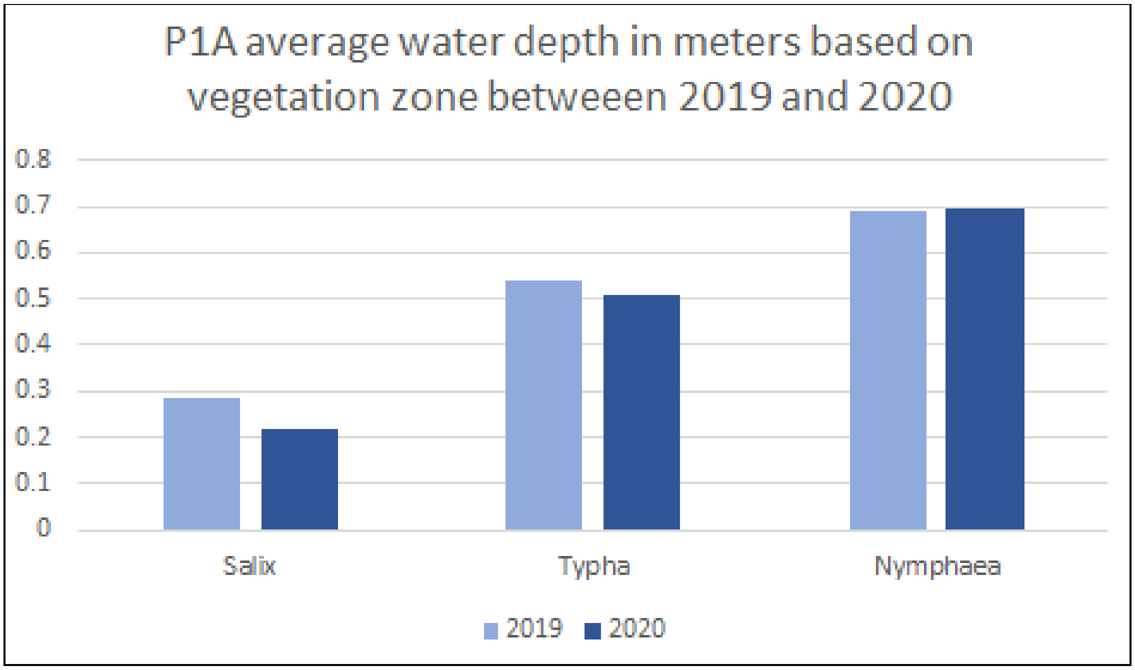


Figure 5.12: Average water depths at 2019 and 2020 P1A macroinvertebrate and vegetation sampling sites. Water depth measurements from both macroinvertebrate and vegetation data were grouped by vegetation type and averaged across all months.

Largemouth Bass

In 2020 we recorded a statistically significant increase in the overall abundance of Largemouth Bass, which was mostly due to a significant increase recorded in P1A. There was a slight increase in the average size of Largemouth Bass recorded in P1A between years, with an average 2019 length of 6.1cm and an average 2020 length of 8.2cm. We suspect that this indicates that in 2020, we recorded growth of the 2019 population, and that the increase in CPUE can be attributed to increased habitat connectivity due to May flooding.

Additionally, other habitat characteristics of P1A in 2020 may have allowed for successful Largemouth Bass persistence. Juvenile sunfish are a primary food source for Largemouth Bass (Steed 2018), and we recorded YOY Sunfish in the top five most abundant fish “species" in P1A in 2020. This high availability of prey likely helped sustain bass populations. Additionally, Largemouth Bass are primarily visual predators, and average turbidity levels in P1A were lower and less variable in 2020 than in 2019. Consequently, Largemouth Bass may have had higher predation success in 2020, making P1A a more sustainable habitat for their populations. However, the 2020 UM-Shiawassee Master’s Project team reported that their turbidity data collection methods were flawed and likely resulted in unreliable data (Lugten et al. 2020). We acknowledge that our hypothesis regarding increased predation success for Largemouth Bass is based on the potentially incorrect assumption that 2019 turbidity data were reliable and accurate.

Northern Pike

We recorded a statistically significant decrease in overall Northern Pike abundance between 2019 and 2020 which was mostly due to declines in unit CPUE in P1A and MN (we did not catch any in MN). Northern Pike are a predacious, generalist species that tend to migrate to shallow spawning sites in the spring (Mecozzi 1989; Lefevre 2012). Though abundances decreased, we recorded an increase in average length in P1A Northern Pike: 17.9cm in 2019 to 25.8cm in 2020. The average length of Northern Pike in MN in 2019 was 53.8cm. Northern Pike tend to leave their nursery wetland ecosystems when they reach a year old and around 30cm in size to seek less intensive thermal regimes as they continue to develop (Mecozzi 1989). Based on the changes in recorded average body length, we suspect that the observed decrease in P1A abundances in 2020 indicates the start of the 2019 year class emigrating from the floodplain unit. Additionally, the smallest individual recorded in 2020 was 17.5cm, indicating that we did not capture any new year class of Northern Pike in our samples. This may indicate that spawning conditions in P1A were too poor for reproduction in spring 2020. Alternatively, the May 2020 flooding could have affected juvenile Northern Pike in two different ways. First, the flooding could have washed juveniles out of the refuge units; or second, the increased connectivity to the river from the flooding facilitated easier passage out of the units by juveniles.

Emerald Shiner

Emerald Shiners *Notropis atherinoides* were only found in P1A in 2020 and showed a statistically significant increase in abundance since 2019. Populations of Emerald Shiners in Lake Huron collapsed due to competition with Alewife *Alosa pseudoharengus* but have since recovered (Schaeffer et al. 2008). Massive late summer and fall migrations upstream into Great Lakes coastal wetlands and smaller, inland lake systems have since been observed (Kapuscinski 2010). We caught Emerald Shiners throughout the entire summer sampling period, though we caught more during the end of July and September sampling periods. It is possible that our sampling captured some of the end of summer and fall migration of Great Lakes Emerald Shiner, but this increase in abundance between 2019 and 2020 is most likely due to standard annual variation. It is also possible that the increase in Emerald Shiners was due to increased connectivity from the May 2020 flooding. Previous studies have indicated that migration is the primary means for population growth of this species in wetlands, which are otherwise too harsh for Emerald Shiners to persist year-round (Kowalski et al. 2014).

Island Biogeography Theory (IBT) and SNWR

Fundamental to fish communities at SNWR is island biogeography theory (IBT), which describes species population dynamics based on the size of the island habitat, distance of the island from the mainland, or a main population source, and extinction rate of species on the island (Angeler et al. 2005). IBT has been extended to many types of ecosystems in which "island" refers to a patch of habitat and "mainland" refers to the nearest source population of the same species. With IBT, we also consider metapopulation dynamics, which recognize that the regional population of a species may be split between separate habitats between which individuals move (Angeler et al. 2005). For SNWR, the floodplain units can be thought of as islands, with the Shiawassee and Saginaw Rivers as one source population, and Saginaw Bay and Lake Huron as another. Connectivity between these two source populations and the refuge units is influenced by both the Shiawassee River and by the refuge control structures.

According to IBT, smaller islands that are farther from the mainland will have lower populations due to decreased resource availability and recruitment (Angeler et al. 2005). We consider the refuge units to be the "smallest island" in summer because it is the time of year when temperature and DO conditions are the most extreme and limiting and when predation by birds is particularly high. We also consider distance from the mainland to "increase" when units are closed to the Shiawassee River, because access is blocked and there is no recruitment. When control structures in MS and MN are closed, as they were throughout both the 2019 and 2020 sampling periods (though MS and MN were both briefly opened in October 2020 before our last sampling effort), fish populations can only increase through reproduction. Additionally, only those species that can avoid local extinction in the units by persisting in high temperature, low DO conditions will maintain viable populations, assuming predation is not unusually high. It is not surprising that hardy species, like Black Bullhead, Green Sunfish, Pumpkinseed, and Bluegill, were some of the most abundant species caught throughout the summer.

For less hardy species, the May 2020 flooding likely increased connectivity between populations, facilitating easier passage from mainland source populations (Shiawassee River) to the floodplain units. We see this reflected, for example, in the higher P1A abundances recorded for Largemouth Bass, Bluegill, and Emerald Shiner, which we attribute to increased connectivity from May flooding. Additionally, we recorded what is likely movement within the regional metapopulation of Gizzard Shad. Though not statistically significant, we observed higher Gizzard Shad abundances in September and October than in any other month sampled. Like Emerald Shiners, Gizzard Shad migrate inshore from Great Lakes waters in fall (Kowalski et al. 2014). Our samples captured indications of seasonal metapopulation connectivity as Gizzard Shad move between Lake Huron and seasonal habitats at SNWR.

Habitat Assessment

Vegetation Zone and Water Quality

We found vegetation zones to be a significant predictor of total site CPUE with SAV zones likely to have higher CPUE values than other vegetation types. SAV provides refuge habitat for juvenile fish and for macroinvertebrates, a common food source for many of the fish species we identified (Miller et al. 2018). The combination of available refuge zones and macroinvertebrate prey may explain the higher CPUE in SAV zones.

Black Bullhead CPUE was higher in SAV zones compared to other vegetation types. This may also be attributed to higher refuge habitat and prey densities in SAV zones, since Black Bullheads are omnivorous and typically prey on macroinvertebrates, particularly as juveniles (Rose 2006) . This could also help explain why Black Bullheads were found to be in the top five most abundant species in MS and MN but not in P1A, where there were no SAV-dominant zones. Future researchers could consider adding synthesis research questions focused on the relationship between unit vegetation zones, macroinvertebrate abundance, and fish community composition.

Of the water quality variables sampled, only average pH and average conductivity (uS/cm) were found to be significant predictors of the CPUE of certain species. In all cases where a species CPUE had a significant relationship with average conductivity, the relationship was negative. The Shiawassee River tends to have higher conductivity due to its increased input of surface runoff containing dissolved solids. Future research should consider conductivity as a focal study point to inform river connectivity management plans for the refuge.

As in 2019, during our final July sampling period, we recorded large numbers of dead fish in nets in *Nymphaea* vegetation zones within P1A. Our water quality recordings for each net set indicated that average turbidity and average DO recorded on these days were lower than the July average for P1A. DO levels dropped between the first net set and the second net set, going from 2.73mg/L on net pull one to 0.83mg/L on net pull two. We attribute fish mortality on the second net pull to this extremely low DO level. We expect to see diurnal fluctuation in DO, as it tracks vegetation photosynthesis and respiration activity, but the levels observed during this sampling effort both fall under the 3mg/L hypoxia threshold for wetlands (Kowalski et al. 2014). We suspect that the constant hypoxic conditions, combined with the extremely low level recorded during the second net pull, resulted in increased physiological stress on the fish in the net, leading to mortality.

Biotic Indices

In the SAV vegetation zone, MS decreased in biotic integrity and P1A increased in integrity between 2019 and 2020. We were unable to compare SAV zone IBI scores for MN because a 2019 score was not generated. Scores for the *Typha* vegetation zone followed the same pattern, decreasing in MS and increasing in P1A. The overall increase in P1A habitat quality is empirically supported by higher abundance of predatory Largemouth Bass, since the presence of top predators and keystone species is typically an indicator of healthy ecological communities. The *Typha* score for MN remained at 40 between sampling years. With the exception of the SAV zone score for MS, our overall scores are consistent with those for Great Lakes wetland habitats in similarly agriculturally-dominated landscapes (Cooper et al. 2018).

IBI scores quantify the level of anthropogenic impact to an ecosystem. The decrease in biotic integrity in MS, reflected in our 2020 IBI scores for fish, macroinvertebrates, and vegetation, indicated a higher influence of anthropogenic factors in that unit. We suspect this decrease to be most likely related, in some way, to the extreme flooding in May 2020. MS was opened to the river to accept floodwaters, which would have brought an influx of nutrients, sediments, and chemical pollutants to the unit. However, it is also equally possible that this change to the status of MS is due simply to annual variation because we would expect to see a similar pattern in MN, which was also opened in May 2020 to accept floodwaters. Should this pattern continue, more research should be conducted to understand differences between MS and MN that make the former more sensitive to human impact.

Study Limitations

We acknowledge the same biases associated with fyke net fish sampling presented by Lugten et al. (2020). These include species escape rates from the net, which we are unable to account for, and within-net predation, which we observed throughout the sampling season. We also acknowledge that compromised fyke nets (for example., by Muskrats *Ondatra zibethicus*) are another source of error, however we excluded data from any compromised nets from our analyses.

Additionally, our fyke netting was limited in two ways during our sampling season. Firstly, due to the COVID-19 pandemic, we were unable to begin our sampling until late June, whereas the 2020 team began in May. We were further restricted by the number of sampling efforts we were able to perform, again due to COVID-19 travel restrictions, leading to an overall smaller net set sample size than was collected by the 2020 team. Secondly, we were unable to perform electrofishing or gill net sampling during this 2020 season due to logistical limitations from the pandemic. Our exclusive use of fyke nets may have insufficiently sampled the fish community due to the water-depth, habitat bias associated with fyke netting (water must be deep enough to cover net cods but shallow enough that the net frame is not entirely submerged).

Finally, some of our statistical analyses comparing our results to those from the 2019 samplings season were limited due to errors found in the analysis of 2019 data.

Management Implications

We identified several variables that could contribute to fish community structure and diversity. Conductivity was found to have a statistically significant negative relationship with the abundances of certain fish species. Since the Shiawassee River tends to have higher dissolved solid input and conductivity, management efforts aiming to increase abundances of certain species should consider the level of unit-river connectivity. However, connectivity to the river also likely increases the abundance of other species and potentially overall diversity. Refuge staff should consider which species are of conservation priority before changing the river connectivity plans for refuge units.

Additionally, the SAV vegetation zone was found to be a significant predictor of increased overall fish abundance at sample sites. Management efforts could focus on promoting growth of this vegetation zone to increase fish abundances. Higher abundance of vegetation may also lead to increased growth and reproduction among fish species in the units; as many of the highly abundant fish use SAV as protection from predators, potential nesting grounds, and scavenge dead plant material for food.

Though we highlighted the potential influences of water depth, river connectivity, and seasonal flooding on the abundances of some fish species, our observation of considerable variation among units and between years indicates that further field data collection and analysis is the most effective management effort at this time. With only two years of sampling data to compare, it was challenging to observe or conclude that trends in the data were due to more than just annual variation. Further, both MS and MN are relatively young units and were only flooded in 2016 and 2017 respectively. The vegetation communities, and those faunal communities that depend on them, are consequently early in their ecological development. It is in the refuge's best management interests to maintain the current habitat management plan while continuing to monitor the floodplain units and record any annual flooding or increased connectivity events in order to build a more robust database.

Overall, our research indicates that the floodplain units of the refuge provide seasonal spawning and feeding habitat for Great Lakes, riverine, and wetland fish species. Additionally, we recorded the likely growth of year classes initially recorded in 2019. Based on the high number of sunfishes, Black Bullhead, and YOY Sunfish, and the changes in species abundances between 2019 and 2020, we can say that these ecosystems are highly productive, though subject to environmental fluctuations. We conclude that the robust fish communities of the refuge support populations of piscivorous birds and other wildlife; and that monitoring of fish communities should continue to further understand those environmental factors that influence community composition.

Recommendations

The Species Accumulation Curves (SAC) from our season near asymptotes at lower average number of species and lower number of sites than those from 2019. We suspect that this difference in SAC results can be attributed to the month in which we began sampling. The 2020 team began sampling fish in May and were able to capture those migratory fish present in spring, but that then may leave the floodplain units. Our team was not able to begin sampling until late June 2020, missing the opportunity to capture springtime species. Species captured by the 2020 team in 2019 that we did not find in 2020 included Banded Killifish *Fundulus diaphanus*, Bluntnose Minnow, Channel Catfish, Fathead Minnow *Pimephales promelas*, Spottail Shiner *Notropis hudsonius*, Tadpole Madtom *Noturus gyrinus*, and Yellow Bullhead *Ameiurus natalis*. Each of these species, with the exception of the Tadpole Madtom, was captured at least once in May and the Spottail Shiner and Yellow Bullhead were also captured in November. From these findings, we recommend that future teams aim to begin sampling as early in the year as possible to capture any migratory species that may be absent later in the season. Additionally, considering that some of the 2019 unique species were also captured in November, we recommend incorporating a later fall sampling effort as well, which we did not do during 2020.

We attributed some of the variation in species abundances between 2019 and 2020 to changes in water levels, though we were unable to perform formal statistical analyses of water depth data. The 2020 team concluded that intra-unit microtopography was the likely cause of variation in ecosystem composition, further reinforcing the need for specific research questions aimed at understanding water depth and floodplain unit topography (Lugten et al. 2020). We recommend that future monitoring groups incorporate water depth into their research questions, field sampling protocols, and statistical analyses. Having these data will potentially help future teams explain variation in fish, vegetation, and macroinvertebrate communities. Water depth data from

2019 and 2020 were collected only at sampling sites and consequently does not account for micro-topography within the structure of each unit. For this reason, we recommend that a comprehensive water depth survey of all vegetation zones within each unit be performed to distinguish true depth variation from micro-topographic differences.

Additionally, both our study and the study for the 2019 field season found evidence of a relationship between conductivity and fish abundances and mortality. We therefore recommend future teams perform more in-depth conductivity analyses within the floodplain units and incorporate conductivity sampling of the Shiawassee River. We also recommend consistent use of DO Loggers and HOBO Depth Loggers on fish nets throughout the entire sampling season to monitor diurnal DO variation which may help explain in-net fish mortality.

Findings across both sampling years indicate that the units are home to an abundance of sunfishes and Black Bullheads during summer months. Of the birds that utilize SNWR habitat, at least 42 species are partly or primarily piscivorous including the Osprey *Pandion haliaetus*, Bald Eagle *Haliaeetus leucocephalus*, Double-crested Cormorant *Phalacrocorax auritus,* and various species of Egret, Rail, Grebe, Gull, Heron, Tern, and Merganser. Future groups should consider collecting data on variation in bird feeding activity between refuge units to determine if there is a positive relationship to abundance of prey fish species.

Finally, we recommend that future teams review the statistical analyses performed in this report prior to developing research questions for their field seasons. This will allow them to craft research questions that can be addressed with available analysis methods that will be comparable to the results presented in this report.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Refuge Ecosystem Variability

Overall, the suite of water quality, vegetation, macroinvertebrate, and fish data generated relatively consistent pictures across the three study units and between seasons. Habitat quality and biotic indices for vegetation, macroinvertebrates, and fish all revealed a 2020 decline in the habitat quality of MS, though classification of the macroinvertebrate score remained within the Mildly Impacted score range. Macroinvertebrate and fish scores for MN and P1A also tell a similar story, indicating maintenance or an improvement of quality in both units. Vegetation scores revealed a decline in quality throughout all units, which was particularly pronounced in MN, which dropped six points on the IBI scale partially due to an influx of invasive species in the *Phalaris* vegetation zone from 2019 to 2020.

Additionally, our data do not indicate that the two most recently reconnected units, MS and MN, are following the exact same restoration and ecosystem development trajectory. The NMDS for macroinvertebrates showed significant differences in the communities in each unit. Though Black Bullhead, Bluegill, and Pumpkinseed were on the top five lists for both MS and MN, the abundances of these species, and others, were notably different between units. Overall, similarities and differences between MS and MN and the reference unit, P1A, were greatly variable. For example, we found numerous invasive species of vegetation in MN that were likely introduced from the May 2020 flooding. However, we did not find invasive species to be as pervasive in MS, as would be expected since both units were open for the flood. Continuing ecological monitoring at the refuge will help staff and researchers understand how variety within and between the study units may be related to ecosystem succession and development.

We identified three likely sources of variability in ecosystem characteristics, structure, and composition across all variables studied. First, we attribute variation in water depth within units to be a likely influential factor on water quality and fish community composition. Similar to the 2019 sampling team, we believe that this intra-unit topographic heterogeneity influences all ecosystem characteristics studied, though most specifically, water quality and fish community composition (Lugten et al. 2020). Water depth can influence water temperature and consequently DO levels, which in turn affects fish movements and survival, particularly for species that are not well-adapted to sometimes hypoxic conditions of wetland habitats.

Second, we believe the historic May 2020 flooding had a significant impact on the ecosystems in each refuge unit. Flooding likely brought an influx of sediments, nutrients, pollutants, invasive species, and native species into the units of study before we began sampling. We have attributed some of the differences in vegetation zone composition, fish species abundances, and water quality parameters between 2019 and 2020 to these flood-induced inputs. However, we did not find substantial nor consistent differences across all parameters and units. The most common fish species and macroinvertebrate families stayed relatively consistent between seasons, with some variation in abundance. Additionally, though vegetation, macroinvertebrate, and fish IBI scores changed in some units, the classifications of those scores mostly stayed the same. This means that despite the change in numeric score in 2020, the ecosystem can be considered in the same condition as was recorded in 2019. This relative consistency, especially considering the degree of flooding and ecosystem disruption, indicates some level of ecosystem stability throughout these floodplain units. Considering the recency of MS and MN's reconnection to the floodplain, this is a notable finding.

Finally, we acknowledge that some of the differences within and among the ecosystems of SNWR between 2019 and 2020 are likely due to simple annual variation, for which we do not yet have a robust enough log of data for comparison. We recommend that the refuge continue to support ecosystem monitoring to establish a usable data log to better understand observed variations.

Overall Research Recommendations

First, we recommend that future monitoring groups establish both their central research questions and their exploratory research questions as early as possible. For example, if a future Master's Project team is assembled in January of a given year, we would encourage that team to establish their research questions by March or April before the field season begins. In contrast, our team spent the months leading up to the sampling season preparing to conduct monitoring in the same way as the previous year. As a result, we spent little time generating our own research questions and sampling plans based on the findings from last year's report. This oversight was exacerbated by uncertainty from the COVID-19 pandemic, as we were unsure if we would be able to perform field sampling during the 2020 season.

Second, we recommend that several changes be made to specific data gathering techniques. We recommend sampling for fish in deeper water whenever time, personnel, and equipment allow. Due to COVID-19 protocols, we were unable to perform electrofishing from a boat and were limited to setting fyke nets only in wadable water, around 180cm maximum depth. When restrictions are lifted and it is safe to sample as a group from a boat, we recommend electrofishing to sample for fish in deeper water (>180cm). Fish data from deeper water will be particularly relevant to monitoring how fish move throughout the refuge, particularly from shallow to deep portions of the floodplain units. We found DO levels varied throughout the seasons and in different locations, particularly in shallow water. We hypothesize that fish were coping with low DO levels in shallow areas by moving to deeper water, or perhaps by leaving the units for more oxygenated water in the Shiawassee River (when control structures allowed). For example, researchers could sample in deep water in July, a month in which DO levels were recorded as particularly low during both the 2019 and 2020 seasons, and compare fish assemblages to those captured in fyke nets on the same days in shallower locations. In addition, electrofishing could allow us to sample from waters nearby the Adaptive Resolution Imaging Sonar (ARIS) camera to ground truth data. If the ARIS camera captures movement of a large number of unknown fish species, electrofishing near the camera could help researchers verify the species and eventually incorporate fish identification into ARIS footage analysis methods.

We further recommend formal water depth sampling within and between the study units.Though water depth was recorded when setting fyke nets and sampling vegetation, our team did not have any complete information on the true depth and topographical variation within each floodplain unit. Instead, we had depth data only for the exact sites where we sampled. These sites were selected randomly and were thus different from previous research teams and not comparable. Similar to findings from the 2019 season, we hypothesized that much of the fish community and water quality variation was due to water depth and topographic heterogeneity throughout the study units (Lugten et al. 2020). A firm understanding of depth and depth variation throughout the entire floodplain unit could help researchers understand the distribution of vegetation, macroinvertebrates, and fish.

We strongly recommend incorporating a more comprehensive method for monitoring DO throughout the season, as this is crucial for understanding ecosystem changes within the study units. One way DO measurements could be improved is by consistently deploying DO and HOBO loggers, which can be left overnight to gather information on diel DO fluctuations. DO loggers were used during the 2019 field season, but we were unable to because the loggers were being serviced. Consequently, we could only record DO when we were physically present at a site and could take a point-in-time water quality measurement with the sonde. Regularly monitoring DO with loggers is particularly useful for analyzing DO trends in the study units overnight, when DO is known to drop dramatically (Lugten et al. 2020). Having these diurnal DO data would have greatly strengthened our data analysis.

Finally, we recommend that future research teams start gathering data in the study units as soon as possible and for as long as possible. We encourage future groups to draft a plan for sampling in the spring and fall, in addition to summer sampling months. Moreover, having clear research questions predetermined for this multi-seasonal sampling will help teams know exactly how much they need to sample in spring, summer, and fall months. Having robust, specific data will help future teams and refuge staff better understand the dynamic ecosystems at SNWR.

Implications for Science and Management

The importance of restoration work occurring at SNWR, particularly given the overall improvement in habitat quality, as evidenced by IBI scores, cannot be understated. The refuge has begun to hydrologically restore areas that were agricultural fields of lesser quality to improve habitat for target waterfowl species (USFWS 2018). We have observed the improvement in habitat for not only waterfowl, but also fish, macroinvertebrates, and native vegetation. Overall, our team found macroinvertebrate and fish biotic integrity to have slightly improved between years in some units. While vegetation IBI scores declined slightly between 2019 and 2020 due to an increase in invasive species presence, the fact remains that a great deal of progress has been made by refuge staff in terms of meeting current and future management and restoration goals at SNWR. The short timeframe during which MN and MS have been restored to a similar level as that of the reference unit P1A is highly encouraging for future restoration efforts at the refuge.

In addition, because we used an IBI score developed specifically for Great Lakes coastal wetlands, SNWR can now be accurately compared to similar systems in the region. This allows for SNWR staff to learn from other similar restoration efforts and vice versa. Likewise, and perhaps most importantly, the use of these scores places SNWR in the overall context of other reference wetland systems in the Great Lakes region. This will contribute to researchers' and managers' better understanding of the role these important ecosystems play in the region.

The restoration monitoring work at SNWR will also help in understanding the length of time required for successful development and restoration of coastal wetlands. Since P1A has been connected to the Shiawassee River since 1958, it serves as a "control" unit, which provides a comparison point for monitoring the amount of time it takes for MN and MS to have a similar quality and species composition. This work will also allow the refuge to monitor and identify the stages of succession that these units go through, particularly in terms of vegetation structure. It is likely that trends in natural annual and seasonal variability will emerge over time, and it will be very useful for SNWR to have yearly data to track this process.

Finally, the historic flooding that occurred in May 2020, which was exacerbated by historically high water levels in Lake Huron, will likely prove to be highly significant for the refuge (U.S. Army Corps of Engineers 2021). Since we were able to conduct sampling of fish, macroinvertebrates, vegetation, and water quality in the aftermath of this event, SNWR will now have what may prove to be a rare dataset. The refuge will be able to compare data from 2019 and all future non-flood years with our 2020 data. It will be useful to know how a severe flood event impacts the system in a given year and those following. This is especially important now, as it is likely that historic flooding events will increase in intensity as climate change progresses (Byun et al. 2019). It is also important to recognize that sampling during both the 2019 and 2020 seasons occurred when water levels in Lake Huron were particularly high, therefore increasing connectivity between waterways and floodplain units (U.S. Army Corps of Engineers 2021). This will allow for interesting comparisons to be made regarding community composition, vegetation structure, and water quality parameters across time at SNWR when water levels in Lake Huron eventually drop.

Furthermore, such shifts in community composition due to water levels and flood events can provide clues regarding how island biogeography theory (IBT) may play a role in the overall ecosystem of the refuge. Individual units within the refuge act as “islands” of habitat for a number of species, which are accessible both through opening of control structure gates and high water levels and flood events. Incorporating this theory can help characterize the role the refuge plays as a fixture in supporting regional fish, macroinvertebrate, vegetation, and bird metapopulations.

REFERENCES

INTRODUCTION

Buchanan, J., Chorbajian, S., Dominguez, A., Hartleben, B., Knoppow, B., Miller, J., Schulze, C., Seiter, C., Chang, Y. 2013. Restoring the Shiawassee Flats: Estuarine Gateway to Saginaw Bay. Master's Project Final Report. *University of Michigan School for Environment and Sustainability.*

Comer, P. J. (1996). Wetland Trends in Michigan since 1800: a preliminary assessment. Environmental Protection Agency.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master's Project Final Report. *University of Michigan School for Environment and Sustainability.*

Davidson, N. C. (2014). How much wetland has the world lost? Long-term and recent trends in global wetland area. *Marine and Freshwater Research*, 65(10), 936-941.

Heitmeyer, M. E., C. M. Aloia, E. M. Dunton, B.J. Newman, and J.D. Eash. (2013). Hydrogeomorphic evaluation of ecosystem restoration and management options for Shiawassee National Wildlife Refuge. Prepared for U. S. Fish and Wildlife Service, Region 3. *Greenbrier Wetland Services Report* 13-07, Blue Heron Conservation Design and Printing LLC, Bloomfield, MO.

U.S. Fish and Wildlife Service. (2001). Shiawassee National Wildlife Refuge: Comprehensive Conservation Plan and Environmental Assessment. U.S. Fish and Wildlife Service, Saginaw, MI.

U.S. Fish and Wildlife Service. (2018). Shiawassee National Wildlife Refuge: Habitat Management Plan. *U.S.* Fish and Wildlife Service & National Wildlife Refuge System*,* 1-105.

Uzarski, D.G. et al. (2016). Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. *Wetlands*, 37(1).

WATER QUALITY MONITORING

Baustian, J. J., Kowalski, K. P., and Czayka, A. (2018). Using Turbidity Measurements to Estimate Total Phosphorus and Sediment Flux in a Great Lakes Coastal Wetland. *Wetlands*, 38(5), 1059–1065.

Cherry, J. A. (2011). Ecology of Wetland Ecosystems: Water, Substrate, and Life. *Nature Education Knowledge,* 3(10),16.

Chumchal, M. (2002). "Cyprinus carpio" (On-line), Animal Diversity Web. Accessed April 09, 2021 at https://animaldiversity.org/accounts/Cyprinus_carpio/.

“Climate: Supplemental Daily Climate Data”. *National Weather Service*. Accessed April 13, 2021 at https://www.weather.gov/dtx/supdata2.

Environmental Protection Agency. (2001). Total Kjeldahl Nitrogen in Water and Biosolids by Automated Colorimetry with Preliminary Distillation/Digestion. Environmental Protection Agency.

Environmental Protection Agency. (2001). The Effect of Climate Change on Water Resources and Programs. Watershed Academy Web. Environmental Protection Agency.

Esri Inc. (2021). ArcGIS Survey123 (version 3.12.277) [Mobile app]. Esri Inc.

Fondriest Environmental, Inc. (2013). “pH of Water.” Fundamentals of Environmental Measurements. Web. https://www.fondriest.com/environmental-measurements/parameters/water-quality/ph/.

Jordan, C., Backe, N., Wright, M.C., and Tovey, C.P. (2009). Biological synopsis of pumpkinseed (Lepomis gibbosus). Can. Manuscr. Rep. Fish. Aquat. Sci. 2886: iv + 16 p.

Kowalski, K. P., Wiley, M. J., and Wilcox, D. A. (2014). Fish assemblages, connectivity, and habitat rehabilitation in a diked Great Lakes coastal wetland complex. Transactions of the American Fisheries Society, 143(5), 1130-114.

Liu, H., Hu, Z., Zhang, J., Ngo, H.H, Guo, W., Liang, S. and Fan, J.. (2016). Optimizations on supply and distribution of dissolved oxygen in constructed wetlands: A review. *Bioresource technology*, 214, 797–805. Journal Article, OXFORD: Elsevier Ltd.

Lugten, E. (2020). Personal Communication during field sampling efforts.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master’s Project Final Report. *University of Michigan School for Environment and Sustainability.*

Lyman, F. E. (1944). Effects of a Flood upon Temperature and Dissolved Oxygen Relationships in Cherokee Reservoir, Tennessee. Ecology (Durham) 25(1), 70-84.

Munn, M.D., Frey, J.W., Tesoriero, A.J., Black, R.W., Duff, J.H., Lee, Kathy, Maret, T.R., Mebane, C.A., Waite, I.R., and Zelt, R.B. (2018). Understanding the influence of nutrients on stream ecosystems in agricultural landscapes: U.S. Geological Survey Circular 1437, 80.

Ovington, J. (1953). Studies of the Development of Woodland Conditions Under Different Trees: I. Soils pH. *Journal of Ecology,* 41(1), 13-34. doi:10.2307/2257097

Rehman, F., Pervez, A., Khattak, B.N. and Ahmad, R. (2017), Constructed Wetlands: Perspectives of the Oxygen Released in the Rhizosphere of Macrophytes. Clean Soil Air Water, 45. https://doi-org.proxy.lib.umich.edu/10.1002/clen.201600054

Rose, C. (2006). "Ameiurus melas" (On-line), Animal Diversity Web. Accessed March 22, 2021 at https://animaldiversity.org/accounts/Ameiurus_melas/.

Rowe, M.D., Errera, R.M., Rutherford, E.S., Elgin, A.K., Pilcher, D.J., Day, J., and Guo, T. (2020). Great Lakes Region Acidification Research. NOAA Ocean, Coastal, and Great Lakes Acidification Research Plan: 2020-2029, https://oceanacidification.noaa.gov/ResearchPlan2020.

Spieles, D. J., and Mitsch W. J. (2003). A model of macroinvertebrate trophic structure and oxygen demand in freshwater wetlands. *Ecological Modelling,* 161(3), 183-194.

Stuber, R. J., G. Gebhart, and O. E. Maughan. (1982). Habitat suitability index models: Bluegill. U.S.D.l. Fish and Wildlife Service. FWS/OBS-82/10.8, 26.

USGS. (2021). National Water Information System. Retrieved April 09, 2021, from https://nwis.waterdata.usgs.gov/usa/

Weaver, T. L. and Fuller, L. M. (2007). Stream-water quality during storm-runoff events and low-flow periods in the St. Clair River/Lake St. Clair Basin, USGS Open-File Report 2007-1201, Washington, D.C.

VEGETATION MONITORING

Chadde, S. (2012). Wetland plants of Michigan: A complete guide to the wetland and aquatic plants of the Great Lakes state. 2nd edition. U.S.A.

Chen, H., Qian, H., Spyreas, G., and Crossland, M. (2010). Native-exotic species richness relationships across spatial scales and biotic homogenization in wetland plant communities of Illinois, USA. *Diversity and Distributions*, 16 (5), 737-743.

Cohen, J.G., M.A. Kost, B.S. Slaughter, D.A. Albert, J.M. Lincoln, A.P. Kortenhoven, C.M. Wilton, H.D. Enander, and K.M. Korroch. (2020). Michigan Natural Community Classification [web application]. Michigan Natural Features Inventory, *Michigan State University Extension,* Lansing, Michigan.

Craft, C. (2015). Creating and restoring wetlands: From theory to practice. Elsevier.

CWMP. (2018). Protocol for Great Lakes Marsh Sampling. Coastal Marsh Vegetation Detailed Sampling Procedures (SOP). *Central Michigan University.*

De Szalay, F. A., and Resh, V. H. (2000). Factors influencing macroinvertebrate colonization of seasonal wetlands: responses to emergent plant cover. *Freshwater Biology*, 45(3), 295-308.

Environment Canada and Wilcox, Douglas A. (2002). "Where Land Meets Water: Understanding Wetlands of the Great Lakes." Government Documents. 37.

Esri Inc. (2021). ArcGIS Survey123 (version 3.12.277) [Mobile app]. Esri Inc.

Freyman, W.A., L.A. Masters, and S. Packard. (2016). The Universal Floristic Quality Assessment (FQA) Calculator: an online tool for ecological assessment and monitoring. *Methods in Ecology and Evolution,* 7(3), 380–383

Glority LLC. (2020). PictureThis - Plant Identifier (Version 3.0.5) [Mobile application software]. Retrieved from http://itunes.apple.com

Heitmeyer, M. E., C. M. Aloia, E. M. Dunton, B.J. Newman, and J.D. Eash. (2013). Hydrogeomorphic evaluation of ecosystem restoration and management options for Shiawassee National Wildlife Refuge. Prepared for U. S. Fish and Wildlife Service, Region 3. *Greenbrier Wetland Services Report* 13-07, Blue Heron Conservation Design and Printing LLC, Bloomfield, MO.

Jude, D. J., and Pappas, J. (1992). Fish utilization of Great Lakes coastal wetlands. *Journal of Great Lakes Research*, 18(4), 651-672.

Larson, J. H., Trebitz, A. S., Steinman, A. D., Wiley, M. J., Mazur, M. C., Pebbles, V., and Seelbach, P. W. (2013). Great Lakes rivermouth ecosystems: scientific synthesis and management implications. *Journal of Great Lakes Research,* 39(3), 513-524.

Liu, H. and Stiling, P., (2006). Testing the enemy release hypothesis: a review and meta-analysis. *Biological invasions*, 8(7), 1535-1545.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master's Project Final Report. *University of Michigan School for Environment and Sustainability.*

Monks, A.M., Lishawa, S.C., Wellons, K.C., Albert, D.A., Mudrzynski, B. and Wilcox, D.A., (2019). European frogbit (Hydrocharis morsus-ranae) invasion facilitated by non-native cattails (Typha) in the Laurentian Great Lakes. *Journal of Great Lakes Research*, 45(5), 912-920.

NOAA. (2020). Lake Superior Manoomin Cultural and Ecosystem Characterization Study. Retrieved from: https://www.1854treatyauthority.org/images/Manoomin_Final.Report_Online.Version_2020.05.29-compressed.pdf.

Puz, MV, Dunton E, Lugten E, Mitchinson O, Sens M, and Vogel, K. (2020). Site-specific Protocol for Inventory and Monitoring of Wetland Vegetation: Shiawassee National Wildlife Refuge. Version 1.0 . Saginaw, Michigan: US Fish and Wildlife Service, Region 3, Bloomington, MN.

Simberloff, D., and Von Holle, B. (1999). Positive interactions of nonindigenous species: invasional meltdown? *Biological invasions,* 1(1), 21-32.

U.S. Army Corps of Engineers. (2021). Great Lakes Update. Volume 208: 2020 Annual Summary. U.S. Army Corps of Engineers, Detroit District.

MACROINVERTEBRATE MONITORING

Blinn, D. W. and Ruiter, D. E. (2013). Tolerance Values and Effects of Selected Environmental Determinants on Caddisfly (Trichoptera) Distribution in *Northwest and North Central Washington, USA.* Western North American Naturalist 73(3), 270-294.

Buchanan, J., Chorbajian, S., Dominguez, A., Hartleben, B., Knoppow, B., Miller, J., Schulze, C., Seiter, and C., Chang, Y. (2013). Restoring the Shiawassee Flats: Estuarine Gateway to Saginaw Bay. Master's Project Final Report. University of Michigan, Ann Arbor, Michigan.

Burton, T. M., Uzarski, D. G., Gathman, J. P., Genet, J. A., Keas, B. E., and Stricker, C. A. (1999). Development of a preliminary invertebrate index of biotic integrity for Lake Huron coastal wetlands. *Wetlands,* 19(4), 869-882.

Brooks, R. T., and Colburn, E. A. (2012). “Island” Attributes and Benthic Macroinvertebrates of Seasonal Forest Pools. *Northeastern Naturalist*, 19(4), 559-578.

Cooper, M. J., Uzarski, D. G., and Burton, T. M. (2007). Macroinvertebrate community composition in relation to anthropogenic disturbance, vegetation, and organic sediment depth in four Lake Michigan drowned river-mouth wetlands. *Wetland*s, 27(4), 894-903.

CWMP. (2019). Great Lakes Coastal Monitoring Program Standard Operating Procedure Macroinvertebrate Sampling and Laboratory Processing. *Great Lakes Coastal Wetland Monitoring Program*.

Duck Unlimited. (2016). Insects: Fueling the Next Generation of Ducks. *Ducks Unlimited Canada*.

Gathman, J., Brady, V., Dobrin, M., Wellard Kelly, H., Hell, R., and Ciborowski, J. (2018). Levels of identification and other taxonomic notes: Great Lakes Coastal Monitoring Project. *Great Lakes Coastal Wetland Monitoring Program*.

Hilsenhoff, W. L. (1995). *Aquatic insects of Wisconsin: Keys to Wisconsin genera and notes on biology, habitat, distribution and species* (No. 3). Natural History Museums Council, University of Wisconsin-Madison.

Jacobus, L. M., Macadam, C. R., and Sartori, M. (2019). Mayflies (Ephemeroptera) and Their Contributions to Ecosystem Services. *Insects,* 10(6), 170-196.

Kirby, J. M. (1982). Prey Utilization Among Four Sympatric Species of Sunfish. Proceedings of the Pennsylvania Academy of Science, 56, 147-150.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master’s Project Final Report. *University of Michigan School for Environment and Sustainability.*

Kennedy, M. A., Sutton-Grier, A. E, Smith, R. F., and Gresens, S. E. (2009). Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. *Terrestrial Arthropod Reviews, 2*, 99-128

MacKenzie, R. A., and Kaster, J. L. (2004). Temporal and spatial patterns of insect emergence from a Lake Michigan coastal wetland. *Wetlands, 24*(3), 688-700.

Scriber, K. E. (2013). An examination of the feeding preferences of the freshwater amphipod Hyalella azteca for aquatic vascular plants and macro algae: The potential roles of prey nutritional quality, chemical, and/or structural defenses. ProQuest Dissertations Publishing. The University of Alabama at Birmingham.

Spieles, D. J., and Mitsch W. J. (2003). A model of macroinvertebrate trophic structure and oxygen demand in freshwater wetlands. *Ecological Modelling,* 161(3), 183-194.

Staton, J. M., Roswell, C. R., Fielder, D. G., Thomas, M. V., Pothoven, S. A., and Hook, T. O. (2014). Condition and diet of yellow perch in Saginaw Bay, Lake Huron (1970-2011). *Journal of Great Lakes Research,* 40(1), 139-147.

Thorp, J. H. and Covich, A. P. (2009). *Ecology and Classification of North American Freshwater Invertebrates, Third Edition*. Elsevier Inc.

USGS. (2002). Assessment of Stream Quality Using Biological Indices at Selected Sites in the Red Clay and White Clay Creek Basins, Chester County, Pennsylvania, 1981-97. USGS Fact Sheet FS-118-02.

Uzarski, D. G., Burton, T. M., and Genet, J. A. (2004). Validation and performance of an invertebrate index of biotic integrity for Lakes Huron and Michigan fringing wetlands during a period of lake level decline. *Aquatic Ecosystem Health & Management*, *7*(2), 269-288.

Voshell, J. R. (2002). *A guide to common freshwater invertebrates of North America* (No. Sirsi) i9780939923878.

Watkins, C. E., Shireman, J. V., and Haller, W. T. (1983). The influence of aquatic vegetation upon zooplankton and benthic macroinvertebrates in Orange Lake, Florida. *Journal of Aquatic Plant Management*, *21*(2), 78-83.

FISH MONITORING

Angeler, David G, and Alvarez-Cobelas, Miguel. (2005). Island biogeography and landscape structure: Integrating ecological concepts in a landscape perspective of anthropogenic impacts in temporary wetlands. *Environmental pollution* (1987), 138(3), 420–424. Journal Article, Oxford: Elsevier Ltd.

Brunson, M. W., and Morris, J. E. (2000). Species Profile Sunfish. *Southern Regional Aquaculture Center Publication No. 724.*

Clemons, E. (2006). "Lepomis cyanellus" (On-line), Animal Diversity Web. Accessed March 30, 2021 at https://animaldiversity.org/accounts/Lepomis_cyanellus/.

Cooper, Matthew J. et al. (2018). An expanded fish-based index of biotic integrity for Great Lakes coastal wetlands. *Environmental monitoring and assessment,* 190(10).

Danylchuk, A. J. and Fox, M.G. (1994). Seasonal Reproductive Patterns of Pumpkinseed (Lepomis gibbosus) Populations with Varying Body Size Characteristics. *Canadian Journal of Fisheries and Aquatic Sciences*, 51(3), 490-500.

Dewey, T. (2012). "Etheostoma nigrum" (On-line), Animal Diversity Web. Accessed March 13, 2021 at https://animaldiversity.org/accounts/Etheostoma_nigrum/.

Esri Inc. (2021). ArcGIS Survey123 (version 3.12.277) [Mobile app]. Esri Inc.

Freshwater Fish of South Carolina. Univ of South Carolina Press. (2009). p. 80. ISBN 978-1-57003-680-4.

Fuller, P., Neilson, M.E., and Hopper, K., (2021). Dorosoma cepedianum (Lesueur, 1818): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=492, Revision Date: 9/12/2019, Peer Review Date: 4/12/2013, Access Date: 3/30/2021.

Fuller, P. and Sturtevant, R. (2019). *Labidesthes sicculus* (Cope, 1865). *U.S. Geological Survey, Nonindigenous Aquatic Species Database*. Available at: https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=318.

Growe-Raney, A. (2011). "Umbra limi" (On-line), Animal Diversity Web. Accessed March 13, 2021 at https://animaldiversity.org/accounts/Umbra_limi/.

Holm, E., Mandrak, N., and Burridge, M.E. (2009). *The ROM field guide to freshwater fishes of Ontario*. ROM.

Jordan, C., Backe, N., Wright, M.C., and Tovey, C.P. (2009). Biological synopsis of pumpkinseed (Lepomis gibbosus). Can. Manuscr. Rep. Fish. Aquat. Sci. 2886: iv + 16 p.

Jude, D.J. and Pappas, J. (1992). Fish utilization of Great Lakes coastal wetlands. *Journal of Great Lakes Research*. Volume 18(4), 651-672.

Kapuscinski, Kevin L. (2010). “Emerald Shiner Migrations”. Blog of Kevin L. Kapuscinski, Ph.D. Accessed March 23, 2021 at https://masquinongy.wordpress.com/2010/10/11/emerald-shiner-migrations/.

Kowalski, K. P., Wiley, M. J., and Wilcox, D. A. (2014). Fish assemblages, connectivity, and habitat rehabilitation in a diked Great Lakes coastal wetland complex. *Transactions of the American Fisheries Society*, 143(5), 1130-1142.

Laarman, P. W. , and Schneider, J. C. (1985). Maturity and fecundity of largemouth bass as a function of age and Size. *Fisheries Research Report No. 1931*.

Larimore, R.W. (1957). Ecological life history of the warmouth (Centrarchidae). Bull. Illinois Nat. Hist. Surv., 27(1), 1-83.

Lefevre, R. 2012. "Esox lucius" (On-line), Animal Diversity Web. Accessed March 23, 2021 at https://animaldiversity.org/accounts/Esox_lucius/.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master's Project Final Report. *University of Michigan School for Environment and Sustainability.*

Mecozzi, Maureen. (1989). "Northern Pike: *Esox lucius*". Wisconsin Department of Natural Resources - Bureau of Fisheries Management. PUBL-FM 707 89.

Miller, Jacob W, et al. (2018). "Fish Community Responses to Submerged Aquatic Vegetation in Maumee Bay, Western Lake Erie." *North American Journal of Fisheries Management*, 38(3), 623–629.

National Oceanic and Atmospheric Association (NOAA) (2019). Low or depleted oxygen in a water body often leads to 'dead zones' - regions where life cannot be sustained. Retrieved March 30, 2021, from https://oceanservice.noaa.gov/hazards/hypoxia/.

Parr, C. (2013). "Lepomis macrochirus" (On-line), Animal Diversity Web. Accessed March 22, 2021 at https://animaldiversity.org/accounts/Lepomis_macrochirus/.

Parr, C. (2013). "Pimephales notatus" (On-line), Animal Diversity Web. Accessed March 13, 2021 at https://animaldiversity.org/accounts/Pimephales_notatus/.

Rose, C. (2006). "Ameiurus melas" (On-line), Animal Diversity Web. Accessed March 22, 2021 at https://animaldiversity.org/accounts/Ameiurus_melas/.

Schaeffer, Jeffrey S., Warner, David M., and O'Brien, Timothy P. (2008). Resurgence of Emerald Shiners Notropis atherinoides in Lake Huron's Main Basin. *Journal of Great Lakes Research*, 34(3), 395–403. ANN ARBOR: International Association for Great Lakes Research.

Sens, M. and Mitchinson, O. (2020). Site Specific Sampling Protocol for Wetland Fish Monitoring: Shiawassee National Wildlife Refuge. Version 1.0. Saginaw, Michigan: US Fish and Wildlife Service, Region 3, Bloomington, MN.

Steed, E. (2018). "Micropterus salmoides" (On-line), Animal Diversity Web. Accessed March 22, 2021 at https://animaldiversity.org/accounts/Micropterus_salmoides/.

Stroud, R. H. summary. (1967). Water quality criteria to protect aquatic 1ife: Am. Fish. Soc. Spee. Pub1. 4, 33- 37.

Stuber, R. J., G. Gebhart, and O. E. Maughan. (1982). Habitat suitability index models: Bluegill. U.S.D.l. Fish and Wildlife Service. FWS/OBS-82/10.8. 26 pp.

Stuber, R. J., G. Gebhart, and O. E. Maughan. (1982). Habitat suitability index models: Largemouth bass. U.S. Dept. Int. Fish Wildl. Servo FWS/OBS-82/10.16. 32 pp.

Tingle, A. (2015). "Pomoxis annularis" (On-line), Animal Diversity Web. Accessed March 13, 2021 at https://animaldiversity.org/accounts/Pomoxis_annularis/.

Uzarski, D.G. et al. (2016). Standardized measures of coastal wetland condition: implementation at a Laurentian Great Lakes basin-wide scale. *Wetlands*. Volume 37(1).

Whitten, Andrya L, DeBoer, Jason A, Solomon, Levi E, Fritts, Andrea K, Fritts, Mark W, Pendleton, Richard M, VanMiddlesworth, T. D, et al. (2020). Spatial variation in bluegill population demographics in a highly modified large river system. *Journal of freshwater ecology*, 35(1), 305–322.

Williamson, K.L., and Nelson, P.C. (1985) Habitat suitability index models and in stream flow suitability curves: Gizzard Shad. U.S Fish and Wildlife Services Biological Reports. 82, 1-33.

Zorn, Troy G., Seelbach, Paul W., and Wiley, Michael J. (2009). Relationships Between Habitat and Fish Density in Michigan Streams. *State of Michigan Department of Natural Resources*. Fisheries Division Research Report 2091.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Byun, K., Cihu, C., and Hamlet, A. F. (2019). Effects of 21st century climate change on seasonal flow regimes and hydrologic extremes over the Midwest and Great Lakes region of the US. The Science of the Total Environment, 650, 1261-1277.

Lugten, E., Mitchinson, O., Puz, M., Sens, M., and Vogel, K. (2020). Reclaiming the Shiawassee Flats: Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem. Master's Project Final Report. *University of Michigan School for Environment and Sustainability.*

U.S. Fish and Wildlife Service. (2018). Shiawassee National Wildlife Refuge: Habitat Management Plan. *U.S. Fish and Wildlife Service* & *National Wildlife Refuge System,* 1-105.

U.S. Army Corps of Engineers. (2021). Great Lakes Update. Volume 208: 2020 Annual Summary. U.S. Army Corps of Engineers, Detroit District.

APPENDIX I

After our water quality, vegetation, macroinvertebrate, and fish datasets are each fully reviewed by USGS, they will be available on USGS ScienceBase-Catalog.

The catalog can be accessed using this link: https://www.sciencebase.gov/catalog/items?q=&filter=systemType%3DData+Release&filter=browseCategory%21%3DData+Release+-+In+Progress.

For questions regarding data access, please email Alexandra Bozimowski with USGS at abozimowski@usgs.gov.

APPENDIX II

The following is a complete list of all vegetation, organized primarily by family, that we sampled and identified during our 2020 season:

Family	Genus	Species
Alismataceae	*Alisma*	*plantago-aquatica*
Alismataceae	*Sagittaria*	*latifolia*
Apocynaceae	*Asclepias*	*tuberosa*
Araceae	*Lemna*	*minor*
Araceae	*Spirodela*	*polyrhiza*
Araceae	*Wolffia*	spp.
Arums	*Lemna*	*trisulca*
Asteraceae	*Bidens*	*cernua*
Asteraceae	*Bidens*	*frondosa*
Asteraceae	*Bidens*	sp.
Asteraceae	*Cirsium*	*arvense*
Butomaceae	*Butomus*	*umbellatus*
Cabombaceae	*Brasenia*	*schreberi*
Ceratophyllaceae	*Ceratophyllum*	*demersum*
Convolvulaceae	*Cuscuta*	*gronorii*
Cornaceae	*Cornus*	*racemosa*
Cyperaceae	*Cyperus*	*strigosus*
Cyperaceae	*Eleocharis*	*palustris*
Cyperaceae	*Eleocharis*	sp.
Cyperaceae	*Schoenoplectus*	*tabernaemontani*
Euphorbiaceae	*Acalypha*	*virginica*
Fabaceae	*Trifolium*	spp.
Haloragaceae	*Myriophyllum*	*sibiricum*
Haloragaceae	*Myriophyllum*	*spicatum*
Hydrocharitaceae	*Elodea*	*canadensis*
Hydrocharitaceae	*Najas*	*minor*
Juglandaceae	*Carya*	*ovata*
Lamiaceae	*Teucrium*	*canadense*
Lentibulariaceae	*Utricularia*	*vulgaris*
Lythraceae	*Lythrum*	*salicaria*
Malvaceae	*Abutilon*	*theophrasti*
Moraceae	*Morus*	sp.
-	*Algae*	spp.
-	*Dead Typha*	-
-	*Dead Unidentified*	-

Nymphaeaceae	*Nymphaea*	*odorata*
Onagraceae	*Epilobium*	sp.
Poaceae	*Echinochloa*	*crus-galli*
Poaceae	*Echinochloa*	*muricata*
Poaceae	*Panicum*	*dichotomiflorum*
Poaceae	*Phalaris*	*arundinacea*
Poaceae	*Setaria*	*viridis*
Polygonaceae	*Persicaria*	*amphibia*
Polygonaceae	*Persicaria*	*maculosa*
Potamogetonaceae	*Potamogeton*	*foliosus*
Potamogetonaceae	*Potamogeton*	*nodosus*
Potamogetonaceae	*Stuckenia*	*pectinata*
Primulaceae	*Lysimachia*	*nummularia*
Ricciaceae	*Riccia*	*fluitans*
Ricciaceae	*Riccia*	*natans*
Salicaceae	*Populus*	*deltoides*
Salicaceae	*Salix*	*amygdaloides*
Salicaceae	*Salix*	*interior*
Salicaceae	*Salix*	*nigra*
Solanaceae	*Physalis*	*longifolia*
Solanaceae	*Solanum*	*dulcamara*
Sapindaceae	*Acer*	*saccharinum*
Sapindaceae	*Acer*	sp.
Typhaceae	*Typha*	*angustifolia*
Typhaceae	*Typha*	*latifolia*
Ulmaceae	*Ulmus*	*americana*
Urticaceae	*Boehmeria*	*cylindrica*
Urticaceae	*Laportia*	*canadensis*
Urticaceae	*Pilea*	*pumila*
Vitaceae	*Vitis*	*riparia*
-	Unknown	small, undeveloped
-	Unknown	small, serrated

APPENDIX III

The following table displays the IVI for each species in each corresponding unit and vegetation zone in which they were sampled. It is important to note that some species did not have IVIs calculated, like canopy trees, because percent cover was not estimated due to time constraints. Those species for which percent cover was not estimated are not included in this table, but are represented in the species list in Appendix II.

Floodplain Unit	**MS**	**MS**	**MS**	**MS**	**MN**	**MN**	**MN**	**P1A**	**P1A**	**P1A**	**P1A**	**P1A**
Vegetation Zone	SAV	*Typha*	Dead *Typha*	Forest	SAV	*Typha*	*Phalaris*	*Nymphaea*	*Typha*	SAV	Mudflat	*Salix*
Abutilon theophrasti							9.5					1.7
Acalypha virginica												11.69
Acer spp.				1.85								
Algae spp.	30.03	23.28	2.92	1.85	22.77			0.39	4.62	35.78		
Alisma plantago-aquatica												3.44
Asclepias tuberosa							1.8					
Bidens cernua							7.26					
Bidens frondosa							1.85					2.6
Bidens spp.												3.33
Boehmeria cylindrica												10.7
Brasenia schreberi						3.05						
Butomus umbellatus							1.95					
Ceratophyllum demersum	39.16	16.35	8.47	26.29	67	14.44		13.89	13.6	41.98	2.23	5.26
Cirsium arvense							5.85					
Cornus racemosa							1.8					4.16
Cuscuta gronorii												5.67
Cyperus strigosus							15.15				12.3	3.37
Dead *Typha*			25.32			7.02			24.1			
Echinochloa crus-galli							1.85					
Eleocharis palustris											28.66	
Eleocharis spp.												
Elodea canadensis	37.8	1.32	12.93		24.3	5.76		0.48	7.54	34.89		
Laportia canadensis												1.63
Lemna minor	5.05	5.52	3.41	49.78		0.39				1.99	40.71	16.15
Lemna trisulca	18.13	4.11	12.36	56.16	11.47	2		0.29			22.17	7.55

Lysimachia nummularia				4.37								6.91
Lythrum salicaria							1.8	0.08				1.77
Morus spp.												1.7
Myriophyllum sibiricum					2.51							
Myriophyllum spicatum					15.01	0.84						
Najas minor	5.35	71.4			11.7	0.74		19		25		
Nymphaea odorata								38.68	11.46	4.78		
Panicum dichotomiflorum												1.84
Persicaria amphibia								0.12	5.16	2.05	6.9	
Persicaria maculosa							3.75				1.09	
Phalaris arundinacea	7.48	1.32	0.97				103.72				7.3	16.45
Physalis longifolia												4.3
Pilea pumila												17.62
Populus deltoides							16.47					
Potamogeton foliosus	2.51	1.37	1.03		5.37	0.96						
Potamogeton nodosus	31.26	17.22	3.25		26.54	3.22		1.16		15.57		
Riccia fluitans			1.09	10.69		0.56		0.06				
Riccia natans			1.46									
Sagittaria latifolia									4.63		8.3	
Salix interior							3.75					
Salix nigra							4.77					40.82
Schoenoplectus tabernaemontani										4.34		
Setaria viridis							5.85					1.77
Solanum dulcamara							3.61					
Spirodela polyrhiza	2.57	3.95	0.97	25.6		0.59		0.79	43.8	7.85	13.8	17.94
Stuckenia pectinata	14.32	21.69	2.13		13.34	6.19		0.67	2.34	21.87		
Teucrium canadense												1.63
Trifolium spp												1.91
Typha angustifolia			0.97			24.84	1.85		57.42			
Typha latifolia									18.24			1.91
Utricularia vulgaris	3.78	11.77	53.38	2.52		2.64			7.08			

Vitis riparia							7.41					3.26
Wolffia spp.	2.57	20.71	8.11	20.88		0.6				3.91	12.53	2.94

APPENDIX IV

The following is a complete list of all macroinvertebrates, organized by order, that were captured and identified to the level of family or genus:

Amphipoda:
Gammaridae *Gammarus*
Hyalellidae *Hyalella*

Arachnida:
Sarcoptiformes *Oribatida*

Coleoptera:
Curculionidae *Lissorhoptrus*
Dytiscidae *Rhantus*
Dytiscidae *Bidessonotuus*
Dytiscidae *Hygrotus*
Dytiscidae *Motus*
Elmidae *Optioservus*
Haliplidae *Brychius*
Haliplidae *Haliplus*
Haliplidae *Peltodytes*
Hydrophilidae *Berosus*
Hydrophilidae *Enochrus*
Hydrophilidae *Hydrochara*
Hydrophilidae *Hydrophilus*
Hydrophilidae *Tropisternus*
Noteridae *Hydrocanthus*
Noteridae *Suphisellus*
Scirtidae *Scirtes*

Decapoda:
Cambaridae *Faxonius*

Rhynchobdellida:
Glossiphoniidae *Alboglossiphonia*
Glossiphoniidae *Batracobdella*
Glossiphoniidae *Glossiphonia*
Glossiphoniidae *Helobdella*
Glossiphoniidae *Theromyzon*
Glossiphoniidae spp.

Isopoda:
Asellidae *Caecidotea*

Diptera:
Ceratopogonidae *Bezzia/Palpomia*
Ceratopogonidae *Serromyia*
Ceratopogonidae *Sphaeromias*
Chironomidae *Clinotanypus*
Chironomidae *Stenochironomus*
Chironomidae *Zavrelimyia*
Chironomidae spp.
Culicidae *Anopheles*
Culicidae *Coquillettidia*
Culicidae *Uranotaenia*
Sciomyzidae *Antichaeta*
Sciomyzidae *Sepedon*
Stratiomyidae *Odontomyia*
Stratiomyidae *Oxycera*
Stratiomyidae *Stratiomys*
Thaumaleidae spp
Tipulidae *Helius*
Tipulidae spp.

Ephemeroptera:
Baetidae *Callibaetis*
Caenidae *Caenis*
Caenidae spp.
Ephemerellidae *Drunella*
Ephemerellidae *Timpanoga*
Metretopodidae *Metretopus*
Siphonutidae *Parameletus*

Trichoptera:
Glossosomatidae *Glossosoma*
Leptoceridae *Ceraclea*
Leptoceridae *Leptocerus*
Leptoceridae *Nectopsyone*
Leptoceridae *Oecetis*
Leptoceridae *Ylodes*
Leptoceridae spp.
Philopotamidae *Wormaldia*
Polycentropodidae *Cernotina*

Hemiptera:
Belostomatidae *Belostoma*
Corixidae *Corisella*
Corixidae *Hesperocorixa*
Corixidae *Palmacorixa*
Corixidae *Ramphocorixa*
Corixidae *Trichorixia*
Corixidae spp.
Hebridae *Hebrus*
Hebridae *Lipogomphus*
Hebridae *Merragata*
Mesoveliidae *Mesovelia*
Nepidae *Ranatra*
Notonectidae *Buenoa*
Notonectidae *Notonecta*
Veliidae *Microvelia*
Pleidae *Neoplea*

Megaloptera:
Corydalidae *Chauliodes*

Sphaeriidae:
Sphaeriidae spp.

Trombidiformes:
Hydracarina spp.
Hydrachnidae spp.

Unranked Gastropoda:
Lymnaeidae *Stagnicola*
Physidae *Aplexa*
Physidae *Physa*
Planorbidae *Physa*
Planorbidae *Heliosoma*
Planorbidae *Laevapex*
Planorbidae *Planorbella*

Odonata:
Aeshnidae *Anax*
Aeshnidae *Basiaeschna*
Coenagrionidae *Amphiagrion*
Coenagrionida*e Coenagrion*
Coenagrionida*e Enallagma*
Coenagrionidae *Ischnura*
Coenagrionidae *Nehalennia*
Coenagrionidae spp.
Corduliidae *Epitheca*
Corduliidae *Neurocordulia*
Corduliidae *Somatochlora*
Corduliidae spp.
Lestidae *Archilestes*
Lestidae *Lestes*
Libellulidae *Erythemis*
Libellulidae *Leucorrhinia*
Libellulidae *Libellula*
Libellulidae *Nannothemis*
Libellulidae *Pachydiplax*
Libellulidae *Perithemis*
Libellulidae *Plathemis*
Libellulidae *Sympetrum*
Libellulidae *Tramea*
Libellulidae spp.
Macromiidae *Macromia*