

UNIVERSITY OF MICHIGAN, SCHOOL FOR ENVIRONMENT AND SUSTAINABILITY

Reclaiming the Shiawassee Flats:

Monitoring During Hydrologic Restoration of the Shiawassee Flats Ecosystem

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EXECUTIVE SUMMARY

In 2016, the US Fish and Wildlife Service (USFWS) completed the restoration of two new wetland units: Maankiki North (MN, opened in 2017) and Maankiki South (MS, opened in 2018) at the Shiawassee National Wildlife Refuge near Saginaw, Michigan. The Refuge sought to reconnect these units, formerly farmland, to the dynamic hydrology of the Shiawassee River, mimicking the function of this area's historic floodplain complex.

In early 2019, staff at the Shiawassee National Wildlife Refuge asked for support from students attending the University of Michigan School for Environment and Sustainability (SEAS) to aid in post-restoration monitoring of the biological conditions in the recently restored Maankiki units and Pool 1A, a wetland unit hydrologically reconnected to the Shiawassee River in 1958. Sampling in 2019 would complement pre-restoration research previously done by UM groups. Sampling techniques were modeled after the Great Lakes Coastal Wetland Monitoring Program and were used to create protocols to guide future studies. This report, organized by the abiotic and biotic factors investigated, represents the culmination of our team's research.

Water Quality describes the chemical, physical, and biological parameters used to measure the tolerance of the wetland units' biological communities.

- Water quality varies by month, distance from the water control structure, vegetation type, and unit.
- Dissolved oxygen decreased throughout the season to levels unsafe for fish, likely due to warming temperatures.
- In the future, water quality monitoring should more closely reflect the GLCWMP methods, focus on nutrient testing, and more data collection from the Shiawassee River and Spaulding Drain.

Vegetation identifies and compares the plant communities within and among wetland units and uses their diversity and abundance to evaluate wetland health.

- Calculations of importance values and dissimilarity indices show decreasing diversity from Maankiki South to Pool 1A to Maankiki North, which has a high abundance and density of invasive *Typha*.
- The Floristic Quality Assessment and Index of Biotic Integrity scored Maankiki South as 'Medium Quality.' Degradation increased from MS to Pool 1A to MN.
- Future research recommendations include the continued implementation of our monitoring protocol, managing the units' flood duration and frequency to mimic the natural flow regime, and the harvesting of *Typha* biomass.

Macroinvertebrates catalogs and compares indicator insect families in response to each unit's water quality, vegetation types, and monthly variation.

- Communities changed throughout the summer following standard life-cycle trends.
- The majority of families found are known to be tolerant to the water quality conditions common to wetlands, such as high turbidity and low DO.
- Future management recommendations include the continued implementation of our monitoring protocol, the use of an elutriator while sampling, identifying individuals to genera, and more closely and accurately categorizing the unit's substrates.

Fish details the different gear types utilized to measure and compare the abundance, composition, and structure of fish communities and the environmental factors shaping these traits within and among units.

- Fish sampling included the use of multiple frame- and mesh-size fyke nets, gill nets, and electrofishing.
- The fish community contained no sensitive species. We found a mix of riverine and wetland species, in addition to abundant juvenile fish, that indicate the wetland units are used for spawning and refuge by species from both ecosystems.
- Future management recommendations include continued monitoring with multiple methods, tailoring methods to target species, and using minnow traps to catch smaller species and juveniles.

We recommend continuous monitoring that incorporates the Shiawassee River and Spaulding Drain to understand how biological communities in the river are using the wetland units, and to provide a comparison of ecological function of restored wetlands to the river. Past, present, and future studies should be analyzed in combination to assist the Refuge in making science-based management decisions.

Worldwide, approximately 35% of wetlands have been lost to agriculture and urban expansion, at a rate that is becoming faster than that of forests (UN Climate Change 2018). These mounting losses make clear the invaluable ecosystem services wetlands provide flood protection, nutrient cycling, water purification, carbon sequestration, food, and shelter (Sierszen et al. 2012). Through the efforts of programs like the Great Lakes Restoration Initiative, the restoration of wetlands has become a national and regional priority. As the threat of climate change and urban expansion continues, monitoring is increasingly important for gaining an enhanced understanding of how development and restoration impact the ecology and ecosystem services of wetlands - and how wetlands can help abate the impairments of Saginaw River and Bay, U.S. Environmental Protection Agency designated Areas of Concern. Monitoring restored wetlands provides an opportunity to investigate the patterns of vegetation distribution, as well as the movement of macroinvertebrates and fish communities. Understanding these spatial and temporal patterns allows us to make observations about the quality of restored habitat and value of restoring ecosystems.

In 2012, a University of Michigan Master's Project Team collected pre-restoration data by monitoring in the Shiawassee River and the remaining farm units that would eventually become the Maankiki Marsh complex. In their subsequent report (Buchanan et al. April 2013), the Master's Project Team provided: 1) a brief history of the Shiawassee Flats with the human and environmental factors responsible for its present conditions, 2) an assessment of the data collected in 2012 which included surveys of water quality, vegetation, macroinvertebrate, and fish communities, and 3) case studies of wetland restorations on other National Wildlife Refuges while identifying potential partners and strategies for future SNWR restoration projects. Later that year, the hydrogeomorphic evaluation of the Shiawassee Flats was completed and created a vision for restoration and management options "to restore specific habitats and conditions within various locations" of SNWR (Heitmeyer et al. 2013). These reports spearheaded the design and construction of the Refuge's recent hydrological reconnection efforts.

In early 2019, following the reconnection of a portion of the Maankiki Marsh complex, Maankiki North and Maankiki South, the U.S. Geological Survey (USGS) and FWS sought interested graduate students from the University of Michigan School for Environment and Sustainability (SEAS) to assist in the post-restoration monitoring of these recently reconnected units. Together FWS, USGS, and our SEAS Master's Project team outlined the objectives necessary to meet the Refuge's post-restoration goals.

These objectives include:

- Assisting the Refuge Biologist in developing mid and long-term monitoring protocols for sampling water quality, vegetation, macroinvertebrate, and fish communities within the restored floodplain areas
- Conducting first-year monitoring of a suite of wetland responses in a series of experimental treatments that assess the effectiveness of methods and equipment, assessing sampling design requirements, and establishing baseline data for the evaluation of restoration treatments
- Comparing the responses of recently reconnected wetland units (Maankiki North and Maankiki South) to a wetland unit reconnected since the 1950s (Pool 1A)

The planning and implementation of monitoring methods employed to fulfill these objectives were modeled after techniques used by the Great Lakes Coastal Wetland Monitoring Program (GLCWMP, Uzarski et al. 2016) and refined based on studies of reconnected Great Lakes coastal wetlands by Dr. Kurt Kowalski (Kowalski et al. 2014), at Ottawa National Wildlife Refuge in Ohio.

The following report details our team's work and findings to the U.S. Fish and Wildlife Service and the University of Michigan School for Environment and Sustainability. Within this report is post-restoration data collected and analyzed for water quality, vegetation, macroinvertebrates, and fish communities. Collectively, our work aims to support Shiawassee National Wildlife Refuge's ongoing restoration efforts by providing this baseline data and offering recommendations for the monitoring and management of the wetland units into the future.

TIMELINE OF WETLAND MANAGEMENT & PROJECT EVENTS

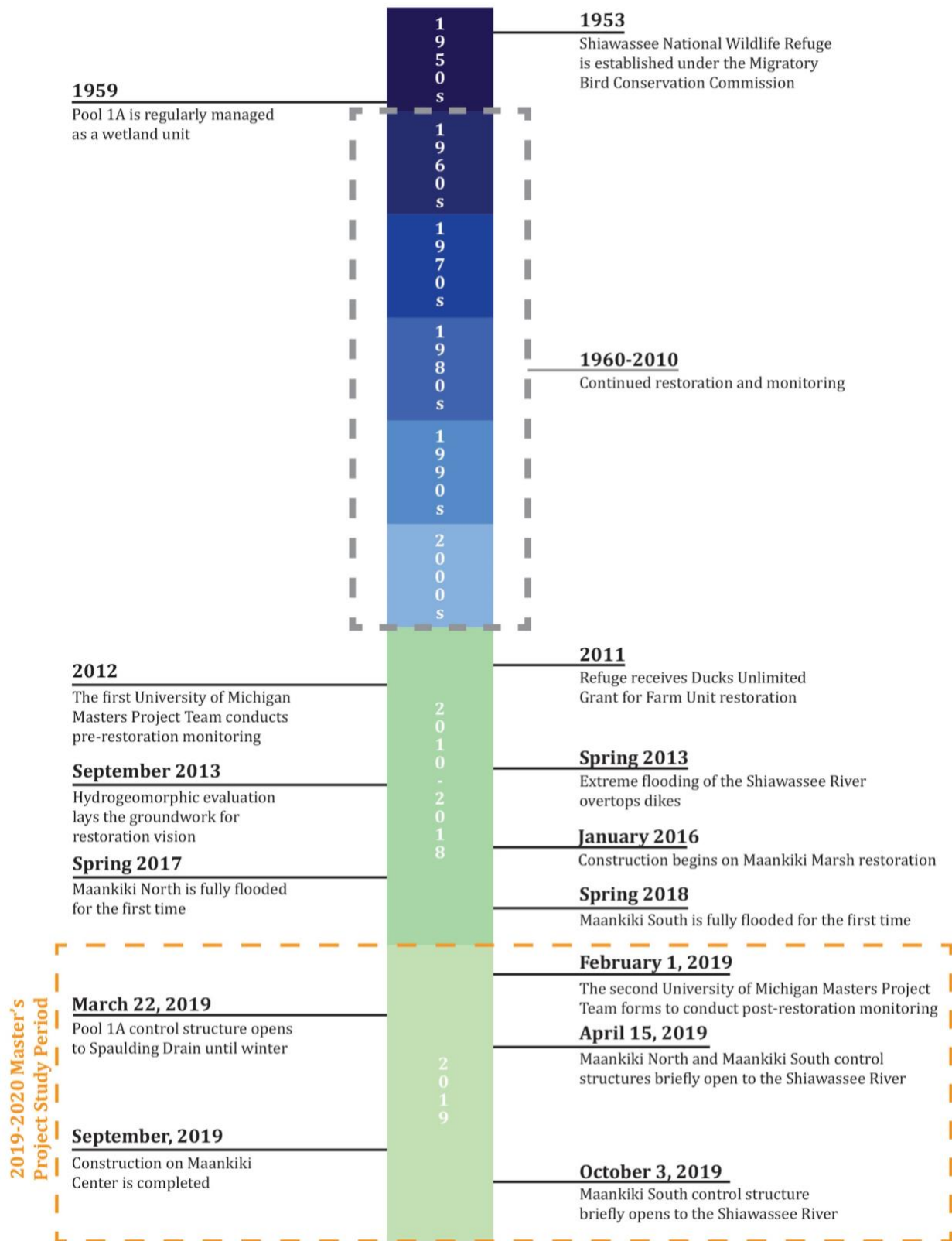


Figure 1.1 A timeline of important SNWR wetland management and UM-SEAS events.

STUDY AREAS

MAP OF SHIAWASSEE NATIONAL WILDLIFE REFUGE

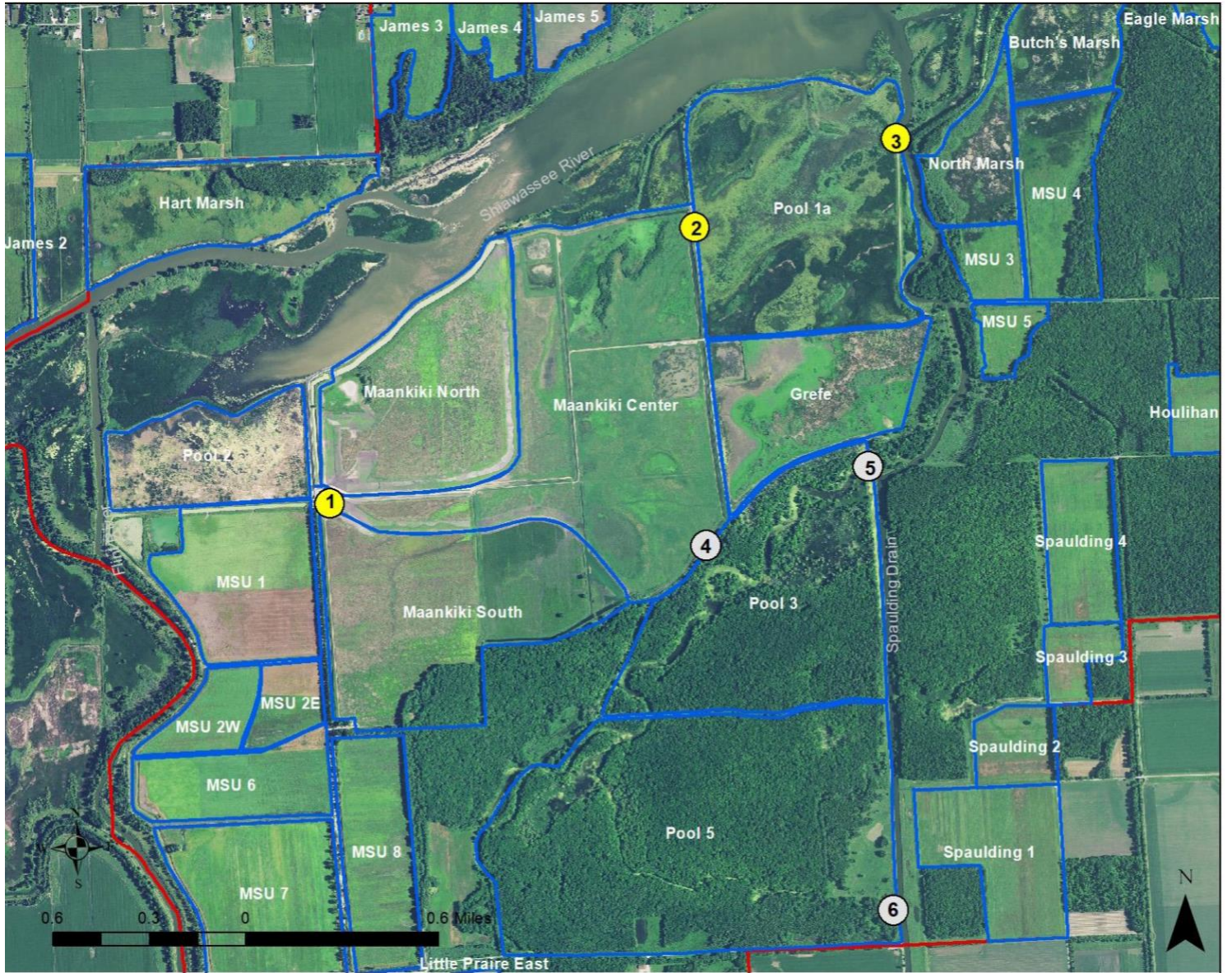


Image 1.2 Aerial image of the Shiawassee National Wildlife Refuge. Yellow circles represent water control structures, white circles represent proposed water control structures.

Wetland Units & Study Area

In 1958, as part of the first phase of restoration, Pool 1A was first connected by a water control structure to the Shiawassee River, and now serves as an example of how wetland units might respond to long term connection to the river. In 2016, two of the remaining agricultural fields were restored as emergent marshes: Maankiki North (MN) and Maankiki South (MS), with Maankiki Center set to be completed by 2020, all together creating Maankiki Marsh. In our 2019 study Pool 1A, Maankiki North, and Maankiki South were the area of focus.

A brief note on terminology: ‘connected’ unit(s) refer to wetlands that have water control structure(s) engineered into their dikes and allow water to enter from the Shiawassee River or Spaulding Drain. ‘Unconnected’ units have no such water control structure(s). When units are ‘open,’ water control structures allow the free passage of water into or out of the units. ‘Closed’ units refer to water control structures that have been managed to retain water within or to keep water from entering their respective wetland units.

Four rivers - the Cass River, Flint River, Shiawassee River, and Tittabawassee River - flow into the SWNR and their conjunction forms the Saginaw River at the northeast end of the Refuge. The Tittabawassee flows in from the north, the Flint from the south, the Cass from the east and the Shiawassee from the west. The stretch of the Shiawassee River flowing through the Refuge becomes much wider and deeper as the other rivers add to its flow. A history of irrigation across the region has created many channels and drains that move water to and around farms. One such modification is Spaulding Drain, a deep canal upstream from the Flint River’s natural conjunction with the Shiawassee River that redirects water through the SWNR before merging back with Shiawassee.

Although the Shiawassee Flats comprise a large floodplain ecosystem, variability in local topography results in different hydrological responses within wetland units. Micro-topographic and hydrologic differences create heterogeneity in water quality and vegetation communities among units which determines the composition of macroinvertebrate, fish, and water bird communities. Differences in communities require different management strategies to support the priorities of the Habitat Management Plan of 2018 (USFWS 2018).

Pool 1A

Reconnected in 1958, Pool 1A remains subject to the hydrology of Spaulding Drain and the Shiawassee River through its water control structure (Image 1.3). The connection to Spaulding Drain is closed in the winter to hold water and provide refuge for overwintering species. This unit is relatively flat with several inches of loose sediment forming its bottom. Depth variation is due to several constructed mounds formerly utilized as nesting habitat for Canadian Geese. These mounds and several perimeter peninsulas act as shallow zones which have allowed the proliferation of willow species across the unit.

Maankiki North

Construction of Maankiki North was completed in 2016, with its control structure (Image 1.4) connected to the Shiawassee River in spring 2017, once ice broke. Construction of the surrounding dikes utilized soil from within the unit resulting in channels around the majority of its perimeter and a deep borrow pit in the northwest corner. Along the eastern edge of this pit is a long row of dead trees used by multiple water bird species for roosting. The unit's center is flat and lacks significant landscape variance. Maankiki North is at a lower elevation than the other sampling units and had the deepest sampling areas in 2019. This unit was opened for several days in late April/early May 2019 before being closed for the remainder of the sampling season.

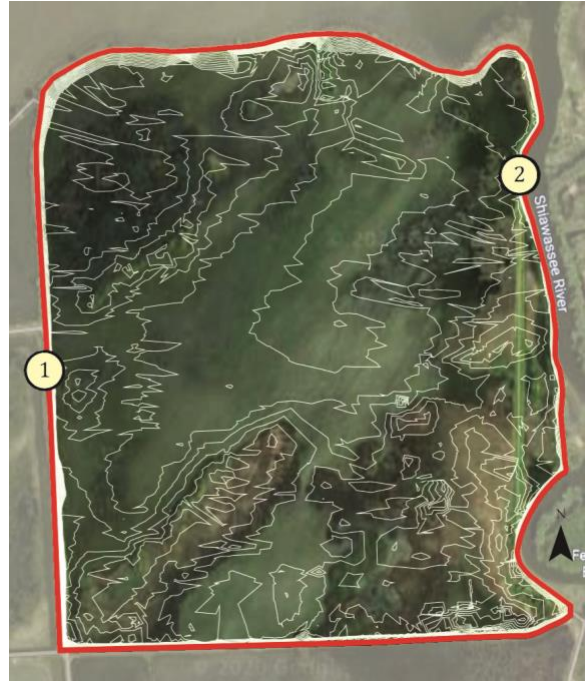


Image 1.3 Aerial photo of Pool 1A. Point #1 represents the water control structure connecting Maankiki Center to Pool 1A. Point #2 represents



Image 1.4 Aerial photo Maankiki North. Point #3 represents the water control structure connecting Maankiki North to the Shiawassee

Maankiki South

Maankiki South was first flooded when ice broke in 2018 and remained connected to the Shiawassee River until May of the same year before the control structure (Image 1.5) was closed again. Construction of the dikes around the west, south, and east sides of this unit utilized soil from within the unit resulting in a channelized interior perimeter. The unit as a whole has more variable micro-topography with a remnant dike that splits the unit east and west. Maankiki South is at a higher elevation above sea level relative to Maankiki North, which results in shallower water across much of this unit's surface.

This has led to several areas that are saturated but not inundated and consist of distinct vegetation. The southeast portion of this unit is a floodplain forest community which contributes a substantial overstory not present in the other sampled units. Similar to Maankiki North, Maankiki South was opened for several days in late April/early May 2019 before being closed. It was open next in early October 2019, while the Shiawassee River was high, to capture water to hold over winter.

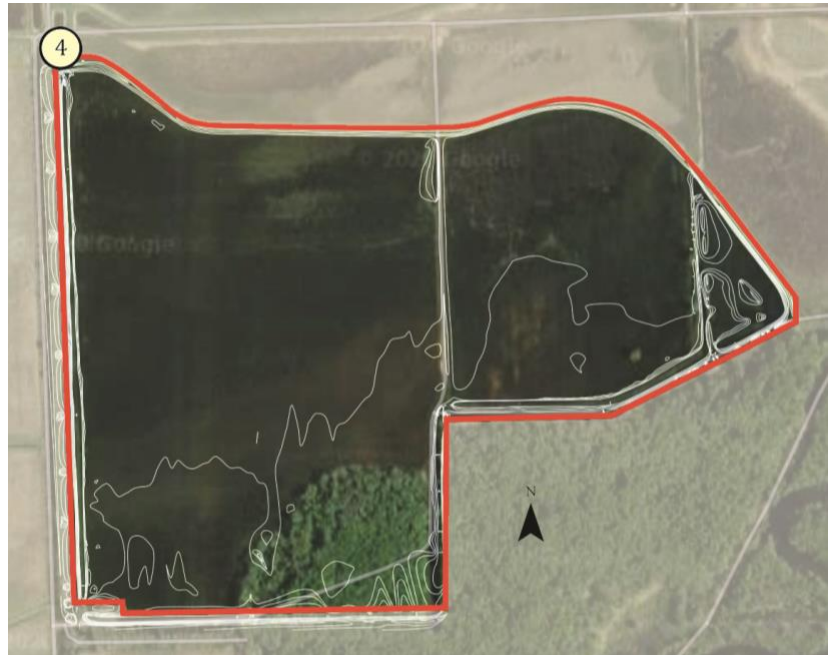


Image 1.5 Aerial photo of Maankiki South. Point #4 represents the water control structure connecting

WATER QUALITY

INTRODUCTION

Water quality is monitored to improve understanding of the factors controlling health and biodiversity of vegetation, macroinvertebrates, fish, and waterfowl populations. Water quality in wetlands is also used as an indicator of ecosystem health (Weaver and Fuller 2007). Water quality is determined by its chemical, physical and biological properties. Parameters of water quality include temperature (°C), dissolved oxygen (mg/L), pH (a measure of hydrogen ion activity), turbidity (a measure of how much suspended material is in the water, measured in FNU), and conductivity (a measure of the ability of water to transmit electric current, measured in $\mu\text{S}/\text{cm}$) (Weaver and Fuller 2007). In areas known to be highly polluted, such as the Saginaw Bay, which was declared an Area of Concern in 1987, studying nutrient levels is also an effective way to analyze ecosystem health and pollution (U.S. EPA 2016). Nitrogen and phosphorus can be limiting nutrients in aquatic ecosystems. Studying nutrient concentrations in wetlands is helpful to understand how hydrologically reconnected areas can enhance or diminish water quality. Restored wetlands may have increased potential to reduce phosphorus, nitrogen, and sediment loading rates (Baustian et al. 2018). Diked wetlands that are hydrologically connected to rivers, such as the three wetland units surveyed at the Refuge in 2019, would be expected to have fluctuations in chemical and nutrient levels as temperature, precipitation, and flow change throughout the year (Baustian et al. 2018).

RESEARCH QUESTIONS

- What is the average temperature, dissolved oxygen, pH, conductivity, and turbidity in each unit?
 - How do these indicators vary by month?
 - How do they vary based on distance from the water control structure (near, intermediate, far)?
 - How do they vary based on vegetation types?
- What is the variation in water quality indicators among units?
 - Are temperature, dissolved oxygen, pH, conductivity, and turbidity significantly different in the units?

METHODS

Multi-Parameter Sonde Sampling

We sampled water quality in the months of May, June, and August in each of the three wetlands: MS, MN, and P1A. Thirty sampling sites in each unit were picked randomly from a 0.5cm x 0.5cm grid overlaying unit maps. Each half centimeter grid box represented approximately 300 ft in the field. We did this by printing full size maps of each unit on 8.5

in x 11 in paper and dividing them into three proportional sections parallel with the water control structure. The three sections were designed to represent distance from the water control structure: near, intermediate, or far. Near sites were within approximately 300 meters of the water control structure, intermediate sites were between 300 meters and 600 meters from the water control structure, and far sites were anything beyond 600 meters. We sampled five sites near to the water control structure, 10 sites from an intermediate distance to the water control structure, and 15 sites that were far from the water control structures.

Our methods are broadly based off of the Great Lakes CWMP, but instead of sampling perpendicular to shore by vegetation zone gradient (i.e., wet meadow, emergent, and submergent) we chose to sample water quality by distance from the water control structures, since there are not distinct depth and gradient vegetation zones in the Refuge units (Uzarski 2016). Vegetation was recorded at each site and consisted of channel, forest, mixed emergent, open water, *Phalaris*, *Salix*, submerged aquatic vegetation (SAV), and *Typha*.

At each site three readings were taken using a YSI EXO III/EXO II hand-held multiparameter sonde. We took our thirty samples in the middle of the water column and waited to record data until all of the parameters stopped changing on the hand-held screen. At each sampling site, we recorded temperature ($^{\circ}\text{C}$), dissolved oxygen (mg/L), turbidity (total dissolved/suspended solids (mg/L)), pH (std units), and specific conductance ($\mu\text{S}/\text{cm}$).

Throughout the sampling season we had two sondes: one for measuring water quality across space, and one for measuring 24-hour cycles. During the months of June and August, for each day that we collected 30 samples from a unit, we also left a multi-parameter sonde in the unit the previous night so that we could record diel changes in water chemistry. For example, if we sampled MS on Monday, on Sunday at 4pm we put one of our two sondes in MS. On Monday around the same time we removed the sonde from MS and placed it in the next unit to be sampled (e.g., MN). The overnight sonde sites were chosen based on their ease of access. In MS the sonde in June was placed near the water control structure, and in August it was placed near the parking lot. The MN sites were consistently near the water control structure. The P1A sites were placed in a far site from the water control structure (Appendix 1.2).

Nutrient Sampling

In addition to the sonde data, we also collected samples to measure nutrient concentration at the structure input and the farthest point away from the structure input. We took two successive 1L water samples at each site and mixed the two samples into one bottle, and

then a second bottle, providing us with two mixed samples. Samples were taken with Polypropylene bottles that were acid-washed and placed underwater at the mid-depth of the water column. In each location and at the same time, we took water quality data with the sonde to examine water quality. After collecting the samples we placed them in a cooler and sent them to [Heidelberg's National Center for Water Quality Research](#) lab for analysis of phosphorus and nitrogen contents.

DATA ANALYSES

Multi-Parameter Sonde

To start visualizing the water quality data, we averaged the water quality data within months in excel. To analyze our water quality data, we performed ANOVAs in R using the packages 'car' and 'dplyr'. In total we had a sample size of 270. We tested the relationships between unit, month, distance, and vegetation and each parameter (temperature, dissolved oxygen, pH, turbidity, and conductivity).

Nutrient Samples

Although we did send our samples in for analysis to Heidelberg, we decided that we did not collect enough information in the field to accurately make conclusions about nutrient concentrations. Our goal was to follow the methods described by Baustian, Kowalski and Czayka (2018) to estimate total phosphorus and sediment flux, but we did not collect enough samples for conclusive analysis. In the results section we provide the plots of the relationship between phosphorus and turbidity, and total nitrogen and turbidity.

RESULTS

Our data showed that water quality varied by month, distance from water control structure, vegetation type, and unit. We used alpha = 0.05 ($p < 0.05$) for statistical significance.

Summary of Averages

Month	May			June			August		
Unit	MS	MN	1A	MS	MN	1A	MS	MN	1A
Temperature (°C)	14.51	15.02	16.31	22.96	23.15	22.82	20.39	21.27	19.12
pH	7.50	7.86	8.01	7.60	7.79	7.99	7.33	7.65	7.16
Conductivity (µS/cm)	383.74	369.57	566.85	339.57	392.65	418.11	348.19	341.34	483.65
Turbidity (FNU)	25.78	64.90	107.77	13.66	16.77	3.68	20.24	12.47	8.73
DO (mg/L)	6.87	8.32	7.71	5.33	7.24	6.86	2.30	5.52	1.88

Table 2.1 Average daytime May, June, and August water quality in Maankiki North and South, Pool 1A (temperature, pH, conductivity, turbidity, dissolved oxygen).

We observed a wide range of values across the sampling season (Table 2.1). Temperature increased from May to June, and decreased from June to August across all units, with Maankiki North notably having the highest average temperature of 23.15°C in June. Average pH varied slightly among the units over time and was lowest in August, with pH ranging from 7.16-8.01. Conductivity (µS/cm) is much higher on average in P1A, ranging from 483.65-566.85 µS/cm, MS and MN range from 339.57-392.65 µS/cm. Average turbidity across units was lower in June and August. DO (mg/L) decreased dramatically throughout the summer, most notably from 6.87 to 2.30 mg/L in MS, and from 7.71 to 1.88 mg/L in P1A.

Nutrient Samples

The water quality samples analyzed by Heidelberg University indicate a general trend that as turbidity (FNU) increases, total phosphorus (mg/L) and total nitrogen (mg/L) increase.

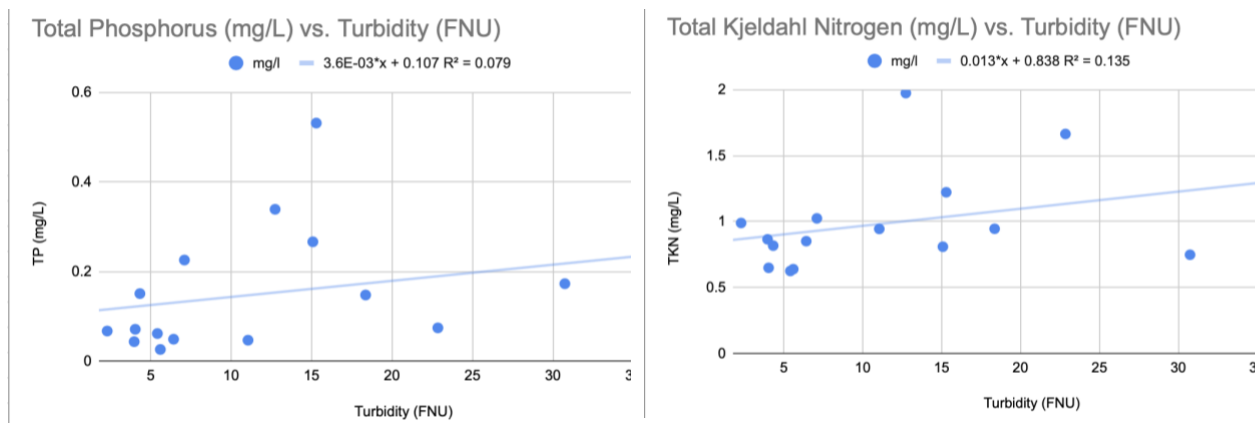


Figure 2.1 Relationship between turbidity and total phosphorus and total nitrogen. As turbidity increased total phosphorus (mg/L) and total nitrogen (mg/L) increased ($R^2 = 0.079, 0.135$ respectively). Total phosphorus ranged from 0.03 to 0.53 mg/L. Total nitrogen ranged from 0.63 to 1.97 mg/L.

Water Quality Parameter	Monthly Variation (May, June, August)	Distance (near, intermediate, far)	Vegetation (channel, forest, mixed emergent, open water, <i>Phalaris</i> , <i>Salix</i> , SAV, and <i>Typha</i>)	Unit (MS, MN, P1A)
Temperature	<0.001	0.044	<0.001	0.586
Dissolved Oxygen	<0.001	0.099	0.033	<0.001
pH	<0.001	0.024	<0.001	<0.001
Conductivity	<0.001	0.803	<0.001	<0.001
Turbidity	<0.001	0.602	<0.001	0.014

Table 2.2 A summary of p-values for tests of significant relationships ($p < 0.05$, bold) between selected physical variables and wetland water quality parameters. It shows the results of general linear model balanced factorial design ANOVAs for temperature, dissolved oxygen, pH, conductivity, and turbidity in Maankiki North and South, and Pool 1A. Data were analyzed by monthly variation (May, June, August), distance from the water control structure (near, intermediate, far), vegetation (channel, forest, mixed emergent, open water, *Phalaris*, *Salix*, SAV, and *Typha*), and unit.

Monthly Variation

Temperature, dissolved oxygen, turbidity, conductivity, and pH clearly varied by month within each unit and showed similar patterns across units (Table 2.1). Temperature increased from May to August by 4.98 degrees Celsius ($p < 0.001$), but temperature in June was higher than in August ($p < 0.001$). Dissolved oxygen was significantly lower in August than in May and June (Figure 2.2) by 4.40 mg/L ($p < 0.001$). Turbidity in June and August was significantly lower than turbidity in May ($p < 0.001$) as shown in Figure 2.3. May conductivity was also greater ($p < 0.001$) than the values recorded in June and August (Figure 2.4). Finally, pH was lower in August than in May and June by 0.41 ($p < 0.001$).

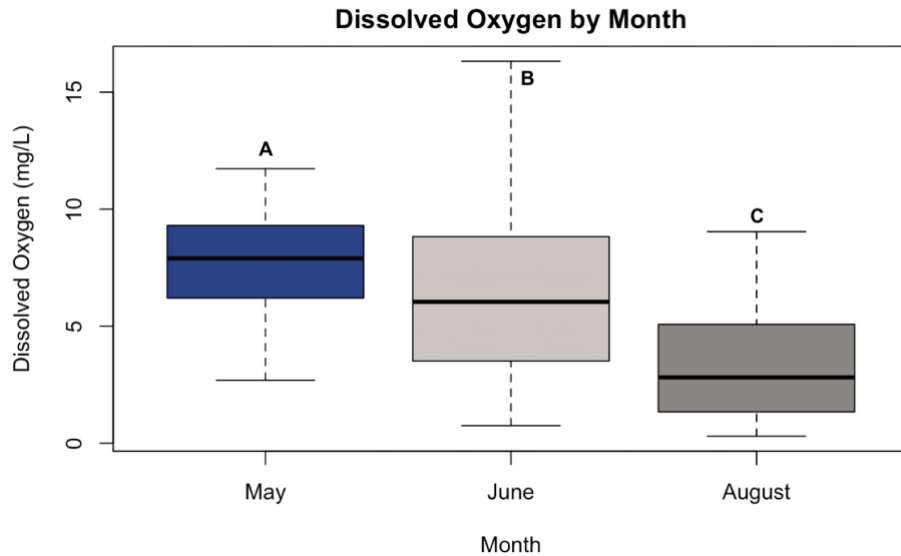


Figure 2.2 Dissolved oxygen (mg/L) levels by month. The different letters and colors each represent significantly different DO levels May>June>August (Tukey-adjusted comparisons).

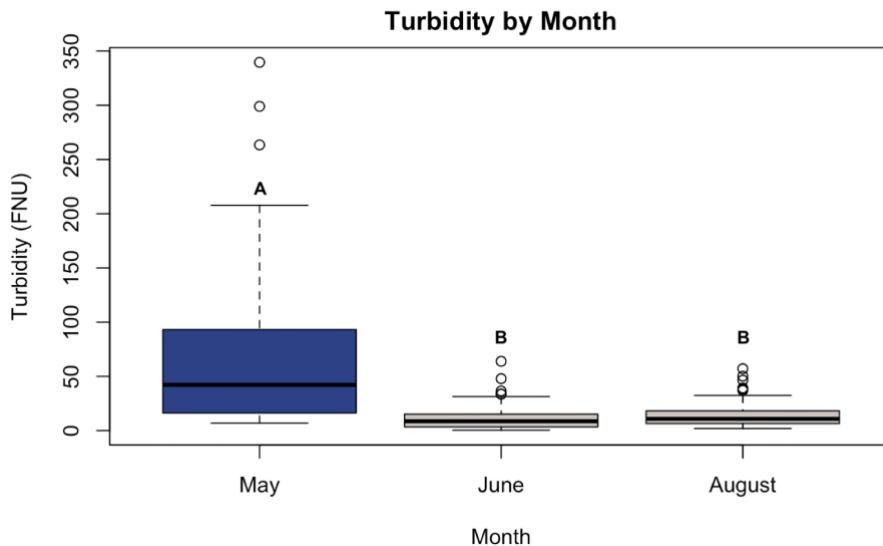


Figure 2.3 Turbidity (FNU) levels by month. The monthly means that share a letter and color are not significantly different from each other (Tukey-adjusted comparisons).

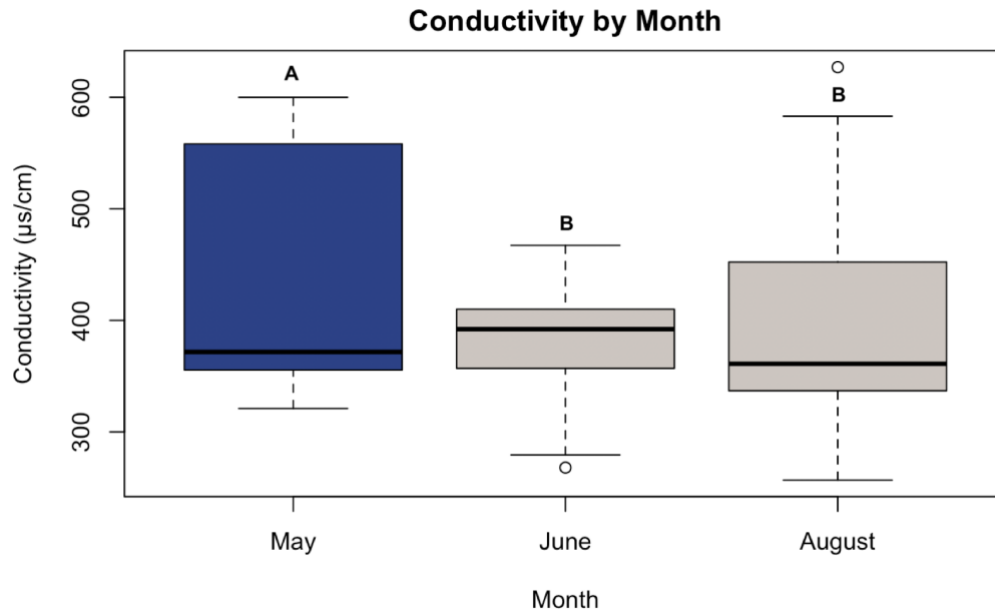


Figure 2.4 Conductivity (FNU) levels by month. The monthly means that share a letter and color are not significantly different from each other (Tukey-adjusted comparisons).

Distance & Water Quality

We found that there was a significant relationship between distance from the water control structure and pH and temperature (Table 2.2). Intermediate distances had lower values of pH than areas that were farther from the structure ($p = 0.04$). Additionally, distance had a significant relationship with temperature ($p = 0.04$), though a Tukey post-hoc test did not show significant comparisons at the $p < 0.05$ level.

Vegetation & Water Quality

Water quality also varied by vegetation type (Table 2.2). *Salix* had lower temperatures than SAV by 4.30 degrees Celsius ($p < 0.001$) and higher levels of conductivity compared to the channel, forest, mixed emergent, SAV and *Typha* vegetation types ($p = 0.02$). Conductivity in *Salix* was not significantly different from conductivity in open water or *Phalaris* veg types. Turbidity levels in *Salix* were also higher than those in the forest, mixed emergent, SAV, and *Typha* types ($p = 0.04$). Lastly, pH was lower in *Typha* than in SAV and *Salix* ($p = 0.02$).

Unit Comparisons

The units displayed significant differences in water quality; even the two recently restored wetlands were distinct from each other (Table 2.2). We found that dissolved oxygen is higher in MN than P1A (1.54 mg/L) and MS (2.19 mg/L) ($p = 0.003$), as shown in Figure 2.5. P1A has significantly higher conductivity levels than MS (143.48 $\mu\text{S/cm}$) and MN

(121.68 $\mu\text{S}/\text{cm}$) ($p < 0.001$), as shown in Figure 2.6. P1A is more turbid than MS by 21.17 FNU ($p = 0.009$). We also found that MS has lower pH than P1A and MN ($p = 0.001$).

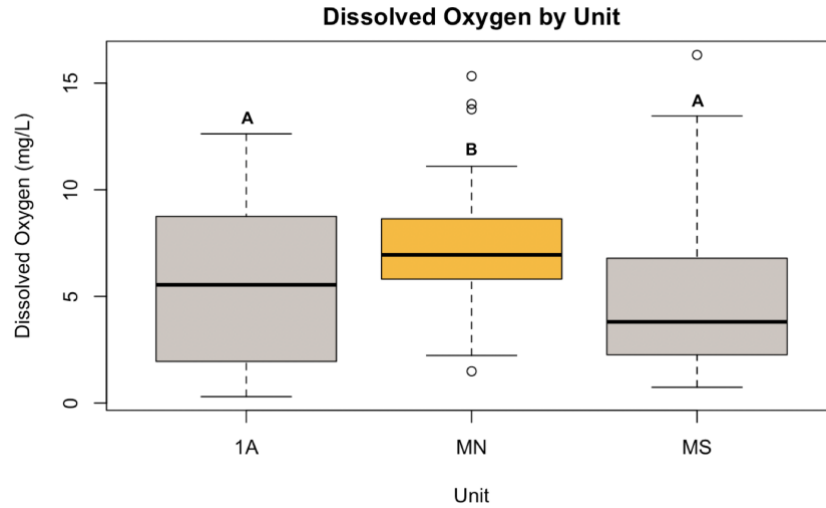


Figure 2.5 Dissolved oxygen (mg/L) levels by unit. The unit means that share a letter and color are not significantly different from each other (Tukey-adjusted comparisons).

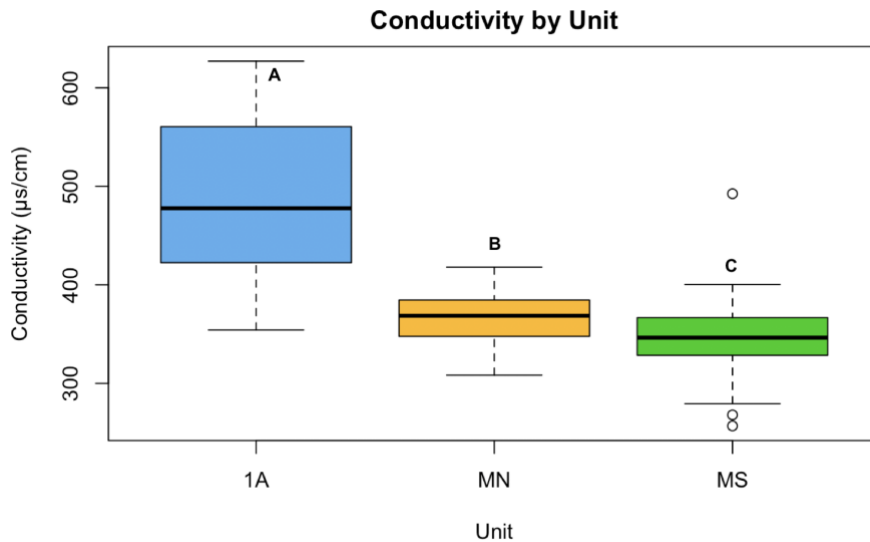


Figure 2.6 Conductivity ($\mu\text{S}/\text{cm}$) levels by unit. The different letters and colors represent significantly different conductivity levels in each unit (Tukey-adjusted comparisons).

DISCUSSION

Main Findings Relative to Literature

Low Dissolved Oxygen in Summer

Dissolved oxygen (DO) in an aquatic system changes with temperature. As temperatures increase, dissolved oxygen concentration decreases. In May, the average daily DO across

units was 7.63 mg/L, whereas in August the average daily DO had dropped to 3.23 mg/L across units. Figures 2.2 and 2.7 show how the DO varied by month and from June to August at our stationary sondes. The ranges witnessed are generally stressful, even lethal for aquatic life. Hypoxic conditions occur below 3mg/L of dissolved oxygen, though stressful levels occur starting around levels of 5mg/L (EPA 2008). Declining oxygen availability, as water warms, is a commonly observed phenomenon in wetlands (Kowalski et al. 2014). At the Crane Creek wetlands, the declines in dissolved oxygen were more severe in the diked units than the open units connected to Lake Erie (Kowalski et al. 2014). We did not observe this at SNWR, as P1A, which was always open, had lower DO levels than MN, which was always closed. Consistently low DO levels restrict the types of organisms that can live in a certain habitat. Our low DO levels throughout the summer, and especially low levels overnight (below 1mg/L in August), suggest that organisms in Shiawassee wetland units must be well adapted to fluctuating oxygen levels or able to move to deeper, cooler water, which holds more dissolved oxygen within the system (USGS 2019).

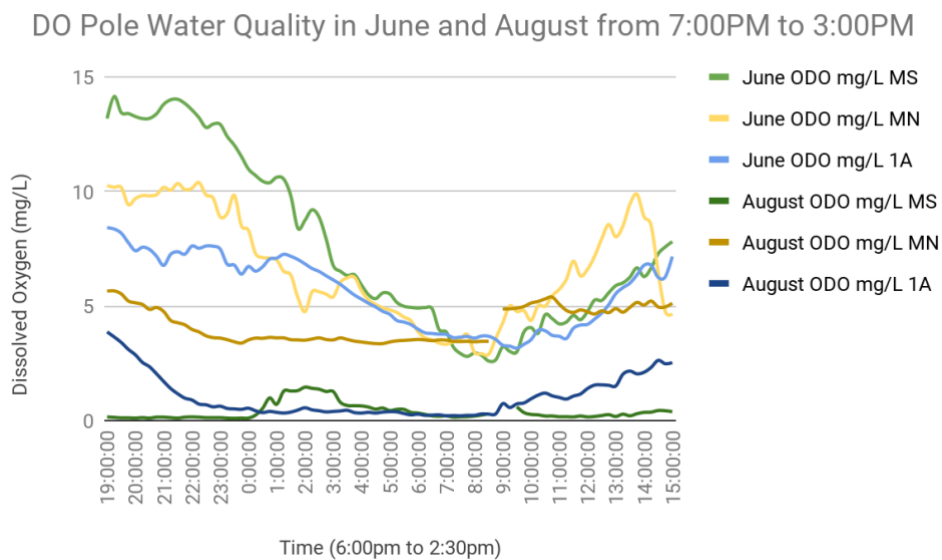


Figure 2.7 Diurnal dissolved oxygen levels varied between the hours of 6:00pm and 2:30pm and decreased from June to August at the stationary poles left overnight.

Distance From Gate

Since MS and MN were closed all summer, we did not observe the impacts of distance from the water control structure on water quality that we had expected. For example, we expected conductivity to be higher near the water control structures if they were open because the majority of soils in the Shiawassee Flats area are clay soils (Heitmeyer et al. 2013), which have higher conductivity due to the presence of materials that ionize in the water (EPA 2012). We expected that conductivity would be higher near the structure where the input to the unit is located due to soil suspension in the water column entering

the units, especially if runoff was also contributing to the flow into the unit. We expected that these suspended sediments, exacerbated by the flow of water into the unit, would increase conductivity near the structure.

Temperature also may have had a significant relationship with distance from the water control structures. The ANOVA for all units showed that distance from the water control structure impacts temperature ($p = 0.041$), but the Tukey post-hoc comparisons were not significant at the ($p < 0.05$). At the ($p < 0.1$) level, near water temperatures were cooler than far ($p = 0.065$). This is likely because we sampled near sites earlier in the morning than intermediate and far sites, though it also could be due to the fact that water is deeper in the channelized areas directly near the water control structures. In P1A, the only open unit, distance did not have a significant relationship with any of our water quality parameters.

Vegetation & Water Quality

The significance of high levels of conductivity in *Salix* compared to the channel, forest, mixed emergent, SAV, and *Typha* vegetation types may be explained by the fact that we only sampled *Salix* in P1A, which is consistently connected to Spaulding Drain. Conductivity, a measure of ions dissolved in water is impacted by types of sediment suspended in the water, thus we speculate that conductivity in P1A is higher in vegetation specific to P1A due to the sediment loading from Spaulding Drain to P1A. The conductivity levels were likely exacerbated by high water levels in May (Figure 2.4). Temperature was significantly lower in *Salix* than in other vegetation types, which may be due to increased canopy cover in addition to seasonal bias, since *Salix* samples were primarily taken during the month of May, our coolest month. Turbidity levels in *Salix* were also higher than those in the forest, mixed emergent, SAV, and *Typha* types ($p = 0.04$). We believe that this could also be due to the fact that *Salix* was mostly sampled in May in P1A when plentiful Common Carp were spawning on the bottom of the water column, resulting in more suspended solids in the water column (Figure 2.3). While water quality data should reflect naturally occurring trends in wetland turbidity, there were so many carp that we were unable to avoid disturbing them when sampling, which combined with spawning activity, created sediment disturbance in the water column.

Unit Comparisons

We expected that the Maankiki units would be more similar to each other than Pool 1A based on the fact that they were more recently restored, and both were closed to the river in 2019. We found, however, that some of our parameters were more similar between Maankiki North and Pool 1A, or between Maankiki South and Pool 1A, which we believe is related to vegetation and depth. While temperatures were not significantly different across units, Maankiki North had higher levels of dissolved oxygen than Maankiki South and Pool

1A (Figure 2.5). We believe that these differences can be attributed to depth: Maankiki North is much deeper than Maankiki South, and deeper, cooler water can hold more dissolved oxygen (USGS 2019).

Significantly, Pool 1A had much higher levels of conductivity than MS and MN (Figure 2.6). Since conductivity is a measure of ions in the water, higher levels of conductivity in P1A were likely representative of its connectivity to the river. Additionally, conductivity in MN was significantly higher than conductivity in MS, which could be due to increased surface-water runoff into MN which has taller dikes than MS. This could also be attributed to the fact that MN was open to the River in 2018, whereas MS was not. Previous Shiawassee projects saw that conductivity in the Shiawassee Flats area ranged from 153 to 977 $\mu\text{S}/\text{cm}$ (Buchanan et al. 2013), and the Crane Creek restoration had conductivity ranging from 106 to 1004 $\mu\text{S}/\text{cm}$ (Kowalski et al. 2014). The conductivity within MS, MN, and P1A ranged from 256 to 627 $\mu\text{S}/\text{cm}$. Conductivity in P1A ranged from 354.2 to 627 $\mu\text{S}/\text{cm}$, 308.3 to 417.93 in MN $\mu\text{S}/\text{cm}$, and 256.8 to 492.47 $\mu\text{S}/\text{cm}$. These numbers are within the ranges of the Crane Creek restored wetlands and are not of concern for fish populations (Kowalski, Personal Communication 2020; Environment Canada).

Turbidity in P1A was higher than in MS by about 20.17 FNU. This is likely because Pool 1A water levels were constantly fluctuating in response to high water levels in the Shiawassee River, leading to an increase in water and sediment disturbance.

Key Findings

While we expected to find that Maankiki North and Maankiki South were most similar to each other regarding water quality based on their recent restoration and disconnection to the river, Maankiki North and Maankiki South only had similar ranges for temperature and turbidity. Interestingly, we saw that there were some similarities between MN and P1A. Maankiki North and Pool 1A both had higher pH and higher conductivity than Maankiki South. We attribute this to variation in connectivity to the river. Though Maankiki South and Maankiki North were both closed in 2019, Maankiki North was open to the Shiawassee River in 2017 *and* 2018, and P1A is constantly connected to the River, so more time connected to the river could have increased these values. Maankiki North also had higher dissolved oxygen than both P1A and MS, likely due to depth. Turbidity values were similar between MS and MN and between MN and P1A, though they were significantly different between MS and 1A. All units had significantly different conductivity levels.

Study Limitations

Although we did our best to mitigate bias in sampling results, it is likely that our water quality data were impacted by various factors both within and outside of our control. To

start, our data were not entirely representative of our sampling season (May to August), as we could not conduct specific site sampling in July due to a broken sonde.

In reflecting on what could be improved upon in the future, we would recommend that when using a multi-parameter sonde, the sonde is left in long enough for all numbers on the sonde to stop changing. Occasionally, the measurements were taken just after the sonde touched the water, which does not give the sonde enough time to adjust to the varying water quality. Three minutes seems to be enough time for numbers to have stopped changing, though we recognize that this does slow down sampling.

We believe that turbidity measures early in the sampling season may not have always been accurate due to us not initially recognizing our own impact on the water clarity as we moved through the water. Turbidity in May was also higher than in the other months, which we believe is due to a combination of Common Carp spawning and high-water levels (Weber and Brown 2011).

Implications for SNWR

Temperature greatly determines the levels of dissolved oxygen available in the wetland units. Although water and air temperature are not something that Refuge managers can decisively control, understanding how DO can negatively impact fish biomass is important. Expectedly, the DO in all of the restored units decreased significantly throughout the summer sampling months. Throughout our sampling, we did not visually notice obvious fish die offs in the units, with the exception of finding dead fish in our fyke nets that experienced stressful dissolved oxygen conditions overnight. We also had a lower catch per unit effort of fish later in the summer. Similar trends were observed in Crane Creek, either representing fish movement within the units to deeper, inaccessible areas, or fish mortality (Kowalski et al. 2014). Connection to the Shiawassee River might help reduce a loss of biomass due to low oxygen conditions and could help to provide refuge to fish.

Although conductivity is clearly influenced by whether a wetland unit is open or closed to the Shiawassee River, all researched units had conductivity measurements that were in a standard range to support fish survival (Kowalski, Personal Communication 2020; Environment Canada).

Future Recommendations

In the future, we would not recommend structuring water quality sampling using the same methods as in the 2019 season; rather we would recommend a combination of the sampling methods from the Coastal Wetlands Monitoring Program (CWMP) and our sampling methods. Sampling thirty random sampling sites within each wetland unit in a day was physically demanding, and at times unproductive. For the 2019 season, sampling

in this manner provided us with the unique opportunity to thoroughly understand a snapshot of the water quality in each unit in every sampling month when the water control structures were closed. However, it is not typical that the units would be closed to the Shiawassee River for more than three months. In measuring water quality, the CWMP protocol takes multi-parameter sonde measurements and nutrient samples at each fyke net location. Fyke net locations are determined by vegetation zones within a gradient from shore, thus so are water quality measures. At the SNWR, sampling in this manner is not feasible as there are no distinct vegetation zone gradients. Therefore, we believe that water quality sampling should still be performed based on three distances from the water control structures: near, intermediate, and far. As recommended by the CWMP though, we believe that water quality should be sampled weekly in conjunction with fyke net sites and macroinvertebrate sites. Sampling in this manner should still account for distances from the water control structure, and if fyke nets or macroinvertebrate sites are not representative of distance, then at least one week each month should be dedicated to sampling water quality by distance from the water control structure. Changing water quality sampling to be weekly at macroinvertebrate and fish sites will likely provide a more holistic picture of water quality throughout the day and across different summer months, in addition to freeing up a week of sampling if water quality no longer has its own sampling week. We had more than 110 fyke nets and over 60 macroinvertebrate quadrats in 2019, and we believe that if water quality was sampled at each net and quadrat (sample size of ~ 180), researchers would be able to analyze water quality without dedicating a full week of sampling to only water quality. It is important to note though that the opening and closing of the water control structures and changing water levels might dictate the need to sample water quality more frequently and in depth, especially if the main research question is to analyze how water quality changes by distance from the water control structure.

Furthermore, in the 2019 sampling season we had intended to collect water depth data using HOBO Data Loggers. While we were able to collect some data, we were not able to make comparisons across units due to the fact that the P1A HOBO Data Logger was lost to the wetland. In the future, we would recommend collecting water depth data more frequently in each unit to analyze how water quality might be changing as water levels change within the units.

If funding is available and the water control structures are open, we recommend that nutrient samples should be taken every time that water quality is sampled. Another method could be to use an automatic nutrient sampler which will be more effective at creating a turbidity-phosphorus curve (Baustian et al. 2018). Nutrient concentrations are valuable indicators of whether restored wetland units are effectively functioning as watershed filters (Mitsch & Gosselink, 2015). Nutrient concentrations are likely to vary as flow into the units changes, highlighting the need for greater emphasis on studying

concentrations throughout the sampling season if units are opened and closed. Nutrient sampling should capture high and low flow conditions in addition to base flow conditions.

Additionally, we would encourage more sampling in the Shiawassee River and Spaulding Drain, near where they each connect to the SNWR wetland units. The water quality in these bodies of water hasn't been extensively studied in over six years, and the previous projects do not have specific water quality data for the Shiawassee River. As such we recommend doing less sampling within the units, and spreading out sampling more between the units, River, and Spaulding Drain if time and resources allow.

VEGETATION

INTRODUCTION

Following the hydrological reconnection of Maankiki Marsh, the Refuge's management goals regarding migratory birds expanded to include the management of vegetation communities, the factors that shape plant community composition, and the influence of vegetation on the ecology of the wetland units (USFWS 2018). Factors that shape wetland plant communities include, but are not limited to hydrology, topography, land-use and seed bank history, disturbance, management practices, (Keddy and Reznicek 1986; Johnston et al. 2009) and water quality (Johnston & Brown 2013). Within the units, the mixture of submerged aquatic and emergent coastal wetland vegetation plays a critical role in the creation of suitable habitat for refuge and spawning of riverine and Great Lakes macroinvertebrate communities (De Szalay and Resh 2000) and fish communities (Jude and Pappas 1992), which in turn affect migratory bird communities who feed on a host of plants, insects, or fish (Wilcox 2002). With a greater emphasis on ecology-oriented management, SNWR needed to monitor the influence of the hydrologic reconnection, and its management, on the composition of the vegetation communities within and among the restored wetland units.

Sampling conducted prior to the reconnection of Maankiki Marsh by a previous UM Masters Project Team (Buchanan et al. 2013) was limited in scope due to resource constraints. Additionally, reconnection of Maankiki North and Maankiki South in 2017-2018 influenced the establishment and proliferation of wetland plant species largely divergent from the farmland and mudflat communities that were observed in 2013. Therefore, our 2019 sampling represents the most comprehensive study of vegetation within the reconnected wetland units at SNWR to date.

RESEARCH QUESTIONS

- How does vegetation vary among the three units?
 - What is the character of the species present?
 - What exotic (non-native) 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 does the structure compare within and among units?
 - How does the composition of each zone compare among units?
 - How does the Abundance of each species (percent cover/frequency) in each vegetation zone compare within and among units?

METHODS

Vegetation methods followed the standardized monitoring techniques developed by the Great Lakes Coastal Wetlands Monitoring Program (Uzarski et al. 2016) and were adapted for SNWR based on research conducted on restored Great Lakes coastal wetlands at Crane Creek in the Ottawa National Wildlife Refuge just east of Toledo, Ohio (Kowalski et al. 2014).

Sampling

Field sampling was conducted in wetland units Maankiki South, Maankiki North, and Pool 1A from August 8, 2019 to August 25, 2019. Stratified random sampling was used to describe the variation in vegetation zones within and among units.

Vegetation Zone Delineation

Prior to sampling, vegetation zones for each unit were determined through a combination of physical observation and aerial photos and later digitized using GIS software (Table 3.1, Images 3.1-3.3 for vegetation zone maps). These zones were described based on areas of a dominant plant species (e.g., *Typha* or *Phalaris*), differential plant structure (e.g., Submerged Aquatic Vegetation or Forest), or distinct habitat types (e.g., Mudflat).

Unit	# of Vegetation Zones	Vegetation Zones	Vegetation Zone Area (acres)	Average Depth (cm)	Total Vegetation Zone Area / Total Unit Area (acres)
Maankiki South	6	Forest	26.43	2.61	275.10 / 288
		Mudflat	5.35	1.00	
		Phalaris	20.60	34.22	
		SAV	49.54	68.94	
		Sparse Typha	10.81	32.91	
		Typha	162.37	40.39	
Maankiki North	3	Phalaris	11.19	12.39	194.90 / 222
		SAV	67.17	113.65	
		Typha	116.54	62.77	
Pool 1A	5	Mudflat	0.39	3.43	306.70 / 322
		Nymphaea	190.94	74.65	
		Salix	13.98	14.60	
		SAV	2.72	56.39	
		Typha	98.67	48.61	

Table 3.1 Characteristics of vegetation zones within the 3 wetland study units.

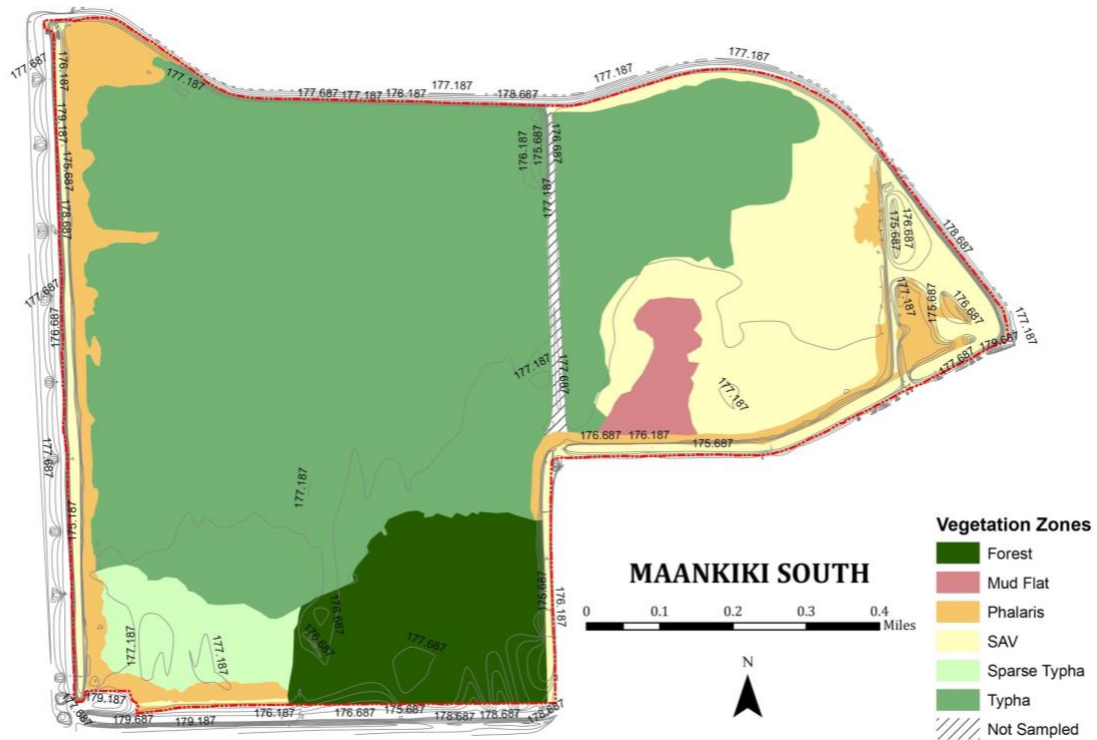


Image 3.1 Maankiki South vegetation zone map. Elevation contours labeled in meters.

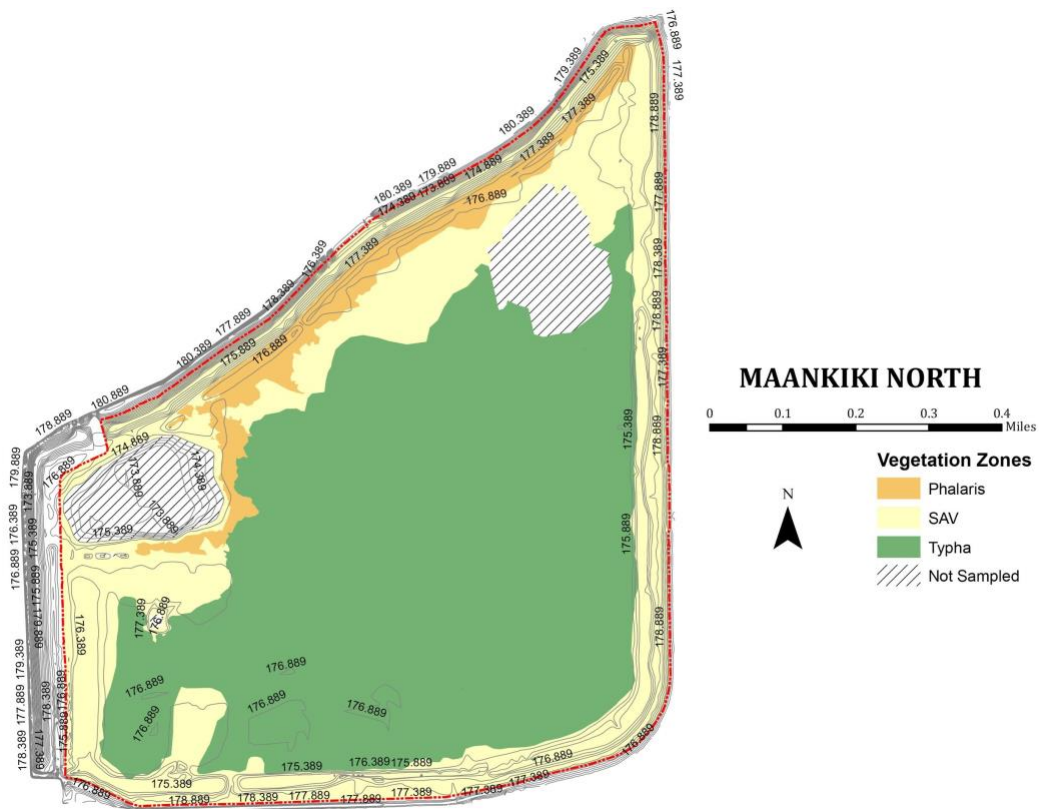


Image 3.2 Maankiki North vegetation zone map. Elevation contours labeled in meters.

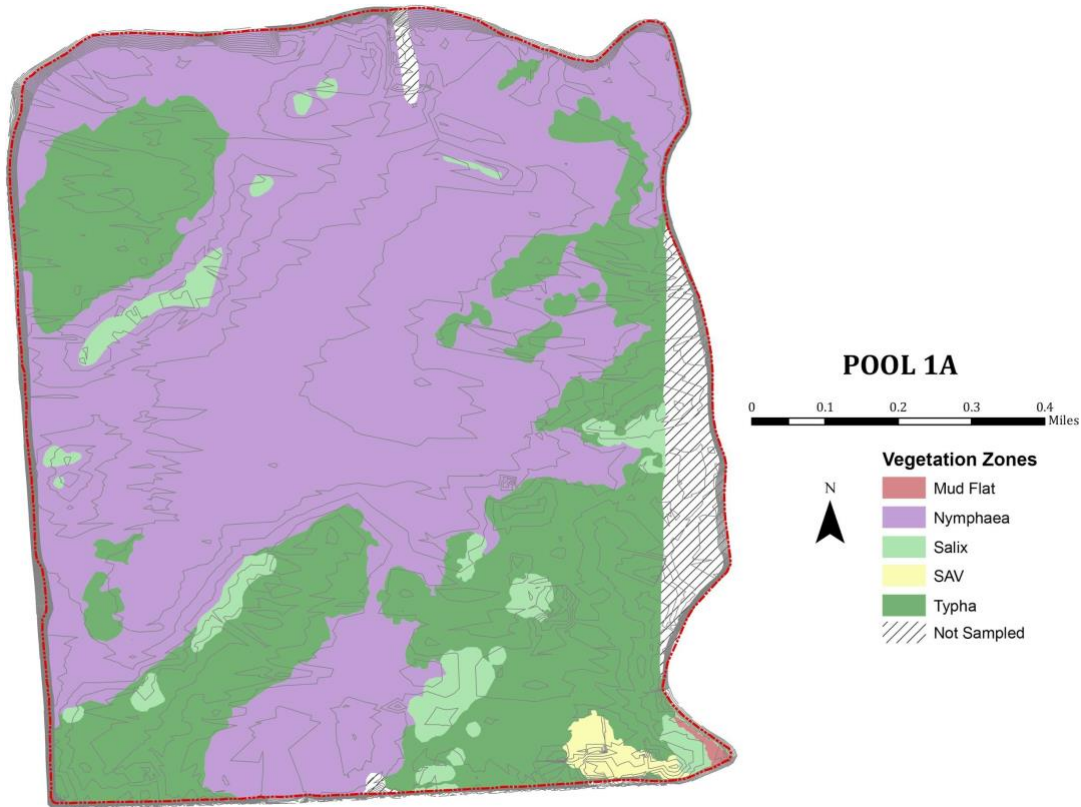


Image 3.3 Pool 1A vegetation zone map. Elevation contours labeled in meters.

Vegetation Survey

Once each vegetation zone was determined, we walked a number of steps determined by a random number generator into the vegetation zone. When that distance was reached, a 1-meter² quadrat was randomly thrown to establish each sampling point. This was done 10 times for each vegetation zone.

Once the quadrat was established, a GPS point was recorded on a Garmin Rino 130 receiver and identified with the wetland unit's name and quadrat number. Water depth was measured in centimeters to the nearest millimeter, for non-inundated areas a measurement of '0 cm' was recorded. Plants within the quadrat were then identified to the species level, or the lowest taxonomic level possible, and each species' percent cover was estimated visually and recorded. One person was responsible for percent cover estimation across quadrats. Any unidentifiable plants were listed on the data sheets with temporary descriptors such as "Unidentified Plant 1", "Unidentified Plant 2," etc. At a minimum, unidentified plants were photographed for distinctive characteristics (e.g., leaves, flowers, or fruiting bodies), and, if possible, collected with a Whirl Pak Bag labeled with the wetland unit's name, the vegetation zone from which the sample was taken, the quadrat number, and the temporary descriptor that matched the data sheet. Those samples were taken to Tony Reznicek at the University of Michigan Herbarium for identification.

For the Forest vegetation zone in Maankiki North and the Salix vegetation zone in Pool 1A, where woody understory or overstory plants were encountered, the quadrat was enlarged to include these species in the survey (Figure 3.1). If the largest species were understory trees (less than ~15 feet tall - see Salix vegetation zone for Pool 1A), a circular quadrat with a radius of 3 meters was measured from the center of the 1 meter² quadrat. Data was collected for herbaceous species as previously described within the 1-meter² quadrat and then collected following the same protocol but expanded for the understory species within the 3-meter radius quadrat. If the largest species were overstory trees (greater than ~15 feet tall - see Forest vegetation zone for Maankiki South), the quadrat was enlarged to a radius of 10 meters and followed the same protocol as previously described for both the 1-meter² quadrat and the enlarged 10-meter radius quadrat. In both instances, the Diameter at Breast Height (DBH) was measured using a tape measure for trees with a diameter larger than 10 cm. Trunk circumferences measured with the tape measure were converted to DBH by dividing by pi once data was electronically entered into a spreadsheet.

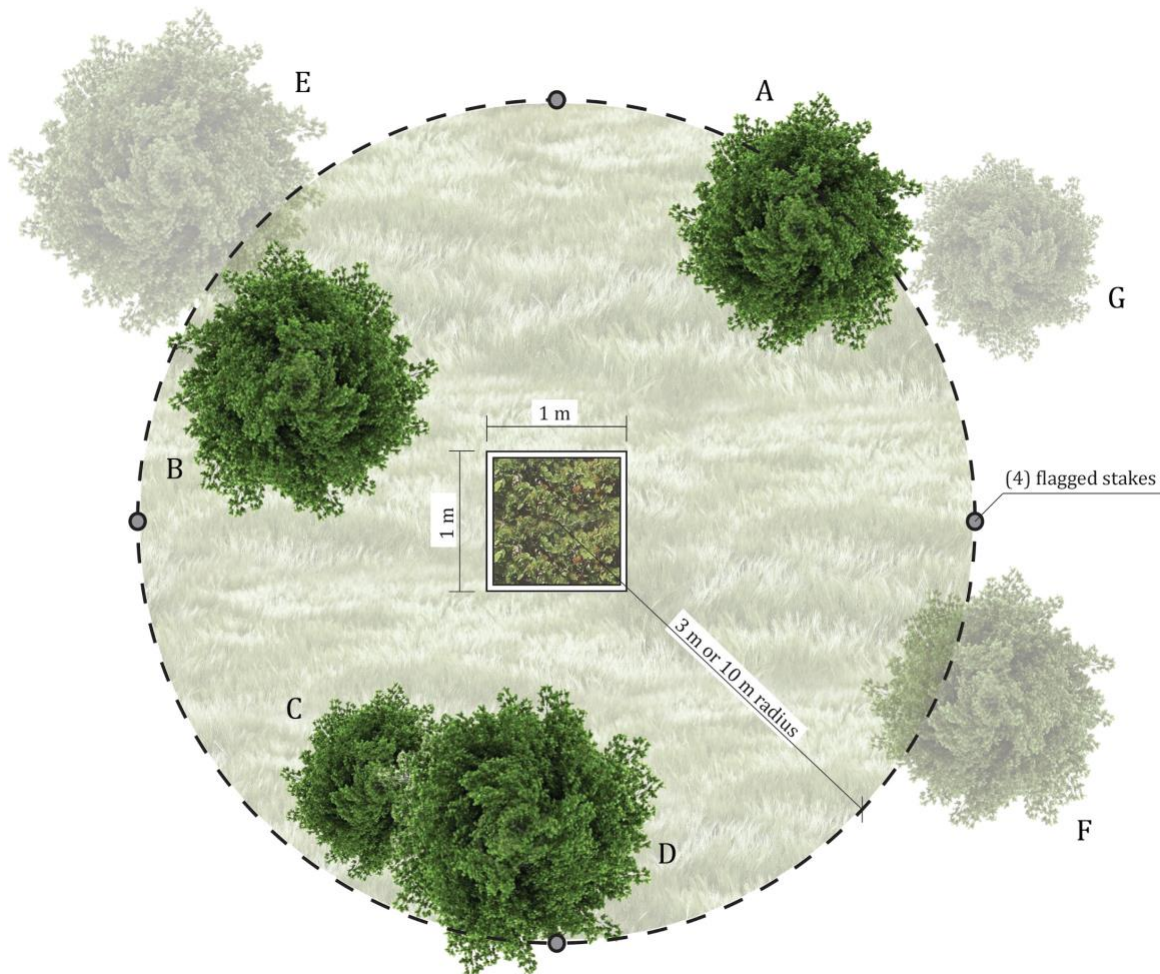


Figure 3.1 Plan diagram of an enlarged quadrat. The 1-meter² quadrat in the center was sampled for groundcover, submerged/floating, and/or emergent species. The enlarged quadrat or 3m or 10m was then sampled and contained only understory or overstory species. Dead trees were not included. Trees whose trunks fell on or within the enlarged boundary (A-D) were sampled. Trees who had canopies, but not trunks, in the boundary were excluded from the enlarged quadrat (E-F). Trees with neither trunk nor canopy in the boundary were not sampled (G).

DATA ANALYSES

The first research question specifically focused on the cataloging of species occurring in each wetland unit and how the composition differed across study units with an emphasis on identifying native, rare, and invasive species. It is our hope that the annual cataloging of wetland plant species will create a master species list specifically for the wetland units that will be used to gauge the change in community composition over time. If rare or new species become established this method will capture this.

Comparative analyses used to analyze and answer our research question regarding differences in plant community structure, composition, and abundance included calculating

Importance Value Indexes (IVI), Non-Metric Multidimensional Scaling ordinations (NMDS) of a Bray-Curtis dissimilarity, Floristic Quality Assessments (FQA), and Indexes of Biotic Integrity (IBI), all of which allow comparison between vegetation zones within and among wetland units.

Importance Value Index (IVI)

Importance value indexes were calculated for every species identified in every vegetation zone in each wetland unit. The IVI is a standard tool used to determine the dominance of plant species in a given area. For our study, the IVI was utilized to identify the dominant species or combination of species, sometimes representing different structural levels, for each vegetation zone. IVI is the sum of the relative frequency and the relative mean cover (Curtis and McIntosh 1951). Following its calculation, the IVI for each species was used as the variable in the Bray-Curtis dissimilarity index to compare vegetation zone compositions within and among units.

The IVI is calculated as follows:

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

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

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

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

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

Non-metric multidimensional scaling (NMDS) Ordination of a Bray-Curtis Dissimilarity Index

An NMDS ordination of a Bray-Curtis dissimilarity index was used to plot the differences of IVIs between species compositions of vegetation zones within and among wetland units in 2D space. Points closer to each other in the 2D plot represent similarly composed vegetation zones. Alternatively, points farther apart represent differently composed vegetation zones. Points are organized across unconstrained axes which are determined

after the statistical test has been run and represent gradients of biotic and/or abiotic factors contributing to the variance in the plotted points.

Floristic Quality Assessment (FQA)

FQAs are a universal tool used to determine the quality of a site by calculating a mean coefficient of conservatism (C), an observed score that calculates the deviation from an expected reference condition, and a floristic quality index (FQI), an adjusted form of C used for “better comparison between large sites with a high number of species and small sites with fewer species” (Herman et al. 2001). C s calculated for specific sites “range from 0 - 10 and represent an estimated probability that a plant is likely to occur in a landscape relatively unaltered from what is believed to be pre-European settlement condition,” with 0 representing a completely degraded site and 10 representing a site with a vegetation community identical to pre-European settlement (Herman et al. 2001). Computing both a site’s C and FQI allows for the standardized comparison of relative degradation across different sites, both within the Refuge or basin wide.

The Universal Floristic Quality Assessment Calculator is an online tool which automatically calculates C and FQI when plant species for a site have been input (Freyman 2016) and can be found at universalfqa.org. FQAs were calculated for each wetland unit as well as the flooded emergent and dry emergent zones of each wetland unit. Dry emergent sites are defined by Uzarski et al (2016) as quadrats with water level measurements less than 1 cm. For complete information on FQAs for Michigan as well as instructions on how to use the FQA Calculator, see Herman et al 2001.

Index of Biotic Integrity (IBI)

Developed by the Great Lakes Coastal Wetland Monitoring Program, the IBI measures a site’s health by combining C values calculated previously using the FQA, with the percent cover and frequency of invasive species. Scores are assigned based on these three parameters for three portions of a study site: the total site, the flooded emergent zone (water level >1 cm), and the dry emergent zone (water level <1 cm). Scores from these nine metrics are added to a final metric that measures the relative cover and frequency of increased nutrient, sediment, and turbidity tolerant submerged aquatic species. The summation of these 10 scores results in a ‘Combined Standardized Score’ which consists of a ‘Combined Numeric Score’ and ‘Combined Descriptive Score’. These combined numeric scores and their associated descriptive scores can be compared across sites within SNWR or with other coastal wetlands throughout the Great Lakes region. Details for computing IBIs can be found on pages 18-26 of the GLCWMP Vegetation SOP located here: greatlakeswetlands.org/docs/QAPPs_SOPs/GLCWMP_Vegetation_SOP_June_4_2018.pdf.

RESULTS

Species Characteristics Within and Among Units

A diverse array of species were characterized among the units with 67 species identified overall (Appendix 2.1). The greatest number of species were identified in Maankiki South (52), followed by Pool 1A (43), and lastly, Maankiki North (17). Many of the species unique to Maankiki South were woody plant species that occurred in the Forest vegetation zone whereas many of the unique species in Pool 1A were wet meadow emergent species identified in the Mudflat vegetation zone. *Myriophyllum spicatum* was the only species unique to Maankiki North. According to the Michigan Natural Features Inventory rare plant database, no rare or endangered species were identified.

Plant community structural analysis for each wetland unit varied across units, but a general pattern emerged. Maankiki South and Pool 1A had similar numbers of species across a range of structural levels while Maankiki North was only comparable in submerged/floating species. Maankiki North also had very few emergent species (4), comparatively, and no other species representing the remaining three structural levels.

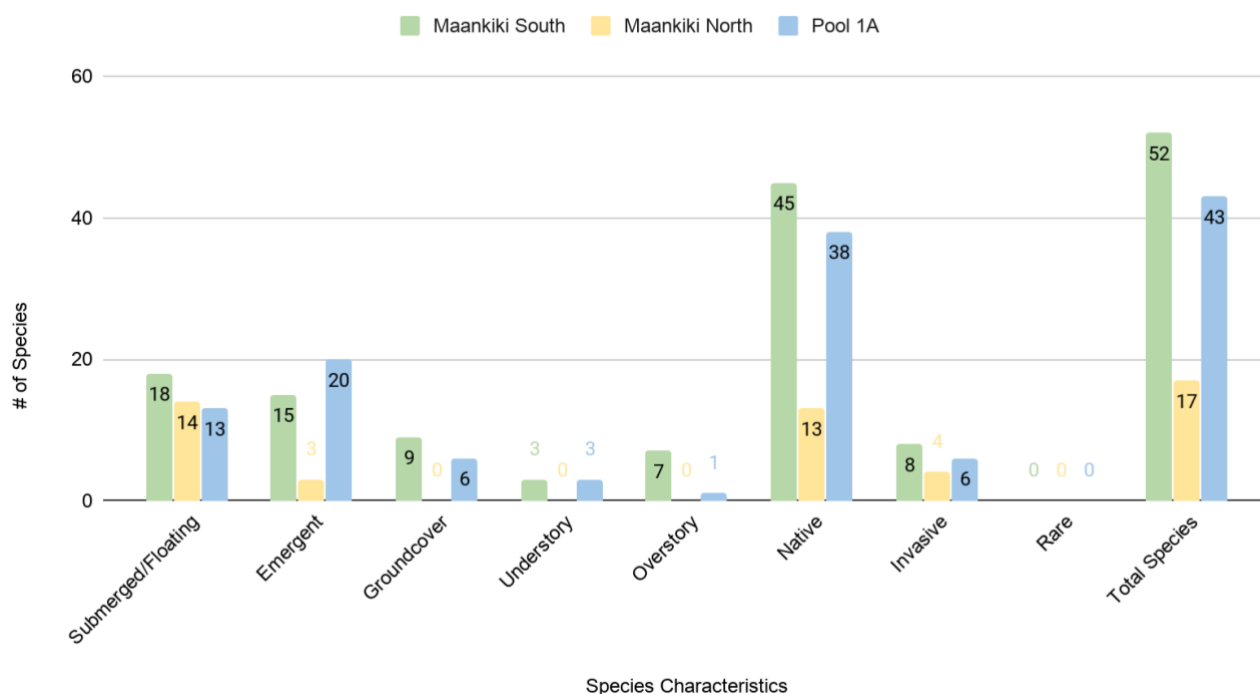


Figure 3.2 Vegetation structure and total species counts among units

Importance Value Index (IVI)

Computing IVIs revealed that each vegetation zone was dominated by a number of species representing different vegetation structural levels (Appendix 2.2). The higher the IVI, the

more frequent and more abundant a particular species is. For example, the Forest vegetation zone in Maankiki South had four major structural species of importance: 1. *Acer saccharinum* (IVI 35.82), a floodplain tree species that represented the overstory level, 2. *Lemna minor* (20.87), a nutrient tolerant species of duckweed that represented the submerged/floating level, 3. *Phalaris arundinacea* (14.15), an invasive grass almost ubiquitous to every vegetation zone in the three study units that represented the emergent level, and 4. *Lysimachia nummularia* (14.12), an invasive creeping plant that represented the groundcover level. While all vegetation zones were not as structurally variable as the Forest, all vegetation zones had at least two structural levels represented by their dominant species (Tables 3.5-3.7). In thirteen of the fourteen vegetation zones, at least one of the dominant species was either categorized as invasive or as a submerged aquatic species that had tolerance to nutrient enrichment, sedimentation, or increased turbidity.

MAANKIKI SOUTH			
Vegetation Zone	Dominant Species	IVI	Structure
Forest	<i>Acer saccharinum</i>	35.82	Overstory
	<i>Lemna minor</i>	20.87	Submerged/Floating
	<i>Phalaris arundinacea</i>	14.15	Emergent
	<i>Lysimachia nummularia</i>	14.12	Groundcover
Mudflat	<i>Alisma plantago-aquatica</i>	90.10	Emergent
	<i>Eleocharis spp.</i>	40.42	Groundcover
	<i>Potamogeton nodosus</i>	22.55	Submerged/Floating
Phalaris	<i>Phalaris arundinacea</i>	78.06	Emergent
	<i>Utricularia vulgaris</i>	31.08	Submerged/Floating
	<i>Lemna trisulca</i>	29.61	Submerged/Floating
	<i>Lemna minor</i>	11.96	Submerged/Floating
SAV	<i>Ceratophyllum demersum</i>	60.63	Submerged/Floating
	<i>Potamogeton foliosus</i>	28.97	Submerged/Floating
	<i>Stuckenia pectinata</i>	19.98	Submerged/Floating
	<i>Lemna minor</i>	13.68	Submerged/Floating
Sparse Typha	<i>Eleocharis spp.</i>	33.57	Groundcover
	<i>Elodea canadensis</i>	26.48	Submerged/Floating
	<i>Potamogeton foliosus</i>	21.43	Submerged/Floating
	<i>Typha angustifolia</i>	15.65	Emergent
	<i>Phalaris arundinacea</i>	11.04	Emergent
Typha	<i>Typha angustifolia</i>	49.00	Emergent
	<i>Utricularia vulgaris</i>	46.74	Submerged/Floating
	Dead Typha	27.56	Emergent

Table 3.5 Importance values for dominant species within the vegetation zones of Maankiki South. Orange species are invasive while bold species are tolerant to nutrient enrichment, sedimentation, and increased turbidity.

MAANKIKI NORTH			
Vegetation Zone	Dominant Species	IVI	Structure
Phalaris	<i>Phalaris arundinacea</i>	87.64	Emergent
	<i>Lemna trisulca</i>	51.32	Submerged/Floating
	<i>Utricularia vulgaris</i>	21.91	Submerged/Floating
	<i>Lemna minor</i>	15.31	Submerged/Floating
SAV	<i>Ceratophyllum demersum</i>	77.63	Submerged/Floating
	<i>Potamogeton nodosus</i>	33.40	Submerged/Floating
	<i>Elodea canadensis</i>	30.40	Submerged/Floating
	Dead <i>Typha</i>	12.20	Emergent
Typha	<i>Lemna trisulca</i>	42.16	Submerged/Floating
	<i>Typha angustifolia</i>	40.01	Emergent
	<i>Riccia fluitans</i>	30.95	Submerged/Floating
	Dead <i>Typha</i>	29.79	Emergent

Table 3.6 Importance values for dominant species within the vegetation zones of Maankiki North. Orange species are invasive while bold species are tolerant to nutrient enrichment, sedimentation, and increased turbidity.

POOL 1A			
Vegetation Zone	Dominant Species	IVI	Structure
Mudflat	<i>Lemna minor</i>	36.61	Submerged/Floating
	Dead <i>Typha</i>	34.98	Emergent
	<i>Bidens cernua</i>	29.17	Emergent
	<i>Phragmites australis</i>	11.14	Emergent
Nymphaea	<i>Nymphaea odorata</i>	72.55	Emergent
	<i>Stuckenia pectinata</i>	47.73	Submerged/Floating
	<i>Potamogeton nodosus</i>	23.70	Submerged/Floating
	<i>Lemna minor</i>	10.48	Submerged/Floating
Salix	<i>Salix interior (OS)</i>	65.95	Overstory
	<i>Lemna minor</i>	28.98	Submerged/Floating
	<i>Phalaris arundinacea</i>	16.24	Emergent
SAV	<i>Stuckenia pectinata</i>	89.10	Submerged/Floating
	<i>Potamogeton nodosus</i>	41.15	Submerged/Floating
	<i>Lemna minor</i>	11.36	Submerged/Floating
	<i>Elodea canadensis</i>	10.52	Submerged/Floating
Typha	<i>Typha angustifolia</i>	63.37	Emergent
	Dead <i>Typha</i>	44.04	Emergent
	<i>Spirodela polyrhiza</i>	39.27	Submerged/Floating
	<i>Lemna minor</i>	15.05	Submerged/Floating

Table 3.7 Importance values for dominant species within the vegetation zones of Pool 1A. Orange species are invasive while bold species are tolerant to nutrient enrichment, sedimentation, and increased turbidity.

Invasive Species

Although total species amounts varied among units, a range of invasive species occurred within every wetland unit. A total of 10 invasive species were identified across all three units (Table 3.2). Three 'Dead' species were evaluated independently of their live counterparts when computing importance values and are included in Table 3.2. *Phalaris arundinacea* was the most widespread invasive, occurring in 10 vegetation zones across all three units, followed by *Typha angustifolia*, which occurred in nine vegetation zones across all three units. Six invasives were identified in only one of the wetland units: Dead *Lythrum salicaria*, Dead *Phragmites*, *Potamogeton crispus*, and *Typha x glauca* (MS), *Myriophyllum spicatum* (MN), and *Persicaria maculosa* (P1A). Similar to total species richness, the greatest number of invasive species were identified in Maankiki South (8), followed by Pool 1A (6), and lastly, Maankiki North (4).

Invasive species IVIs ranged greatly across vegetation zones both within and among units. Generally, vegetation zones dominated by their namesake invasive species had the highest IVI of any vegetation zones. *Phalaris arundinacea* had the highest coverage in each *Phalaris* zone found in Maankiki North (87.64) and Maankiki South (78.06). A combination of *Typha angustifolia* (MS: 49.00; MN: 40.01; P1A: 63.37) and Dead *Typha* (MS: 27.56; MN: 29.79; P1A: 44.04) had the highest IVI in each *Typha* zone across all three units. Common wetland invasive species of concern, such as *Lythrum salicaria*, *Myriophyllum spicatum*, *Phragmites australis*, and *Typha x glauca*, were observed in very few quadrats and most often at low coverages.

Invasive Species	Vegetation Zone	IVI	Unit
<i>Butomus umbellatus</i>	Typha	1.53	MS
	SAV	2.76	MN
Dead <i>Lythrum salicaria</i>	Phalaris	1.70	MS
Dead <i>Phragmites</i>	SAV; Sparse Typha; Typha	2.45; 1.28; 1.53	MS
Dead <i>Typha</i>	Phalaris; SAV; Sparse Typha; Typha	3.56; 5.37; 6.42; 27.56	MS
	SAV; Typha	12.20; 29.79	MN
	Mudflat; Salix; Typha	34.98; 5.57; 44.04	P1A
<i>Lysimachia nummularia</i>	Forest	14.12	MS
	Salix	1.69	P1A
<i>Lythrum salicaria</i>	Phalaris	1.74	MS
	Salix; Typha	3.12; 2.11	P1A
<i>Myriophyllum spicatum</i>	SAV	6.49	MN
<i>Persicaria maculosa</i>	Mudflat	1.66	P1A
<i>Phalaris arundinacea</i>	Forest; Mudflat; Phalaris; Sparse Typha; Typha	14.12; 2.52; 78.06; 11.04; 1.49	MS
	Phalaris; SAV	87.64; 2.70	MN
	Salix; SAV; Typha	16.24; 2.84; 2.03	P1A
<i>Phragmites australis</i>	Sparse Typha; Typha	4.19; 4.50	MS
	Mudflat	11.14	P1A
<i>Potamogeton crispus</i>	Sparse Typha	1.32	MS
<i>Typha angustifolia</i>	Sparse Typha; Typha	15.65; 49.00	MS
	Typha	40.01	MN
	Mudflat; Typha	2.53; 63.37	P1A
<i>Typha x glauca</i>	Sparse Typha; Typha	1.32; 1.61	MS

Table 3.2 List of identified invasive species, the vegetation zones in which they were present, the importance value indexes (IVI) for that species in each vegetation zone in which it occurs, and the wetland unit in which those vegetation zones occur.

Non-metric Multidimensional Scaling (NMDS) Ordination of a Bray-Curtis Dissimilarity Index

The NMDS ordination of the Bray-Curtis Dissimilarity shows similarities in specific vegetation zones among units with a range of differences found between most of the zones (Figure 3.3). These similarities are a result of a gradient of water depth (y-axis) and canopy cover (x-axis). Vegetation zones that are plotted closer together share greater similarities in their community compositions than zones that are plotted farther apart. For example, all three units have a *Typha* zone and the composition of these three zones are more similar to each other than to any other zone within their respective units. The *Phalaris* zone, shared between Maankiki North and Maankiki South also share similar species compositions. The Sparse *Typha* and SAV zones of Maankiki South are most similar to the Nymphaea zone of Pool 1A. Disparities between the SAV zones of Maankiki North and Pool 1A are largely due to depth. The Forest and Mudflat zones of Maankiki South and the Mudflat and *Salix* zones of Pool 1A are the most dissimilar zones and appear mostly at the extreme end of one or both axes.

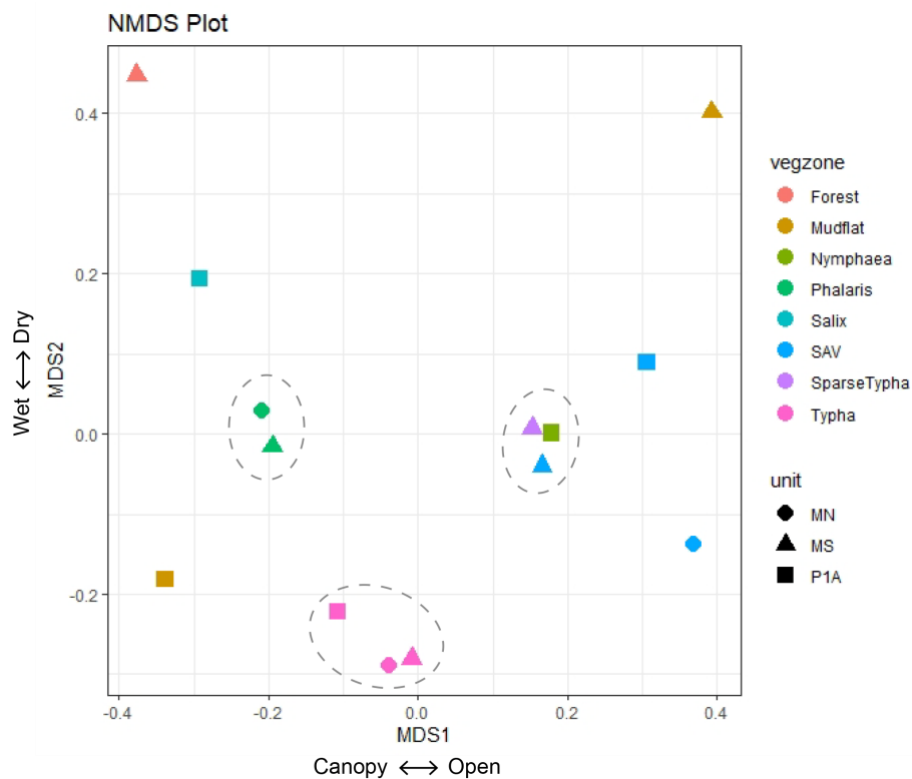


Figure 3.3 NMDS ordination of a Bray-Curtis Dissimilarity. The chart plots the differences between plant species' IVIs against one another. Vegetation zones with more similarity in their composition are plotted more closely (i.e., *Typha*, *Phalaris*, Sparse *Typha*, *Nymphaea*, and SAV zones, all circled). Axes are determined after the ordination has been created and represent a gradient of biotic or abiotic factors contributing to the dissimilarity between vegetation zones.

Note: Dashed ellipses are added for clarity and are not statistical.

Floristic Quality Assessment (FQA)

Input of wetland unit species into the FQA Calculator shows a clear pattern of degradation among sites. All units diverge notably from the ideal reference score of 10. Maankiki South and Pool 1A are identical in terms of their mean *C* score (3.5). When adjusted to FQI for the total number of species, Maankiki South has a slightly higher score (23.5) than Pool 1A (21.6). Maankiki North's scores represent the most degradation, with a mean *C* of 2.9 and an FQI of 11.2. Species totals are less than the observed unit totals in this output because the FQA calculator does not provide scores for 'Dead' species or nonvascular plants.

Unit	Forb	Grass	Sedge	Shrub	Vine	Tree	Native Species	Total Species*	Mean C with adventives**	FQI Value (Natives Only)
Maankiki South	31	3	1	0	2	8	39	45	3.5	23.5 (25.0)
Maankiki North	14	1	0	0	0	0	11	15	2.9	11.2 (12.9)
Pool 1A	28	3	3	1	1	2	34	38	3.5	21.6 (22.7)

Table 3.3 FQA Calculator output for each wetland unit. Mean *C*s show significant deviation from the reference score of 10 with Maankiki South and Pool 1A having identical values of 3.5. Maankiki North shows the most degradation with a Mean *C* of 2.9. When scores are adjusted to FQI, Maankiki South has the highest score at 23.5, followed closely by Pool 1A with 21.6, and lastly, Maankiki North with 11.2.

*The FQA does not factor in 'Dead' or nonvascular species and therefore, total species across each unit are less.

**The term 'adventives' refers to invasive species.

Index of Biotic Integrity (IBI)

IBI scores show a similar pattern of degradation among the wetland units as calculated by the FQA. Maankiki South had the highest IBI score (22, Descriptive Score 'Medium'), followed closely by Pool 1A (20, Descriptive Score 'Low/Medium'), and finally, Maankiki North (14, Descriptive Score 'Low'). Areas where water levels measured less than 1 cm were not observed in Pool 1A. Therefore, the Dry Emergent and Flooded Emergent Zone (marked in red in Table 3.4 below) were not calculated and instead, the Total Site score was adjusted to the 50-point scale of the other units.

Unit	Total Site			Dry Emergent Zone (water level <1 cm) *			Flooded Emergent Zone (water level >1 cm)			Submergent Coverage	Total IBI	Descriptive Score
	Invasive Cover	Invasive Frequency	C	Invasive Cover	Invasive Frequency	C	Invasive Cover	Invasive Frequency	C	Tolerant Submergents		
Maankiki South	1	0	3	3	1	5	1	0	3	5	22	Medium
Maankiki North	1	0	1	0	0	5	1	0	1	5	14	Low
Pool 1A	1	1	3							5	20	Low/ Medium †

Table 3.4 IBI scores among wetland study units. Total IBI scores are the sum of scores for invasive cover, invasive frequency, and calculated C throughout the total site, the dry emergent zone, and the flooded emergent zone. These scores are then combined with the sites overall tolerant submergent coverage to produce a ‘Total IBI’ and ‘Descriptive Score.’ Per the GLCWMP Vegetation SOP, Descriptive Scores range from Very Low (0-5), Low (6-20), Medium (21-40), and High (41-50)

*This zone was not observed in Pool 1A.

† Although Pool 1A’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 Maankiki North, but slightly more degraded than Maankiki South.

DISCUSSION

Wetland Community Types within SNWR Units

The wetland study units at the SNWR function as a hybrid of wetland types commonly observed within the Great Lakes basin. They act as lacustrine wetland systems when seiche activity from Lake Huron causes flooding (Albert et al. 2005; Burton 1985); they act as riverine wetland systems when the drainage of the Shiawassee River causes water level fluctuations (Albert et al. 2005); and they act as diked wetland systems when the closing of their water control structures disconnect them from their natural hydrology (Wilcox 2005). Lacustrine and riverine systems influenced by natural hydrologic patterns develop a commonly observed gradient of vegetation communities perpendicular to the shoreline (Keddy and Reznicek 1986; Wilcox and Meeker 1991; Keddy 2010). This gradient follows the elevation contours away from the water body and results in the subsequent major mono-dominant vegetation zones of submergent, emergent, and wet meadow vegetation (Uzarski et al. 2016). As a result of diking, this gradient is not observed in the SNWR wetland units. In lacustrine systems, submergent and emergent vegetation are generally variable, but become more diverse, or emerge - as is the case with wet meadow species - with decreased wave energy (Albert et al. 2005). Riverine systems typically have variable emergent and wet meadow communities, which usually contain wild rice, with submergents only occurring in low flow conditions (Albert et al. 2005). Characteristic

mono-dominant vegetation zones consist of *Schoenoplectus*, *Typha*, and *Nymphaea* in emergent zones (Cooper et al. 2018), *Calamagrostis*, *Carex*, and *Scirpus* in wet meadow zones (Keddy and Reznicek 1986), and a varying combination of species in submerged zones (Cooper et al. 2018).

Wetland plant communities are often negatively impacted by the effects of diking. Wetland systems that have been diked are often more degraded by invasive plant species relative to undiked systems (Herrick and Wolf 2005) due to increased proximity to agricultural and urban land use (Houlahan et al. 2006; King et al. 2007; Loughheed et al. 2001; Trebitz and Taylor 2007). These land uses alter conditions in water quality that allow invasives to thrive: increased sediment, increased turbidity, and increased nutrients (Loughheed et al. 2001). Paired with the disturbance caused by the construction within the dikes, these conditions are optimal for the proliferation of advantageous and tolerant species like invasives. While the diked wetland units at SNWR lack the classic natural gradient, an array of micro-environments within the wetland units (Heitmeyer et al. 2013) allows different vegetation succession in units where topographic heterogeneity exists or has been created by construction or the managed hydrology. This observation, in conjunction with the influence of the surrounding agricultural and urban land uses, explains the different diversity and levels of degradation among plant communities within the wetland units and between Maankiki South and Maankiki North, in particular, despite their similar management strategies (USFWS 2018).

Landscape and Hydrologic Variation, and Plant Diversity

Topographic and bathymetric variation within Maankiki South created six separate vegetation zones, the largest diversity occurring in the Forest and Sparse *Typha* zones, each with 24 species. Woody taxa such as *Acer saccharinum* and *Ulmus americana*, typical of floodplain forest communities (Barnes and Wagner 2004; Putt 2019), account for the greatest diversity within this vegetation zone. Importance values show the Sparse *Typha* zone is less dominated by dense *Typha angustifolia* (15.65 for the Sparse *Typha* zone compared to 49.00 for the *Typha* zone). This lack of dense canopy allows for the growth of more varied emergent species (Albert and Minc 2004) like *Alisma plantago-aquatica*, *Peltandra virginica*, and *Ludwigia palustris* and a plethora of submerged or floating aquatic species. It also allows for more invasive emergent species like *Phalaris arundinacea*, *Phragmites australis*, and *Typha x glauca*. The *Phalaris* and *Typha* zones are characterized mainly by their namesake invasive emergents and a series of tolerant submerged or floating species, which grow in response to the impaired water quality (Albert and Minc 2004). Predictably, many of these submerged and floating species also occur in the SAV vegetation zone.

The lack of shallow topographic variation within Maankiki North, coupled with the unit's alteration from dike construction and deviation from the historical flood regime, allowed for the establishment and proliferation of the invasives like *Phalaris arundinacea* and *Typha angustifolia* (Green and Galatowitsch 2001; Frieswyk & Zedler 2007). These invasives outcompete native plant species, creating monocultures and lowering the overall site diversity (Albert et al. 1988). Importance values show that for the *Phalaris* vegetation zone, *Phalaris arundinacea* was the only observed emergent species and dominated this zone (IVI of 87.64). Similarly, the *Typha* zone was dominated by emergent *Typha angustifolia* and Dead *Typha* (IVI of 40.01 and 29.79). The lack of diversity within the emergent portion of these zones contributed to the overall lack of diversity within this unit. The remainder of the unit consists of deep channels (Heitmeyer et al. 2013) colonized by submerged or floating aquatic species which make up most of its structural diversity. Most of these species are tolerant of poorer water quality measures such as increased nutrients, sedimentation, and turbidity (Albert and Minc 2004).

Plant diversity in Pool 1A is similar to Maankiki South despite having less topographic variation (Heitmeyer et al. 2013) and a smaller number of vegetation zones. This can be attributed to the management of Pool 1A, which only closes the water control structure for the winter (USFWS 2018) and subjects vegetation to the natural water level fluctuations of the Shiawassee River (Wilcox and Meeker 1991). Diversity in Pool 1A is greatest in the Mudflat vegetation zone. Strangely, this was not actually a shallow water mudflat, rather it was a floating mat of dead *Typha* stalks that provided structure and shallow water conditions for a host of typical mudflat species to grow upon. Similar to Maankiki South's Forest zone, much of the diversity in the *Salix* zone came from woody taxa and a series of emergent species which take advantage of the topographic variation created by the constructed water bird nesting mounds where the *Salix* zone occurs throughout the unit.

Indicators of Wetland Health

Mean *C*s calculated for the three wetland study units were comparable to, but generally lower, especially in Maankiki North, than those calculated by Comer (1991; 1993) and Cvetkovic and Chow-Fraser (2011) for other coastal wetlands within Saginaw Bay's open embayments and Wigwam and Wildfowl Bay along Lake Huron. Wilcox and Meeker (1991) note that unregulated water fluctuations create more diverse plant communities, which, in conjunction with its lack of topographic variation, may explain the lower *C* in Maankiki North. In addition, diked wetlands often contain more taxa and higher densities of invasive species (Herrick and Wolf 2005) and may act as nutrient traps (Herrick et al 2007), promoting the expansion of invasives and nutrient-tolerant species generally corresponding to lower *C*-values (Herman et al. 2001). The mean *C* calculated for Bradleyville Bay (GLCWMP 2018) is lower than both Maankiki South and Pool 1A. Other studies (Albert et al. 1988) found that 12 Saginaw Bay coastal wetlands' FQI scores were

lower than 20, making them more degraded than Maankiki South and Pool 1A, and are most comparable to Maankiki North. IBI scores determined by the GLCWMP show a strong decreasing disturbance gradient from south to north although several wetlands around Saginaw Bay range in ecological degradation, from 'low quality' to 'high quality' (Uzarski 2016). Having wetland units across this range is not concerning given these other assessments and the topographic variation found within the units.

Management Implications

Hydrologic reconnection is not without negative consequences. Depending on management practices, new connections between river and wetland units may lead to increased nutrient loading (Herrick et al 2007) and increased turbidity, whether through sedimentation (Albert and Minc 2004) or turbidity increasing agents like the Common Carp (Lougheed et al 1998). Connections may also increase the spread of invasive plant species which can have detrimental effects for other biotic communities, specifically fish (Shrank and Lishawa 2019). One invasive species of concern is the Eurasian Watermilfoil (*Myriophyllum spicatum*), only identified in Maankiki North. Eurasian Watermilfoil has a suite of ecological impacts: it forms dense mats which outcompete native aquatic vegetation, it produces anoxic conditions below these mats which may lead to physiological stress in invertebrate and fish communities, and it offers no food source to water birds in addition to inhibiting their ability to prey on fish (Getsinger et al 2002). While the number of observed patches was minimal, opening all the water control structures may lead to its rapid spread throughout the wetland units. Allowing low water levels in the units during the river's low flow seasons may also promote the growth of *Typha x glauca* (Lishawa 2010), a hybrid invasive rarely observed during our monitoring but becoming increasingly widespread throughout the Great Lakes (Trebitz and Taylor 2007).

In contrast to the risk of increasing pathways for the spread of invasive vegetation, managing the water control structures to mimic natural fluctuations can also alleviate the negative ecological effects of diking. Diking can lead to the development of monocultures, accumulation of organic material that would otherwise be flushed, and increased temperatures which could promote algae growth, decrease submergent vegetation growth, and therefore, lower dissolved oxygen content (Chow-Fraser 1998). For monocultures that have already developed in the three wetland units, specifically *Typha angustifolia*, studies by Lishawa et al. (2015) show that the harvesting of *Typha spp.* biomass promotes greater plant diversity in Great Lakes coastal wetlands. This management strategy, in conjunction with hydrology management strategies that mimic natural processes (Poff et al. 1997; Wilcox 1999), specifically flood duration and frequency (Casanova and Brock, 2000), can lead to greater aquatic vegetation diversity (Wilcox and Meeker 1991). Managing for greater vegetation diversity aids a wide range of water birds, migratory or otherwise, who utilize wetlands not only for food resources and nesting areas but also for areas that lack

human disturbance and provide cover from inclement weather (Gray et al. 2013). The NMDS shows the widest range of habitat types in Maankiki South with areas of habitat open, sheltered by canopy, inundated or merely saturated. Conserving the existing vegetation variation and promoting greater variation in other units can provide more resources to water birds as well as other species who utilize wetlands for some form of their life history like invertebrates, mammals, and herps (Bolen and Robinson 2003). Additionally, the reduction of *Typha x glauca* and *Typha angustifolia* and the subsequent increase of native species can lead to an increase in abundance and richness of fish species (Schrank and Lishawa 2019).

Limitations

While stratified random sampling is a standard method, it is meant to provide a general representation of plant species and is not intended to capture representatives of all plant species that may exist in each wetland unit. Therefore, species may exist in the wetland unit that were not captured by our sampling effort. Additionally, as we are not wetland plant experts, it is possible that we may have misidentified certain species. Inaccurate visual estimates of percent cover could occur if multiple people across teams are estimating percent cover. Kercher et al (2003) shows that percent cover visual estimates across teams is relatively comparable but suggests both observer calibrations and incorporating quality controls to ensure reliability. However, we sought to maintain consistency across quadrats by having the same person always determine percent cover.

Recommendations

We recommend the continued implementation of our sampling protocol for future monitoring. Overall, we believe we provided an accurate depiction of vegetation within the sampling units. Annual monitoring for 5-10 years should be utilized to capture the effects of Refuge hydrologic management strategies and interannual variation. In the future, it would be optimal to consistently sample the same wetland units - as well as new units, to judge how management strategies have affected the existing plant communities and how these communities change over time with special emphasis on the change in invasive species. Greater sampling effort (more than 10 quadrats) should be considered for large areas of vegetation or vegetation zones that occur in smaller pockets across a large area (e.g., the *Nymphaea* or *Salix* zone of Pool 1A). However, care should be taken in determining the balance between the total number of units to sample and the sampling effort required for those units as time and funds will certainly affect one or the other.

Specifically, for the hydrological management of the units to support the vegetation community, management should mimic the natural flow regime of the Shiawassee River (Poff et al. 1997). Studies from Wilcox and Meeker (1991), Wilcox (1999), and Casanova and Brock (2000) show that natural processes, specifically flood frequency and duration,

play the largest role in plant community diversity. Having the wetland units function as a floodplain also provides additional benefits as described in Chow-Fraser (2011).

Similarly, the harvesting of cattail (*Typha spp.*) biomass within the wetland units, which is already something SNWR manages elsewhere on the Refuge, could lower the coverage of invasive cattail while also promoting the growth of diverse native species (Lishawa et al. 2015). In addition, if invasive cattails persist, studies by Mitchell et al. (2011) suggest a greater decline in plant diversity. Harvesting could alleviate these time-dependent negative effects. Special monitoring should be implemented as cattails are removed, to capture whether there is an increase in native species and to thwart any early colonization by other invasives. Similarly, the monitoring and swift removal of less ubiquitous invasives like Eurasian Watermilfoil, will help to deter their spread during periods of connection to the Shiawassee River and to additional wetland units. A suite of mechanical, biological, and chemical removal options are thoroughly detailed in Getsinger et al. (2002) and can guide management strategies based on the Refuge's effort and cost constraints.

MACROINVERTEBRATES

INTRODUCTION

The study of aquatic invertebrates is an important piece in understanding the health of a wetland ecosystem due to the role invertebrates play in wetland food chains and as excellent indicators of ecosystem health. The presence or absence of invertebrates can be indicative of long-term patterns in water quality parameters such as dissolved oxygen concentrations, turbidity, and pH (Cooper 2007).

The study of macroinvertebrates is of importance to the Shiawassee National Wildlife Refuge for their role in food chains and as indicators. The Refuge was created for the protection of migratory birds. Many migratory bird species depend directly on invertebrates as a food source (McParland and Paszkowski 2006) and invertebrates feed the fish that piscivorous bird species depend on. Additionally, invertebrate communities can be used to understand the effect of restoring the Refuge wetland units and historical hydrology of the area. Through the summer of 2019, we sampled macroinvertebrate communities to measure the response of macroinvertebrates to restoration efforts within the Refuge.

RESEARCH QUESTIONS

- How does the abundance and diversity of invertebrate communities vary across months?
- How does vegetation structure influence the abundance and diversity of invertebrate communities?
- How does water quality influence the abundance and diversity of invertebrate communities?
- Does the invertebrate community vary based on treatment (open and closed)?
- Does the invertebrate community vary based on time since restoration?
- What does the invertebrate community imply about the success of restoration?

METHODS

We sampled macroinvertebrates once a month, May through August, with a second sampling effort in July. An effort was made to standardize the time passed between sampling efforts in order to capture the most representative collection of invertebrates.

Sample sites were stratified by vegetation type. These types included submerged aquatic vegetation (SAV), emergent vegetation, open water, *Typha*, *Phalaris*, *Salix*, and forest. At each site, three one-meter quadrats were placed along a transect one meter apart. Before sampling for invertebrates, site characteristics such as a visual assessment of the total percent cover of vegetation, substrate characteristics, and water depth were taken for each quadrat. A triplicate of water quality parameters was also recorded. A .5mm mesh dip net

was used to sweep nine times within each quadrat. The full water column was sampled including substrate, vegetation, and any present open water. All sweeps were combined into a gridded enamel tray. All large and highly visible individuals were collected regardless of location. Then grid numbers were randomly selected and were thoroughly picked before the next grid section was examined. This effort continued for a combined effort across participants of 30 minutes. Individuals were identified to family level, sorted, and counted.

DATA ANALYSES

All counts were standardized based on unit effort. In most cases, effort was considered the number of sites sampled in a specific unit, vegetation type, or unit of time.

We performed ANOVAs and linear regressions in R using packages ‘car’ and ‘dplyr’ to statistically test for differences among communities based on location, vegetation, treatment, and time using an alpha value of 0.05 ($p=0.05$). Dot plots with error bars were used to characterize abundance over time. We used an ordination of Bray-Curtis dissimilarity index to compare the similarity or difference in species composition between sites and units.

Aquatic invertebrate surveys can be used in the analysis of wetland health and resource availability. Index of Biotic Integrity (IBI) scores created by the Great Lakes Coastal Wetlands Monitoring Program can be used as an indication of disturbance and recovery in a system (Uzarski 2004). This analysis is based on vegetation types. Since some of our vegetation types didn’t have specific IBI criteria, we used the IBI criteria for the type closest in vegetation structure (Uzarski 2004).

RESULTS

In total, we sampled 69 sites and collected and identified 7,587 individual invertebrates. We found 43 families across the 3 units, although not all families were found in each unit. Maankiki South had 41 families, Maankiki North had 32 families, and Pool 1A had 36 families. Eighty-six percent of individuals identified were within 9 families: Caenidae, Hyalellidae, Chironomidae, Coenagrionidae, Corixidae, Pleidae, Physidae, Hydrachnidae, and Belostomatidae. Of those, the first four were most dominant (Table 4.1).

Overall Abundance

Family	Total CPUE
Caenidae	28.0
Hyaellidae	21.9
Chironomidae	17.0
Coenagrionidae	10.8
Corixidae	6.1
Pleidae	3.1
Physidae	2.8
Hydrachnidae	2.8
Belostomatidae	2.4

Family	Common Name	MS CPUE
Caenidae	Mayfly	54.3
Chironomidae	Chironomid	18.1
Hyaellidae	Scud	13.4
Coenagrionidae	Narrow Winged Damselfly	11.8
Corixidae	Water Boatmen	4.5

Family	Common Name	MN CPUE
Hyaellidae	Scud	37.4
Chironomidae	Chironomid	17.6
Caenidae	Mayfly	17.3
Coenagrionidae	Narrow Winged Damselfly	11.9
Belostomatidae	Giant Water Bug	2.4

Family	Common Name	P1A CPUE
Hyaellidae	Scud	20.6
Chironomidae	Chironomid	15.1
Corixidae	Water Boatmen	11.4
Coenagrionidae	Narrow Winged Damselfly	8.4
Pleidae	Pygmy Backswimmer	6.4

Table 4.1 Top 9 families for all sampling and top 5 dominant families and associated CPUE for each sampled unit. Highlighted families were found in all units.

	Monthly Variation	Vegetation Type	Water Quality
CPUE	0.562	<0.001	0.714
Number of Families	0.186	0.054	0.303

Table 4.2 P-values for tests of significant relationships between selected physical variables and invertebrate CPUE and the number of families at a site. Significant ($p < 0.05$) values are in bold.

Monthly Variation

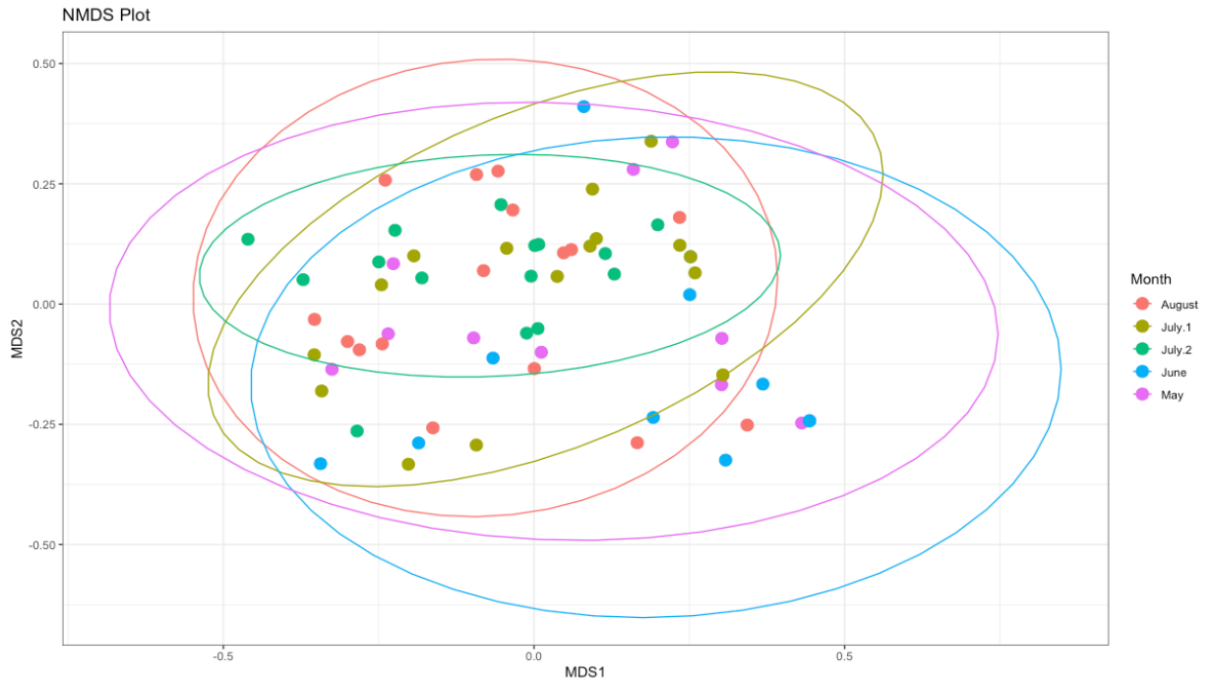


Figure 4.1 NMDS of Bray-Curtis dissimilarity index for invertebrate sampling between sites in our study units. Labels and ellipses show sites sampled in the same month. Ellipses show similarity of community composition by site gathered by month.

We found there to be little difference between sites over time; ellipses around monthly samples overlapped in ordination space (Figure 4.1). May and June appear to have the highest variability in community composition; May and June samples had the greatest spread in ordination space. Later months appear to be more similar in community composition; later months were more closely clustered in ordination space. The abundance of Caenidae decreased in Maankiki South in July. Chironomidae abundance decreased slightly in all three units sampled in July.

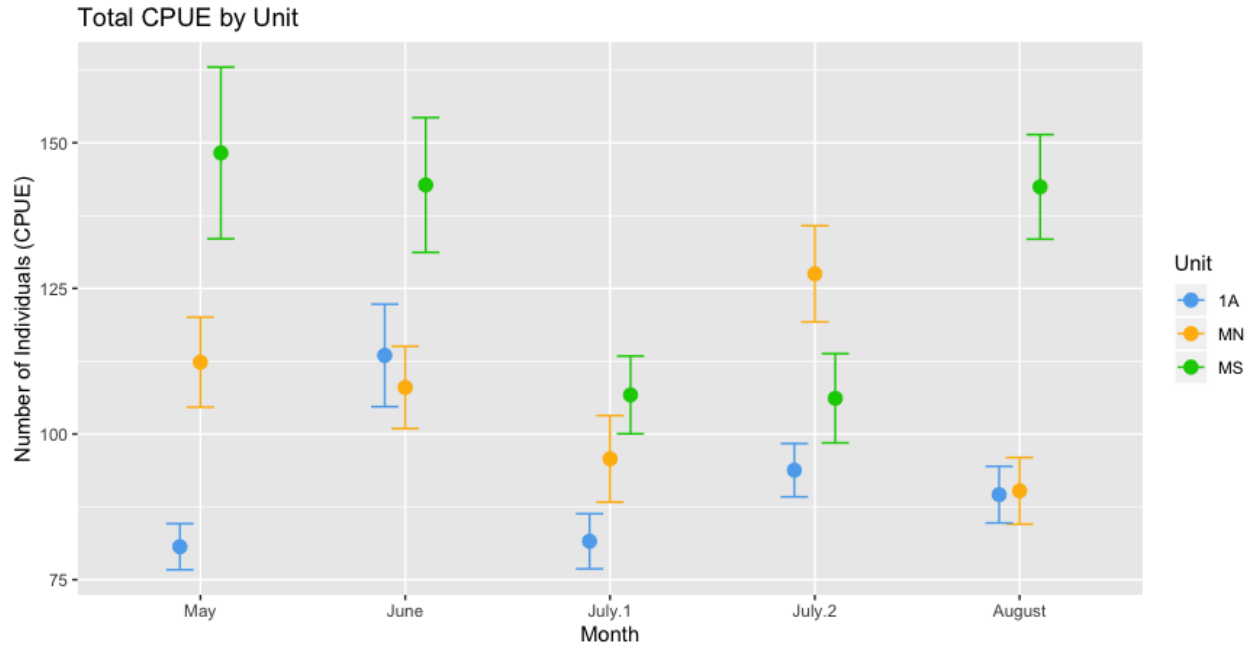
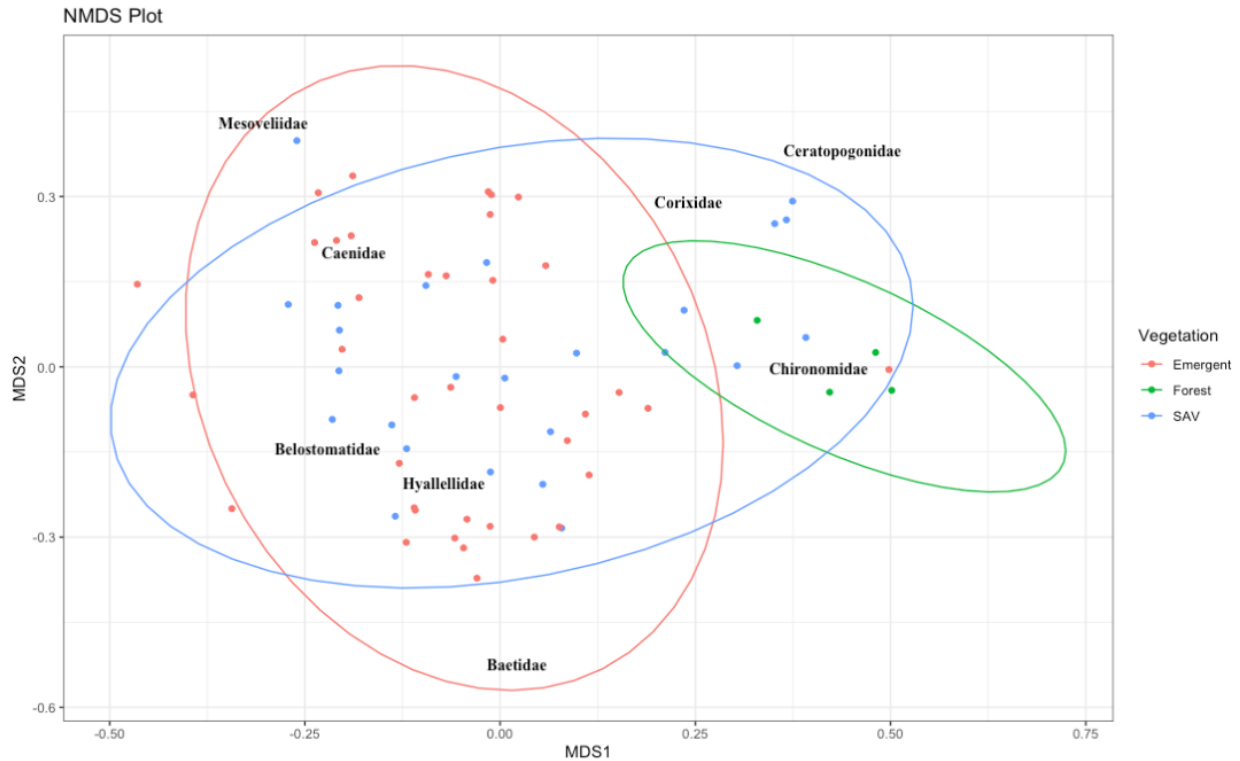


Figure 4.2 Dot and whisker plot showing change in CPUE over summer months by unit. Dots represent the mean CPUE of each unit during that sampling time. Whiskers represent the standard deviation.

There were no significant differences between months (ANOVA, $p = 0.562$). There were, however, trends that could be significant with more studies over time. In most months, Maankiki South has the highest CPUE. P1A had the lowest. CPUE for all units was lowest in both July sampling efforts (Figure 4.2).

Vegetation



Decreasing presence of SAV →

Figure 4.3 NMDS of Bray-Curtis dissimilarity index labeled by dominant vegetation structure. Ellipses indicate similarity by vegetation structure. Points indicate samples and proximity to family names indicate composition.

Vegetation type had a significant impact on the CPUE for a site ($p < 0.001$). The forest vegetation type had lower CPUE than mixed emergent ($p = 0.002$), open water ($p = 0.001$), *Phalaris* ($p = 0.037$), and *Typha* ($p = 0.026$). The vegetation types characterized by emergent structure (*Phalaris*, *Typha*, and mixed emergent) had the highest CPUE in all units (Table 4.3). Forested types only occurred in Maankiki South and consistently had the lowest CPUE of all vegetation types.

Vegetation type did not have a significant impact on the number of families found in each type. When sorted by vegetation structure (emergent, forest, and submerged aquatic vegetation), rather than vegetation type, forest structure occupied a significantly restricted ordination space in comparison to emergent and SAV structure classes was characterized by Chironomidae (Figure 4.3). Emergent and SAV shared similar community composition.

Vegetation Type	MS	MN	P1A
Mixed Emergent	178.6	n/a	100.2
<i>Typha</i>	141.8	112.4	92.6
SAV	106.85	106	86.4
<i>Phalaris</i>	137.8	93	n/a
Forest	28	n/a	n/a
<i>Salix</i>	n/a	n/a	92
Open Water	287	130	n/a

Table 4.3 Total CPUE for each vegetation type

Water Quality

Water quality parameters had no statistically significant relationship with the composition or abundance of invertebrate communities (Table 4.2).

Treatment and Age

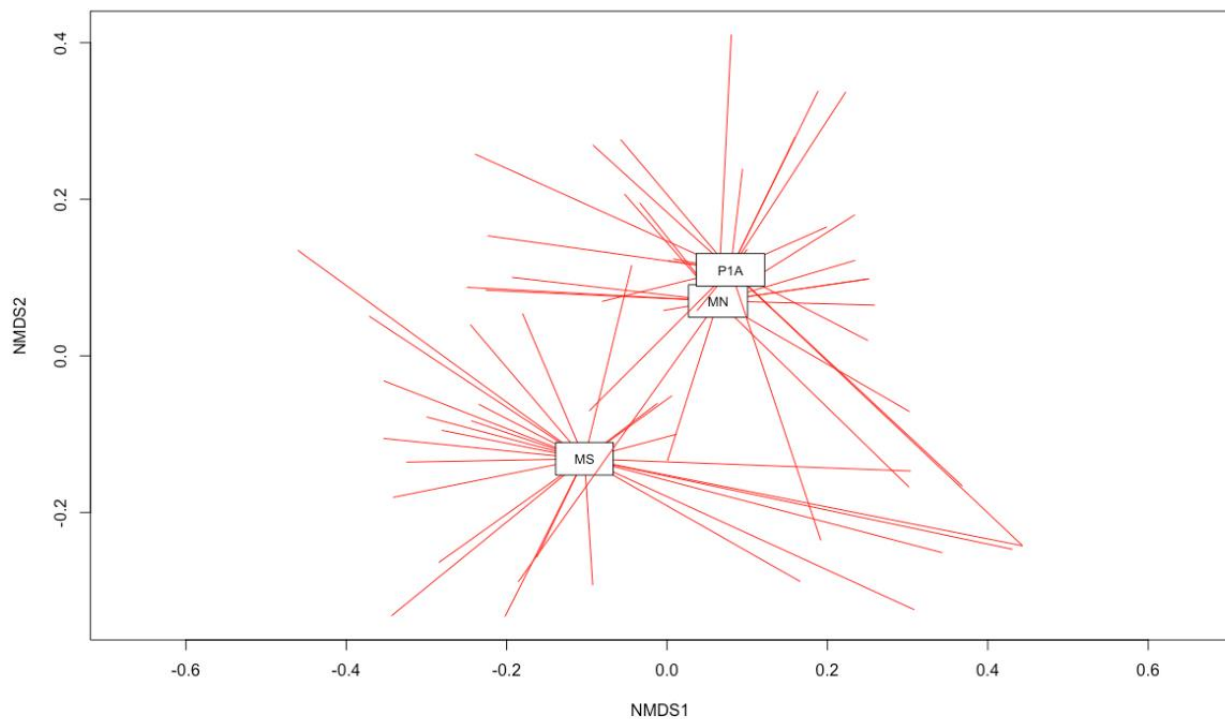


Figure 4.4 NMDS plot of Bray-Curtis dissimilarity Index labeled with the centroid of each unit. Tips of red lines indicate individual samples in each unit.

Maankiki North and Maankiki South, the two most recently restored units with the same history of connection to the river in 2019 were not similar to each other in community

composition (Figure 4.4). Maankiki North and Pool 1A had very similar community compositions despite different treatment conditions and age since restoration.

Restoration Success

Index of Biotic Integrity (IBI) scores for aquatic invertebrates are an indication of anthropogenic disturbance in a wetland. Maankiki North and Maankiki South were both rated as Moderately Degraded with scores of 31 and 37, respectively. Pool 1A received the highest score of 39 which receives a grade of Mildly Impacted.

DISCUSSION

Major Findings Relative to Literature

Over 86% of individuals we collected are in families commonly associated with riverine wetlands in the Great Lakes including Chironomidae, Gammaridae and Hyalellidae, and Planorbidae (Cooper et al. 2007; MacKenzie et al. 2004). Many of these families are considered to be tolerant to poor water quality conditions such as high levels of suspended sediment or fluctuating levels of dissolved oxygen as is expected of wetland invertebrates (Hilsenhoff 1995).

When all units are combined, it appears that monthly variation had little impact on the community composition. This is not true on an individual unit level. The abundance of individuals in MS decreased in both July sampling efforts, likely due to the emergence of families such as Caenidae who emerge from the water in transition from their larval to flying adult stage during this time of the summer (Voshell 2011). Maankiki South had a larger percentage of Caenidae caught throughout sampling, and changes in their abundance due to emergence could be what changed the abundance of invertebrates overall. Additionally, past studies on invertebrate communities in the Refuge have found that increased channel distance, defined as the distance traveled for dispersal between habitat patches, inhibits the dispersal and success of individuals, although dispersal through flight was less affected (Pollock 2016). Maankiki South has fewer deep-water channels surrounding the unit than MN, which is defined by its deep-water habitats. This may have encouraged the dispersal success and emergence of individuals from Maankiki South and decreased the overall abundance of individuals in that unit.

Despite dominance by invasive *Typha*, the emergent vegetation structure had the highest abundance of individuals in all units (Table 4.2). Many of the most commonly caught families such as Caenidae and Hyalellidae feed on decaying vegetation (Voshell 2011). Additionally, those families, as well as Coenagrionidae which was also largely abundant in our sampling, use vegetation as habitat (Voshell 2011). Studies show that invertebrates prefer habitat with high amounts of surface area for protection from predation and

attachment locations (Watkins et al 1983). Emergent vegetation may also prohibit predation on invertebrates by fish due to the difficulty in navigating dense *Typha* and *Phalaris* stands (Gardner et al. 2001). The vegetation structure and type did not have an impact on the number of families found in each type, likely due to the same nine families evenly dominating the majority of our catch in all of the units.

We had hypothesized that, due to their similar age since restoration and management throughout the summer months of 2019, MS and MN would have the most similar community structure. Unexpectedly, we found that MN and Pool 1A were very similar in community composition and MS was dissimilar from both (Figure 4.4). There are many abiotic and biotic factors we suspect influenced the abundance of invertebrates and community composition between units. We have collected data on the influence of location, time, and vegetation type, but there are other factors including individual unit characteristics such as age since restoration, unit size, and microtopography that make pointing to a single determining factor of abundance and community composition difficult.

Previous project groups at the Refuge studied invertebrate communities in the channel that now leads into Maankiki North, South, and the newly finished Center unit. This site was called Farm Unit 1. They found 22 families in the unit dominated by Coenagrionidae, Dogaelinotidae (contains Hyalellidae), and Caenidae (31%, 28%, and 13% respectively) (Buchanan et al. 2013). We identified 19 of those 22 families in our sampling which indicates that dispersal is largely dominated by individuals found within Shiawassee flat wetlands. Chironomids were dominant in our sampling but were so infrequently found and were so small in past sampling of the Farm Unit 1 that they were not counted and were simply marked as 'present.' Chironomids are highly tolerant so this is not necessarily an indication of increased wetland health since restoration but does indicate a change in overall community. These differences could also be due to sampling error as Chironomids are often an important and highly productive part of wetland ecosystems (Catherine Riseng, personal communication, 2020).

Implications for Management

Invertebrates as Indicators

Many aquatic invertebrate indices are created for streams and lakes with water quality and conditions that are generally different than that in wetlands (Hilsenhoff 2017). These indices are generally not representative of wetland health. Wetland Index of Biotic Integrity (IBI) scores are generally understood to be an accurate representation of wetland health based on macroinvertebrate communities, as these scores are created with the understanding that wetlands generally contain species more tolerant of low oxygen levels and high levels of suspended solids and pollutants (Uzarski et al. 2004). Wetland IBI scores,

especially when created regionally as done by the Great Lakes Coastal Wetland Monitoring Program, can be very accurate indicators of wetland health determined by invertebrate community composition. We suggest these scores as the most accurate way to determine the status of invertebrate communities through time and in response to management strategies.

In our study, Pool 1A had the highest IBI score of 39, which is designated as only mildly impacted by anthropogenic disturbance. Maankiki North and South, 31 and 37 respectively, were both rated as moderately degraded by anthropogenic disturbance. There are many potential factors that may account for the differences across units in IBI scores that may impact management decisions: age since opening, hydrologic management technique (opened/closed control structures), and micro-topography within the unit, and all variables previously discussed. We are unable to attribute the difference in scores to a single one of these factors, especially because the scores do not differ greatly, but groups could consider more specifically studying these questions in the future.

Invertebrates in the Food Chain

Invertebrates are often considered the link between primary producers and fish and waterfowl in wetlands (McParland and Paszkowski 2006). As the Refuge's main goal is the protection of migratory birds, it is important to ensure enough food in the form of invertebrates is available to waterfowl and to the fish waterfowl may feed on. The Refuge may consider making decisions to manage wetland units to encourage the growth of emergent vegetation structure which we found to have a higher abundance of invertebrates. Future studies may take an interest in analyzing food chain dynamics of the wetland units.

Sampling Recommendations

We recommend our sampling methods, as detailed above, for future characterization of aquatic invertebrates in the Shiawassee National Wildlife Refuge. Our techniques collected a large number of individuals that were representative of communities in the wetland units and allowed for analysis of questions surrounding abundance, diversity, and management implications.

Our method of study contains some limitations based on sampling and analysis that could be addressed in future years for more precise analysis. During sampling, future groups might use an elutriator to filter out invertebrates sampled in sediment and standardize the collection of smaller invertebrates. Smaller invertebrates, such as *Daphnia*, are common sources of food for fish (Wu and Culver 1994). Additionally, we would recommend identification of individuals to genera. This allows for analysis based on even more specific life history traits of individuals in the community. We chose to identify to family based on

our level of experience in macroinvertebrate identification, but it would be ideal if future groups had the resources and skills to identify further.

Studies done by Cooper and Uzarski (2007) found that the depth of organic substrate plays a large role in determining community composition as it influences food and habitat available. Future studies may consider more closely and accurately categorizing the substrate to determine if this is true within Refuge wetlands.

FISH

INTRODUCTION

Wetlands are used by many fish species for spawning habitat and seasonal refuge (Jude & Pappas, 1992). Assessment of the fish communities found within the Shiawassee National Wildlife Refuge will provide information on both the health of the overall aquatic community and the Refuge species of priority, Yellow Perch. Within the recently completed Maankiki wetland units, the fish community's use of the wetlands could be an indicator for habitat health and success of restoration. During the spring and summer of 2019, our team developed a monitoring protocol and implemented initial first-year monitoring for fishes within the wetland units Maankiki North, Maankiki South, and Pool 1A.

The overarching goal of this project is to develop a standardized sampling protocol to be implemented for several years in order to compile a long-term dataset that can be used to detect ecological trends. We determined that fyke netting, which is regularly used in wetland research, provides the most complete representation of the fish community. The sampling framework we designed is based on methods from the Great Lakes Coastal Wetland Monitoring Program (GLCWMP) out of Central Michigan University (Uzarski et al. 2016). Sampling occurred through the open water months of spring, summer, and fall. Our surveys provide initial data which, when built upon by future monitoring, will become a dataset on which managers can rely to make evidence-based monitoring and management decisions.

RESEARCH QUESTIONS

How do fish assemblages vary among the 3 units during the sampling months of 2019?

- What is the variation in composition, abundance, and size-structure of the community among and within the units through time?
 - What species were found in each unit?
 - How does the composition compare among units?
 - How do Indices of Biotic Integrity compare among units?
 - How does the composition of each unit compare between months?
 - What is the abundance of each species in each unit?
 - How does size class and trophic level width vary among units?
- What environmental variables determine community composition and species abundance among and within the units through time?
- What is the variation in size class, composition, abundance, and structure between different sampling methods?

METHODS

Fyke Nets

Fyke netting is a passive collection method that relies on fish behaviors and movement to catch fish throughout a diurnal cycle of behavior. Fish travel along the edges of dense vegetation when moving between feeding and refuge areas. A fyke net consists of four separate mesh components; a lead, two wings, and the fyke trap. The lead and wings are attached to the frame of the fyke trap net and held taut by stakes driven into the sediment. The fyke trap consists of a long cylindrical net that ends in an open cone and has several open cones within to segment the net. These cones are called cods and fish are removed from the end cod by lifting the frame opening out of the water and sifting the fish through the internal cods to the cod end. When fish swimming along the edge of vegetation hit the net lead, they swim to deeper water for refuge. The wings serve to funnel the fish that travel down the lead into the trap, which is designed so that fish can enter but not leave the net.

Upon arrival at a wetland unit, preliminary assessment of water level and vegetation types was used to dictate the areas suitable for net setting. Fyke nets were set along the edges of dominant vegetation types within the wetland unit to capture the fish communities that travel along and within those vegetation types. To select a site within the suitable area, a random number generator was used to determine a number of steps taken along the vegetation type to walk and set the net. Each site for fish data consisted of two consecutive 24-hour fyke net sets. Typically, nets should not be set in water significantly deeper than the net frame or fish will be able to swim over the trap opening. Nets also cannot be placed in water depths that fall below the internal cods of the net or fish will not be able to move into the trap portion of the net.

Four total nets were available to be used per unit, one large frame - small mesh, one large frame - large mesh, one small frame - small mesh, and one small frame - large mesh. Depth determined the frame size, but mesh was selected randomly to prevent size bias. A large frame net with a height of 1m was put in deeper water than a small frame net with a height of 30 cm. If an appropriate water depth for a fyke frame size was not present within a unit (too shallow or too deep), those frame sizes were not used in that sampling effort. Differently sized frames allow differently sized fish to enter the trap, some fish are too large to get into the trap portion of the 30cm frame and smaller cod openings in the net. Smaller mesh sizes obscure more of their environment and net contents and are easier for fish to see. Larger mesh nets are less noticeable but allow for smaller fish to escape (Portt 2006).

Study Area

Three wetland units within the Shiawassee National Wildlife Refuge were monitored during the open water season of 2019. During each sampling event, we attempted to set four fyke nets in an assortment of vegetation types and depths, to provide an accurate representation of the fish community of each unit.

Maankiki South is one of two units recently reconnected to the Shiawassee River, and has a large variety of habitats. We sampled most frequently on the western side of the unit, in wadable water among *Typha* and *Phalaris* vegetation types (Figure 5.1). Sampling was focused in these areas as they contained the dominant habitat types within the unit and were easily accessible. Sampling also occurred on the eastern side of the unit, where wading access was restricted due to a deep channel along the unit's edge. Fyke nets were not consistently set within the forest in the unit due to low dissolved oxygen and high mortality after one sampling event.



Figure 5.1 The area in red displays the area fyke nets were set in Maankiki South.

Maankiki North was open to the river in 2017 and is entirely bordered by deep, unwadable channels. This unit has three fish habitats; the channels, shallow *Phalaris*, and a large expanse of *Typha* that comprises the majority of the unit. High water levels in 2019 restricted sampling to large frame fyke nets. Nets were set in the southwest corner and on the northern edge of the unit along random accessible points of the *Typha* and *Phalaris* vegetation zones (Figure 5.2).

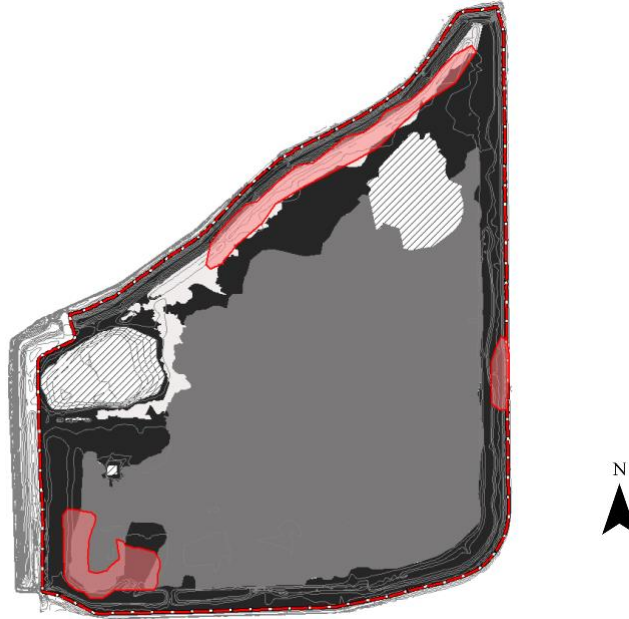


Figure 5.2 The area in red displays the area fyke nets were set in Maankiki North.

Pool 1A was constructed in 1958 and has been connected to the river during open water seasons ever since. Throughout the sampling season, nets were set along every side of the unit (Figure 5.3). Because of the connection to the river, water level fluctuations had a larger impact on the accessibility of sampling areas. In the spring, high water levels limited fyke net use to only large frame nets, and during one sampling event, prevented netting in Pool 1A altogether. As water levels went down, small frame nets were eventually usable within the unit. In Pool 1A, emergent vegetation types were more spatially dispersed, resulting in multiple distinct sites available. Sites consisted of patches of *Typha* and *Salix* surrounded by SAV, as well as areas of SAV with the lead staked at the diked edges of the unit.

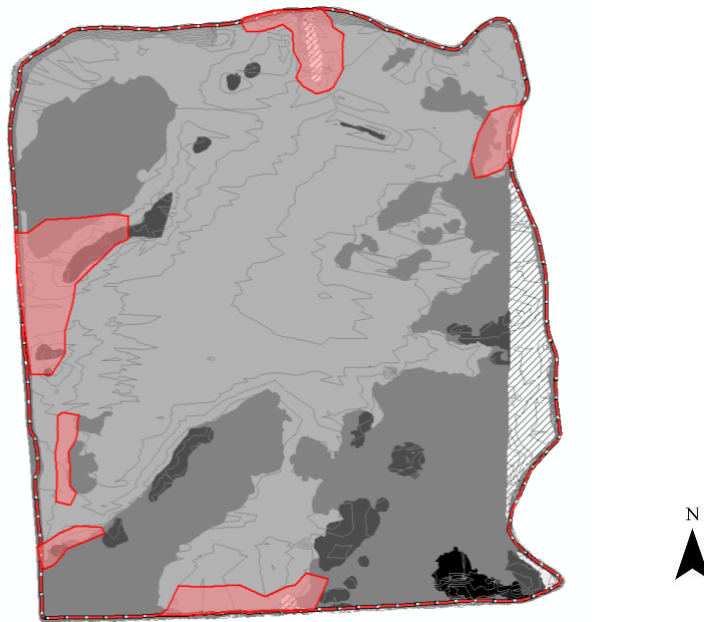


Figure 5.3 The area in red displays the area fyke nets were set in Pool 1A.

Sampling Schedule

Sampling in 2019 began the first full week of May and occurred every two weeks through August. Additional sampling took place in September in Pool 1A, and November in all three units. Sampling in November coincided with the temporary opening of the water control gates for Maankiki South and Maankiki North. Late season sampling was intended to align with fall fish migration from the Saginaw River or Bay and measure any fish movement into the wetland units for seasonal refuge.

Sampling Protocol

Nets were set with the lead perpendicular to the edge of the designated vegetation type or edge of the unit, and the wings at approximately 45-degree angles from the lead. Leads ran into the vegetation far enough to intercept fish movement along the edge of the vegetation type boundary. Once set, nets remained in place for 48-hours to capture the daily movement cycles of fish. Nets were pulled and fish were removed twice, every 24-hours, resulting in two days of data for each net set.

At the net set, a triplicate of water quality parameters including temperature, pH, conductivity, turbidity, and dissolved oxygen were collected using an EXO water quality sonde. Frame characteristics such as lead length, frame size, and mesh size were recorded. Additional information such as time, date, vegetation type, cloud cover and precipitation, wind strength, and air temperature were also recorded.

After 24-hours, the water quality parameters were recorded again, and the net was pulled. The captured fish were removed through the cod end into a bucket and brought to shore for processing. The fyke net was then reset in the same place to be checked after another 24-hours. Personnel working on the net pull and fish ID were noted on the datasheet. The length of the first 30 individuals of each species was measured and recorded. If more than 30 individuals of a species were present in a net, then a random sample of 30 individuals was measured and the remaining individuals counted and examined for DELTs (deformities, eroded fins, lesions, and tumors) before release. After captured fish were identified and total length was measured, they were released far enough away from the net site, so they were not immediately recaptured. We repeated these steps for the second sample and removed the fyke nets from the unit.

If a fish was unidentifiable and personnel were certain it is not a species of concern, a voucher specimen was retained, within the allotment of the MI-DNR Collectors Permit, to be brought to an expert. We euthanized voucher specimens with MS-222 and preserved the fish in 70% ethanol.

Nets left out for long periods of time could be subject to harsh weather, tampering by animals, or a net falling apart. If this resulted in a hole in the net below the surface of the water beyond the first cod or the cod end was open, the net was considered to be compromised. In the event of a hole, nets were repaired with a sewing kit at the site. Nets were also compromised if the cod at the end became opened. In these circumstances, fish may have entered the net but would have been able to leave, so the catch per unit effort is potentially biased. Data from these nets may be used for species presence/absence accounts, but not abundance calculations.

Identification:

It is important that the sampling team establish field identification standards for the sampling season. Use of a dichotomous key and field guides is suggested. The 2019 team preferred the use of *The ROM Field Guide to Freshwater Fishes of Ontario*, Book by Erling Holm, Mary Burrige, and Nicholas Edward Mandrak. As we are not fish identification experts, it is possible that identification may have been unreliable. This is a reason for standardizing identification rules within the team, so fish species are at least identified as the same species.

An example of standard identification rules used in 2019 are as follows:

“A fish was determined to be a Bluegill when the colored spot on the operculum was solid black. A fish was determined to be a Green Sunfish if the colored spot on the operculum has a white border and the fish has green lightning markings on its cheeks. A fish was determined to be a Pumpkinseed if the colored spot on the operculum had an orange spot.”

DATA ANALYSES

Species Accumulation Curve

A species accumulation curve was created for each unit to investigate whether the sampling effort had collected a representative sample of the species present in the fish community.

Catch Per Unit Effort

Catch per unit effort (CPUE) was calculated for each species in each unit. CPUE is a measure of relative abundance calculated as the ratio of the total number of fish of each species caught divided by the number of sampling efforts. Constraints on sampling effort during the sampling season altered the amount of sampling effort and attention each unit received. Calculating the CPUE for each species among units and time controlled for variation in sampling.

$$\text{Catch per unit effort CPUE} = \text{fish counts} / \text{fyke net sets}$$

Variance of CPUE

We performed ANOVAs to determine whether abundance of each species varied across units and across months. We used Tukey HSD post-hoc tests to determine how abundance was different across wetland units and months, in addition to the significance of the variation. An ANOVA determined whether the various combinations of frame and mesh sizes caught significantly different fish abundances. A Tukey HSD post-hoc test detailed which net types were different from the others. We also performed an ANOVA of CPUE and environmental variables within units and across time to determine whether water quality data held a significant relationship with the abundance of fish caught within each unit.

Variance of Length

A multiple linear regression of average length was performed to determine the pattern, if any, of length increase for each species through the sampling season and between units, indicating growth. ANOVAs were used to determine whether sizes were different among units, and among months. A Tukey HSD post-hoc test determined how length varied by wetland units and months. To visualize the distribution of lengths, a violin plot was created for each comparison. The violin plots show the range of lengths along the y-axis and for each category on the x-axis, the “violin” gets wider with the frequency of each length.

Variance of Fyke Nets

We performed ANOVAs to test whether the various combinations of frame and mesh sizes caught significantly different sizes of fish, which is an expected trend with fyke nets. A Tukey HSD post-hoc test detailed which net types caught different size classes from the others.

Shannon-Wiener Diversity Index for Fish

We calculated the Shannon-Wiener Diversity Index (SWDI), which is a measure of diversity that combines species richness and relative abundances, for each surveyed wetland unit. As a measure of the relative richness and evenness, the SWDI is a simple calculation to determine the demographic shape of the community. A low index indicates a community dominated by just one or a few species; a zero means there is only one species present. A higher index represents a community with a more balanced species composition. Most communities fall between 1.5-3.5 on the index.

Index of Biotic Integrity for Fish

Individual IBI Scores were calculated for each wetland unit, modeling the CWMP-QAPP. With these calculations, the fish communities of the SNWR wetland units can be compared to that of other Great Lakes coastal wetlands. IBIs are stratified by vegetation type and calculated based on weighted scores for different categories of fish, which are specific to each vegetation type. Examples of these categories include nonnative, benthic, nest spawners, high or extra high temperature spawners, large or extra-large lengths, tolerance levels, Cyprinidae, and diet (Cooper 2018). Since not all of our vegetation types are represented in the CWMP, we fit our vegetation types into SAV or *Typha* based on the structure of the habitat they create (e.g., mixed emergent was counted under *Typha*).

Bray-Curtis Non-Metric Multidimensional Scaling Model for Fish

To compare the fish communities sampled with a fyke net, we calculated a Bray-Curtis dissimilarity index matrix of the differences in community composition among sampling efforts. A Bray-Curtis index ranges from 0 to 1, where 0 means the two sites have the same composition, and 1 means the two sites do not share any species. These indices can then be plotted in a Non-Metric Multidimensional Scaling Model (NMDS) that can graphically display the scores in your matrix in 2D space. Points are organized across unconstrained axes of variation which can be identified to be associated with biotic and/or abiotic factors after statistical analysis of variables. Finally, labeling the plot by unit or species, depending on individual research interests, can help display the variation among unit compositions.

SAMPLING WITH ADDITIONAL METHODS

Due to the high-water levels in 2019, as well as the design of the constructed units, some areas of the wetland units were not accessible for fyke net sampling. To investigate whether we had an accurate representation of the fish community in the wetland, we decided to incorporate two other types of wetland sampling: gill netting and electrofishing.

Gill Netting

In order to construct the wetland units in the Shiawassee National Wildlife Refuge, soil from within the unit was excavated to create the surrounding dike. This left borrow pits and channels along the edges of the Maankiki units that are too deep for fyke nets. These deep-water refuges potentially harbor fish that don't travel into the shallow water where we used fyke nets. In deeper water fish surveys, gill nets are commonly used. A gill net is a long nylon net suspended in the water column that fish do not see, swim into and become entangled within. The mesh of the gill net is designed so that fish swimming into a net can get their heads through but not their bodies. As a fish tries to back out of the net, its gills get caught on the mesh and the fish becomes trapped in the net.

We set gill nets for two hours in the deep-water channels where there was space for the full 150 feet of net to be set. We selected transects through sparsely vegetated open water around two meters deep. We sampled once in the morning, setting nets in both MS and MN and twice in the evening, setting nets in MS one night and MN one night, for a total of eight net sets. These times were chosen in order to align with the diurnal patterns of fish movement (Portt et. al. 2006). Nets were set with weights on the bottom corners and buoys on the top corners so that the net remained taut in the middle of the water column while set. The net was gradually released into the water from the boat, while the boat was paddled forward. We used an experimental mesh which was composed of small, medium, and large sized mesh in order to catch a wider distribution of fish species and sizes.

At the net set and just before the net was pulled, we collected water quality parameters including temperature, pH, conductivity, turbidity, and dissolved oxygen using an EXO water quality sonde. We also recorded the time, date, vegetation type, weather, wind strength, and air temp. After two hours, the nets were pulled back into the boat a short length at a time so the fish could be removed, identified, measured, and released back into the water. The length of the first 30 individuals of each species was measured and recorded. We examined all fish for DELTs (deformities, eroded fins, lesions, and tumors) before release and noted if individuals were dead.

Electrofishing

In the deeper water we also used an electroshocking catamaran for an electrofishing survey. This catamaran is a simple platform on detachable floats that has poles that project from the front of the vessel. The cathodes hang from the poles and the anode hangs off the platform. The electrical component has settings that the operator can adjust to the environmental conditions including water conductivity and depth. We received assistance from Justin Chiotti from the USFWS Fisheries Division because this method requires a certified electrofishing operator.

We determined the areas suitable for electrofishing based on the regions that were unwadable and also contained no emergent vegetation. An electrofishing transect consisted of ten minutes of effort. At the start of the transect, water quality parameters including temperature, pH, conductivity, turbidity, and dissolved oxygen were collected using an EXO water quality sonde. During each sample, the operator rowed the catamaran and controlled the electrical settings, while one of our team members stood at the front, dip-netted fish as they were shocked, and deposited them in a cooler. After the transect was complete, the transect length in seconds was recorded along with electrical settings including voltage, amps, DC pulse per second, and percent power. Additional information such as time, date, depth, vegetation type, weather, wind strength, and air temperature were also recorded.

Fish were then brought to shore, identified, measured, and released. If more than 30 individuals of a species are caught, then a random sample of 30 individuals were measured and the remaining individuals counted and examined for DELTs (deformities, eroded fins, lesions, and tumors) before release.

Electrofishing transects were sampled four times in MS, four times in MN, once in Pool 1A, twice in the Shiawassee River, and once in Spaulding Drain. The Shiawassee River and Spaulding Drain samples were taken adjacent to Pool 1A.

Variance of Methods

We used ANOVA to test whether the various sampling methods caught significantly different sizes of fish. A Tukey HSD post-hoc test detailed which sampling methods caught different size classes from the others. To visualize the distribution of lengths, a violin plot (described above) was created for each comparison. Additionally, the species richness of each method was compared to inform future sampling.

RESULTS

Throughout the sampling season, a total of 113 uncompromised fyke nets were set. Spatial and temporal variation in water depth led to an unequal distribution of samples per unit. We set 51 nets in Maankiki South, 27 in Maankiki North, and 35 in Pool 1A. Although gill netting and electrofishing were also used, the following results come from the fyke net sampling only, unless otherwise noted (starting at Figure 5.15). Across all units a total of 26 species were identified. There were 22 species caught in Maankiki South and in both Maankiki North and Pool 1A there were 19 species caught (Table 5.1). MS had three species unique to that unit, Pool 1A had one unique species, and MN had no unique species. There were a total of 8,855 individual fish caught and identified to the species level. There are two non-native species found in fyke nets, Common Carp and Goldfish (Table 5.2). All units

reach the asymptote of their calculated species accumulation curves with the number of samples taken in the 2019 sampling season (Figure 5.4). This tells us that the sampling schedule we followed captured a complete picture of what species were present in the wetland units, and that additional fyke net sampling would be unlikely to capture new species.

	MS	MN	1A
# of Net Sets	51	27	35
# of Species	22	19	19
# of Fish	4938	2780	1137
Species Unique to Unit	Bluntnose Minnow White Crappie Central Mudminnow	None	Channel Catfish

Table 5.1 Summary of fyke net results that displays the number of net sets in each unit, the number of species caught in each unit, the total number of fish identified in each unit, and which species were unique to each unit.

Common Name	Scientific Name	Game Fish	Riverine Fish	Wetland Fish	Rare	Non-Native	Total CPUE	MS CPUE	MN CPUE	P1A CPUE
Banded Killifish	<i>Fundulus diaphanus</i>	N	N	Y			0.42	0.67	0	0.4
Black Bullhead	<i>Ameiurus melas</i>	Y	N	Y			51.43	56.78	91.37	12.86
Black Crappie	<i>Pomoxis nigromaculatus</i>	Y	Y	N			0.93	0.92	1.48	0.54
Bluegill	<i>Lepomis macrochirus</i>	Y	N	Y			5.45	7.88	4.59	2.57
Bluntnose Minnow	<i>Pimephales notatus</i>	N	N	Y			0.053	0.12	0	0
Bowfin	<i>Amia calva</i>	N	N	Y			1	0.61	0.70	1.8
Brown Bullhead	<i>Ameiurus nebulosus</i>	Y	N	Y			0.04	0.04	0.11	0
Central Mudminnow	<i>Umbra limi</i>	N	N	Y			0.008	0.02	0	0
Channel Catfish	<i>Ictalurus punctatus</i>	Y	Y	N			0.07	0	0	0.23
Common Carp	<i>Cyprinio carpio</i>	Y	Y	N		Y	0.19	0.1	0.29	0.26
Emerald Shiner	<i>Notropis atherinoides</i>	N	Y	N			0.08	0.18	0	0.03
Fathead Minnow	<i>Pimephales promelas</i>	N	N	Y			0.23	0.41	0	0.14
Gizzard Shad	<i>Dorosoma cepedianum</i>	N	N	Y			0.37	0	0.29	0.97
Golden Shiner	<i>Notemigonus crysoleucas</i>	N	N	Y			0.24	0.37	0.25	0.06
Goldfish	<i>Carassius auratus</i>	N	N	Y		Y	0.11	0.1	0.11	0.17
Green Sunfish	<i>Lepomis cyanellus</i>	Y	N	Y			3.24	6.35	0.70	0.86
Johnny Darter	<i>Etheostoma nigrum</i>	N	Y	N			0.01	0.02	0.03	0
Largemouth Bass	<i>Micropterus salmoides</i>	Y	N	Y			0.32	0.25	0.40	0.37
Northern Pike	<i>Esox lucius</i>	Y	Y	N			0.27	0.16	0.40	0.34
Pumpkinseed	<i>Lepomis gibbosus</i>	Y	N	Y			12.08	21.51	1.51	6.54
Spottail Shiner	<i>Notropis hudsonius</i>	N	Y	N			0.03	0.06	0.03	0
Tadpole Madtom	<i>Noturus gyrinus</i>	N	N	Y			0.02	0.02	0.074	0
Warmouth	<i>Lepomis gulosus</i>	Y	Y	N			0.50	0.06	0.14	1.43
White Crappie	<i>Pomoxis annularis</i>	Y	N	Y			0.02	0.06	0	0
Yellow Bullhead	<i>Ameiurus natalis</i>	Y	N	Y			0.03	0	0.03	0.09
Yellow Perch	<i>Perca flavescens</i>	Y	Y	N			0.25	0	0.33	0.57

Table 5.2 Species caught by fyke nets during the sampling months of 2019. Species are categorized as game (Y) or non-game (N) species, as wetland (Y) or non-wetland species (N), as riverine (Y) or non-riverine (N), as well as species that are rare or non-native to the Great Lakes Region.

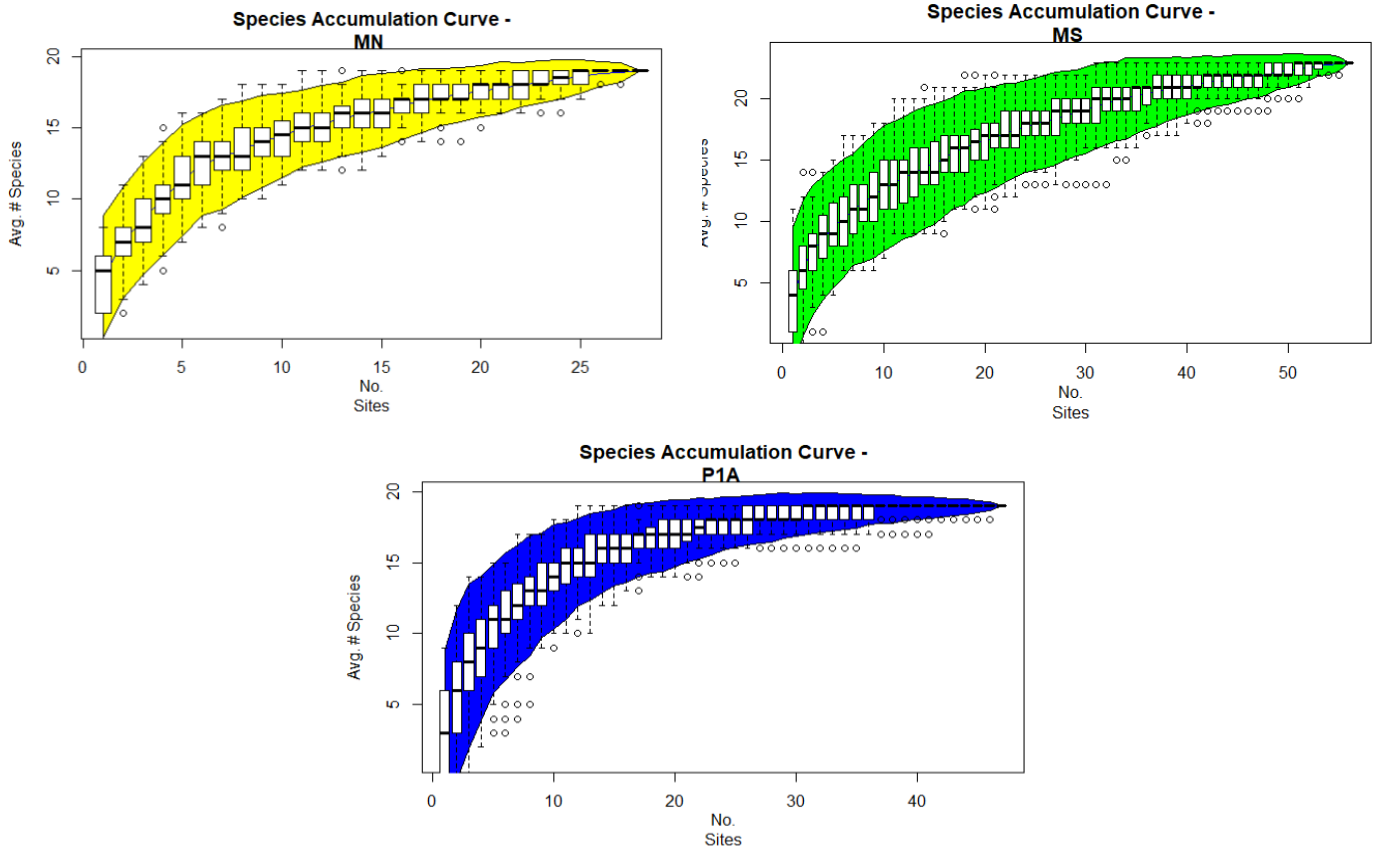


Figure 5.4 Species accumulation curves for each unit. The species accumulation curve displays the number of samples needed to collect an accurate representation of the fish community in each unit.

Abundance by Species

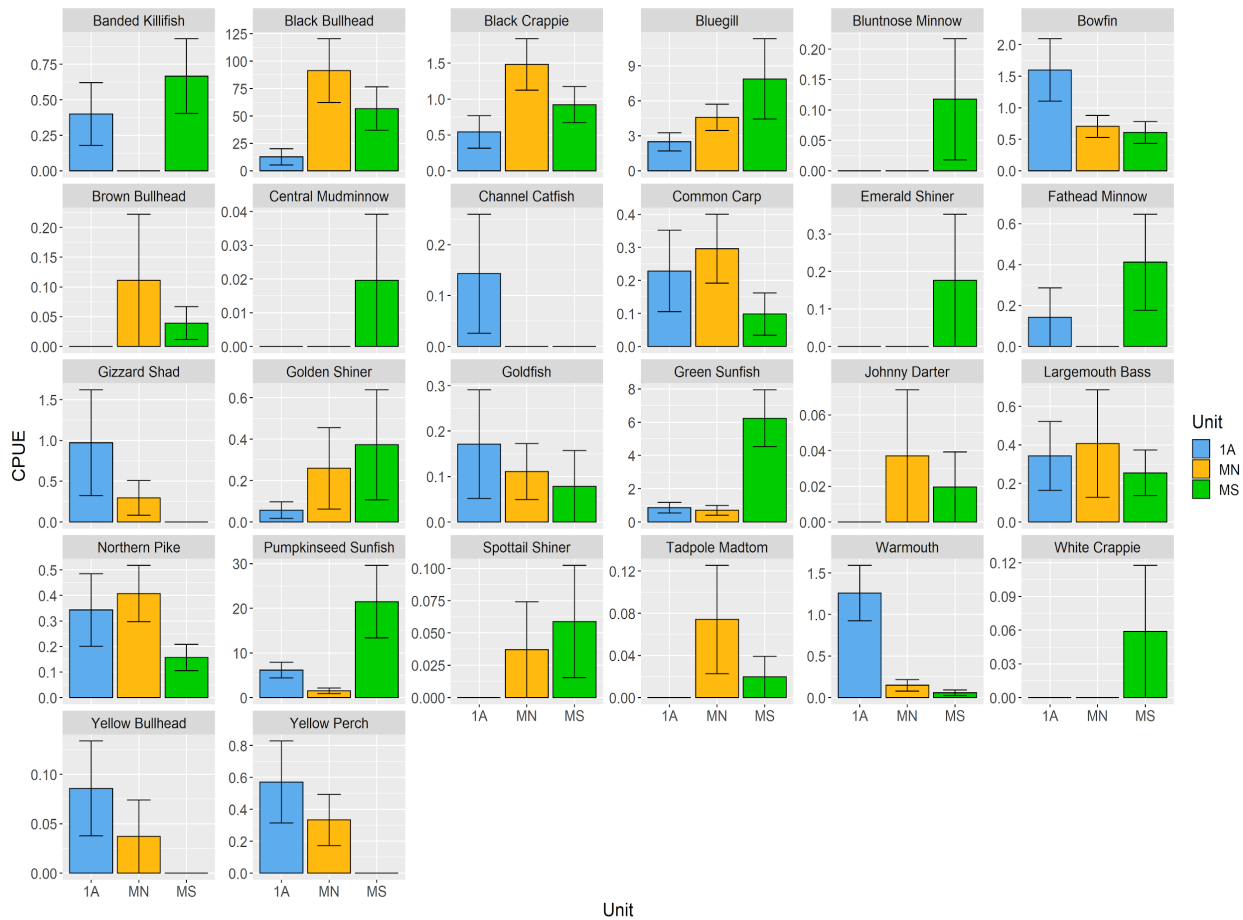


Figure 5.5 Catch per unit effort for each species across the wetland units. Each species has its own scale for the x-axis, CPUE.

All species differed in abundance across units. (Table 5.2, Figure 5.5). Black Bullhead across all of the units had the highest CPUE. The Fish Species of Special Interest for the SNWR, the Yellow Perch, was found in Pool 1A and Maankiki North, but not in Maankiki South. Emerald Shiner, Bluntnose Minnow, White Crappie, and Central Mudminnow were found only in MS. Channel Catfish were only found in Pool 1A. MN had no unique species found.

	Month	Unit	Vegetation	Avg. Temp	Avg. pH	Avg. Cond	Avg. Turb	Avg. DO
CPUE	0.209	0.121	0.032	0.987	0.788	0.780	0.988	0.363
Species composition	0.775	0.244	0.574	0.215	0.967	0.741	0.897	0.727
Number of species	0.748	0.690	0.066	0.119	0.944	0.330	0.549	0.718

Table 5.3: P values for ANOVAs of CPUE, Species Composition, and Number of Species with environmental variables. Significant results ($p < 0.05$) are shown in bold.

Influence of Water Quality

ANOVAs showed water quality parameters to have no statistically significant relationship with the abundance or composition of fish communities (Table 5.3).

Influence of Vegetation

ANOVAs showed that vegetation types had no statistically significant relationship with the composition of fish communities, or the number of species caught in a sample ($p < 0.05$). ANOVAs did show that sampling vegetation types had a significant effect on the CPUE ($p = 0.032$). Mixed emergent vegetation types had higher CPUE than *Salix* ($p = 0.025$, Tukey HSD) and *Typha* ($p = 0.033$, Tukey HSD) vegetation types.

Influence of Unit

ANOVAs showed that wetland units had no statistically significant relationship with the abundance or composition of fish communities (Table 5.3).

Influence of Month

ANOVAs showed that month had no statistically significant relationship with the abundance or number of species caught. Species composition was not considered over time as MS and MN were closed for the majority of the sampling season, therefore new species would not have the opportunity to enter the units (Table 5.3).

Length

Figure 5.6 displays the distribution of fish lengths by unit, calculated using the average length of each species per site. On average, fish were significantly longer in MN than in MS ($p = 0.003$), but there is no significant difference in the length of fish between MS and P1A or MN and P1A.

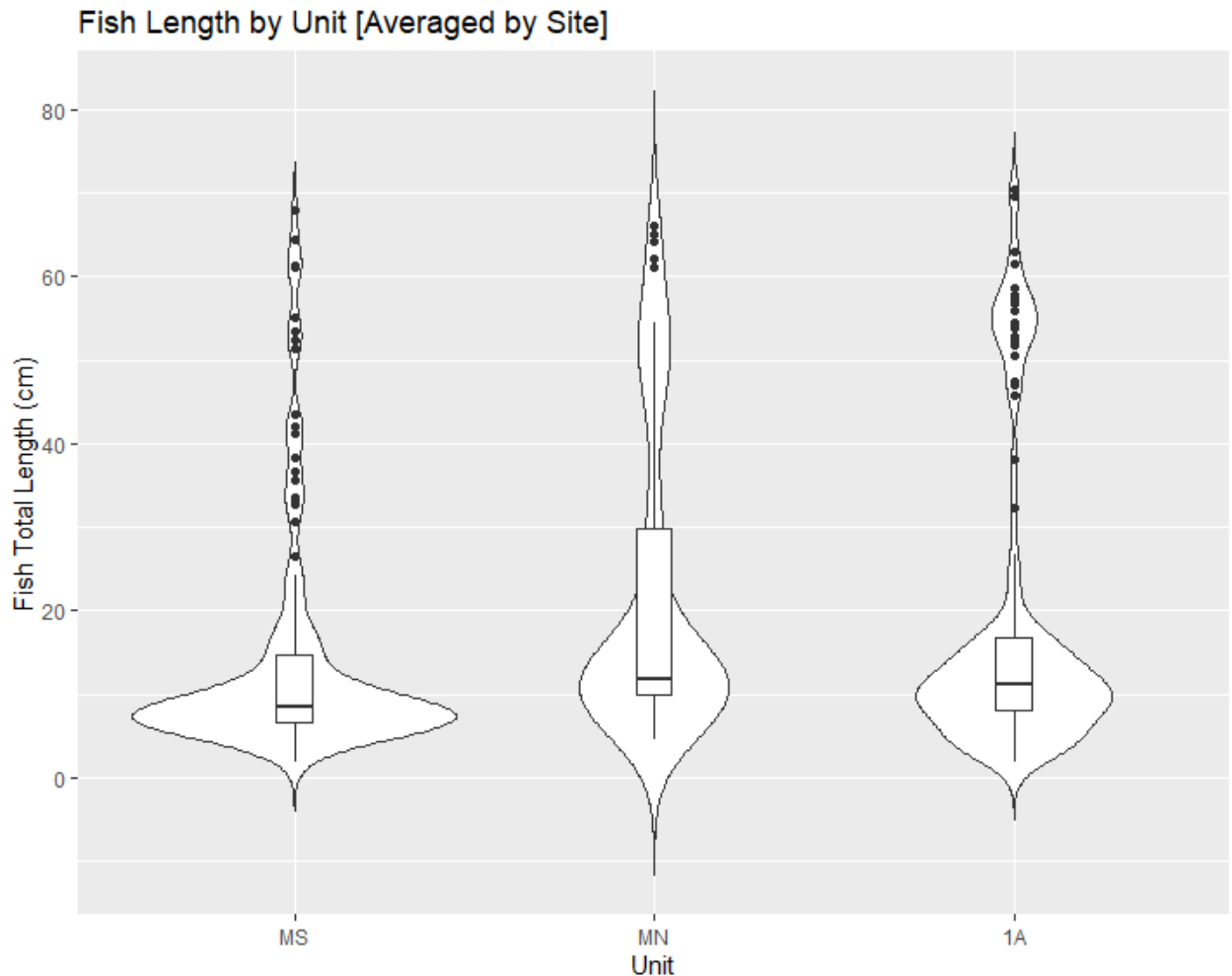


Figure 5.6 A violin plot showing the distribution of fish lengths by each unit. The width of the plot at each length shows the frequency of that size. Data were plotted using an average length of each species by site.

ANOVAs of the length of fish through time for each unit

ANOVAs were used to compare average fish length in sites across months. The length of fish in Maankiki South got steadily larger throughout the sampling period (Figure 5.7). Fish lengths in August and November were significantly longer than earlier months in MS (Figure 5.7). Maankiki North fluctuated without a clear pattern. November had significantly longer fish lengths compared to all sampled months except May. May had significantly longer fish length compared to July and August (Figure 5.8). Pool 1A showed a general decrease in size throughout the months except November. May had significantly longer fish lengths compared to all other months. November is significantly different from all months except June. September lengths are smaller than all months except June (Figure 5.9). When all units are considered together, length was significantly different across months (Table 5.4) though there is no clear pattern through time (Figure 5.10).

Unit	Month
All Units	0.002
MS	0.058
MN	0.157
P1A	0.027

Table 5.4 Results of general linear model balanced factorial design ANOVAs comparing fish length by month for all units, and each individual unit. Significant results ($p < 0.05$) are shown in bold.

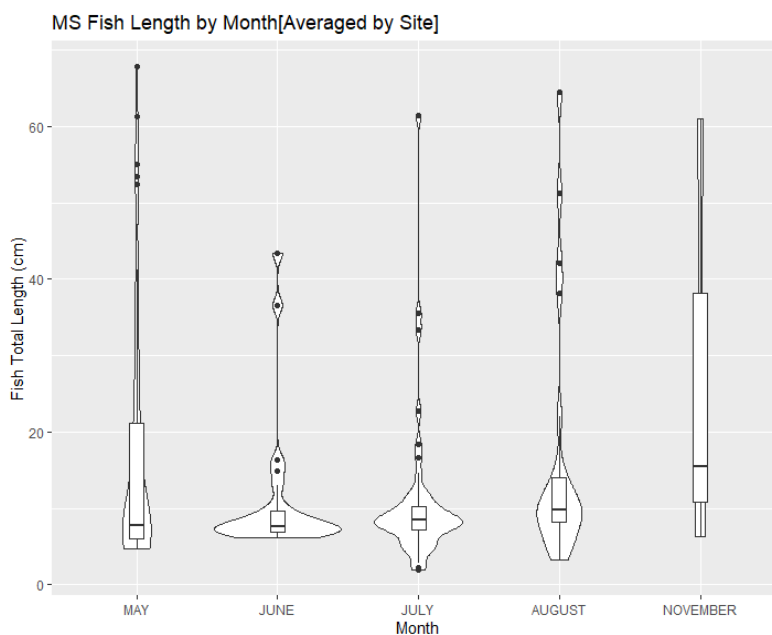


Figure 5.7 Violin plot of fish lengths in Maankiki South by sampling month. Plots are wider where lengths are more frequent. Box and whiskers within the “violins” show the average length and quartiles for each month.

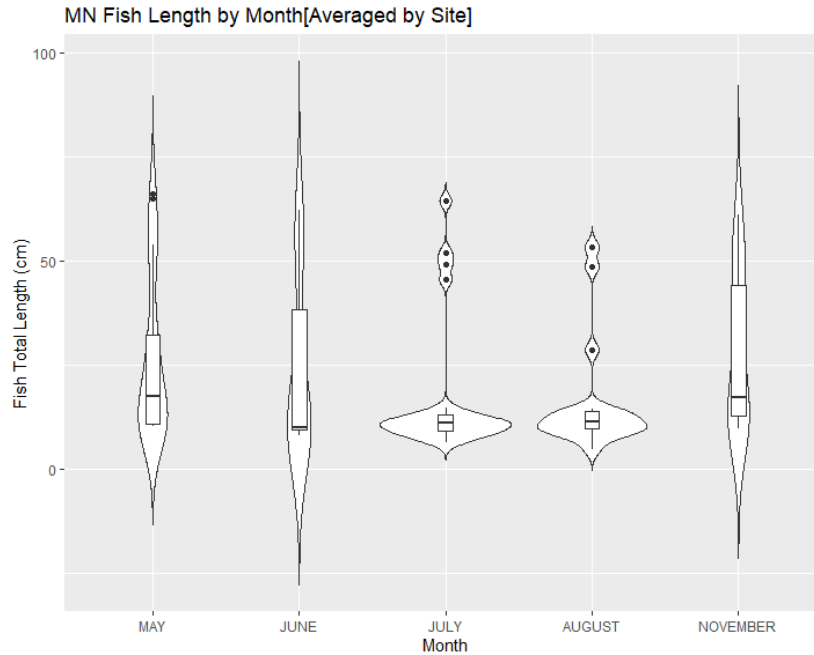


Figure 5.8 Violin plots of fish lengths in Maankiki North by sampling month. Plots are wider where lengths are more frequent. Box and whiskers within the “violins” show the average length and the quartiles for each month.

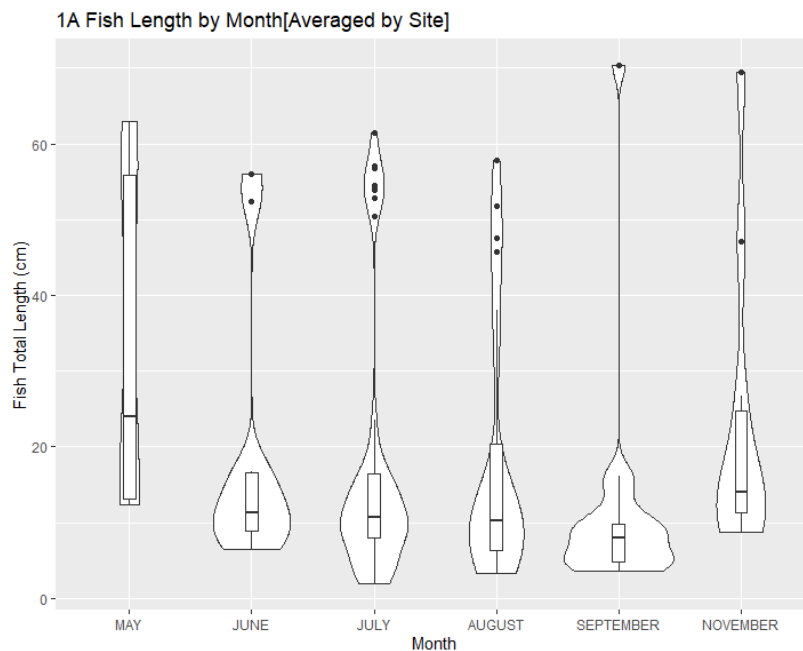


Figure 5.9 Violin plots of fish lengths in Pool 1A by sampling month. Plots are wider where lengths are more frequent. Box and whiskers within the “violins” show the average length as well as the quartiles for each month.

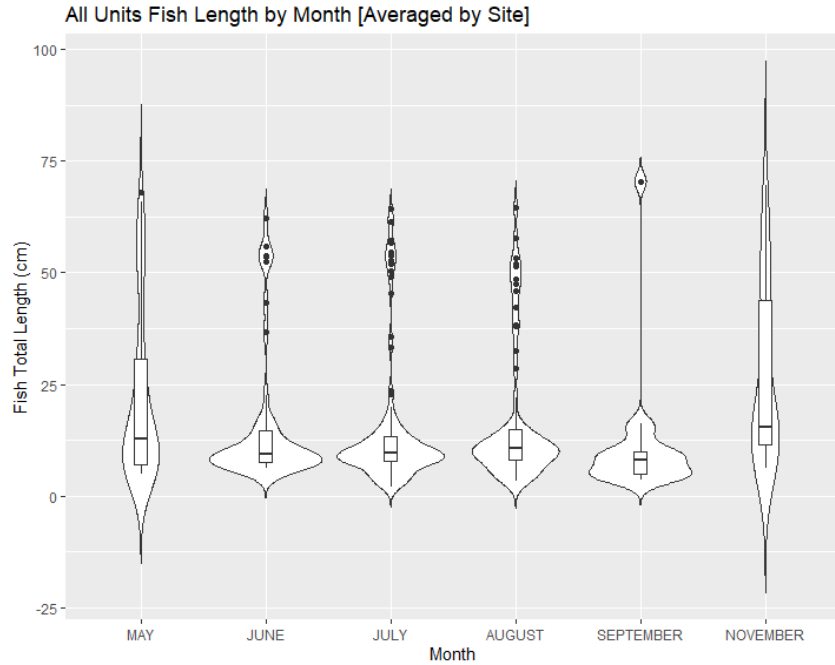


Figure 5.10 Violin plots of fish lengths in Maankiki South, Maankiki North, Pool 1A, and all units by sampling month. Plots are wider where lengths are more frequent. Box and whiskers within the “violins” show the average length and the quartiles for each month.

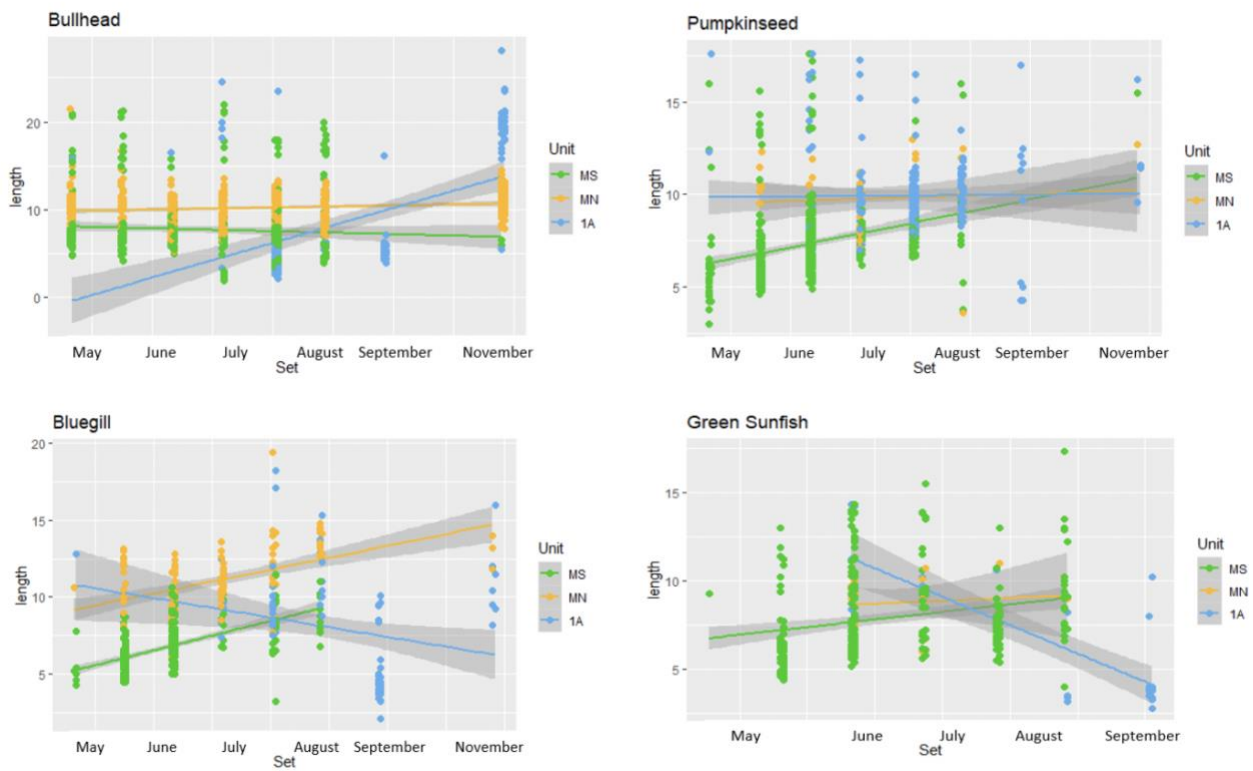


Figure 5.11 Linear regressions of the four most abundant species in the wetland units. The length is displayed on the y-axis and is reported in centimeters, the x-axis displays the set month.

	Black Bullhead	Pumpkinseed	Bluegill	Green Sunfish
Unit	<0.001	<0.001	<0.001	0.003
Month	<0.001	<0.001	<0.001	<0.001
Unit and Month	<0.001	<0.001	<0.001	<0.001

Table 5.5: P-values for terms in a general linear model of species lengths with unit, the month fish was caught, and the interaction between unit and month the fish was caught. Significant results ($p < 0.05$) are shown in bold.

All sunfish had positive growth in Maankiki South through the sampling season (Figure 5.11). Black Bullhead populations in Maankiki South and North were a stable size, but in Pool 1A they were longer by the end of the summer. Bluegill got longer in Maankiki North, but smaller in Pool 1A. Green Sunfish also got smaller in Pool 1A through the sampling season.

Indices

Shannon-Weiner Diversity Index of each unit

Shannon Weiner Diversity Indices for Each Unit

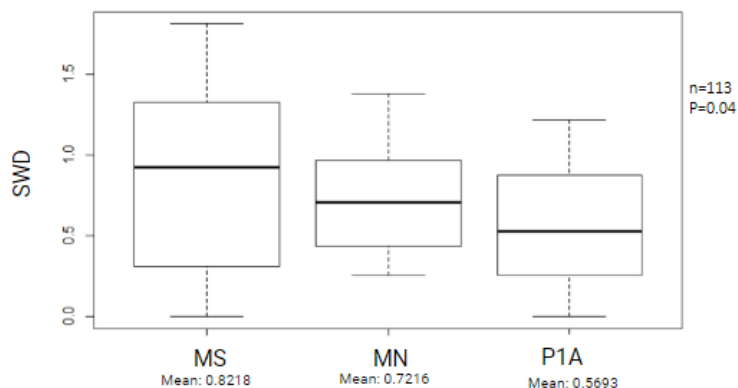


Figure 5.12 The average Shannon-Weiner Diversity Index for all of the fyke net sampling sites in each unit.

The Shannon-Weiner Diversity-Index was significantly lower in Pool 1A (SWDI = 0.57) than in MS (SWDI = 0.82, $p = 0.033$, Tukey HSD). The two Maankiki units did not differ in SWDI ($p = 0.622$, Tukey HSD). Maankiki North and Pool 1A units did not differ in SWDI ($p = 0.388$, Tukey HSD) (Figure 5.12).

IBI Score calculated to model the CWMP-QAPP

Unit	IBI Score SAV	IBI Score Typha
Maankiki South	54.54	55
Maankiki North	NA	40
Pool 1A	22.72	35

Table 5.6 The Index of Biotic Integrity scores calculated for each wetland unit and specific vegetation zones in accordance with the Coastal Wetlands Monitoring Protocol.

IBI scores range from 0-100 and represents the level of anthropogenic impact at a site. Scores near zero have a lower habitat quality and scores near 100 have a higher habitat quality. Maankiki South has the highest IBI scores and Pool 1A had the lowest (Table 5.6). An IBI score for Maankiki North in SAV could not be calculated because sampling in SAV in MN was not possible with fyke nets.

Fyke Net Analysis of Variance

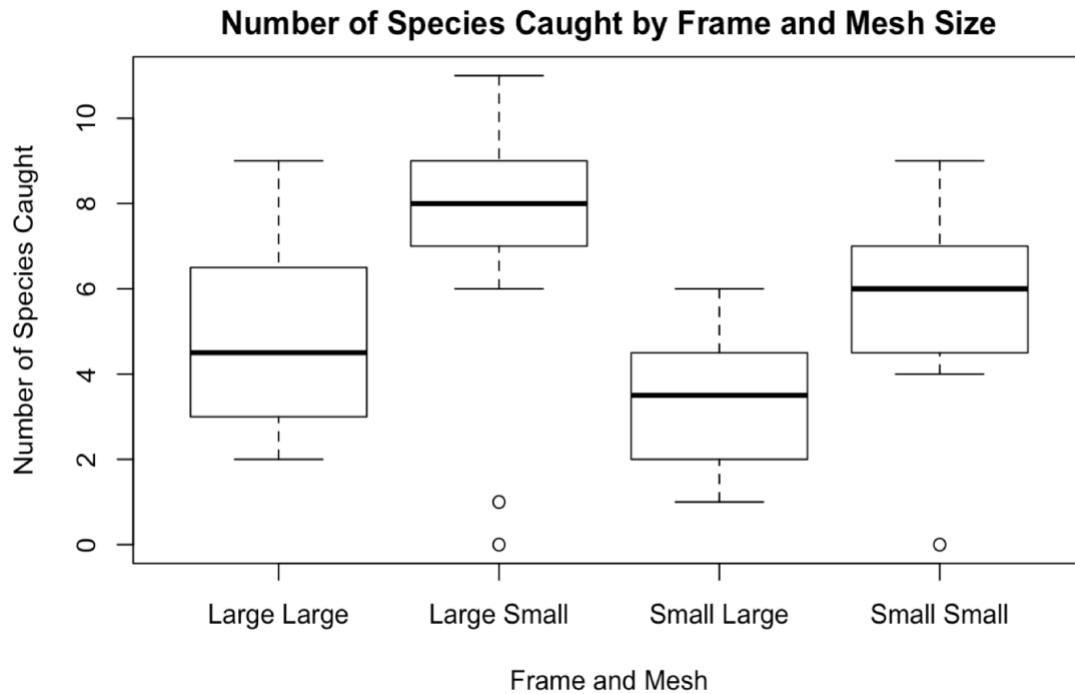


Figure 5.13 Boxplot of the number of species caught in each fyke net type.

	Frame Size	Mesh Size	Frame - Mesh
Abundance Per Site (CPUE)	0.45	<0.001	0.006
# of Species	0.03	<0.001	<0.001
Length	<0.001	<0.001	<0.001

Table 5.7 A summary of p-values from general linear model balanced factorial design ANOVA results of the effect fyke net frame, mesh, and specific frame-mesh combination type have on the CPUE per site, the number of species per site, and the length of individuals per site. Significant p values ($p < 0.05$) are bolded.

Frame size had no significant effect on the CPUE per site but did on the number of species caught and size (Figure 5.13). Large frame nets caught more species and longer fish. Small mesh nets caught a higher CPUE across the board, more species and longer fish than large mesh nets. The frame-mesh size of nets together also had a significant effect on the various measures of the catch (Table 5.7).

Ordination of Bray-Curtis Dissimilarity Index

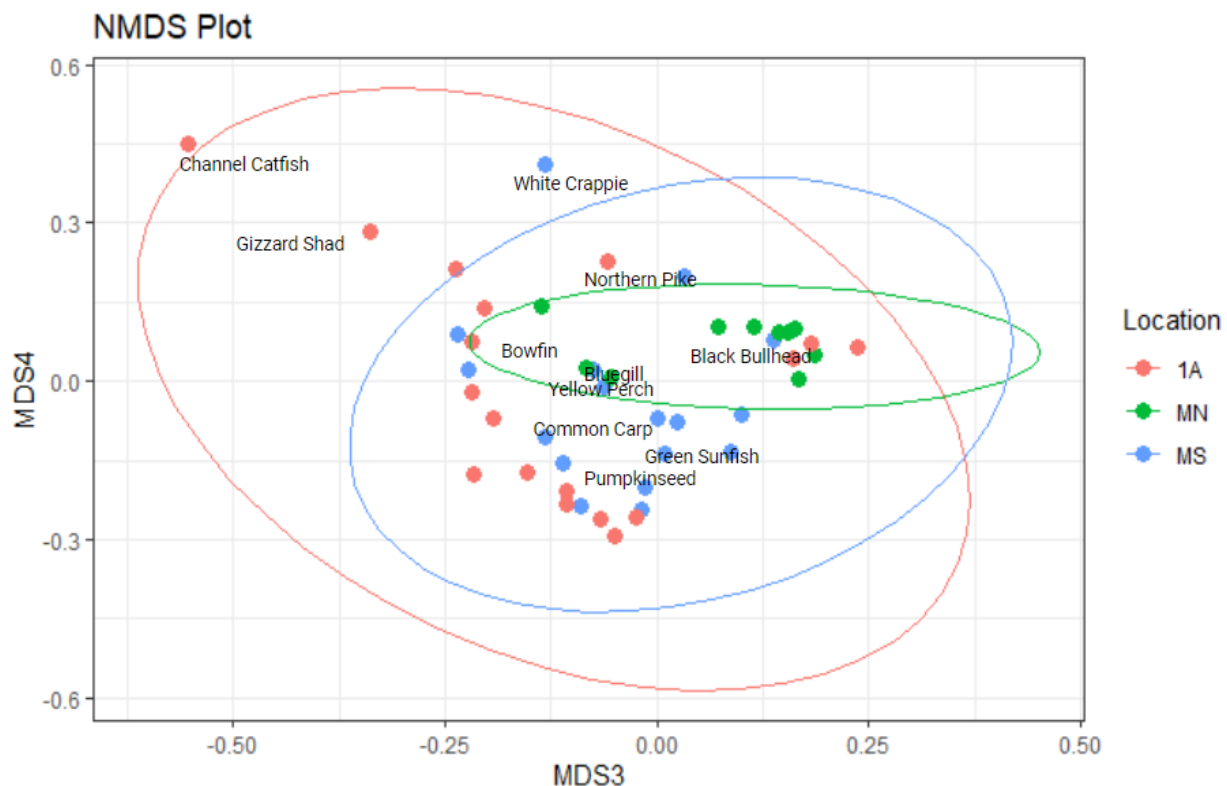


Figure 5.14 An NMDS ordination of a Bray-Curtis dissimilarity index. The graph depicts the differences in the species composition of each sampling site. Sampling sites with similar compositions are plotted closer to one another. Sites plotted near species names are likely to have those fish species. The unit ellipses indicate the area of the plot in which a new sample site within a unit would be expected to occur.

Overlap in the ellipses around sites within each unit reflects similarity between units in overall species composition (Figure 5.14). Sites in Maankiki North occurred within a relatively narrow ellipse reflecting little variation in composition; Pool 1A's large ellipse area indicates a far more varied catch across the unit. All of the unit ellipses overlap at the center of the ordination where sites dominated by the most commonly caught species (Black Bullhead, Bluegill, Green Sunfish, and Pumpkinseed) occurred. Sites plotted in the upper left of the ordination space reflect those samples with relatively rare species. For example, the sampling point near the Channel Catfish in Pool 1A was the only time we caught Channel Catfish.

VARIATION BY METHOD

Species by Method

Only one species, the Bigmouth Buffalo, was caught by electrofishing and setting gill nets and not in the fyke nets (Figure 5.15).

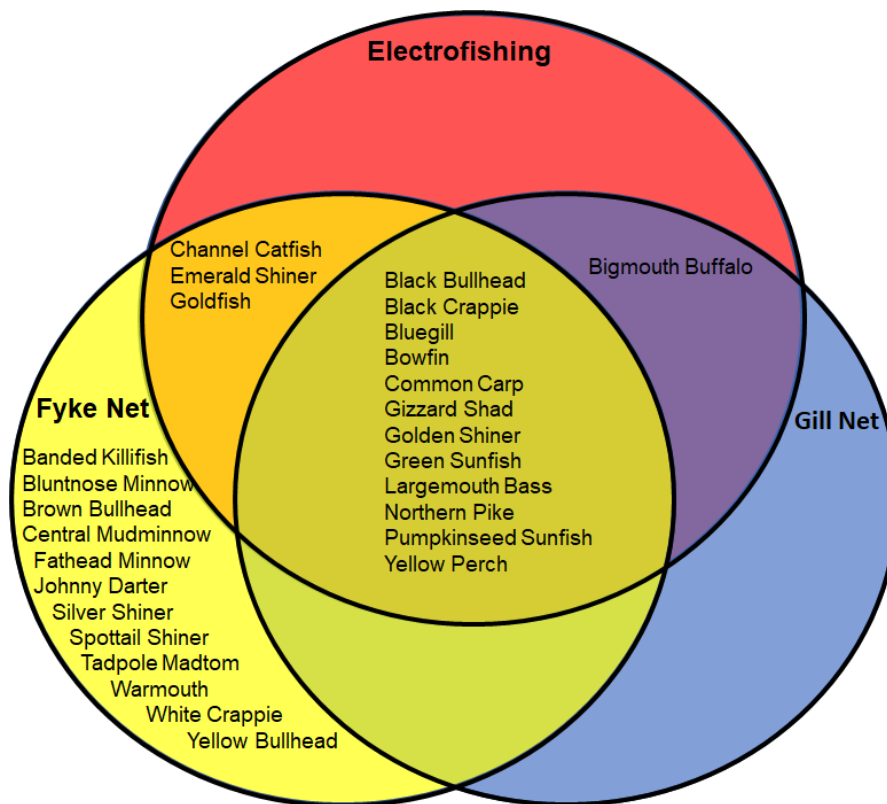


Figure 5.15 Venn diagram visualizing the species caught in each method: fyke nets, electrofishing, and gill nets.

Common Name	Scientific Name	Also Caught in Fyke nets	Caught by all Methods	MS Electro fishing CPUE	MS Gill netting CPUE	MN Electro fishing CPUE	MN Gill netting CPUE	P1A Electro fishing CPUE	P1A Gill netting CPUE	Game SP	Riverine SP	Wetland SP
Bowfin	<i>Amia calva</i>	Y	Y	0.12	0.33	0	0	0.31	NA	N	N	Y
Emerald Shiner	<i>Notropis atherinoides</i>	Y	N	0	0	0	0	0.21	NA	N	Y	N
Bluegill	<i>Lepomis macrochirus</i>	Y	N	0.25	0	0.02	0	0.1	NA	Y	N	Y
Johnny Darter	<i>Etheostoma nigrum</i>	Y	N	0	0	0	0	0.1	NA	N	Y	N
Fathead Minnow	<i>Pimephales promelas</i>	Y	Y	0.05	4.66	0.05	1.8	0	NA	N	N	Y
Goldfish	<i>Carassius auratus</i>	Y	Y	0.05	2.66	0	1.2	0	NA	N	N	Y
Golden Shiner	<i>Notemigonus crysoleucas</i>	Y	Y	0.05	1	0	0.6	0	NA	N	N	Y
Central Mudminnow	<i>Umbra limi</i>	Y	Y	0.07	1	0.02	0.4	0	NA	N	N	Y
Black Bullhead	<i>Ameiurus melas</i>	Y	N	0.29	0	0	0.2	0	NA	Y	N	Y
Bluntnose Minnow	<i>Pimephales notatus</i>	Y	Y	0.17	1	0	0.2	0	NA	N	N	Y
Gizzard Shad	<i>Dorosoma cepedianum</i>	Y	Y	0.05	0.33	0.02	0.2	0	NA	N	N	Y
Largemouth Bass	<i>Micropterus salmoides</i>	Y	Y	0.02	0.66	0.07	0.2	0	NA	Y	N	Y
Yellow Perch	<i>Perca flavescens</i>	Y	N	0	0	0	0.2	0	NA	Y	Y	N
Bigmouth Buffalo	<i>Ictiobus Cyprinellus</i>	N	Y	0.61	1.33	0	0	0	NA	Y	N	Y
Brown Bullhead	<i>Ameiurus nebulosus</i>	Y	Y	0.07	1	0	0	0	NA	Y	N	Y
Green Sunfish	<i>Lepomis cyanellus</i>	Y	Y	0.02	0.66	0	0	0	NA	Y	N	Y

Table 5.8 Species caught by gill nets and electrofishing during the sampling months of 2019. Species are categorized as game (Y) or non-game (N) species, as wetland (Y) or non-wetland species (N), as riverine (Y) or non-riverine (N). Also lists whether the fish was caught in fyke nets or in all three sampling methods. The bigmouth buffalo was the only species of fish not caught in a fyke net during 2019 sampling.

Size by Method

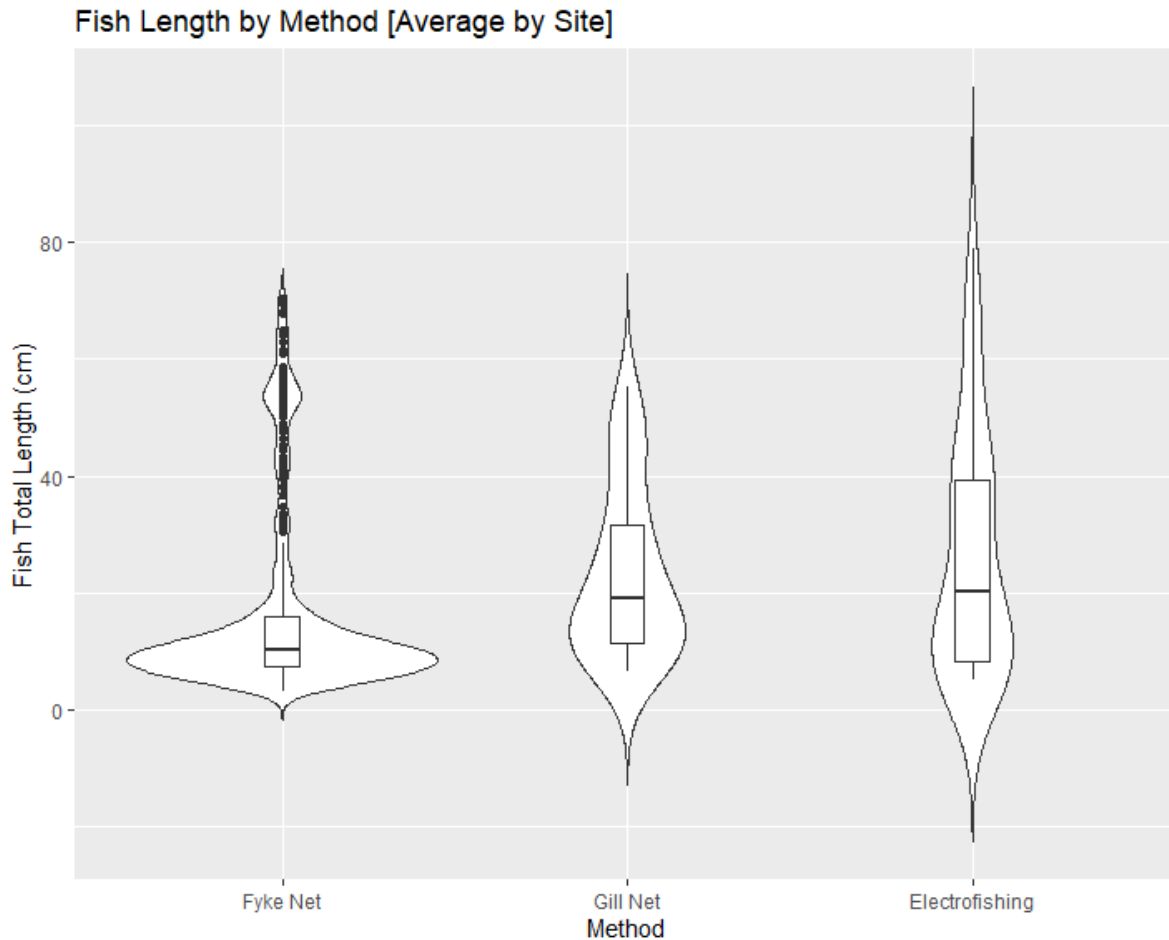


Figure 5.16 A violin plot of fish lengths across the sampling methods. The width of the plot at each length shows the frequency of that size. These were calculated using an average length of each species by site.

Overall, the sampling method had a significant effect on the length of fish caught. Fish caught in fyke nets were significantly smaller than fish caught by electrofishing (Tukey HSD post-hoc test, $p = 0.029$). Fish caught by gill netting were not significantly different in length from fish caught by fyke nets or electrofishing (Figure 5.16).

To illustrate the differences in size by species, dot plots were created, one for species with individuals caught that were longer than 40 cm and one for those that had all individuals seen under 40 cm (Figures 5.19, 5.20). For the smaller species, almost all methods overlapped in fish lengths showing similar sizes across methods (Figure 5.17). However, Largemouth Bass caught in fyke nets were smaller than those caught in gill nets, and Yellow Perch caught in fyke nets were larger than those caught in gill nets.

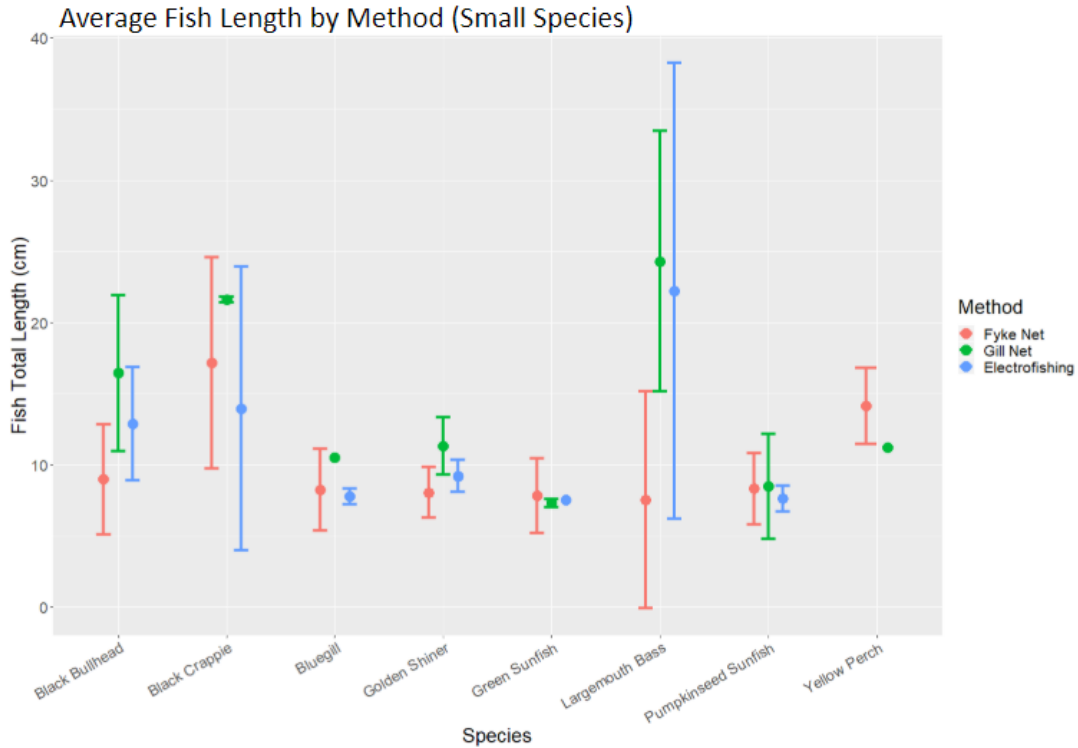


Figure 5.17 A dot and whisker plot showing the length of individuals by species for each sampling method. The dot is the average length of the species caught using a certain method and the whiskers represent one standard deviation each. This graph is for the smaller length species, up to 40cm, caught in multiple methods.

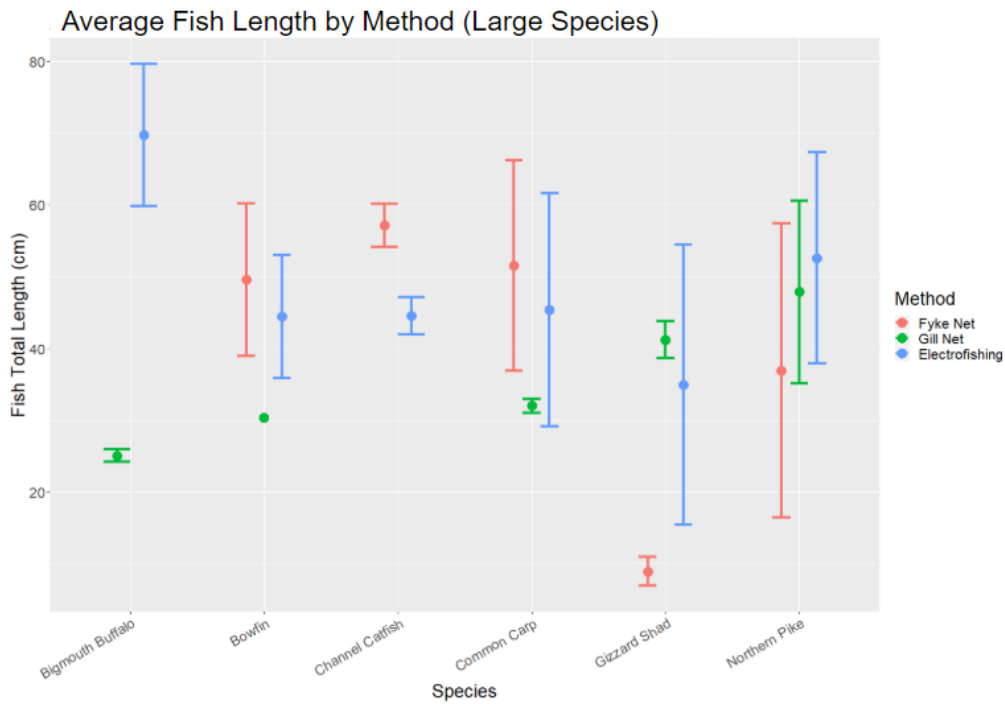


Figure 5.18 A dot and whisker plot showing the length of individuals by species for each sampling method. The dot is the average length of the species caught using a certain method and the whiskers represent one standard deviation each. This graph is for the larger length species, up to 80 cm, caught in multiple methods.

Among the larger species there is less overlap, with distinct differences in sizes across methods for Bigmouth Buffalo, Channel Catfish and Gizzard Shad (Figure 5.18). Bigmouth Buffalo were significantly larger in electrofishing samples than gill net samples, and not found in fyke nets. Channel Catfish were significantly smaller in electrofishing samples than fyke net samples, and not found in gill nets. Gizzard Shad were significantly smaller in fyke net samples than in gill net samples and electrofishing samples, which were not significantly different from each other. The gill net sample of Bowfin was smaller in length than the fyke net and electrofishing samples, but there was only one individual caught in gill nets.

DISCUSSION

Main Findings for Abundant Species

Black Bullhead

The most abundant fish species caught across the sampling season was the Black Bullhead (*Ameiurus melas*). A sturdy fish that thrives in turbid, low oxygen environments, Black Bullhead are benthic insectivores that are known to be able to raise the turbidity of an entire pond (Rose 2006). Black Bullheads are important intermediate predators connecting benthic invertebrates to fish and birds higher on the food web (Rose 2006). A high population of Black Bullheads helps convert the primary production captured by the lower trophic levels into biomass available to predator species (Rose 2006). The average length of Black Bullhead in the open wetland unit Pool 1A increased through the summer, with the longest of the specimens caught over the sampling season caught in Pool 1A. In Maankiki North, the Black Bullhead population was observed to have a skin condition resulting in the deaths of many individuals observed washed up along the shores of the unit. Sample Black Bullheads were collected and sent to the Aquatic Animal Health Laboratory (AAHL) at Michigan State University for analysis. The AAHL reported that the bullhead sent in had a systemic infection of *F. columnaris*, which was found in their skin, kidneys, and brains.

In the closed Maankiki units the large populations of bullhead were a stable size for the sampling season, with the exception of newly hatched young, swarms of which were caught in Maankiki South during two sampling efforts. High populations can limit the size of individuals. Black Bullhead reach maturity at around 17 cm (Rose 2006), and although in the Maankiki units the average length was below 10 cm throughout the summer, Maankiki South had many individuals above 17 cm. Individuals above the average length of maturity and small individuals (2-3 cm) caught and observed in schools of young-of-year indicate the population in Maankiki South is reproducing. A lack of either of these sized individuals

in Maankiki North supports the idea that the Black Bullhead population was not reproducing.

Sunfishes

The next three most abundant species caught in 2019 were sunfishes: the Bluegill, the Green Sunfish, and the Pumpkinseed. All three of these species displayed growth in average length in Maankiki South over the sampling season. The Bluegill and Green Sunfish average lengths also grew in Maankiki North, but they shrank in Pool 1A, possibly due to the recruitment of juveniles to a catchable size. There was no trend in the populations of Pumpkinseed in Maankiki North and Pool 1A.

Bluegill reach maturity after three years or when they're longer than 7.5 cm (Paulson 2004). The population in Maankiki South had an average length of 5 cm in May growing to an average of 8 cm by July. Pumpkinseed sunfish reach maturity after 2 years or after reaching 5 cm in length (Danylchuk 1994). Pumpkinseed sunfish in Maankiki South grew from an average length of 6 cm in May to an average length of 8 cm by July. Green Sunfish reach maturity after 3 years in Michigan and are between 4-7 cm long (Carlander 1977). Green Sunfish in Maankiki South grew from an average length of 7 cm in May to an average length of 9 cm by July. The Bluegill population in Maankiki South was the only sunfish population whose average length at the beginning of the sampling season was below the average length at maturity. The populations of both Pumpkinseed and Green Sunfish start the sampling season with large outlier individuals and a large portion of the population below the average age of maturity. The large outliers continued in our catch throughout the season and the smaller individuals increased in size over time.

These three sunfish are nest spawners, moving sediment to create circular depressions in shallow water. Sunfish will congregate their nests in groups of multiple species and can interbreed (Carlander 1977). In the east side of Maankiki South there is a large section of open shallow water that was recently treated with a pesticide to remove invasive *Phragmites*. During water quality sampling in May we noted a large number of groups of depressions in this shallow water that fit the description of these nests. Both the growing populations and finding these nests leads our team to posit that Maankiki South is also being used for sunfish spawning.

Common Carp

A common pest in wetlands is the naturalized species Common Carp. The Common Carp has been in the Great Lakes region since the 1800s. They primarily feed on aquatic plants and, in large populations, are major causes of disturbance in wetland ecosystems. This is due to their feeding habits which result in ripping up and destroying vegetation (Stuart et al. 2006). They also spawn in high densities, stirring up the water and increasing the

turbidity of entire areas (U. Boon Swee 1966). They become reproductive as early as 3 years old and live 15-20 years (U. Boon Swee 1966). Common Carp spawn in water temperatures of 15-20°C, their eggs hatch in 4 days, and can produce one million eggs in a breeding season (Nico 2019). Common Carp CPUE was highest in Pool 1A in May, coinciding with observed visual abundance of carp breeding in shallow water. This May spawning event in Pool 1A had a higher CPUE than the closed units, then the CPUE in Pool 1A dropped off for the rest of summer. Water quality measures were unavailable in the beginning of the sampling season, May, so we cannot be sure of water temperature within Pool 1A at that time. We also cannot determine if the presence of spawning Common Carp had any effect on the turbidity of Pool 1A because of a lack of May water quality data. We can, however, compare it to the average temperature of Pool 1A we were able to capture later in May 16.31°C, which falls within the reproductive temperature range. The temperature, in addition to the observed spawning behavior, supports the use of Pool 1A as spawning habitat for Common Carp. However, we captured no young-of-year or juvenile Common Carp using fyke nets or our other methods in 2019.

Wetland Units for Reproduction by other Fishes

Some less abundant species may have also been breeding in the wetland units. Migratory species are known to travel to Michigan's coastal wetlands to spawn or for a nursery area (Jude 1992). Gizzard Shad are a large river and bay species but use wetlands as a nursery (Jude 1992). In Pool 1A we caught juvenile Gizzard Shad in September and November. Northern Pike and Bowfin are known to migrate into wetlands to breed (Jude 1992). Northern Pike are known to migrate to wetlands in early spring to reproduce and then the young migrate among wetlands to use them as a nursery (Jude 1992). In the Maankiki units, adult Northern Pike were caught throughout the sampling season, but in Pool 1A Northern Pike were only caught in July, where there were three adults and nine juveniles caught. Our team hypothesizes that the Northern Pike in the Maankiki units came in to breed during the brief period the units were open in April and became trapped when the unit control structures were closed. The Northern Pike may have moved into Pool 1A after the population of Common Carp decreased. Common Carp are known to increase the turbidity of habitats and Northern Pike are not as active in turbid environments (Engström-Öst 2008). Bowfin, which commonly live and reproduce in Michigan wetlands were the fifth most abundant species of fish caught. At the beginning of the sampling season, Bowfin in all surveyed units displayed bright turquoise-green underbellies and fins, then lost that color as the summer progressed. Males turn this color during their mating season (Thomas 2007), but in neither the open Pool 1A or the closed Maankiki units did we find any juvenile or young-of-year Bowfin. It is unclear why there was no evidence in the collected data that there were any young Bowfin successfully living in the wetlands, even though we found reproductive Bowfin in both open and closed units. Bowfin are competitive and solitary after they leave the nest (Emmerson 2004). Therefore, this species

could have produced offspring that either left the open unit or were quickly outcompeted in the closed units, which were much more densely populated with competitive genera like bullheads and sunfish.

Habitat Assessment

Observation of Larval Fish

Our samples did not include young-of-year individuals for any of the fish known to reproduce in wetlands, except for Black Bullhead. However, the team observed many schools of small and larval fish throughout the summer months, even identifying Largemouth Bass and an Emerald Shiner smaller than 3cm. Data on the presence of young-of-year fish would support the idea that fish are using the SNWR as a spawning ground and nursery. This demographic of the community was not sampled by fyke nets particularly well, so it is suggested that those interested in determining the amount of young in SNWR turn to the use of minnow traps. These small traps limit the size of fish let in and because they have a smaller mesh, they can trap smaller fish.

A Comment on Water Quality

Fish sampling in the forest of Maankiki South was only attempted in May, when the forest was entirely inundated. Water levels limited sampling to a small frame fyke net and all fish caught were dead at net pull except for a Bowfin. The DO in the forest was below 1 mg/L, which is considered anoxic, for all three measures at net set and both net pulls. Because of the mortality rate and small CPUE in this vegetation type, this was the only sampling effort to take place in the forest so as to limit sampling mortality. The water levels in the forest dropped throughout the summer until most of the forest was no longer accessible to fyke netting. During a year with lower water levels or if the gate had been open and allowed for fluctuations in the water levels in the unit, the forest may have not had enough water for sampling at all.

Fish sampling in Maankiki South and Pool 1A had similar mortality problems at the end of July and throughout August. Net set and pull water quality measures were not extreme: the DO was above 1 mg/L and the temperature was only above 25°C at one sample site. In Maankiki South a portion of fish in each sample were found dead in the net, but in Pool 1A often entire samples were deceased. In these units the average day temperature was around 20°C. The average DO for Maankiki South was 2.30 mg/L and the average DO for Pool 1A was 1.88 mg/L, both averages are above 1 mg/L. We suspect that the DO in these units drops below 1 mg/L for long enough to cause the mortality. Notably, the fyke net set on July 30 near the control gate in Pool 1A contained dead fish and there were several dead fish observed in the *Typha* vegetation zone the net was set in. The dissolved oxygen in at this location at the net set was 6.74 mg/L and pull was 7.58 mg/L, both healthy DOs. The

conductivity of this site was very high, above 1500 uS/cm. Conductivity is a measure of ions dissolved in water, commonly salts. This die off could have been caused by a spike in salinity, as both fish trapped in the net and fish observed in the area were dead.

Differences in Indices

Maankiki South had the highest average Shannon-Weiner Diversity Index (SWDI) and the highest IBI scores among the units. The range for a Shannon-Weiner Diversity Index goes from 0-5, and the average for Maankiki South was 0.8218. This is a low number compared to other ecosystems as scores considered normal fall between 1-3 (Kwak et al. 2007). Maankiki North had the next highest SWDI of 0.7216, lower than Maankiki South because it was less species rich. Pool 1A had the lowest Shannon-Weiner Diversity Index of 0.5603. Since the SWDI is calculated both by richness and evenness, average scores could be lower because of a high number of samples dominated by one or few species, or with a high variety of composition among samples.

IBI scores within the units were calculated for either the SAV vegetation type or the *Typha* vegetation type and can range from 0-100. Most of the wetlands surveyed between 2011 and 2015 in the Saginaw Bay received a degraded score using the same IBI metrics (Uzarski 2016). The maximum score that can be given for an IBI in *Typha* is 63 (Uzarski 2016). Maankiki South IBI scores were in the fifties for both vegetation types. These IBIs are in line with the expected IBIs for Saginaw Bay and close to the maximum scores for these types. In Maankiki North there were no fish samples that could be used for calculating an IBI for SAV and the score for the *Typha* type was 40, which is not particularly lower than sites in Saginaw Bay (Cooper et al. 2018). Pool 1A had the lowest scores of 22.72 in SAV and 35 in *Typha*. These scores are poor and lower than the expected scores for a historically restored unit. Since IBI is a score that measures the impact of anthropogenic changes, invasive species greatly impact the results and are a heavily weighted metric in the calculation. Common Carp were extremely common in Pool 1A and their presence lowers the score considerably.

Are these good indices?

It is unclear how suitable the data were for a SWDI, as infrequent sampling may not have captured a reliable profile of the wetland units. Although species accumulation curves for the units reach their asymptote, the sampling protocol may not have captured an accurate measure of the relative abundance of each species. Our IBIs were adjusted to fit into the CWMP IBIs for SAV and *Typha*, as there are not standard IBIs for each sampled vegetation type. CWMP scientists are currently working on publishing IBIs for other vegetation types (Uzarski, personal communication, 2020). Future projects should reference revised IBIs for the various vegetation types sampled.

Comparing Methods

Fyke Net Variation

Variation in length of fish across net types can indicate how fish use each habitat. Because wetlands are known as potential nursery habitat, (Jude and Pappas 1992) small fish can indicate where these nurseries are located within a unit. The smaller fish sizes found in small frame nets suggest that at the SNWR the shallow areas are important for juvenile fish. The significantly longer fish in large frame fyke nets are likely due to the deeper habitats accessible with these nets. When temperatures rise and DO falls, larger fish find thermal refuge and higher DO in deeper water (Kowalski et al. 2014)

We expected to find smaller fish in small mesh nets because these nets are more easily seen and avoided by large fish (Kowalski, personal communication, 2019). However, significantly larger fish were found in small mesh nets. This may be correlated with the higher CPUE caught in small mesh nets. With the exception of swarms of juvenile fish, we would expect a higher number of individuals to show a greater variation in length. While the small mesh is theoretically more visible to longer fish than large mesh, they may have been drawn to the net by small fish already caught, as we know that net predation does occur (Breen & Ruetz 2006). This cannot be determined from our data, however, because we do not know the order in which fish entered the nets.

Small mesh nets had higher CPUE, likely due to lower escape rates through the smaller openings. Frame size had no impact on CPUE, meaning that the smaller space containing fish in small frame nets does not affect the number of fish caught. This also relates to how many fish live in each habitat type, because the frame size correlates to water depth. The similar CPUE between frame sizes over the sampling season suggests that overall, the same number of fish occupy the shallow habitats as the deeper habitats.

More species are found in large frame nets, which is consistent with the bias of fyke nets. Larger species are less likely to fit in or enter small frame nets and were not frequently observed in small frames in our study. Small species can easily fit in either frame size and were seen often in both frame sizes. For mesh size, the higher number of species in small mesh nets is likely due to reduced escape of smaller species through the mesh.

Supplementary Methods

Studies have shown that the different sampling methods, and using different gear specifically, can be varied in likelihood of catching certain fish species and result in sampling bias (Porreca et al. 2013; Cvetkovic et al. 2012). Contrary to this, we only found one unique species with the alternate methods of gill netting and electrofishing and many unique species using fyke nets. This could be due to limitation in suitable sampling areas

for gill netting and electrofishing within the wetland units. For this reason, fyke nets are the most appropriate as the primary sampling gear in these units.

Within a species, significantly different lengths were seen between the three collection methods. The most notable length differences between the methods are that of Gizzard Shad and Bigmouth Buffalo. The smaller Gizzard Shad found in fyke nets show that this species may use shallow habitats for refuge during growth (Kowalski et al. 2014), while the larger individuals caught in gill nets live in deep areas. Bigmouth Buffalo were largest in electrofishing samples and smaller in gill nets, it is likely that the very large individuals caught with electrofishing were too large to fit into the gill net mesh. The differences in size compositions may be associated with the variation in habitats that are able to be sampled with each method. Because fyke nets are depth limited, it is possible there was an under sampling of the largest fish found in deeper water when only fyke nets are used.

Limitations and Error

According to previous studies there are three primary sources of bias when using fyke nets. These include species specific escape rates, net predation, and length of time the net was in the water (Breen & Ruetz 2006). Our nets were all set for 24-hours per pull; however, we acknowledge the bias presented by escape rates and the possibility of predation within the nets (Brady et al. 2007). Additionally, another source of sampling was compromised nets. Compromised nets resulted from holes created by the fish themselves, or unintentionally caught animals such as muskrats or turtles attempting to escape.

Each sampling method has limitations in terms of sampling bias or habitat access. Fyke nets may not allow sampling in deep habitats where large fish reside. Gill nets can only sample for fish that fit through the mesh. Electrofishing is limited by access with the catamaran. Because of these disadvantages when used individually, all three gear types are needed to fully sample the units.

Implications for Management

A greater understanding of where fish live and how they utilize the environments within units will allow for informed management decisions. With the knowledge of where fish nurseries are, the Refuge can avoid disturbance of important habitats. Our study primarily found nurseries for sunfishes, Black Bullhead, and Largemouth Bass. Supporting the sunfish and bass populations could be important to supplementing the game fish in the region. Based on the locations in which these juveniles were found, the creation of shallow regions with vegetation for refuge would likely promote nurseries in regions where they currently are not seen, such as Maankiki North (Stephenson 1990). Minnow traps would help to collect additional data on which fish are spawning in the units, by catching juveniles too small to be retained in fyke nets.

Deep regions of open water or submerged aquatic vegetation, on the other hand, promote the presence of large fish, as well as thermal refuge during warmer months for fish of all sizes. If managers seek to create a diverse fish community such as is seen in Maankiki South, steps should be taken to manipulate the topography to create varied depths and habitat types.

Sampling Method Recommendations

Across the sampling season, multiple methods should be used to collect a representative sample of the fish community. Due to the bias of gears towards certain species, as well as their use in varied habitats, a mixed methods approach will capture the greatest variety of fish sizes and species. Our results indicate that even if there is one target species, it may be necessary to vary methods in order to gather a representative sample of its population. If a certain species is of interest, then Refuge managers should assess which method or methods best capture those individuals. For fyke nets specifically, a combination of mesh sizes and frame sizes are necessary to access different fish habitats and get a range of species, lengths, and CPUE. Due to the potential for fyke net escape and the observations of small fish in the water that were never seen in the nets, minnow traps may be required to catch the smallest species and juveniles. These can also be used to access varied depths, and therefore account for different habitats these smaller fish may utilize.

After each sampling season using the methods described above to sample all possible habitats, species, and lengths, these results can be used to determine the health of the wetland community as well as the way the riverine community utilizes a unit. The accumulation of this data across multiple sampling seasons will allow for the assessment of management practices and changes in ecosystem health over time.

RECOMMENDATIONS & OVERALL CONCLUSIONS

IMPLICATIONS FOR SCIENCE

Once farmland, the converted units of Maankiki North, Maankiki South, and Pool 1A represent nature's ability to recolonize an area. Although the IBIs and FQIs for the units characterize the restored units as degraded, that is not to say that they are not being utilized as coastal wetlands and providing essential habitats and ecosystem services that otherwise would not have existed if the wetlands had not been converted from farmland. When considered in the context of the Refuge's past, even low IBI scores show improvement and function of the new wetland units. The wetlands are host to riverine and wetland fishes, diverse wetland macroinvertebrates, and both native and nonnative plant species, similar to many other restored wetlands in Michigan. Restoring these units showed that simply returning water to an ecosystem that historically was a wetland (before it was farmland) is enough to create an environment that can support vegetation, macroinvertebrates and fish. The differences in biological communities in the wetlands demonstrate that the process of *how* coastal wetland units are restored can have serious implications for the types of biodiversity that can be supported. Maankiki North, a deep, open water wetland, could not provide the habitat for more diverse submerged aquatic vegetation, as it was heavily channelized from the creation of the dikes. On the contrary, Maankiki South had such varied topography that it could support a diverse range of plant and fish species. Variations in topography (depth) and connectivity to the river appear to be the most influential factors on the biodiversity of wetlands. Age of the wetland did not appear to impact how biodiverse it was. Future restoration of coastal wetlands in the Great Lakes Region should factor in topography and connectivity when determining how to restore farmlands to coastal wetlands.

MANAGEMENT IMPLICATIONS

Surveying Schedule

Time spent surveying each community is time that cannot be used to survey other taxa and locations. It is important to schedule sampling around times important for various biological communities. Vegetation is best sampled in late July or early August at the peak of the growing season, when anatomy commonly used for identification is most prominent. Invertebrates are best sampled throughout the summer to capture life cycle variation of multiple families. Fish should be sampled throughout the open water season, but special attention should be paid to events such as migration for spawning and winter refuge as well as to significant fluctuations in water temperature and changes in water quality and quantity. It should be the responsibility of Refuge managers to prioritize which communities are surveyed based on management goals should these phenological cycles occur simultaneously.

Refuge Variability

Diversity of the wetland units appears to be driven by their topographic variation, as different portions of the unit are exposed to different hydrologic regimes. Yearly water level fluctuations influence the development of available vegetation habitats, which can support variable and robust communities of invertebrates and fishes. Refuge managers can control the amount of water entering each unit over time by manipulating when the water control structures are open and closed and should determine when to open and close them by prioritizing different biological communities.

It is our recommendation to expand surveys to include measures of water depth, riverine habitats, and patterns of historical management within units. Refuge managers should be able to analyze the suite of biological responses to environmental conditions to determine if and when units should be connected to the river. Therefore, stronger analysis of abiotic variables would support conclusions made about communities within the wetland units and the decisions Refuge managers can make that affect the ecology of the units. Additionally, river sampling, both in the Shiawassee and Spaulding Drain, should be included in future studies to compare riverine and wetland communities and assess the use of the wetland units by communities in the watershed.

As hydrologic management strategies are implemented and natural variables fluctuate, continued yearly monitoring is recommending to capture and analyze the influence interannual variation has on the recovery and health of the wetland units. In some instances, ecosystem function and structure fail to recover to reference levels, take decades to do so, or fall into alternative stable states (Moreno-Mateos et al. 2012). Similarly, not all the biologic communities respond in the same timeframe (Warren et al. 2002) with recovery contingent on many factors such as soil moisture (Rohal et al. 2019) or elevation and plant community composition (Meyer et al. 2008). However, the recovery of biodiversity has been correlated with improved ecosystem services (Meli et al. 2014). Yearly monitoring can capture this data and allow Refuge managers to adaptively manage wetland units as conditions change or, as studies have shown, meet management goals ahead of their estimated trajectory (Hinkle and Mitsch 2005).

CONCLUSION

Conclusions and recommendations detailed within this report are the result of data collected during one sampling season, May 6th to November 3rd, 2019. This data captured baseline biologic conditions following the first-year hydrologic reconnection of Maankiki North and Maankiki South. Due to the combination of high-water levels and continued construction in Maankiki Center, Maankiki North and South remained closed to the Shiawassee River for practically the entire sampling season. During future sampling, with the completion of Maankiki Center, we expect all three Maankiki units to be open to the

river for more days than were observed during our sampling season. Under these conditions, Pool 1A may also be open to Maankiki Center in addition to Spaulding Drain, connecting all the restored units to one another and simulating the natural function of this floodplain complex. As the historic flow of the Flint River is restored and new wetland units, like Ferguson Bayou, are reconnected, the dynamics of the entire floodplain complex, and the Shiawassee River, will need to be monitored. With this in mind, sampling the range of current and future reconnected wetland units for several years should be done to refine the recommendations listed herein.

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CONCLUSION

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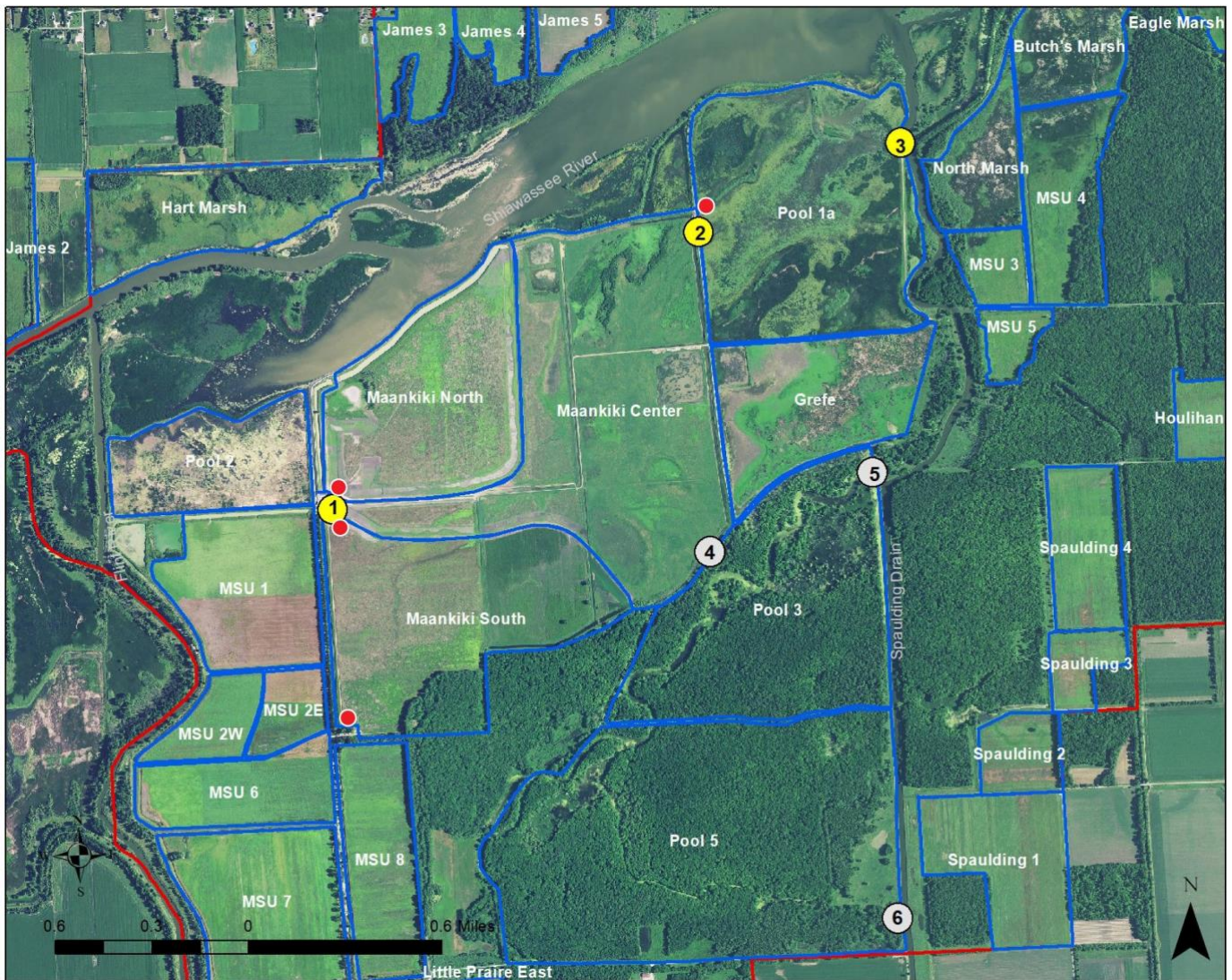
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APPENDICES

APPENDIX 1.1: Water Quality Data

Please visit the University of Michigan's Deep Blue website to view the water quality data titled "SNWR_WaterQuality2019" that was used to perform analyses.

APPENDIX 1.2: 24-Hour Sonde Locations



Red dots represent where sondes were left overnight in June and August to measure water quality.

APPENDIX 2.1: Plant Species List by Unit

Note: Dashes (-) are dead species not counted towards unit totals

Species	Maankiki South	Maankiki North	Pool 1A
<i>Acer saccharinum</i>	x		
<i>Acer spp.</i>	x		x
Algae spp.	x	x	x
<i>Alisma plantago-aquatica</i>	x		x
<i>Bidens cernua</i>	x		x
<i>Boehmeria cylindrica</i>	x		x
<i>Butomus umbellatus</i>	x	x	
<i>Carya ovata</i>	x		
<i>Celtis occidentalis</i>	x		
<i>Ceratophyllum demersum</i>	x	x	x
<i>Cicuta bulbifera</i>			x
<i>Cyperus strigosus</i>			x
Dead <i>Lythrum salicaria</i>	-		
Dead <i>Phragmites</i>	-		
Dead <i>Populus</i>	-		
Dead <i>Typha</i>	-	-	-
<i>Eleocharis palustris</i>	x		x
<i>Eleocharis spp.</i>	x		x
<i>Elodea canadensis</i>	x	x	x
<i>Elodea nuttallii</i>	x		
<i>Epilobium ciliatum</i>	x		
<i>Fontinalis spp.</i>	x		x
<i>Fraxinus spp.</i>	x		
<i>Galium triflorum</i>			x
<i>Impatiens capensis</i>	x		x
<i>Juglans nigra</i>	x		
<i>Laportea canadensis</i>	x		
<i>Leersia oryzoides</i>	x		x
<i>Lemna minor</i>	x	x	x
<i>Lemna trisulca</i>	x	x	x
<i>Ludwigia palustris</i>	x		

Species	Maankiki South	Maankiki North	Pool 1A
<i>Lysimachia nummularia</i>	x		x
<i>Lythrum salicaria</i>	x		x
<i>Myriophyllum spicatum</i>		x	
<i>Najas flexilis</i>	x		x
<i>Najas minor</i>		x	
<i>Nymphaea odorata</i>			x
<i>Peltandra virginica</i>	x		
<i>Penthorum sedoides</i>	x		x
<i>Persicaria amphibia</i>			x
<i>Persicaria maculosa</i>			x
<i>Persicaria punctata</i>			x
<i>Persicaria virginiana</i>	x		
<i>Phalaris arundinacea</i>	x	x	x
<i>Phragmites australis</i>	x		x
<i>Populus deltoides</i>	x		
<i>Potamogeton crispus</i>	x		
<i>Potamogeton foliosus</i>	x	x	
<i>Potamogeton nodosus</i>	x	x	x
<i>Quercus bicolor</i>	x		
<i>Riccia fluitans</i>	x	x	x
<i>Ricciocarpus natans</i>	x		
<i>Sagittaria latifolia</i>	x		x
<i>Salix interior</i> (OS)			x
<i>Salix interior</i> (US)			-
<i>Salix nigra</i>			x
<i>Salix spp.</i>	x		x
<i>Schoenoplectus tabernaemontani</i>			x
<i>Scutellaria galericulata</i>			x
<i>Scutellaria lateriflora</i>			x
<i>Sparganium eurycarpum</i>			x
<i>Spirodela polyrhiza</i>	x	x	x
<i>Stuckenia pectinata</i>	x	x	x
<i>Teucrium canadense</i>	x		

Species	Maankiki South	Maankiki North	Pool 1A
<i>Toxicodendron radicans</i>	x		
<i>Typha angustifolia</i>	x	x	x
<i>Typha x glauca</i>	x		
<i>Ulmus americana</i>	x		
<i>Utricularia minor</i>	x		
<i>Utricularia vulgaris</i>	x	x	x
<i>Vitis riparia</i>	x		x
<i>Wolffia spp.</i>	x	x	x
Totals	52	17	43

APPENDIX 2.2: Importance Value Indexes

Note: Dead species are included in total species counts

MAANKIKI SOUTH						
Species	Vegetation Zone					
	Forest	Mudflat	Phalaris	SAV	Sparse Typha	Typha
Submerged aquatic vegetation or floating leaf vegetation						
<i>Algae spp.</i>			7.77		5.32	
<i>Ceratophyllum demersum</i>			2.04	60.63	7.55	1.69
<i>Elodea canadensis</i>				4.56	26.48	
<i>Elodea nuttallii</i>				2.34		
<i>Fontinalis spp.</i>			1.70	4.56	7.51	3.38
<i>Lemna minor</i>	20.87		11.96	13.68	1.28	8.98
<i>Lemna trisulca</i>	1.55		29.61	2.34	14.22	12.20
<i>Najas flexilis</i>				2.34		
<i>Potamogeton foliosus</i>		5.18		28.97	21.43	
<i>Potamogeton nodosus</i>		22.55		4.56	15.22	9.57
<i>Riccia fluitans</i>	7.58					4.59
<i>Ricciocarpus natans</i>			6.81			5.96
<i>Spirodela polyrhiza</i>	3.19		5.11	7.53		1.49
<i>Stuckenia pectinata</i>			1.81	19.98	3.69	3.34
<i>Utricularia minor</i>						1.85
<i>Utricularia vulgaris</i>			31.08	18.42	12.02	46.74
<i>Wolffia spp.</i>			11.93		1.28	1.53
Emergent						
<i>Alisma plantago-aquatica</i>		90.10			2.46	
<i>Bidens cernua</i>		2.80				
<i>Boehmeria cylindrica</i>	11.32					
<i>Impatiens capensis</i>	4.85					
<i>Ludwigia palustris</i>					1.28	
<i>Peltandra virginica</i>					1.64	
<i>Penthorum sedoides</i>				7.32		
<i>Persicaria virginiana</i>	5.17					
<i>Sagittaria latifolia</i>		8.40				

MAANKIKI SOUTH (continued)

Species	Vegetation Zone					
	Forest	Mudflat	Phalaris	SAV	Sparse Typha	Typha
Trees, groundcovers, or vines						
<i>Acer saccharinum</i>	35.82	2.45				
<i>Acer spp.</i>	15.10					
<i>Carya ovata</i>	13.46					
<i>Celtis occidentalis</i>	1.89					
Dead <i>Populus</i>		7.56				
<i>Eleocharis palustris</i>		2.45		2.34	2.59	3.02
<i>Eleocharis spp.</i>		40.42		12.62	33.57	6.95
<i>Epilobium ciliatum</i>	1.55					
<i>Fraxinus spp.</i>	1.55					
<i>Juglans nigra</i>	1.89					
<i>Laportea canadensis</i>	9.48					
<i>Leersia oryzoides</i>	1.64					
<i>Populus deltoides</i>	7.37	7.98	5.11		1.28	
<i>Quercus bicolor</i>	5.47					
<i>Salix spp.</i>		7.56				
<i>Teucrium canadense</i>	1.89					
<i>Toxicodendron radicans</i>	1.55					
<i>Ulmus americana</i>	16.62					
<i>Vitis riparia</i>	1.89					
Invasive submerged aquatic vegetation floating leaf species						
<i>Potamogeton crispus</i>					1.32	
Invasive emergent species						
<i>Butomus umbellatus</i>						1.53
Dead <i>Lythrum salicaria</i>			1.70			
Dead <i>Phragmites</i>				2.45	1.28	1.53
Dead <i>Typha</i>			3.56	5.37	6.42	27.56
<i>Lythrum salicaria</i>			1.74			
<i>Phalaris arundinacea</i>	14.15	2.52	78.06		11.04	1.49
<i>Phragmites australis</i>					4.19	4.50
<i>Typha angustifolia</i>					15.65	49.00

MAANKIKI SOUTH (continued)						
Species	Vegetation Zone					
	Forest	Mudflat	Phalaris	SAV	Sparse Typha	Typha
<i>Typha x glauca</i>					1.32	1.61
Invasive trees, groundcovers, or vines						
<i>Lysimachia nummularia</i>	14.12					
Species Richness	24	12	15	17	24	22

MAANKIKI NORTH			
Species	Vegetation Zone		
	Phalaris	SAV	Typha
Submerged aquatic vegetation or floating leaf vegetation			
Algae spp.	2.94	3.27	
<i>Ceratophyllum demersum</i>	5.18	77.63	3.24
<i>Elodea canadensis</i>		30.40	1.62
<i>Lemna minor</i>	15.31		8.09
<i>Lemna trisulca</i>	51.32	8.09	42.16
<i>Najas minor</i>		9.76	
<i>Potamogeton foliosus</i>			1.62
<i>Potamogeton nodosus</i>		33.40	9.50
<i>Riccia fluitans</i>	5.49	2.70	30.95
<i>Spirodela polyrhiza</i>	10.22		8.09
<i>Stuckenia pectinata</i>		3.27	5.25
<i>Utricularia vulgaris</i>	21.91	7.32	18.01
<i>Wolffia</i> spp.			1.67
Invasive submerged aquatic vegetation or floating leaf vegetation			
<i>Myriophyllum spicatum</i>		6.49	
Invasive emergent species			
<i>Butomus umbellatus</i>		2.76	
Dead Typha		12.20	29.79
<i>Phalaris arundinacea</i>	87.64	2.70	
<i>Typha angustifolia</i>			40.01
Species Richness	8	13	13

POOL 1A					
Species	Vegetation Zone				
	Mudflat	Nymphaea	Salix	SAV	Typha
Submerged aquatic vegetation or floating leaf vegetation					
<i>Algae spp.</i>			1.31		
<i>Ceratophyllum demersum</i>		9.99	7.25	9.90	7.85
<i>Elodea canadensis</i>		1.74		10.52	
<i>Fontinalis spp.</i>			1.35		
<i>Lemna minor</i>	36.61	10.48	28.98	11.36	15.05
<i>Lemna trisulca</i>	12.68	5.22	2.66		2.00
<i>Najas flexilis</i>		7.40		2.90	
<i>Potamogeton nodosus</i>		23.70		41.15	2.00
<i>Riccia fluitans</i>	3.33	1.74			2.00
<i>Spirodela polyrhiza</i>	5.02	14.05	10.67	11.36	39.27
<i>Stuckenia pectinata</i>		47.73		89.10	2.00
<i>Utricularia vulgaris</i>					2.69
<i>Wolffia spp.</i>		3.52		2.84	6.36
Emergent					
<i>Alisma plantago-aquatica</i>	3.39				
<i>Bidens cernua</i>	29.17				
<i>Boehmeria cylindrica</i>	3.33		2.78		
<i>Cicuta bulbifera</i>	13.09				
<i>Galium triflorum</i>	1.66		1.35		
<i>Impatiens capensis</i>			1.31		
<i>Nymphaea odorata</i>		72.55	6.91		7.20
<i>Penthorum sedoides</i>			2.70		
<i>Persicaria amphibia</i>	1.85	1.87	6.24		2.03
<i>Persicaria punctata</i>			3.20		
<i>Sagittaria latifolia</i>	5.27			3.15	
<i>Schoenoplectus tabernaemontani</i>	0.83			14.87	
<i>Scutellaria galericulata</i>	3.51				
<i>Scutellaria lateriflora</i>	3.39				
<i>Sparganium eurycarpum</i>	5.37				

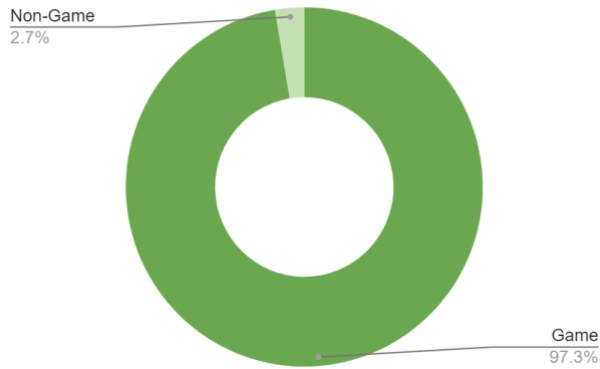
POOL 1A (continued)					
Species	Vegetation Zone				
	Mudflat	Nymphaea	Salix	SAV	Typha
Trees, groundcovers, or vines					
<i>Acer spp.</i>	1.66				
<i>Cyperus strigosus</i>	7.75				
<i>Eleocharis palustris</i>	5.34				
<i>Eleocharis spp.</i>	5.59				
<i>Leersia oryzoides</i>			1.69		
<i>Salix interior</i> (OS)			65.95		
<i>Salix interior</i> (US)			17.69		
<i>Salix nigra</i>			5.05		
<i>Salix spp.</i>	0.86				
<i>Vitis riparia</i>			6.31		
Invasive emergent species					
Dead <i>Typha</i>	34.98		5.57		44.04
<i>Lythrum salicaria</i>			3.12		2.11
<i>Persicaria maculosa</i>	1.66				
<i>Phalaris arundinacea</i>			16.24	2.84	2.03
<i>Phragmites australis</i>	11.14				
<i>Typha angustifolia</i>	2.53				63.37
Invasive trees, groundcovers, or vines					
<i>Lysimachia nummularia</i>			1.69		
Species Richness	24	12	22	11	15

APPENDIX 3.1: Invertebrate Families

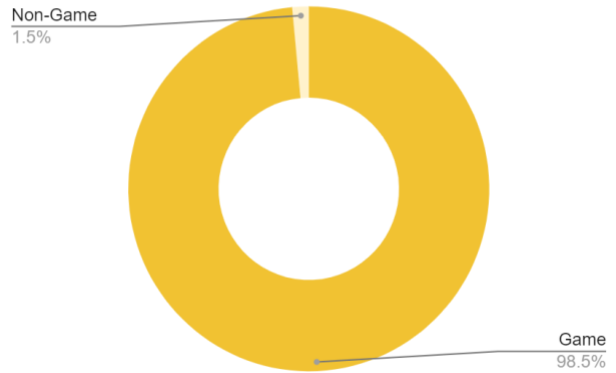
Please visit the University of Michigan’s Deep Blue website to view the macroinvertebrate data titled “SNWR_Macroinvertebrates2019” that was used to perform analyses.

APPENDIX 4.1: Game Species Unit Composition

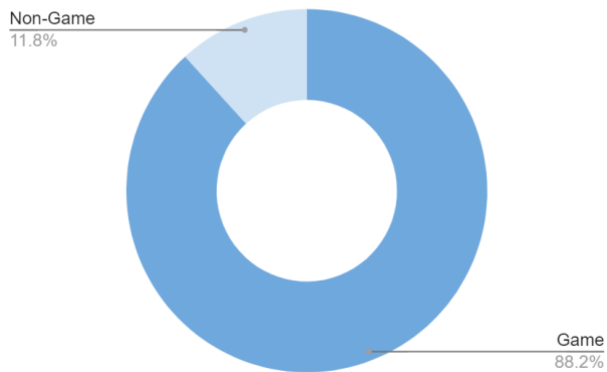
MS Game Fish



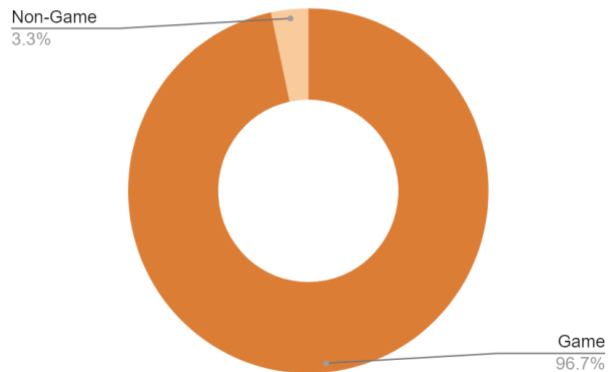
MN Game Fish



P1A Game Fish



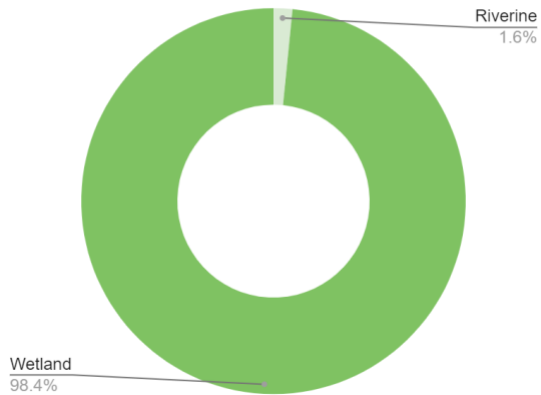
Total Game Fish



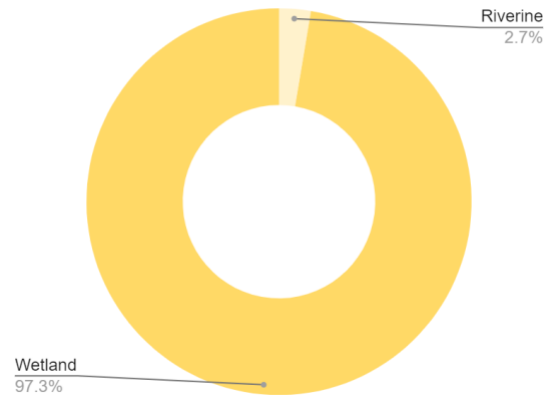
Game species percent composition of the wetland units. The majority of the CPUE of each wetland unit consists of game species. A game fish species is one that anglers value for recreational fishing according to the Michigan DNR.

APPENDIX 4.2: Wetland and Riverine Species Composition

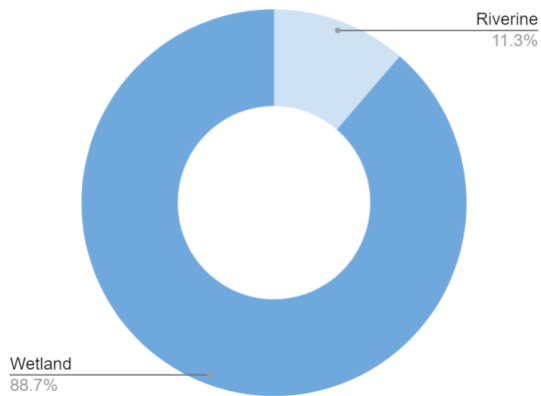
MS Fyke Net CPUE



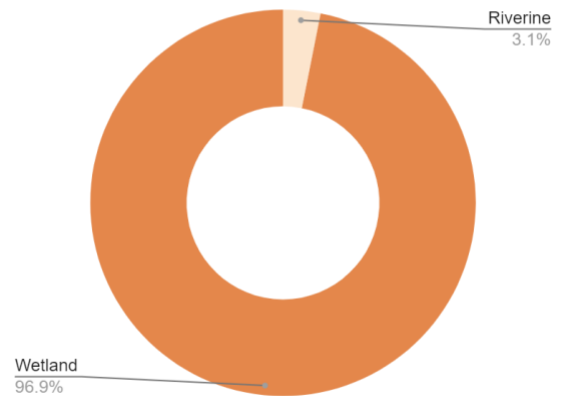
MN Fyke Net CPUE



Pool 1A Fyke Net CPUE



All Units Fyke Total



Wetland and riverine species percent composition of the wetland units. The majority of the CPUE of each wetland unit consists of mostly wetland species. Distinctions between the wetland/riverine species are based on those made by Jude and Pappas (1992).