UNIVERSITY OF MICHIGAN, SCHOOL FOR ENVIRONMENT AND SUSTAINABILITY

Restoring the Shiawassee Flats:

The Fourth Year of Ecological Monitoring Post Hydrologic Restoration at Shiawassee National Wildlife Refuge

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

April 2023

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ACKNOWLEDGEMENTS

We would like to offer our sincerest gratitude for the people, plants, invertebrates, and fish that helped to make this project possible. Thank you to our loved ones who have supported us through this journey. Our advisor, Paul Seelbach, has offered continuous support throughout our project. His dedication to our success and professional growth is not lost on us. We thank Paul for his guidance and expertise on all things fish and congratulate him on his well-earned retirement.

Additionally, we would like to thank our partners and clients U.S. FWS and USGS. Our Shiawassee National Wildlife Refuge family Eliza Lugten, Eric Dunton, and Scott Simmons have dedicated numerous hours of their time to keeping our team on track. We appreciate their willingness to get their hands dirty (actually) alongside our team, as well as offer encouragement. Their passion and commitment to the success of the refuge inspire us.

We offer thanks to USGS partners Sasha Bozimowski and Kurt Kowalski, both of whom have provided us valuable guidance and feedback. Your assistance and hands-on leadership helped us grow as professionals and create a rich team experience.

We would like to acknowledge those who offered behind-the-scenes support, efforts sometimes invisible to us. We are grateful for the time spent preparing for sample collections in the field, data preparation, analysis support, and the general thought-provoking conversation.

Lastly, we would like to thank all the clients for treating our team with respect, not only as students, but as equals who are making a meaningful contribution to Shiawassee National Wildlife Refuge.

EXECUTIVE SUMMARY

Our research took place at Shiawassee National Wildlife Refuge (SNWR) where recent hydrologic reconnection has restored a large area of floodplain wetland in the Shiawassee Flats region, where four major rivers meet before flowing into the Saginaw Bay. In this fourth and final year of post-restoration monitoring, our project team sought to assess the ecological health of the recently restored wetlands in comparison to reference conditions and previous years. Through a partnership with the U.S. Fish and Wildlife Service and U.S. Geological Survey, we were able to effectively carry out four months of ecological research monitoring centered around the study of four indicators of overall wetland health: water quality, vegetation, aquatic macroinvertebrates, and fish.

Our goals for 2022 were to evaluate ecological conditions indicative of the wetland's overall ecosystem health shortly after restoration in order to demonstrate the impact and value of SNWR's restoration investment. By collecting and analyzing a robust set of ecological data along with comparing conditions across years, our team is able to provide recommendations to refuge managers and provide the data to support this refuge's standing as a wetland restoration model for the Great Lakes region.

Water Quality: We analyzed five key water quality parameters: average temperature, average pH, average conductivity, average turbidity, and average dissolved oxygen, for variation across months, wetland management unit, and vegetation type. Additionally, we analyzed nutrient samples to characterize the relationship between turbidity and dissolved Nitrogen and Phosphorus concentrations throughout SNWR. Finally, we visually compared water quality and nutrient levels across all four years of post-restoration monitoring.

- We found consistent trends in water quality across 2019-2022; conductivity is higher in the Shiawassee River (SHR) and Pool 1A (P1A) than in Maankiki units, and Maankiki South (MS) consistently holds the lowest dissolved oxygen levels.
- In 2022, water quality varied significantly across season, management unit, and vegetation type which in turn impacts community assemblages and seasonal movements of invertebrates and fish which impact higher trophic levels.
- Dissolved oxygen (DO) is possibly the most salient determinant of species guilds among our five parameters and limits the range of more sensitive taxa within the refuge making preservation of areas of higher DO important to preserving overall species diversity.
- We found a significant correlation between pH and dissolved oxygen which is related to rates of autotrophy and heterotrophy.

- Turbidity still cannot be used as a reliable predictor for Phosphorus and Nitrogen concentrations, but in the future, using flow data as another predictor variable may help elucidate the relationship between Lake Huron seiche dynamics and upstream nutrient levels, or conversely the impact of upstream river water on nutrients at SNWR.
- Recommendations for management include increased sampling of water quality in certain vegetation types to better assess spatial heterogeneity, incorporating flow data into nutrient analysis, as well as preserving areas of high dissolved oxygen that are correlated with specific vegetation types.

Vegetation: We surveyed and identified the plant community at SNWR with the goal of describing the vegetative structure of each of the four wetland management units, as well as the overall ecological health of the vegetative community within the sampled area. We performed 190 individual quadrat samples, collecting between 35 and 55 sample points in each wetland management unit, and performed a variety of statistical analyses in order to compare and contrast biotic indices in the wetland management units amongst dominant vegetation zones, individual units, and among overall sampling years (2019-2021). We found that:

- The ecological health of SNWR's 4 wetland management units, while relatively low when compared with pristine pre-colonial reference conditions, is fairly high in the context of regional wetland quality.
- Indices of Biotic Integrity (IBI) scores have decreased since 2021 as a result of interannual discrepancies in data analysis and interpretation.
- Maankiki Center (MC), the most recently connected wetland management unit, had the lowest IBI assessment, and its 2022 metrics differed greatly from analysis performed in 2021.
- Floristic Quality Analysis (FQA) and Floristic Quality Index (FQI) scores have increased in every unit since the 2021 sampling season.
- Based on our data and results, we provide future recommendations for the management of these wetland management units, including the prioritization of invasive species management via removal and intentional flooding.

Aquatic Macroinvertebrates: We describe variations in macroinvertebrate abundance and community composition in SNWR and compare our findings to previous monitoring seasons, 2019-2021. Data is analyzed by unit, month, and vegetation zones of sampling.

- We collected macroinvertebrates from 55 sites and obtained fewer total individuals than previous years but maintained similar taxa richness.

- The month of sampling contributed to the total abundance of macroinvertebrates collected, likely due to seasonal changes in the environment that trigger transitions in life-cycle stage.
- P1A, the ‘reference’ unit, had a community composition different than that of the Maankiki Marsh units, which is likely attributable to invertebrate dispersal ability and time-since-reconnection (as it is the oldest restored unit).
- Vegetation type had little impact on macroinvertebrate abundance or composition across all units.
- When calculating macroinvertebrate IBIs, all wetland management units were considered “mildly impacted.” This represents an increase in IBI values from the previous sampling season (2021).

Fish: We monitored and surveyed wetland management units to characterize how fish populations are influenced by seasonal variation and interaction of other biotic and abiotic factors. Our research goals include assessing wetland health with IBI scores across wetland management units over the months of May, June, July, August, and October of 2022. Two gear types, fyke netting and electrofishing, were used to sample a wide variety of habitats within the wetlands, spanning various vegetation zones and water depths. There were a total of 108 fyke nets set and 31 electrofishing sites surveyed during the summer 2022 field season. We conducted statistical analysis to determine the implications of the data collected from monitoring. Statistical analysis allowed for an in-depth look at fish species abundance, community composition, and an assessment of the ecological wetland health.

- Despite dynamic filters that constrain the species pools of SNWR, the restored wetlands have recovered relatively quickly.
- The most recently restored wetland management unit, MC, is still more similar to SHR showing that there is a stage of colonization where species must compete to establish in the previously inaccessible habitat.
- IBI score assesses overall health of wetland management units based on species composition which ranged from moderately impacted (>45-50) to degraded (<36). Over the past four years, fish IBI has generally improved in the wetland management units.
- We surmise that the high fish abundance and various assemblages that we found support complex trophic interactions and high-quality ecosystem services. Trophic interactions between vegetation and macroinvertebrates are important factors in determining species composition and abundance.
- Island biogeography theory plays an important role in understanding the importance of unit connectivity and explains ecological variation by collective time of unit connection.

In the final year of post-restoration monitoring, our team was able to incorporate past years' data alongside data from 2022 to comprehensively characterize ecological conditions shortly after restoration. Overall, our findings suggest a successful response of biotic communities to hydrologic reconnection and enable us to make recommendations that support further habitat improvements and biodiversity.

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INTRODUCTION

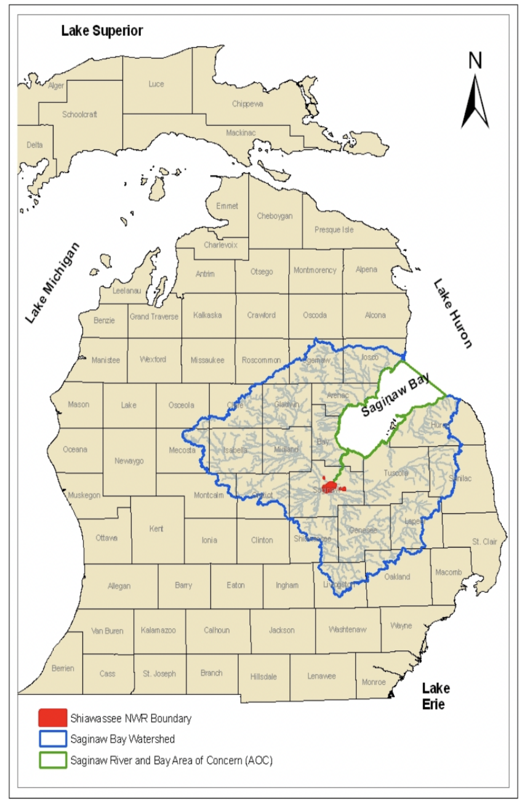


Figure 1: Map of the Saginaw Bay Watershed, illustrating the position of Shiawassee National Wildlife Refuge as a key focal point in terms hydrologic flow and connectivity in the region.

Influenced by the hydrologic dynamics of Lake Huron, and sitting at the confluence of four major rivers, the Shiawassee National Wildlife Refuge (SNWR) consists of 10,000 acres of protected land and important floodplain, representing an essential remnant of an increasingly fragmented ribbon of Great Lakes coastal wetlands. Historically consisting of more than 75,000 acres of hardwood swamp, emergent marsh, and wetland prairie, the setting of SNWR has undergone significant change since European influence arrived nearly 250 years ago (MNFI, 2022). Drained and converted to farmland as a result of the Swamp Lands Act of 1850 (Dahl & Allord, 1996), the wetlands at what is now SNWR, also called the Shiawassee Flats, spent the majority of the 19th and 20th centuries disconnected from the neighboring Shiawassee River. The flats remained primarily as farmland until the year 1953 when the newly formed U.S. Migratory Bird Conservation Commission authorized the initial annexation of 2,300 floodplain acres for the protection of crucial stopover habitat for migratory bird species (Department of the Interior, 1953). In response, the Shiawassee National Wildlife Refuge was established with the intention of rehabilitating this vast floodplain area as refugia for local and migratory waterfowl. Within 6 years of establishment, SNWR was able to enact a hydrologic-based restoration of the first ecological unit, Pool 1A (P1A), by connecting it to the Shiawassee River. Through the rest of the 20th century, SNWR continued to gradually expand its property and pursue the conversion of historic agricultural land into ecologically valuable wetlands and what we now know as the Maankiki units.

Located 18 miles inland from Saginaw Bay, Lake Huron, the Shiawassee National Wildlife Refuge is hydrologically unique because of its relationship with Lake Huron and its distinct role within the Saginaw Bay watershed (Figures 1 & 2). SNWR sits within the Shiawassee Flats, a historic glacial lake plain with a unique location and topographic qualities creating extremely complex and interesting hydrology. The Flats are at an extremely low elevation (about

176-181m), only slightly higher than that of Saginaw Bay (175m). This low elevation, which extends from Saginaw Bay upstream to the Shiawassee flats, makes the entire system inextricably linked to Lake Huron and its seiche dynamics; as displacement from wind currents creates uneven water distribution, the waters of Lake Huron move from one side of the lake to the other. The associated pressure and water levels generated from this activity are often high enough to force water to flow up the (very flat) Saginaw River in the opposite direction of traditional downriver flow. This means that SNWR and its surrounding areas feel the effects of Lake Huron's hydrologic dynamics as if they were located just a few miles from Huron's shoreline, making the refuge a true freshwater estuary with high levels of productivity, allowing the area to be a crucial spawning ground for migratory Great Lakes fish species (Buchanan et al., 2013; Larson et al., 2016).

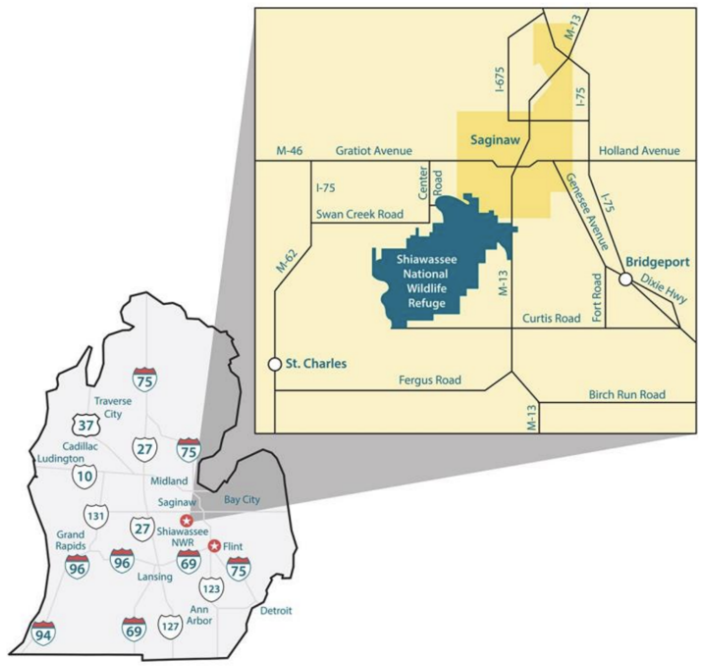


Figure 2. Location of Shiawassee National Wildlife Refuge relative to Saginaw and surrounding cities within Michigan's lower peninsula.

In addition to its proximity to Saginaw Bay, the Shiawassee Flats are centrally and strategically located within Michigan's largest watershed. The Flats lie at the intersection of a multi-river system, namely the Cass, Flint, Shiawassee, and Tittabawassee rivers; which converge to form the Saginaw River, flowing out to Saginaw Bay and Lake Huron roughly 20 miles downstream (Figure 1). The Saginaw River watershed drains roughly 12% of Michigan's land area, making the SNWR a focal point of a staggering river discharge (U.S. Fish and Wildlife Service, 1999). This means that the Shiawassee Flats and SNWR's restored floodplains can contribute tremendously to local flood mitigation. For example, during the historic 2020 flooding that resulted from the collapse of the Sanford and Edenville dams on the Tittabawassee River, water levels across the 10,000-acre refuge raised roughly 10 feet; protecting downriver property, agriculture, and livelihoods from destruction (Ducks Unlimited, 2020).

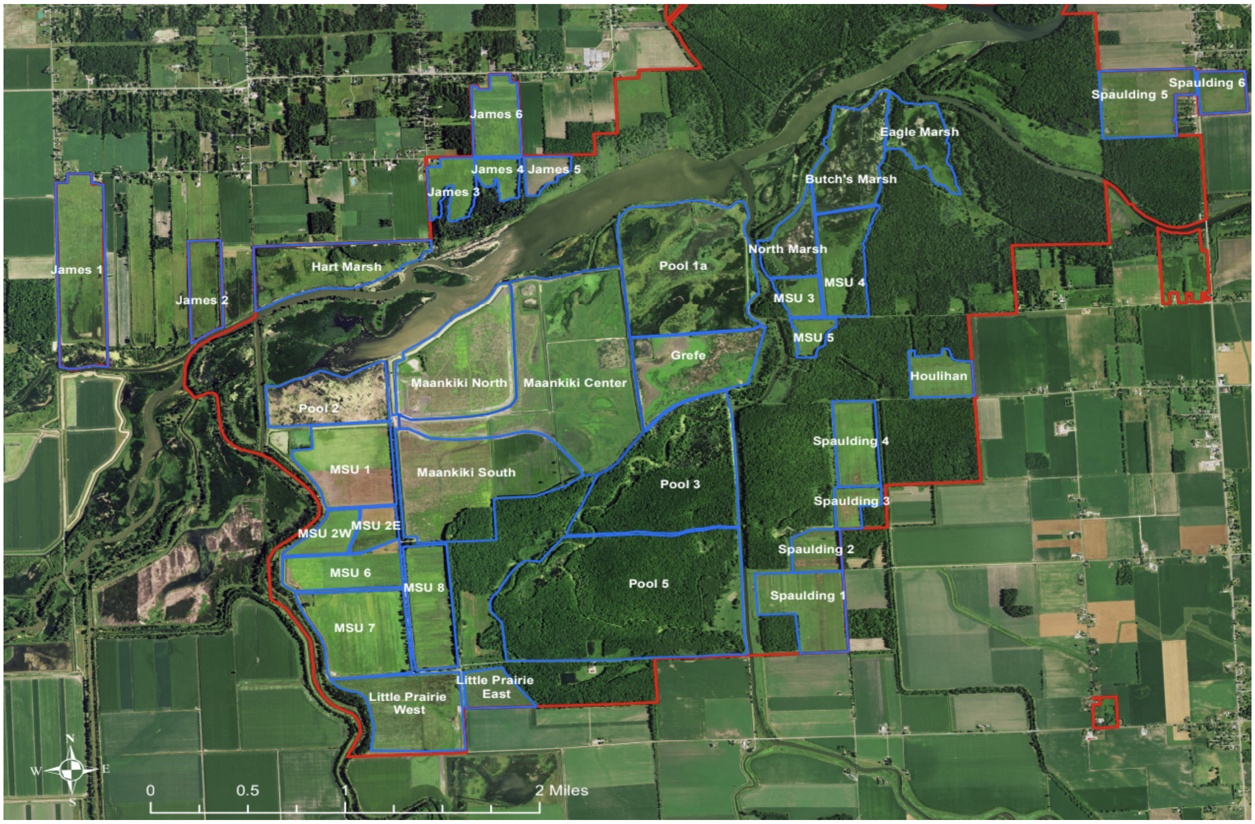


Figure 3. Aerial view of named wetland management units (blue boundaries) within the SNWR (red boundaries).

The Shiawassee Flats, and SNWR, provide a broad range of ecosystem services to the surrounding area (Figure 3). Coastal wetlands like the Shiawassee Flats provide supporting ecosystem services that help regulate climate in both the sequestration of carbon and the fixing of nitrogen into N_2, making them essential habitats with necessary biological tools in the fight against climate change (Debusk, 1999; Kusler and Christie, 2011). Their anaerobic soils and saturated hydrologic conditions slow the process of decomposition, trapping gasses like carbon dioxide and methane and sequestering decomposing organic waste on a magnitudes-longer scale than they might persist in other ecosystems (Dronova et al., 2021). Shiawassee National Wildlife Refuge's wetlands also host a variety of native plant species, many of which are highly effective in the absorption and storage of inorganic nutrients like phosphorus and nitrogen. These nutrients, used in agricultural fertilizers enter watersheds via runoff and have the potential to create biological hazards (such as harmful algal blooms) in freshwater environments, making the refuge's floodplain area critical in the function of water filtration for downriver aquatic environments, including Saginaw Bay. SNWR also supports provisioning ecosystem services by providing habitat for migratory waterfowl and spawning Great Lakes fish species. In turn, these wetland plants provide food for breeding and migrating waterfowl and provide the basis for aquatic food webs that support important Great Lakes fishes such as Yellow Perch *(Perca* *flavescens)*. Additionally, primary and secondary producers, vegetation and macroinvertebrates respectively, create trophic links to primary and secondary consumers, including many of the fish and bird species found at SNWR and throughout the Saginaw Bay watershed (Baxter et al., 2005). In turn, aquatic and riparian food webs have a direct link through piscivorous and insectivorous species. In fact, 75 to 90% of Great Lakes fish species use coastal wetlands for part of their life cycle including economically valuable species present as SNWR such as Northern Pike *(Esox lucius)*, Walleye *(Sander vitreus)* and Yellow Perch *(Perca flavescens)* (Sierszen et al., 2012).

Heavily reliant upon artificial dikes to prevent overbank flooding events from the neighboring Shiawassee, Flint, and Tittabawassee Rivers, agricultural fields within the boundaries of SNWR were actively farmed as recently as 2016. In 2011, the US Fish and Wildlife Service, in conjunction with Ducks Unlimited and the Great Lakes Restoration Initiative (GLRI), received approximately 1.5 million dollars in funding to hydrologically reconnect the farm field floodplains to the Shiawassee and Flint rivers (U.S. Fish and Wildlife Service, 2018). The recent, and ongoing, restorative actions at SNWR focus on the restoration of former agricultural lands and enhancement of existing wetland management units via hydrological reconnection of diked floodplains wetland management units with the Shiawassee River. Controlling flows within the refuge is done via water control structures that allow refuge managers to open and close specific wetland management units to the river to achieve desired habitat conditions despite fluctuating water levels. Managers use these mechanisms to provide a variety of shallow and deep water habitats which provide critical habitat for species at SNWR. Specific control decisions depend on seasonal conditions and management objectives. While an undisturbed, "pristine" floodplain ecosystem lacks such control mechanisms, the level of systemic degradation across Shiawassee's floodplain habitats today necessitates active management. By controlling water levels in the refuge, biologists can mimic seasonal hydrology to provide quality habitat, especially as climate change and low water levels provide added stress to many wetland species. Three SNWR wetland management units, Maankiki North, Maankiki South, and Maankiki Center, were reconnected to the Shiawassee River in 2017, 2018, and 2020, respectively.

To capture the ongoing changes in the biological communities of restored SNWR floodplain wetlands since its 2016 restoration, this project continued the University of Michigan School for the Environment and Sustainability's (UM SEAS) relationship with the U.S. Fish and Wildlife Service as its final year of post-restoration monitoring. To quantify and understand the effects of the reconnection, the USGS and the University of Michigan began annual post-restoration monitoring in 2019. Four years of biological monitoring, including collecting, measuring, and documenting fishes, aquatic macroinvertebrates, plant communities, and water quality, have been completed. While SNWR still functions primarily to provide quality habitat to migratory birds like waterbirds (ducks, geese, swans, shorebirds, wading birds), passerines, neo-tropical songbirds, and a variety of other wildlife species, increased research and examination of the refuge's influence on local aquatic biodiversity and hydrology are now beginning to broaden its regional importance.

The long-term intent of this project is to illustrate and evaluate the efficacy of coastal wetland restoration in freshwater environments so that SNWR might serve as a model for other floodplain restoration projects in the Great Lakes region. Through the fourth and final year of post-restoration monitoring, summer/fall 2022, the goals of our study were to: (1) demonstrate the impact and value of SNWR's restoration investment; (2) evaluate ecological conditions indicative of the wetland's overall ecosystem health after only a few years of recovery; and (3) provide recommendations to SNWR leadership for effective wetland management moving forward (4) serve a wetland restoration model for the Great Lakes region.

We aimed to quantify conditions and associated variation for an array of biotic and abiotic characteristics across several SNWR wetland management units, to understand and characterize how, (and how quickly), hydrologic reconnection impacts wetland conditions and wildlife communities. In discussing variables such as water quality, vegetation distribution, aquatic macroinvertebrate populations, and the seasonal and spatial distribution of fish communities; we hope to fully elucidate the ecological and holistic benefits of hydrologic reconnection on wetland communities.

STUDY SITES

Within SNWR, 36 identified wetland management units are delineated based on hydrologic criteria and habitat management goals. Unit classifications at SNWR include floodplain forests, deep water pools, moist soil wetland management units, emergent marshes, prairies, cropland, and riverine areas (U.S. Fish & Wildlife Service, 2018). Wetland management units classified as deep-water pools or emergent marshes are contained by earthen dikes, which prevent the natural flow of surface water (e.g., in or out from neighboring rivers) and can limit the dispersal of certain species. To remedy this, water control structures (gates within dikes) have been implemented in some wetland management units, allowing refuge managers to strategically control water flow between wetland management units.

The focus of our study was the four wetland management units that were included in the 2016-2020 restoration initiative: Maankiki South (MS), Maankiki Center (MC), Maankiki North (MN), and Pool 1A (P1A). The oldest site of hydrological reconnection, P1A, houses the first water control structure on the refuge (operational starting in 1958), connecting this unit to the Shiawassee River. Because this unit has maintained a consistent hydrologic connection with the river for over six decades, it acts as a study control site for potential ecological conditions and variability. The Maankiki Marsh units, MS, MC, and MN, are the subject of the recent

(2016-2019) hydrologic reconnections through the addition of several water control structures connecting these adjacent wetland management units with the Shiawassee River.

Since the restorative actions began in 2016, refuge management has tracked the response of biotic communities across the wetland management units to monitor progress toward habitat management goals. A *Typha*-dominated wetland, MS, underwent its first flooding event in 2017. MN, like MS, underwent its first whole flooding event in 2017 and is also dominated by *Typha*. Maankiki Center's water control structure was completed in 2019, and the unit is dominated by woody vegetation, emergent wetland plants, and *Phalaris*. Lastly, P1A is dominated by *Typha* and *Nymphaea*, and managers attempt to leave this water control structure open to allow for natural fluctuations in water level. Interestingly and important to SNWR's mission to protect habitat for migratory waterfowl, P1A seasonally hosts the highest concentration of water bird species, potentially related to its long-term connection with the Shiawassee River (U.S. Fish & Wildlife Service, 2018).

Within the four wetland management units, we took monthly measurements of three aquatic ecosystem elements (water quality, aquatic macroinvertebrates, and fish). Vegetation sampling was carried out in August only, as this is towards the end of the growing season. At that time, plant communities and individual plant structures (such as flowering bodies) are more fully developed, making for easier identification. In addition to the four wetland management units, we also sampled fish in the Shiawassee River (SHR) and Spaulding Drain (SPD). The collection and measurement of fish within SHR occurred using two different methods per month: electrofishing and fyke netting. In SPD, collection and measurement occurred only once per month using electrofishing.

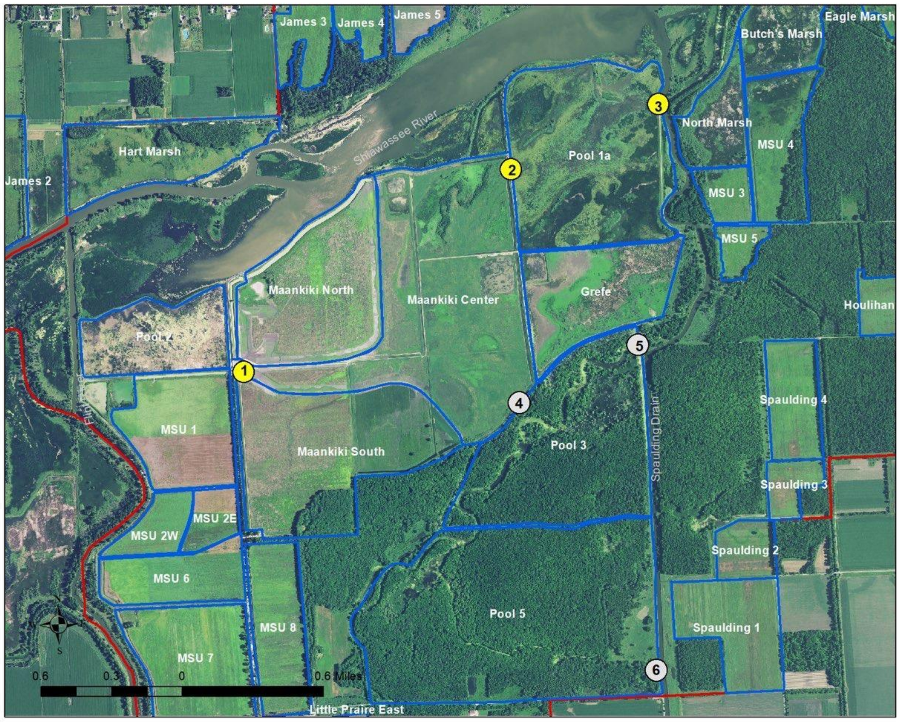


Figure 4. Aerial view of SNWR and water control structure points. Yellow points denote existing structures and gray points denote future structures

OVERALL METHODS

To characterize current biological conditions of restored wetland management units and make comparisons to previous monitoring years, we analyzed four key parameters that can broadly indicate wetland ecosystem health: water quality, vegetation, aquatic macroinvertebrates, and fish. Our field sampling methods were generally adapted from protocols developed by the Great Lakes Coastal Wetland Monitoring Program (CWMP), with changes made by previous master's project teams to best sample Shiawassee's unique habitats. Individual sampling protocols were developed for each monitoring parameter by SNWR staff, with additional adaptations by previous UM-SEAS project teams (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). Some sampling equipment was used across multiple parameters. Water quality parameters were recorded with a YSI EXO 3 handheld multiparameter sonde at all macroinvertebrate and fish sampling sites. Additionally, the quadrat ($1m^2$) used to standardize spatial sampling coverage was utilized for macroinvertebrate and vegetation sampling.

Sampling was conducted during summer months, May through August of 2022. We conducted electrofishing several days per month in June, July, and August; collected aquatic macroinvertebrate samples in May, June, July, and August; recorded vegetation presence in August; and carried out fyke netting for two consecutive 24-hour periods during May, June, July, and August; and in October to assess seasonal shifts in fish assemblages. Gill netting occurred in February, March, April, May, June, and July. In-situ water quality measurements were collected at each site before sampling for fish and invertebrates, therefore sampled in every sampling month.

DATA MANAGEMENT

Except for our nutrient samples which were collected in the field and shipped to a water chemistry laboratory for analysis, all data were recorded in the field using the ArcGIS online data collection platform, Survey123, via iPads or smartphones (ESRI Inc., 2022). Data underwent quality assurance and control (QA/QC) by our partners at the SNWR and U.S. Geological Survey, Great Lakes Science Center. QA/QC procedures included inspecting invertebrate data for unlikely identifications, and examining quantitative data such as water quality measures and fish counts for outliers potentially due to human entry error. Data were then exported as Microsoft Excel files for final analyses. QA/QC for invertebrate identification followed a similar process, except that invert identifications were initially noted on paper in the lab and then entered into Survey123, which was subsequently followed by an internal review to ensure that all data were entered correctly. Excel files were then further restructured using Microsoft Access if necessary based on the guidance of Biological Technician Eliza Lugten and USGS Ecologist Alexandra Bozimowski. Once the data structure was appropriate based on standards set by USGS and FWS guidelines, these files were converted to .csv so they could be readily used in R Studio statistical software. Most R code used in our statistical analysis was adapted from versions drafted by the previous year's field team and can be found in Appendix I (Conrad et al., 2022).

WATER QUALITY MONITORING

INTRODUCTION

Water quality is crucial in dictating overall wetland structure and health, and the abiotic factors that determine it ultimately influence what types of biotic communities can exist. Recently, measuring nutrient levels has been particularly important in the Great Lakes region and beyond, as it is a proxy for understanding and predicting harmful algae blooms (Baustian et al., 2018). Phosphorus loads in tributary rivers have been shown to predict the extent of harmful algal blooms in Lake Erie, which can significantly affect human health (Bertani et al., 2016). Furthermore, coastal floodplain wetlands like SNWR have the ability to retain nutrients, thus acting as a net nutrient sink, reducing overall loads into the great lakes (Carter et al., 2022). Water quality typically has a positive association with biodiversity making it an essential area of focus for wildlife managers (Weaver & Fuller, 2007). At SNWR, water quality is dynamic and influenced by many factors, including wetland depth and degree of connectivity with the Shiawassee River. Initially, P1A was connected to the Shiawassee River via water control structures; recent hydrologic connections of Maankiki North, South, and Center make much of the floodplain wetlands subject to outside influences. Water levels and water quality in the upstream rivers and downriver Saginaw Bay, as well as nearby Lake Huron, influence water conditions in wetlands at Shiawassee. However, these effects are still being measured and quantified, and comparing recently reconnected wetland management units to P1A provides valuable insight.

During the 2022 summer field season, we replicated past years' sampling techniques by conducting in-situ measurements of 5 key water quality parameters at various sites throughout the refuge. Our team took water quality readings at sites chosen for electrofishing, fyke netting, and invertebrate sampling. Variables were: temperature, pH, conductivity, turbidity, and dissolved oxygen. Additionally, nutrient samples were taken to track changes in Nitrogen and Phosphorus levels between locations and across years and to characterize the relationship between turbidity (a likely indicator) and nutrient levels.

Research Objectives

- *Characterize water quality parameters among wetland management units, months, and vegetation zones.*
- *Investigate potential correlations between temperature, pH, conductivity, turbidity, and dissolved oxygen.*
- *Determine the impact of month, wetland management unit, and vegetation type on our five water quality parameters.*
- *Determine the relationship between turbidity variation and nutrient levels (phosphorus and nitrogen) in the wetland management units, control structures, and Shiawassee River, and evaluate the potential for using turbidity as a proxy for nutrient concentrations*
- *Evaluate annual variation in nutrient levels at each monitoring location between 2019 and 2022*

METHODS

Sonde-Based Field Sampling of WQ Parameters

We followed procedures for measuring water quality parameters developed by SNWR staff and used by previous master's project student teams (Vogel et al., 2021). In situ water quality measurements were conducted using YSI EXO 3 handheld multiparameter sonde devices, which measured: temperature in degrees Celsius, pH in a standard unit, conductivity in µS/cm, turbidity in FNU, and dissolved oxygen in mg/L. We took water quality measurements at electrofishing, fyke netting, and invertebrate sampling locations (Table 1 and 2). At each sampling location, depth was recorded using a marked PVC pipe. Date, unit, latitude and longitude coordinates, and vegetation type were also recorded. Sonde readings were triplicated, with the average values recorded to account for potential sources of single-sample error. For example, we occasionally stirred up the benthic sediment by walking along the bottom of a sample site, which often induced significant increases in turbidity. Surprisingly, distinct thermoclines appeared at multiple sampling sites despite their relatively shallow depth and limited surface area (Gorham & Boyce, 1989). Significant temperature variations in a narrow depth range necessitated multiple measurements at each site.

Nutrient Sampling

We collected nutrient samples at all four wetland management units, the Spaulding Drain and Shiawassee River once each month from May to July. Sample jars were put in an ice chest before being sent to Heidelberg's National Center for Water Quality Research via FedEx for analysis of a wide variety of nutrient levels. Additionally, sonde sampling was performed at each nutrient sampling location in a manner consistent with sonde sampling for other monitoring types. The primary use of the laboratory nutrient data we received is to analyze the relationship between turbidity and two important nutrients, Total Phosphorus (TP) and Total Kjeldahl Nitrogen (TKN).

Data Management

Data for water quality collected in the field via handheld sonde was then input into ArcGIS's Survey123 platform via iPad. These data were then subjected to quality assurance and quality control measures (QA/QC) by our SNWR and USGS research partners to produce final data sets in Microsoft Excel that included variables: month, unit, vegetation type, and our five key parameters: average temperature, average pH, average conductivity, average turbidity, and average dissolved oxygen. Samples analyzed by the Heidelberg University National Center for Water Quality Research were QA/QC'd by our partners for sample site accuracy, providing data on turbidity and nitrogen and phosphorus concentrations in Excel.

Data Analysis

We performed all data analyses using the open-source platform R and adapted code developed by Conrad et al. (2022). The code used to perform statistical tests and produce boxplots was developed by Kuiran Zhang (previous student team member) and Alexandra Bozimowski (USGS) before being modified to fit this year's data and include a general linear model for the first time. We first produced box plots illustrating variation in water quality by vegetation, month, and unit to help visualize variation in conditions across space and time at the refuge.

Correlation Analysis

We used Pearson Correlation Analysis to investigate the potential correlation between our five independent variables: Temperature, pH, conductivity, turbidity, dissolved oxygen as well as depth in cm. This tool can help managers infer overall conditions from a single measure by quantifying the relationship between variables. The R package "Hmisc" was used.

ANOVA

Using our full dataset of 249 in situ water quality observations, we ran three sets of five one-way ANOVAs, (15 in total), to analyze the effects of three independent variables: month, monitored location, and vegetation zone on our five water quality parameters. Assumptions of normality and equal variance between samples were tested using Levene's test and "qqplot" to visually test for normality. To interpret the results of our ANOVA, we then utilized Tukey-HSD tests to analyze which independent variables varied significantly from one another concerning their influence on the dependent variables. R packages "carData", "car", "dplyr", "tidyverse", and "ggstatsplot" were used.

General Linear Models and Linear Regressions

Due to the non-linear nature of our nutrient data we used generalized linear models (Family = "Gamma", Link = "Log") in addition to traditional ordinary least squares linear regressions to find models that best fit our data. For each form of linear model, we created models using both Total Phosphorus (TP) and Total Kjeldahl Nitrogen (TKN) concentrations as response variables and turbidity, (FNU), as the explanatory variable, in order to characterize the relationship between turbidity and nutrient levels using data from 2019-2022. R packages "lmtest", "car" and "dplyr" were used.

Table 1. Number of samples collected by management unit and numerical month. SPD is underrepresented due to it not being sampled for vegetation, aquatic macroinvertebrates, or fish. However, it is connected directly to SHR and can therefore be viewed as a river unit.

Unit						Month					Total Samples
SHR	**SPD**	**P1A**	**MS**	**MC**	**MN**	**5**	**6**	**7**	**8**	**10**	
43	6	29	64	58	49	46	60	57	62	24	249

Table 2. Number of samples collected by dominant vegetation type. Vegetation type abbreviation are as follows: M.E. (mixed emergent), SAV (Submerged Aquatic Vegetation), R.B. (River Bulrush). *Nymphaea*, River Bulrush, and *Phalaris* represent relatively small percentages of overall wetland vegetation cover, while over half of sampled sites were SAV.

Vegetation Type							
Open Water	**M.E.**	**SAV**	***Typha***	***Nymphaea***	**R.B.**	**Forest**	***Phalaris***
47	15	133	30	4	5	13	2

RESULTS

ANOVA Summary

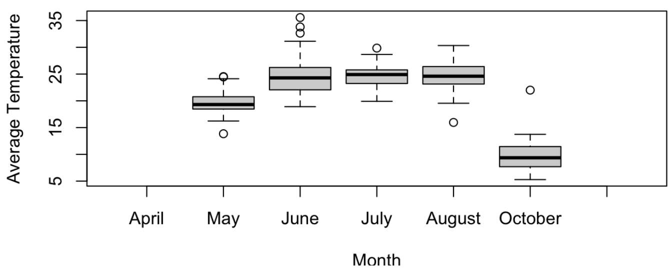
We ran fifteen One-way ANOVAs to analyze whether independent variables of month, vegetation zone, and wetland management unit had significant impacts on each of our five water quality parameters. Overall, we found significant differences among groups in fourteen of fifteen analyses; with only evaluation of the impact of month on turbidity not yielding a statistically significant result (Table 3).

Table 3. Each water quality parameter is a dependent variable; Month (April-Oct), Vegetation Zone (Submerged Aquatic Vegetation (SAV), Typha, Nymphaea, Forest, Phalaris, River Bulrush, Mixed Emergent Vegetation, Salix), and Unit (MC, MN, MS, P1A, SHR, and SPD) are independent variables. Significance codes are: [0, 0.001] " *** ", (0.001, 0.01] " ** ", (0.01, 0.05] " * ", (0.05, 0.1] "." P-values less than 0.05 are highlighted in bold. F-values represent the ratio of between sample variation over within sample variation, parenthetical values represent between groups' degrees of freedom and within groups' degrees of freedom, respectively. All three independent variables significantly influence each of the five dependent variables with the exception of sampling month and turbidity.

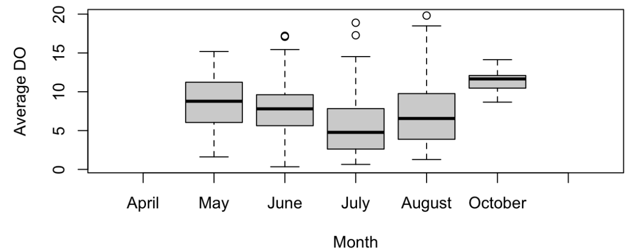
Water Quality Parameter	**Monthly Variation**		**Wetland Unit Variation**		**Vegetation Zone Variation**	
	F-value	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Temperature (°C)	F(4,244)= 160.2	**<2e-16 *** **	F(5,243) = 2.901	**0.0145***	F(7,241)= 2.711	**0.0101 ***
DO (mg/L)	F(4,244) = 11.85	**8.17e-09 *** **	F(5,243) = 7.807	**7.82e-07 *** **	F(7,241)= 7.979	**1.03e-08 *** **
pH	F(4,244) = 3.053	**0.0176 ***	F(5,243) = 11.95	**2.35e-10 *** **	F(7, 241)= 5.504	**6.93e-06 *** **
Conductivity (uS/cm)	F(4,244) = 11.58	**1.27e-08 *** **	F(5,243) = 57.49	**<2e-16*****	F(7, 241)= 7.924	**1.19e-08 *** **
Turbidity (FNU)	F(4,244) = 0.392	0.814	F(5,243) = 4.102	**0.00136 ** **	F(7, 241)= 3.408	**0.00172 ** **

Monthly Variation

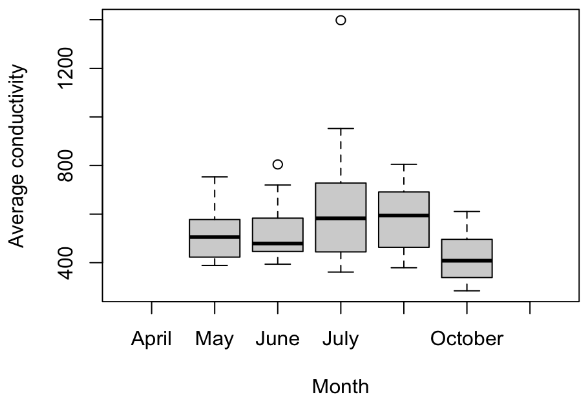
Sampling month significantly impacted temperature, dissolved oxygen, pH, and conductivity (Table 3). While variation among the summer months was relatively modest for these four parameters, including October data revealed a dramatic seasonal shift in abiotic conditions between summer and fall at SNWRs wetlands (Figure 5). Notably, temperature and conductivity fell considerably between the summer months and October, while dissolved oxygen showed a marked increase (Figure 5). Temperatures varied significantly across all months, stepping up from May to June, then holding steady through August, with the high temperature of 35°C occurring in June (Figure 5a). The low temperature occurred in October at 5°C (Figure 5a). Dissolved oxygen decreased from May to July, where it bottomed out around 5 mg/L before increasing to its October peak of over 10 mg/L; therefore showing a strong seasonal pattern (Figure 5b). Conductivity varied modestly yet significantly across most months, showing a slight rise throughout the summer before plummeting in October, with the maximum outlier value occurring in July at 1398 (Figure 5c). On the other hand, pH varied little between months averaging slightly below 8, with only October experiencing a significantly greater pH than July (Figure 5d). No significant differences were found between months for turbidity and multiple high outlier values were found each month (Figure 5e).



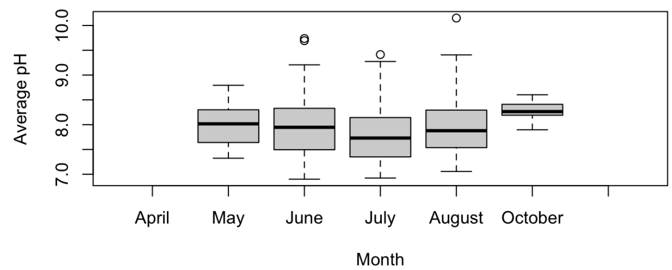
(a)



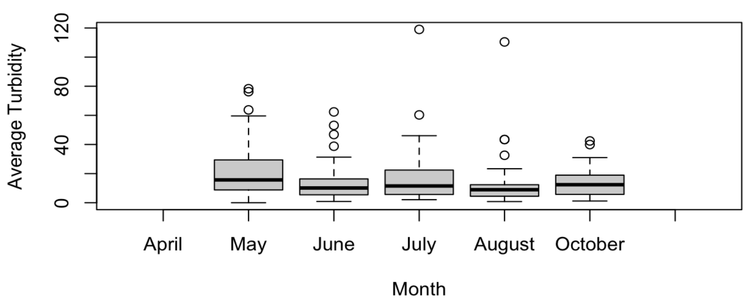
(b)



(c)



(d)



(e)

Figure 5. Boxplots display sampled values for five water quality parameters (y-axis) by month (x-axis). Black bars within gray boxes represent median values, and the boxes represent the interquartile range from the 25th to the 75th percentile (IQR), and the lower and upper whiskers mark Q1 - 1.5 x IQR and Q3 + 1.5 x IQR respectively. Points above whiskers are outlier values. Plots are: (a) average temperature(°C); (b) average dissolved oxygen (mg/L); (c) average conductivity (μS/cm); (d) average pH; and (e) average turbidity (FNU). Temperature, DO and conductivity show especially strong seasonal trends. No significant differences were found between turbidity and month.

Variation by Wetland Unit

All five water quality parameters varied significantly with respect to the wetland management unit (Table 3). Temperature varied the least across all parameters, only differing significantly between P1A and MS as well as between P1A and SHR, with P1A having the highest average temperature (Figure 6a). pH varied significantly across several wetland management units, with MS having lower values than MN, MC, and SHR (Figure 6b). Conductivity varied significantly across almost all wetland management units, with sites adjacent to or within the Shiawassee River- SHR, P1A, and SPD - having significantly higher average conductivity values than most other wetland management units (Figure 6c). Turbidity did not vary as widely across wetland management units, with only the least turbid unit, MS, showing significantly lower average values than MC, MN, and P1A (Figure 6d). Dissolved oxygen levels showed a similar trend to turbidity with MS anoxic waters significantly lower in average DO than all other wetland management units, excluding SPD (Figure 6e).

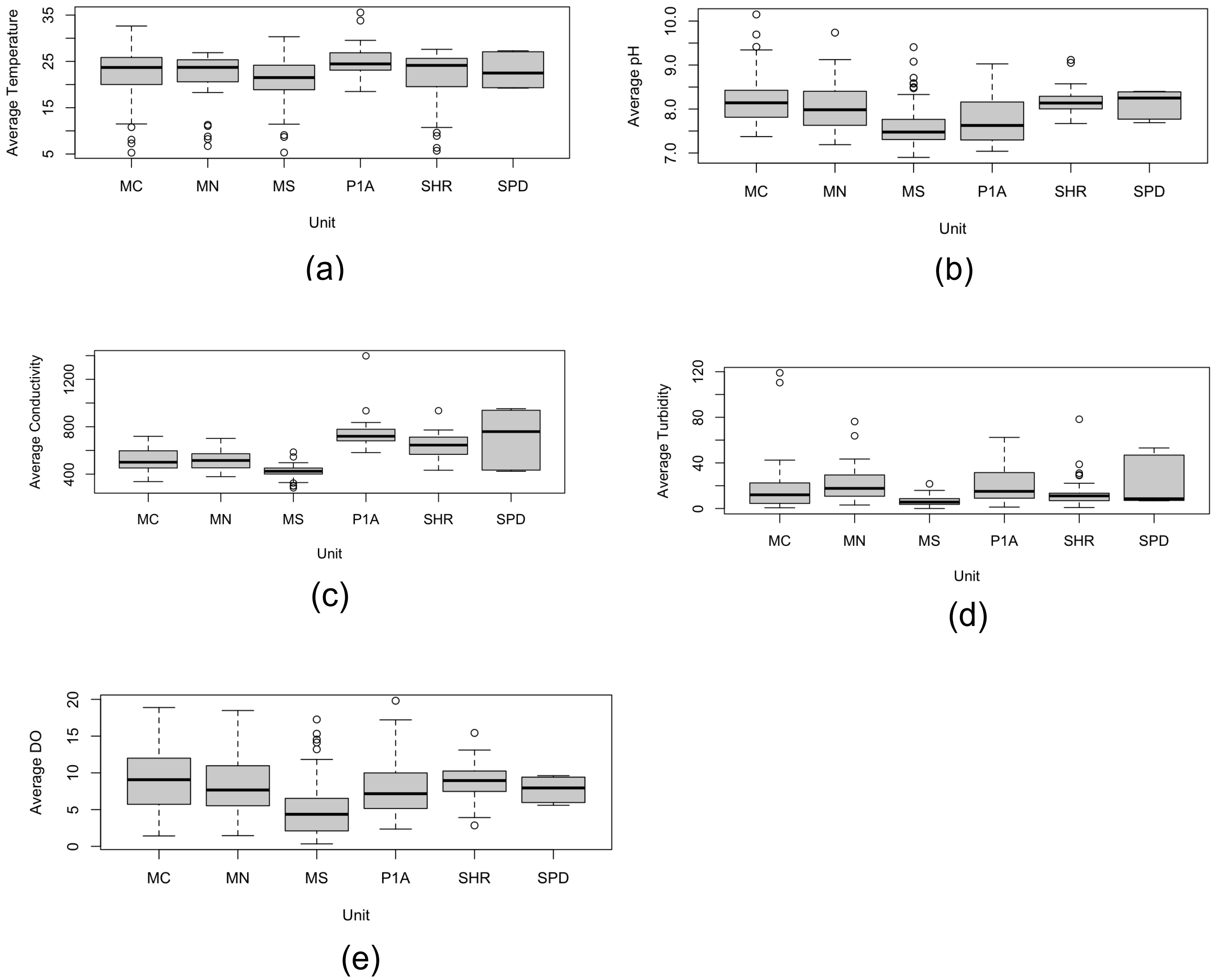


Figure 6. Boxplots display sampled values for five water quality parameters (y-axis) by wetland management unit (x-axis). Abbreviations refer to wetland management units and river locations: MN, MS, MC, P1A, SHR and SPD. Black bars within gray boxes represent median values, and the boxes represent the interquartile range from the 25th to the 75th percentile (IQR), and the lower and upper whiskers mark Q1 - 1.5 x IQR and Q3 + 1.5 x IQR respectively. Points above whiskers are outlier values. Plots are: (a) average temperature(°C); (b) average pH; (c) average conductivity (μS/cm); (d) average turbidity (FNU); and (e) average dissolved oxygen (mg/L). pH, conductivity, and dissolved oxygen show the strongest relationships with the wetland management unit.

Variation by Vegetation Type

All water quality parameters varied significantly across vegetation zones. Temperature varied the least across zones, with no group significantly different from any other despite overall statistical significance (Table 3), (Figure 7a). Dissolved oxygen, pH, and conductivity, on the other hand, varied significantly across dominant vegetation types (Table 3). pH was highest at *Phalaris* sites and lowest at Forest sites (Figure 7b). As a result, Forest sites differed significantly from most other vegetation types in average pH (Figure 7b). Average conductivity varied significantly among vegetation types, with *Nymphaea* exhibiting the highest average conductivity and Mixed Emergent the lowest (Figure 7c). For dissolved oxygen levels, Forest once again diverged significantly from other vegetation types, with levels significantly lower than at Mixed Emergent, Open Water, *Phalaris* , River Bulrush, SAV, and *Typha* sites (Figure 7d). Vegetation type also significantly impacted average turbidity, with Open Water sites associated with significantly higher turbidity levels than Mixed Emergent and Forest (Figure 7e).

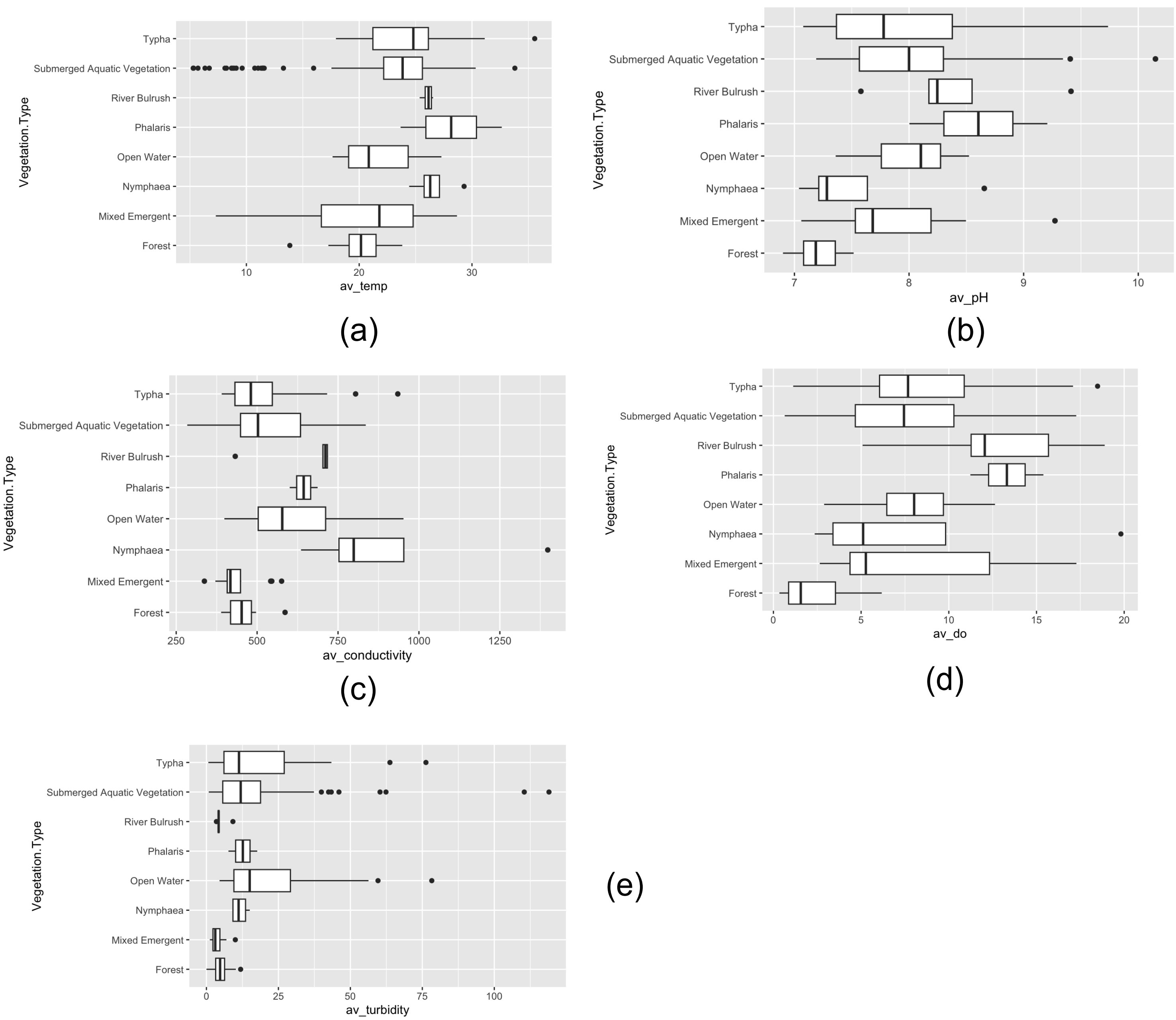


Figure 7. Water quality parameter by dominant vegetation type. Boxplots display sampled values for five water quality parameters (x-axis) with the prefix "av_ " representing average, temp representing temperature, and do representing dissolving oxygen. Y-axis labels display the dominant vegetation zone. Black bars within gray boxes represent median values, the boxes represent the interquartile range from the 25th to the 75th percentile (IQR), and the lower and upper whiskers mark Q1 - 1.5 x IQR and Q3 + 1.5 x IQR respectively. Points above whiskers are outlier values. Plots are: (a) average temperature(°C); (b) average pH (mg/L); (c) average conductivity (µS/cm); (d) average dissolved oxygen; and (e) average turbidity (FNU). DO, pH, and conductivity varied most significantly with vegetation type.

Correlation Analysis

We used a Pearson correlation analysis to identify significant correlations between pairs of our five water quality parameters. We observed significant correlations for four variable pairs (table 4) (figure 8). We found strong correlations between pH and dissolved oxygen ($r= 0.84$, $p<0.000$), and conductivity and temperature ($r= 0.43$, $p<0.000$) (table 4). We also found weak correlations between turbidity and dissolved oxygen ($r= 0.15$, $p<0.05$) and pH and conductivity. ($r= -0.18$, $p<0.01$). While all of these correlations are statistically significant, the latter 2 had correlation coefficients that were too small (<0.2) to warrant consideration.

Table 4. Pearson correlation coefficient table for water quality parameters. Significance codes refer to the statistical significance of a correlation between two parameters: 0 " ***, "0.001 " **, "0.01 " *, "0.05 ". Statistically significant values are bolded ($p < 0.05$). Four pairs of variables were significantly correlated; DO and pH, conductivity and temperature, turbidity and conductivity, and pH and conductivity. pH and DO showed the strongest correlation.

	Average Temperature	Average Dissolved Oxygen	Average pH	Average Conductivity	Average Turbidity	Average Depth
Average Temperature	1.00	-0.10	0.01	0.43***	0.00	-0.13
Average Dissolved Oxygen		1.00	0.84***	-0.06	**0.15**	-0.05
Average pH			1.00	-0.18*	0.07	0.10
Average Conductivity				1.00	0.22**	-0.11
Average Turbidity					1.00	-0.09
Average Depth						1.00

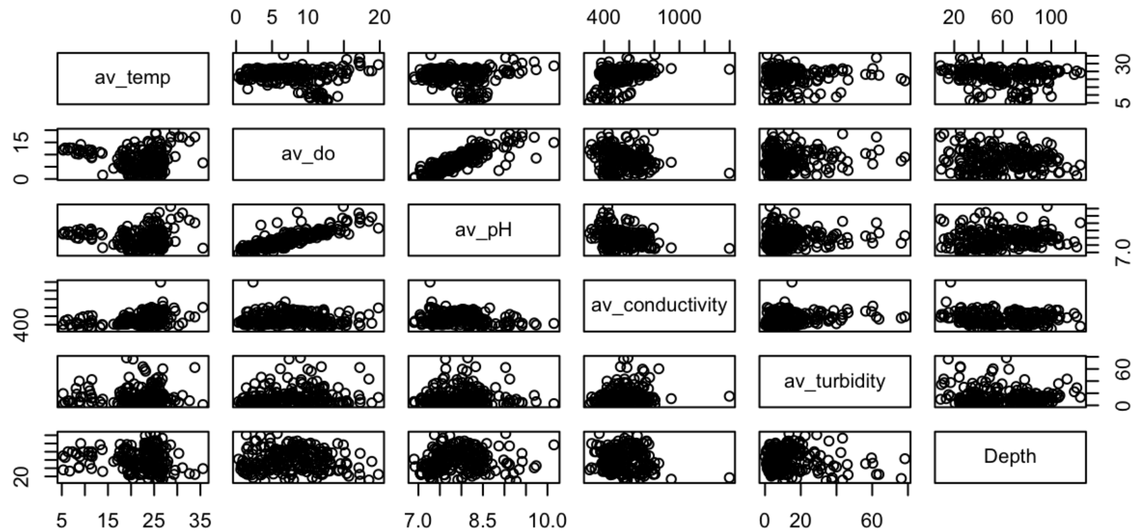


Figure 8. For all samples, N=249, left and right y-axes show wetland management units for the corresponding variable, and top and bottom x-axes show wetland management] units for the parameter labeled below or above, respectively. Strong correlations are indicated by scatter plots with slopes approaching 1, either negative or positive. Abbreviations refer to water quality parameters: average temperature (av_Temp), average dissolved oxygen (av_do), average conductivity (av_conductivity), average turbidity (av_turbidity), and average depth (Depth). DO and pH show the strongest linear relationship.

Cross Comparison of 2019-2022

In 2022, overall seasonal patterns for water quality parameters followed 2019-2021 trends with a few exceptions. Although data were not available for every month across all years, in 2022 water temperature trend followed the seasonal pattern of previous years, with temperature rising from spring to summer to a peak of roughly 25°C. In 2022, temperature held steady from June to August before precipitously declining in October to a low of just below 10°C (Figure 9). Where pH data were available, three out of four years showed slight declines in pH between spring, late summer, and fall months. In contrast to other years, in 2022 pH rose from July to August, then again in October (Figure 10). Conductivity remained relatively constant across months in 2020 and where data were available in 2019 while displaying more variation across months in 2021 and 2022 (Figure 11). Average turbidity showed only slight variation in some years, and significant variation in others. Most notably, in 2019, the May average was near 70 FNU, more than double the second-greatest value across all years (Figure 12). Overall, turbidity seems to decline very slightly until late summer before increasing slightly in the fall (Figure 12). 2022 was no exception to this trend (Figure 12). Dissolved oxygen was the least consistent of the five parameters across years. In 2022, we found that DO declined steadily from May to July, increased dramatically from July to August, then spiked from August to October (Figure 13). In other years, DO increased between late summer and fall and bottomed out in July or August (Figure 13).

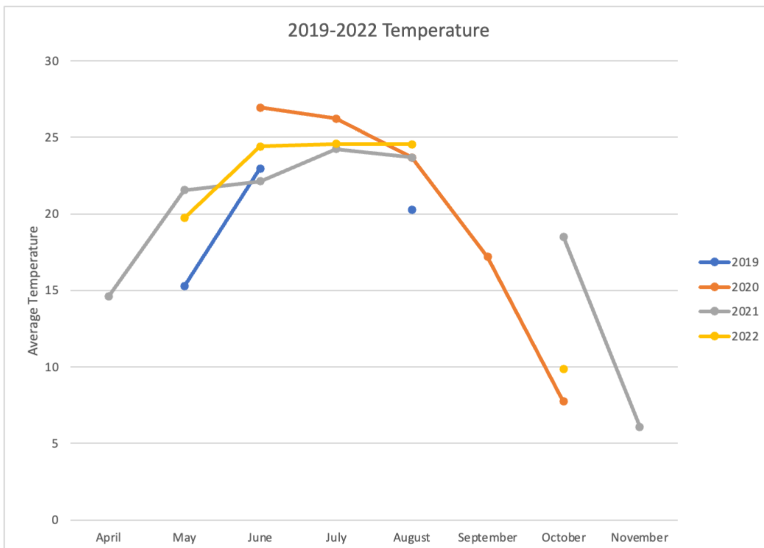


Figure 9. Calendar months are labeled on the x-axis and average temperature (°C) on the y-axis. Years are identified by color: blue (2019), orange (2020), gray (2021), and yellow (2022). Data are only available for three months in 2019. Generally, temperatures rise until mid-summer before dropping sharply in the fall across all years.

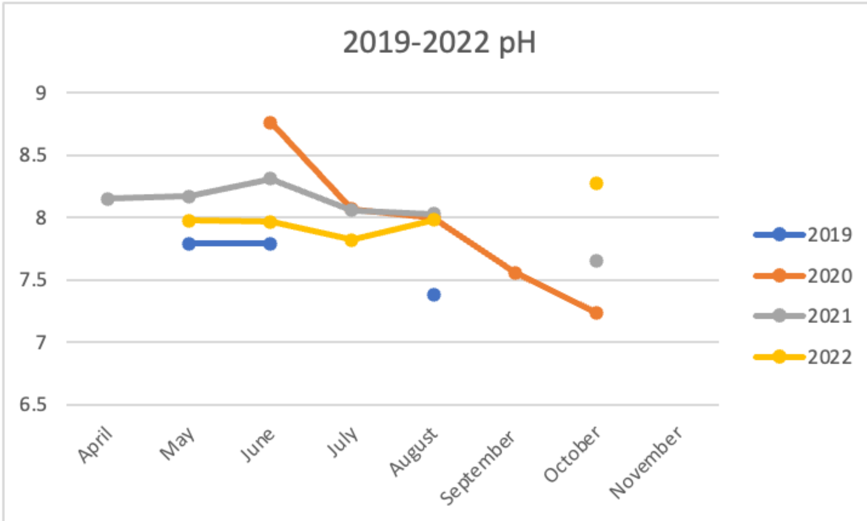


Figure 10. Calendar months are labeled on the x-axis and average pH (°C) on the y-axis. Years are identified by color: blue (2019), orange (2020), gray (2021), and yellow (2022). Data are only available for three months in 2019. pH stayed fairly constant across all years except for for in 2022 where it showed a steady negative trend from spring to fall.

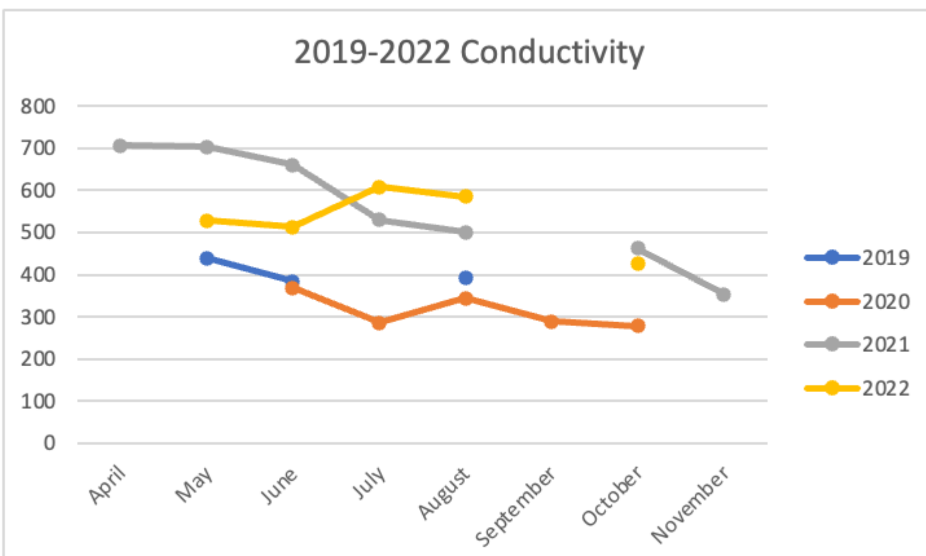


Figure 11. Calendar months are labeled on the x-axis and average conductivity (uS/cm) on the y-axis. Years are identified by color: blue (2019), orange (2020), gray (2021), and yellow (2022). Data are only available for three months in 2019. Conductivity showed a steady negative trend in 2021 while remaining fairly consistent across the other three years.

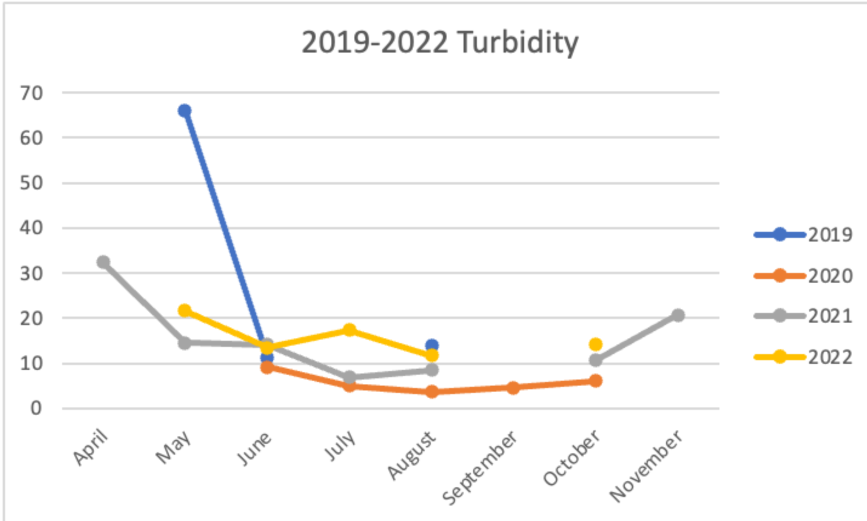


Figure 12. Calendar months are labeled on the x-axis and turbidity (FNU) on the y-axis. Years are identified by color: blue (2019), orange (2020), gray (2021), and yellow (2022). Data are only available for three months in 2019. Note the extremely high average value for may of 2019, generally, turbidity showed a u-shaped pattern across the months.

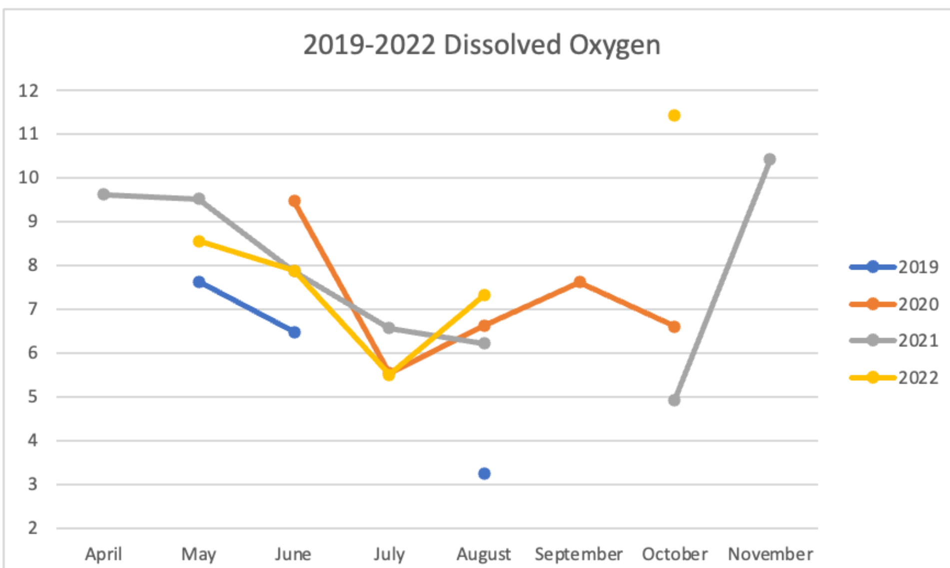


Figure 13. Calendar months are labeled on the x-axis, and dissolved oxygen mg/L on the y-axis. Years are identified by color: blue (2019), orange (2020), gray (2021), and yellow (2022). Data are only available for three months in 2019. Dissolved oxygen levels show significant variability across months with a less clear trend than other variables.

Linear Models between Turbidity and Nutrients

Using both general and traditional linear models, we could not make predictions of TP or TKN concentrations based on turbidity. While both general linear models had beta coefficients with p-values below 0.0001, the beta coefficient values were exceedingly low, below 0.2 in both cases (Table 5 and Table 6). The two linear models had statistically significant p-values yet low R-squared values below 0.2, indicating that our turbidity data explained relatively little variation in nutrient levels.

Table 5. Two general linear model summaries (glm; family: Gamma, link: log): turbidity as an explanatory variable for Total Phosphorus (TP) and Total Kjeldahl Nitrogen (TKN) nutrient and turbidity data from 2019 to 2022. Significance codes are: 0 " *** ", 0.001 " ** ", 0.01 " *, "0.05". Both models indicate weak, yet significant relationships between turbidity and nutrient levels.

Model	Data	AIC	Beta coefficient	
			Intercept	Turbidity
GLM TP & Turbidity	2019-2022 Nutrient Data	-186.43	**-1.988426*** **	**0.014348** **
GLM TKN & Turbidity	2019-2022 Nutrient Data	79.588	**-0.005598**	**0.012126 *** **

Table 6. Two regression model summaries (linear model, lm; ordinary least squares, OLS): turbidity (Turb) as an explanatory variable for total phosphorus (TP) and total Kjeldahl nitrogen (TKN) nutrient and turbidity data from 2019 to 2022. Significance code: 0 " *** ", 0.001 " ** ", 0.01 " *, "0.05". Both models show weak coefficients and model fit despite finding a significant relationship between turbidity and nutrient levels.

Model	Model Significance (p-value)	R^2		Beta Coefficient	
		Multiple R^2	Adjusted R^2	Intercept	Turbidity
Lm (OLS) log(TP) & Turbidity	0.0001784***	0.1437	0.1343	-2.166036***	0.014675 ***
Lm (OLS) log(TKN) & Turbidity	4.983e-05***	0.1958	0.1852	-0.033431	0.010245 ***

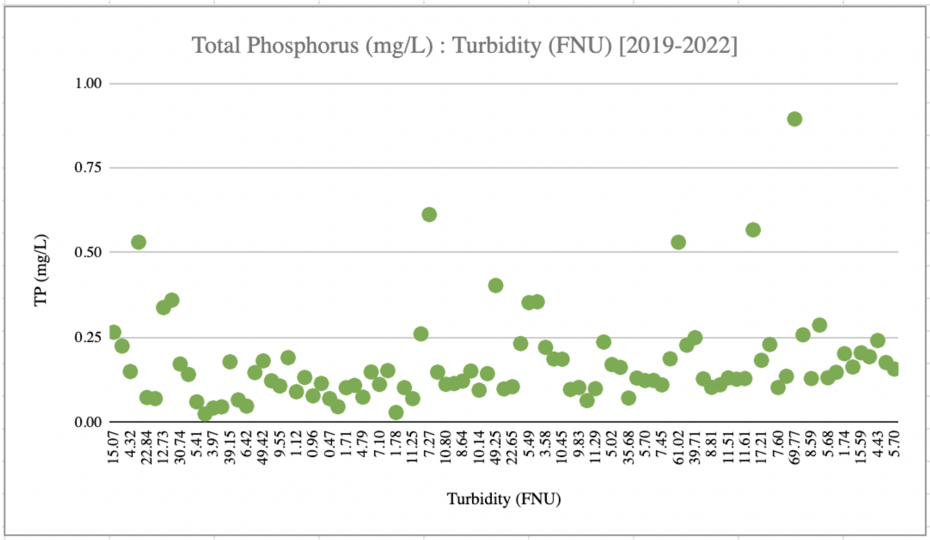


Figure 14. (Formazin Nephelometric Unit, FNU, x-axis) as an explanatory variable of total phosphorus (TP, mg/L, y-axis), using all available data. Sampling locations (not labeled) include all four wetland management units, SHR, and SPD, measured May-Aug, 2019-2022. A very weak positive correlation is shown above.

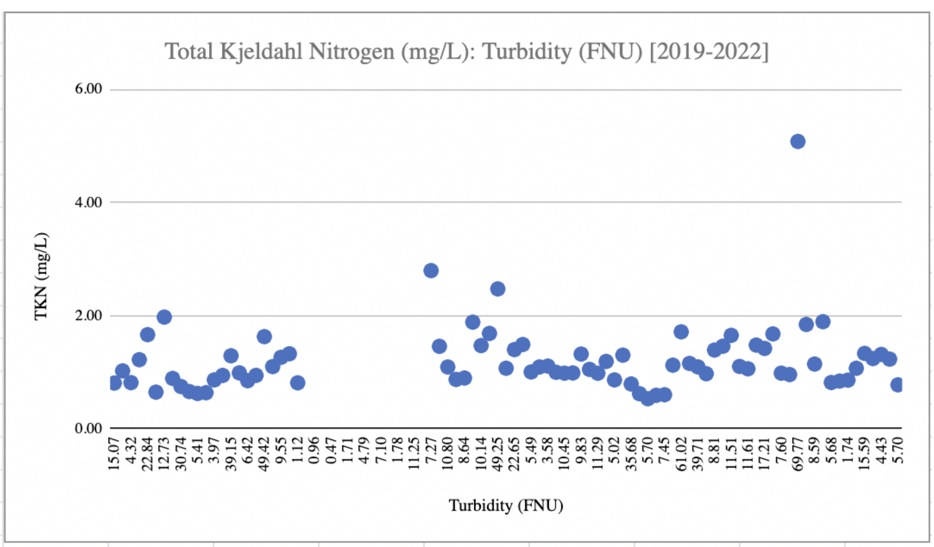


Figure 15. Formazin Nephelometric Unit (FNU), (x-axis), as an explanatory variable of total Kjeldahl nitrogen (TKN, mg/L, (y-axis), using all available data. Sampling locations (not labeled) include all four wetland management units, SHR, and SPD (MC, MN, MS, P1A, SHR,

SPD), measured May-Aug, 2019-2022. Note the weak positive correlation and the gap indicates a lack of TKN data across much of 2020.

Annual Trends in Nutrients

TKN levels were overall much higher than TP concentrations during 2019-2022, with both nutrients showing significant variability across the four sampling years especially in MS and P1A. Yearly unit TKN concentrations varied from 0.79 to 2.33 mg/L (Figure 16). P1A had the greatest variability in TKN concentrations showing an especially significant increase from 2020 to 2022. TP levels followed a similar overall pattern to TKN. Like TKN, P1A showed the most significant variability in TP levels representing a low value of 0.065 and a high of 0.37 mg/L (Figure 17). MS and SHR showed more variability between years and did not follow a clear pattern, while P1A and MC increased across the years, and MN more or less held constant (Figure 17).

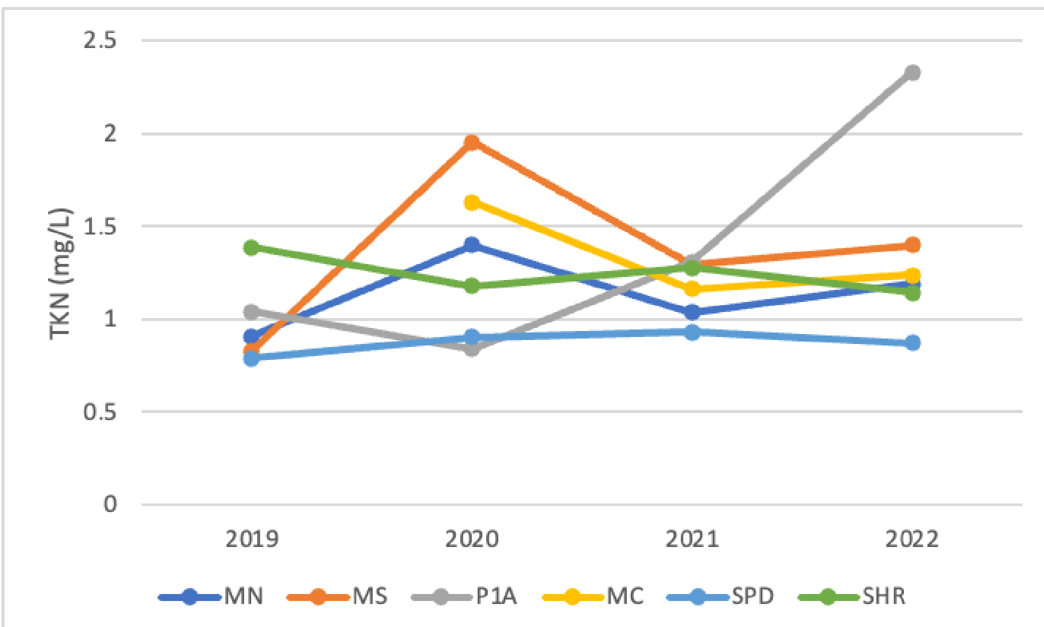


Figure 16. Average annual total Kjeldahl nitrogen, (y-axis), for all sampling locations, (x-axis), 2019-2022. Variation in total phosphorus (TKN, mg/L) in each wetland management unit and river location for 2019-2022, note that MC only has three sampling years. P1A shows a significant increase in TKN levels between 2020 and 2022, while other wetland management units oscillate slightly but overall remain fairly consistent.

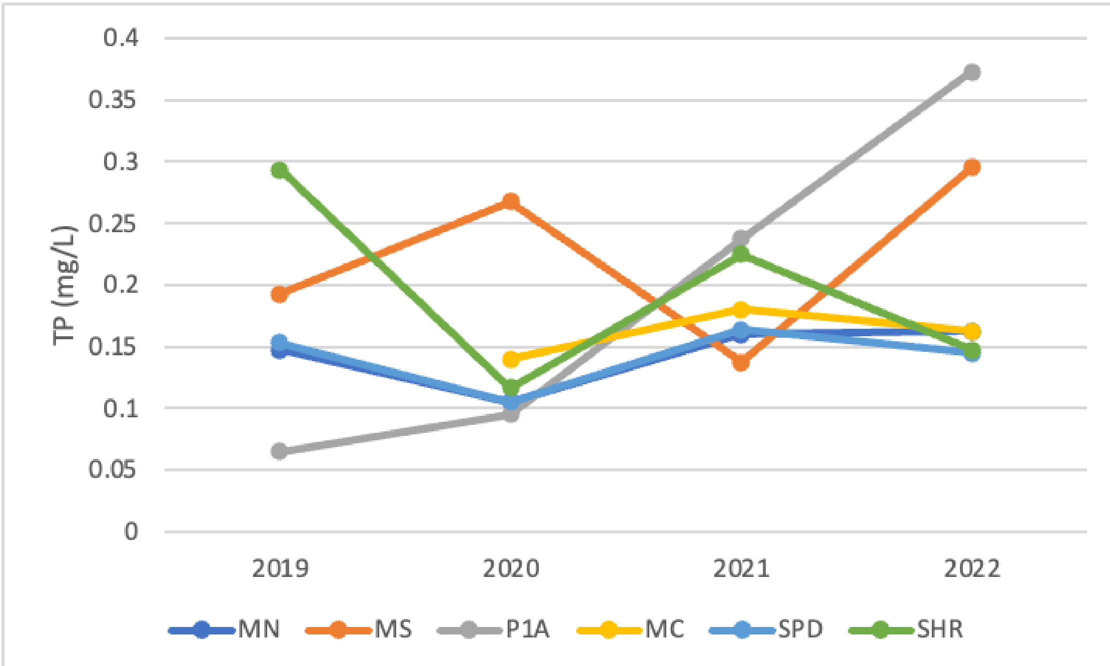


Figure 17. Average annual total phosphorus (y-axis) for all sampling locations (x-axis), 2019-2021. Variation in total phosphorus (TP, mg/L) in each wetland management unit and river location for 2019-2022, note that MC only has three sampling years. TP levels show significant yearly fluctuation, especially in MS, SHR, and P1A.

DISCUSSION

Multi-year and seasonal trends in Water Quality

Looking at water quality trends from a broader temporal scale, it is clear that water quality conditions in the three recently restored wetland management units, MS, MN, and MC, are influenced not only by their connectivity status with the Shiawassee River, but also by their own unique characteristics. Depth, vegetation cover type, and other factors impact each unit's condition, in addition to river conditions and connections, making SNWR floodplain wetland management units a dynamic and diverse environment. Across all four years of monitoring, conductivity has been consistently higher in SHR and P1A than in Maankiki units, illustrating that higher conductivity river water does not entirely inundate the Maankiki units via water control structures. Higher conductivity in these wetland management units may result from anthropogenic inputs from the surrounding watershed, which increases the concentration of ions, increasing conductivity (EPA, 2012). On the other hand, DO levels show more variation among wetland management units and years, where MS consistently had the lowest levels, while other pools varied considerably from year to year. Temperature varied annually based on season but did not significantly vary between wetland management units in three of four years of sampling. However, in 2022, P1A was significantly warmer than the other wetland management units in part due to the exceedingly shallow conditions of this wetland management unit. The impacts of direct river connections versus indirect connectivity via a distribution basin may explain the similarity between Shiawassee River sampling sites and the longest connected wetland management unit, P1A, that show similarities in conductivity and temperature across years.

Nutrient and Turbidity Relationship

Similar to past project years, we could not predict total phosphorus (TP) and Total Kjeldahl Nitrogen (TKN) concentrations from turbidity levels. We joined previous teams in attempting to replicate the results found in Baustian et al. (2018), where total suspended sediments and turbidity could be used to predict TP concentrations. However, unlike their study site, which took place at Pool 2B and Crane Creek at Ottawa National Wildlife Refuge, less than 2 miles from Lake Erie, our sampling occurred almost 20 miles upstream from the mouth of the Saginaw River at Saginaw Bay. Their study found that TP spikes were closely linked to Lake Erie seiche events, where water entered the refuge's wetlands at a high velocity, and turbidity increased dramatically (Baustian et al., 2018). While Lake Huron also experiences similar seiche events, SNWR's distance from the lake decreases the intensity of their impact, limiting the dramatic influx of water into its wetlands, conditions likely causing turbidity and nutrient levels to rise dramatically. This likely accounts for the inability to predict nutrient levels from turbidity alone. In the case of SNWR wetlands, the majority of turbidity variation may be due to factors within the refuge as opposed to watershed scale events, which would have a greater impact on TP and TKN.

Despite the overall inability of our models to predict nutrient levels from turbidity, in 2022, we did observe some suggestive events; four-year highs in both TP and TKN in P1A coincided with high turbidity levels. On June 22, a nutrient sample from P1A showed four-year highs in TKN (5.08 mg/L) and TP (0.89 mg/L), along with the second-highest recorded turbidity reading across all four years at 69.77 FNU. This single reading greatly impacted our overall averages for P1A in 2022 and underscores the potential link between extremely high turbidity events and nutrient concentrations, while in times of typical, fluctuating turbidity levels, no strong relationship seems to exist at SNWR. With a relatively small sample size of just six nutrient readings per month in 2022, this single reading helps explain the seasonal trend we observed, where June experienced the highest average nutrient levels, while May, July, and August produced fairly consistent, lower nutrient concentrations of roughly 0.17 mg/L TP and 1.2 mg/L TKN. We also examined USGS river flow data from the Holland Street Gage in Saginaw, MI to look for a potential link between flow direction and turbidity/nutrient levels. We looked specifically at the ten highest sampled turbidity events in P1A and SHR/SPD over 2019-2022 to see whether flow direction may be impacting other variables at sites most impacted by Lake Huron seiche dynamics. In these ten samples, TP was above average in six cases and TKN in seven. Interestingly, in only two of these ten samples was river flow negative, indicating flow was opposite from typical conditions flowing upriver from Saginaw Bay to the refuge. In these two instances, this flow direction as measured in Saginaw likely indicates that water was flowing into SNWR from downstream, indicating potential seiche events. During our highest nutrient event on June 22, 2022 however, flow was positive, raising the possibility that nutrient levels at SNWR are more impacted by upstream tributaries than the downstream effects of the Saginaw Bay. Integrating flow data, (both for magnitude and direction), into future analysis of nutrient samples may reveal a relationship between these variables.

Water Quality's Impact on Biotic Communities

Water quality significantly impacts community composition and the overall ecological state of Great Lakes wetlands (Chow-Fraser et al., 1998). While all five of our water quality parameters impact biotic communities; dissolved oxygen, temperature, and pH have the most direct impact on community composition and overall ecosystem health (Saari et al., 2018); (Gomez et al., 2021) (EPA, 2023). Dissolved oxygen (DO) levels are critical to the survival of a wide variety of aquatic organisms, with some tolerating hypoxic conditions better than others (Saari et al., 2018). During our sampling season, DO mainly stayed above five mg/L, except within MS and forest sites. At levels of 2-5 mg/L DO, moderate hypoxia occurs where physiological or biochemical stress occurs in fish and aquatic invertebrates (Saari et al., 2018). Juvenile Largemouth Bass (*Micropterus salmoides)* experience 50 percent mortality rates when DO falls to between 1.53-2.28 mg/L, and juvenile Northern Pike (*Esox lucius*) experience these mortality rates when DO fall to 1.73 mg/L. Less DO-sensitive species, such as Yellow Perch (*Perca flavescens)*, experience this level of mortality when DO falls to 0.55 mg/L and Channel Catfish (*Ictalurus punctatus)* at 0.79 mg/L. In addition to lethal effects, low DO can also cause sublethal effects in fish such as impacted metabolism, feeding, or swimming (Tang et al., 2020). These sublethal effects occur at higher DO concentrations. For example, in our commonly caught Bluegill (*Lepomis macrochirus)*, 50 percent or greater mortality occurs at DO concentrations of 1.15 mg/L, while 50 percent or greater individuals show sublethal effects when concentrations fall to 1.5 mg/L (Tang et al., 2020). Similarly tolerant, warm water adapted Black Bullhead (*Ameiurus melas)* experience these levels of sublethal effects at 1.99 mg/L (Tang et al., 2020). More sensitive cool water adapted species at SNWR are impacted at higher DO levels with Yellow Perch (*Perca flavescens)* and Walleye (*Sander vitreus)* populations negatively affected by similar levels of sublethal effects at 3.41 and 3.77 mg/L DO respectively, making significant areas of the refuge unsuitable for these species in midsummer (Tang et al., 2020). In fact, at some points during the summer, sampling sites in MS fell below 1.0 mg/L DO, a possible explanation for why this unit had the second lowest catch per unit effort (CPUE). While DO levels clearly affected fish, aquatic invertebrates proved more resilient to extreme conditions. MS held the highest family-level diversity and CPUE of invertebrates caught in 2022. This may be explained by habitat diversity within MS and the tolerance of many aquatic invertebrates to low DO. For example, *Hyalella azteca*, in the genus of our highest CPUE taxa in 2022, does not reach 50 percent mortality until DO levels fall to 0.70 mg/L (Saari et al., 2018). Similarly, Chironominae, another one of our most commonly caught taxa, is very tolerant of low DO (Chow-Fraser et al., 1998)

While temperature can also profoundly affect species survival and presence in aquatic environments, our data suggest that temperatures alone in SNWR's wetlands likely did not act as a limiting factor for most commonly caught warm water fish species and invertebrates (Lyons et al., 2009). Bluegill, Common Carp (*Cyprinus carpio)*, and Largemouth Bass, some of the most commonly caught fish species in 2022, are all warm water adapted species that have critical maximum temperatures above 34°C (Lyons et al., 2009); (Gomez et al., 2021). We recorded just one average temperature reading above this threshold. Even in July, our warmest month, the average water temperature was slightly below 25 degrees Celsius, indicating that DO instead of temperature likely was a more significant factor in determining species presence. In 2022, pH generally ranged from 7-9, which falls in the safe range for most wildlife (EPA, 2023).

Another interesting finding corroborating previous teams' reports is the strong correlation between pH and DO. While no chemical relationship exists between the two variables, the two co-vary and can be used to infer rates of autotrophy to heterotrophy (O'Boyle et al., 2013). Mechanistically, primary producers can drive the positive relationship between DO and pH by uptaking CO_2, which increases pH, and releasing oxygen which increases DO (O'Boyle et al., 2013). This mechanism is useful when looking at trends of pH and DO over a period of time, allowing for the inference of overall trophic status, which can be applied to SNWR's wetlands.

Assumptions and Limitations

Of our fifteen one-way ANOVAs, ten failed Levene's test of equal variance between groups despite using log and square root transformations. We chose to still perform the ANOVA to keep our results comparable to past project teams, which also used ANOVA. In the future, we recommend using a more robust statistical test, such as the Welch F test, as homogeneity of variance violations are shown to have significant impacts on Type I error rates and statistical power for detecting population mean differences (Kim & Cribbie, 2018).

Additionally, the relatively small sample size at some vegetation zones has the potential to skew data. For example, *Phalaris* and River Bulrush (*Bolboschoenus fluviatilis*), the vegetation types with the highest DO levels only, were sampled two and five times, respectively. Furthermore, forest sites, while more frequently sampled, were only sampled in one location in MS where the water was at times very shallow, potentially explaining the extremely low DO levels there.

Management Implications and Future Recommendations

SNWR's wetlands experience a wide range of abiotic conditions due to seasonal shifts in water quality, as well as the diverse habitats they provide, allowing for areas of high DO refugia for more sensitive taxa. It is therefore essential to preserve this distinct heterogeneity existing among wetland management units and vegetation zones allowing diverse populations of invertebrates and fish to survive harsh summer conditions in the wetland management units. These invertebrates and fish in turn provide food for many of the refuge's charismatic bird species. Managing for River Bulrush *(Bolboschoenus fluviatilis)* and *Phalaris* vegetation zones may be beneficial to achieving this diversity as these zones were associated with the highest DO levels.

Furthermore, tracking water quality parameters in the Shiawassee River over the course of an entire year would better reveal trends in water quality tied to seasonal shifts in precipitation, evapotranspiration, temperature, and other factors. Given the influence of the river on wetland management units, especially P1A, a more comprehensive sampling protocol incorporating data from all months would allow for a thorough understanding of river conditions. These data would help inform decisions to open and close water control structures to best accommodate wildlife's seasonal needs, such as spawning fish, based on river conditions.

Finally, integrating flow data from Shiawassee and Saginaw River gauges may help managers better predict nutrient levels at SNWR. Baustian et al. (2018) showed that flow rates influence nutrient levels in a coastal wetland system. Long-term flow data as an explanatory variable alongside turbidity may help more accurately predict nutrient levels than turbidity alone. Specifically, it would be interesting to analyze the overall frequency in which negative (upriver) flow rates occur at SNWR and whether there is an association between the occurrence and magnitude of these events and higher nutrient and turbidity levels. More frequent nutrient sampling would have to be conducted to build a representative sample that can be compared to flow rates in an analysis such as a multiple linear regression.

VEGETATION MONITORING

INTRODUCTION

This year's team surveyed and analyzed SNWR's vegetative community throughout the first week of August 2022 to characterize the vegetative structure of each wetland management unit and the biotic health of the floodplain wetland. Because some of the study wetland management units were farmed as recently as 2016, the refuge's inundation of the floodplain wetland management units created expansive new wetland habitats with the potential to be populated by a mosaic of native and non-native vegetation species (as illustrated by the prior three years of monitoring) (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). In areas that have undergone such sudden ecological change, factors including diversity of species, quality of soil and water, breadth of regional seed dispersal, local hydrology, and topography determine resulting species presence and abundance (Keddy and Reznicek, 1986; Johnston and Brown, 2013). The presence of rare and endemic species helps to indicate a healthy system and, in restored areas, is the result of effective management practices and continued mitigation of anthropogenic disturbance. The presence of indicator species (e.g., those tolerant or intolerant of known stressors) helps identify potential pollutants, like excess nutrients such as phosphorus or nitrogen. They also allow us to confirm findings based on metrics that influence plant communities, such as water turbidity, soil saturation, and temperature.

Wetland vegetation serves as the primary source of structure and sustenance for the aquatic, terrestrial, aquatic, and avian species that call the wetlands home. Annual monitoring of the refuge's vegetative species allows us to better understand better how yearly changes in precipitation, disturbance, and temperature induce vegetative community shifts and, in turn, how this impacts other biotic indices at SNWR. These findings cumulatively help us to understand the patterns of succession that occur in newly restored wetland floodplains, creating a baseline for how vegetative communities re-emerge after long disruptions by legacy agriculture. Fish, birds, macroinvertebrates, amphibians, and mammals all rely upon a range of aquatic vegetation species found at SNWR for habitat and food. Submerged aquatic vegetation (SAV) and emergent vegetation are crucial for the success of many of the refuge's macroinvertebrate and fish species, providing structure, associated food sources and organisms, and habitat for spawning and rearing (Jude and Pappas, 1992; De Szalay and Resh, 2000). These vegetative species also provide productive food sources for waterfowl and other migratory species, producing the essential habitat for which the refuge was founded (Wilcox et al., 2002). The research objectives listed below guided our vegetation sampling process throughout the field season.

Research Objectives

- *Determine and identify the variety of species present in the four wetland management units and map their subsequent vegetative zones.*
- *Gain an understanding of how vegetation community structure and species abundance differed between both vegetation zones and within a given wetland management unit.*
- *Describe and categorize the differences in abundance, structure, and community composition between the four wetland management units and their vegetation zones.*
- *Determine variations in the prevalence of non-native, native, and rare vegetative species between wetland management units.*

METHODS

The surveying methods utilized in our summer 2022 vegetation sampling were adapted from the Great Lakes Coastal Wetland Monitoring Program (Uzarski et al., 2017). These methods were then altered to be site specific to SNWR by the summer 2019 monitoring team, based on research conducted by Kurt Kowalski, of the USGS (Kowalski et al., 2014) (Lugten et al., 2020). The changes were made with the intention of producing consistent multi-year data that would help to guide temporal water control structure decision-making and the management of critical emergent-vegetation habitat conditions within the refuge. As a result, the methods utilized in the sampling protocol in the summer of 2022 were identical to those used in the 2021 vegetation survey (Conrad et al., 2022).

We performed a week of vegetation sampling, from August 1st to August 5th, 2022. Sampling has always been conducted at the end of July or during the first week of August in an attempt to standardize and control conditions, as well as to ease the degree of difficulty for field identification, as this is the time of year when many of the herbaceous plants on the refuge are flowering.

Zone Delineation

The process of vegetative survey and analysis began by attempting to delineate the vegetative zones on the refuge utilizing a combination of the ArcGIS application and Google maps satellite imagery. Using these tools, we identified vegetation zones in each of the four wetland management units by determining dominant species and primary community structure (Table 7). Vegetation zones were either distinguished based on community type (i.e., Mudflat, Submerged Aquatic Vegetation) or the dominant species present within that zone (i.e., *Typha angustifolia,* or *Phalaris arundinacea*). The largest zone in each wetland management unit was assigned fifteen random sample points, while the remaining zones were each assigned ten randomly selected sample points, producing a total of 190 randomly generated sample points for the specific vegetation zones labeled amongst each of the four floodplain pools (Figure 18). These zones consisted of various dominant vegetation types, including: *Typha*, Forest, *Salix*, *Nymphaea*, submerged aquatic vegetation (SAV), emergent vegetation, Mudflat, and *Phalaris*.

We assigned the location of these points randomly using ArcGIS software, with the only constraint being that the minimum distance between points was 10m, while the maximum distance for our larger zones was 100m. Often, for our smaller vegetation zones, random sample points were often separated by only the ten-meter distance. We traveled on foot to these sample points, occasionally utilizing a single-hulled Jon boat for deeper locations. We were infrequently presented with obstacles such as extreme water depth, mud, and unnavigable vegetation that prevented our team from reaching the exact GPS coordinates. In these cases, the team navigated to the closest possible position to the GPS coordinates in question and took the samples in that location.

Table 7. Vegetation characteristics throughout all four SNWR wetland management units. Includes total acreage, average depth, total acreage of the specific vegetation zone, as well as the number of zones per unit.

<table>
<tr><th>Unit</th><th>Number of Vegetation zones</th><th>Vegetation Zones</th><th>Vegetation Zone Area (Acres)</th><th>Average Depth (cm)</th><th>Total Vegetation Zone in Acres</th></tr>
<tr><td rowspan="5">Maankiki Center</td><td rowspan="5">5</td><td>Typha</td><td>49.97</td><td>9</td><td rowspan="5">835.8</td></tr>
<tr><td>Mixed Emergent</td><td>458.85</td><td>16.33</td></tr>
<tr><td>Phalaris</td><td>157.45</td><td>0</td></tr>
<tr><td>Salix</td><td>45.97</td><td>13.4</td></tr>
<tr><td>SAV</td><td>123.56</td><td>34.6</td></tr>
<tr><td rowspan="5">Maankiki South</td><td rowspan="5">5</td><td>Typha</td><td>305.17</td><td>38.53</td><td rowspan="5">513.7</td></tr>
<tr><td>Mixed Emergent</td><td>9.76</td><td>16.8</td></tr>
<tr><td>Forest Overstory</td><td rowspan="2">51.04</td><td>2.8</td></tr>
<tr><td>Forest Understory</td><td>6.2</td></tr>
<tr><td>SAV</td><td>147.73</td><td>42.1</td></tr>
<tr><td rowspan="3">Maankiki North</td><td rowspan="3">3</td><td>Typha</td><td>174.28</td><td>0</td><td rowspan="3">406.7</td></tr>
<tr><td>Phalaris</td><td>14.15</td><td>0</td></tr>
<tr><td>SAV</td><td>218.27</td><td>34.7</td></tr>
<tr><td rowspan="4">Pool 1A</td><td rowspan="4">4</td><td>Typha</td><td>188.93</td><td>3</td><td rowspan="4">593.92</td></tr>
<tr><td>Nymphaea</td><td>304.85</td><td>24.47</td></tr>
<tr><td>Mudflat</td><td>86.17</td><td>4.1</td></tr>
<tr><td>Salix</td><td>13.97</td><td>0</td></tr>
</table>

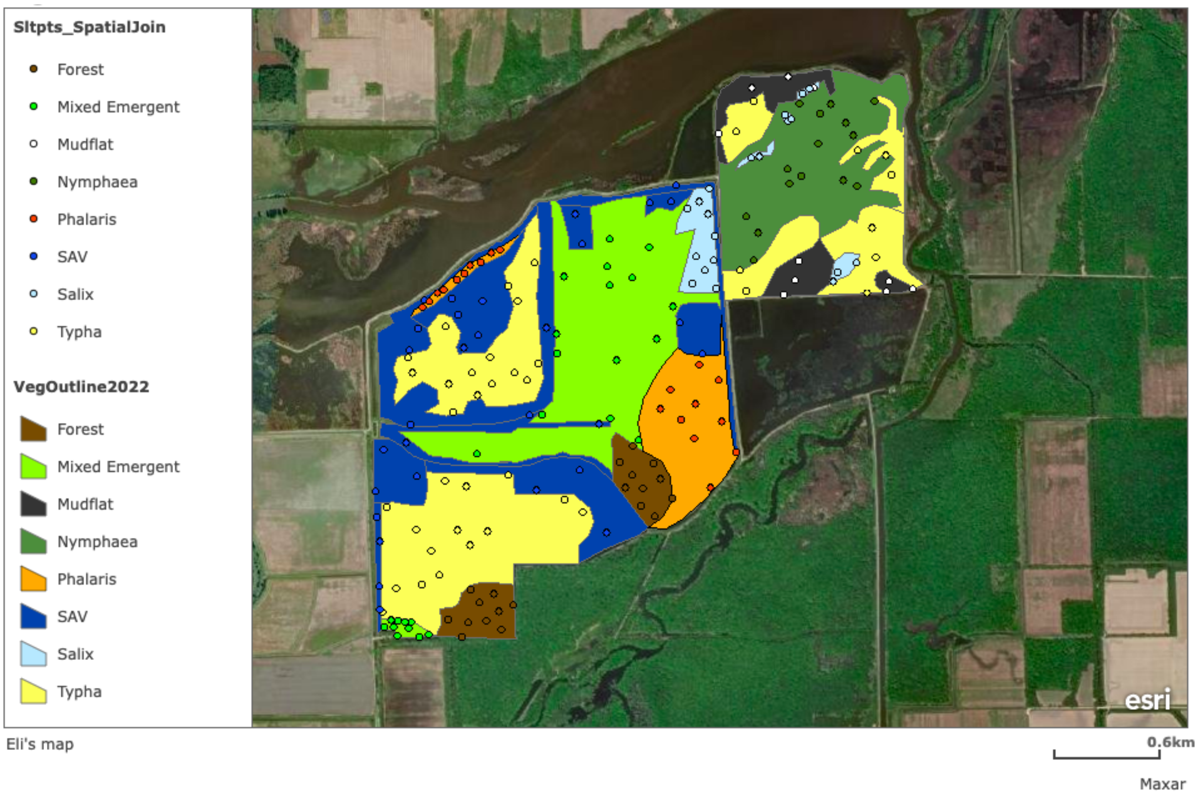


Figure 18. Map of all sampling quadrats for vegetation observed during the 2022 season. Includes zone delineation as well as all 190 quadrat locations, coded to their specific vegetation zone by sampling point and overall background coloration.

Field Surveying

We began the *in situ* sample process by uploading our detailed vegetation zone map into the ArcGIS application service, Survey123. The Survey123 app was made available to us on iPads provided by SNWR, and these iPads had cellular capability so that we were able to use them while taking samples in the field.

Utilizing the Survey123 map as an active GPS, we then picked a sample point and navigated to the GPS coordinates, approximating our location as closely as the sensitivity on the map would allow. Not only was Survey123 crucial in locating and pinpointing exact sample locations, but the application also then allowed us to categorize and record metrics of importance in each quadrant. This made the documentation of the metrics of interest both feasible and streamlined. Metrics include: the depth of water, type of vegetation zone, exact GPS coordinates, the types of species recorded, the percentage of vegetative coverage, the time of sample, and site information.

Once we had arrived at a sample location, we then utilized a traditional random quadrat sample method to determine where to survey. A 1-meter by 1-meter quadrat constructed from PVC piping was deployed at random over the surveyor's shoulders to determine which 1x1 meter area would be sampled (Figure 19). When the plot was chosen, we proceeded with cataloging the data of interest. This began with water depth, which we measured to the nearest centimeter, and recorded a 0 if the area was dry or on land.

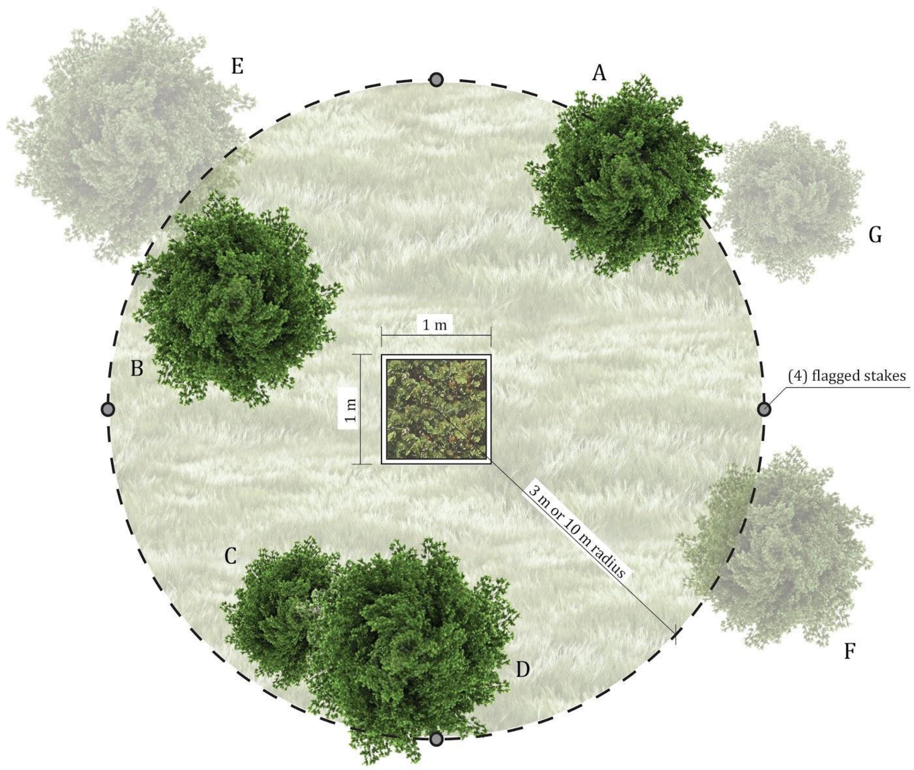


Figure 19. An example of an enlarged, meter-by-meter sampling quadrat, which was the extent of sampling for groundcover, submerged/floating, and wet meadow/emergent species. In forest surveys, the quadrat was then enlarged to a circle with a radius of 5m in order to sample any understory or overstory species that were nearby. Only trees who had a portion of their trunk fall into the enlarged quadrat were sampled (A-D below), while those that only had their canopy enter the quadrat were excluded (E-G).

We then moved to identify the species that were present, along with their associated vegetation coverage within the sampling quadrat. Species were observed and documented by layer, starting from the surface of the water and slowly working down to the sediment layer. In situ plant identification was also done primarily through visual ID, and was supplemented as needed by the use of another iPad application, entitled "Picture This Plant Identifier" (Glority LLC), to confirm related or unknown species. Team members worked in groups of three and frequently had the assistance of Eliza Lugten, an SNWR Biological Technician, to confirm difficult identifications. Plants were identified down to the lowest taxonomic unit possible, which in almost every case was Genus/species.

We also assessed total coverage for each species inside the quadrat simultaneously with each species identification. Team members went through organized practice prior to the vegetative survey, assessing coverage in practice quadrats at the beginning of vegetation week. This allowed the team to calibrate and sync coverage assessments, with the goal of ensuring that each individual was quantifying vegetation cover from the same base reference point in a similar manner. Coverage of each vegetative species was assessed in a top-down manner, meaning species either floating on the surface or at the top of the quadrat were identified and had their coverage assessed first. If there were multiple species at the top of the quadrat, we began with the most abundant species in the quadrat in the hopes of minimizing visual disturbance for the submerged vegetation. Species' coverage was then entered into Survey123 as a percentage, derived from the average of the percentages given by each of the team's members (e.g., a team whose members assigned coverages of 80%, 85%, and 90% would then enter 85% as the cover for that species). The species that had the broadest coverage were entered into Survey123 first. Percentage totals for a single quadrat often exceeded one hundred percent, as the quadrats were analyzed in stratified layers, and more than one species could have 95%+ cover depending on the layer in which it was located.

Our sampling process differed slightly for sample points that were placed in our terrestrial vegetation zones, namely: *Phalaris*, Forest Overstory, and Forest Understory. In quadrats that were entirely absent of water, species identification and total coverage was determined from the bottom up to ensure that smaller, less dominant species at ground level were not overlooked during the survey.

For our overstory forest sites, we expanded the quadrat radius to ten meters in order to capture and document species that exceeded 4.6 meters in height. Conversely, if the species within the adjusted ten-meter radius were deemed as understory (<4.6 meters), then the species observed and documented were only those present with the initial one-meter by one-meter quadrat. Height estimates to determine whether a species was above or below the 4.6-meter height threshold were done using a measuring tape. For the Overstory species that were documented (e.g. *Fraxinus americanus, Salix interior*), radii were estimated utilizing Diameter at Breast Height (DBH).

Data Analysis

After field data collection and thorough quality assurance/quality control from Eliza Lugten and Alexandra Bozimowski, FWS and USGS, respectively, we analyzed data from our summer vegetation sampling utilizing a series of data summaries and statistical tests. These tests were chosen to specifically with the aim of addressing and providing answers to the four research objectives we set for our 2022 sampling.

In order to best assess and quantify the overall structure and health of the vegetative communities on the refuge, we calculated the following metrics:

- Important Value Index, which was utilized to identify species dominance and community structure.
- Non-metric Multidimensional Scaling, used to quantify and assess significant differences between the distinct vegetation zones.
- Floristic Quality Analysis using the Floristic Quality Index, which helps to quantify the ecological state of the vegetative zones using the species that exist there.
- Index of Biotic Integrity, to understand the degree to which invasive species are affecting the refuge and to gain a holistic view of the health of the plant community in each unit.

Important Value Index

The goal of calculating the important value index score (IVI) for each species was to understand the organisms that were most dominant in each of the wetland management units we evaluated, as well as to better discern the impact individual species might have on the surrounding community. We calculated an Important Value Index score for every single species that was observed on the refuge during 2022 vegetation sampling. We arrived at a final IVI value by adding the relative frequency of a certain species with the relative abundance of that species in the unit and zone in which it was present. The full formula for IVI calculations can be found below (Formula 1) (Curtis and McIntosh, 1951). Each IVI value represents the degree of influence a particular species had on its surrounding vegetation.

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

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

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

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

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

Formula 1. Important Value Index formula for all species calculations. Demonstrates how numeric values were determined for frequency, relative frequency, abundance, relative abundance, and ultimately, the final calculation for IVI scores.

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

Utilizing the IVI values calculated in our first analysis, NMDS testing allowed us to distinguish and compare the difference between plant communities in all of the dominant vegetative zones by examining the overall environmental composition in these zones. Non-metric Multidimensional Scaling or NMDS ordination visually illustrates the similarities and differences between variables calculated by the Bray-Curtis Dissimilarity index. Following the completion of the Bray-Curtis Dissimilarity Index, points representing each unique vegetative zone are plotted on an x, y-axis, and their coordinates are determined by a series of biotic and abiotic factors. The NMDS plots the values of each vegetative zone in 2D space, utilizing the linear distance between each point to illustrate the collective difference in community composition between various vegetative zones. Points on the graph that fall more closely together are thus more similar in terms of their overall composition, whereas a longer linear distance between points indicates more significant difference. For our project, NMDS ordinations were created using R code written by Kuiran Zhang (previous student team member) and Alexandra Bozimowski (USGS), which utilized the "vegan", "ggplot2", and "ape" packages in its construction (Appendix I). This code will be documented as a part of the addendum to this report and will be saved with this final product.

Floristic Quality Analysis (FQA)

After cataloging all present species at SNWR, we used Floristic Quality Analysis as the primary method by which to calculate and understand the environmental health and quality of the study site. While FQA produces a variety of values indicating overall quality and ecosystem health, we focused on two of particular interest for our vegetative survey of SNWR; the mean conservatism index (C score) and the Floristic Quality Index, or FQI.

The mean conservatism index or C score is calculated for each individual site based on a traditional one-to-ten scale that evaluates the probability that the species observed there would also be found in a "landscape relatively unaltered from what is believed to be pre-European settlement condition" (Herman et al. 2001). In this case, the number 0 is representative of an incredibly degraded site with little or no resemblance to pre-European community structure, while a 10 is categorized as identical to those same pre-European sites.

The Floristic Quality Index applies a numeric, comprehensive value to the quality and health of an ecosystem while also incorporating the mean C value. FQI is directly calculated by weighting the mean C of an ecosystem by the species richness present there. More precisely, it is the square root of the species richness multiplied by the mean C value. While the mean C is a useful metric, it can sometimes be deceiving when comparing two natural areas, as two areas with similar C values can have vastly different species richness (Freyman et al. 2016).

The Floristic Quality Assessment our team conducted was achieved using the Universal FQA Calculator tool, which stores a comprehensive database of plant species for each ecoregion. Utilizing the 2014 Michigan region for the background plant database, our unit species lists were entered into the calculator and a Floristic Quality Assessment was performed on each of the wetland management units.

The only issue with producing the FQA utilizing the Universal FQA Calculator and the 2014 Michigan database was the potential for a certain species in our 2022 assessment to be absent in the 2014 Michigan database. This occurred for a wide variety of reasons, one being that as the calculator was solely designed for vascular plant species. As a result, dead plants, recent non-natives, and non-vascular species were modified to the FQI analysis due to an absence of regional data. Absent species or species of a taxonomic order that was too high for the FQA Calculator were entered as the closest discernible species existing in the Michigan region, or occasionally, as in the case of Dead *Typha*, left out of the FQA.

Index of Biotic Integrity (IBI)

We calculated a wetlands vegetation Index of Biotic Integrity, or IBI, as the final portion of SNWR vegetative data analysis for the purpose of providing a holistic vegetation evaluation. The IBI metric is based on an additive calculation that takes into account both the percent cover and frequency of invasive species, as well as the C values of each wetland management unit that were calculated during the floristic quality assessment (Conrad et al., 2022; Puz et al., 2021).

We began by dividing each of the four wetland management units into three individual categories; the entire site (which consisted of the whole unit's species list), the wet meadow / dry emergent (WMDE) species section (species found in <1cm of water), and finally the flooded emergent/submerged (FES) species section (species observed in >1cm of water) (Conrad et al., 2022; Puz et al., 2021). The mean C index, the total invasive cover, and the invasive frequency were then calculated individually for each of these specific categories

These nine scores were then assessed in conjunction with a final metric that quantifies the relative frequency and relative cover of tolerant submerged aquatic species to create a comprehensive IBI value (Puz et al., 2021). This final metric was calculated utilizing only fully submerged vegetative zones, namely *Nymphaea* and Submerged Aquatic Vegetation (SAV). Each of these ten metrics then had its score assessed on a standardizing 0 to 5 scale, based on whether the metric reflects a healthy or unhealthy ecological state, with zero being the poorest quality and five being the most pristine (Conrad et al., 2022). The combination of these ten individual standardized scores was then summed to a cumulative IBI score between 0 and 50 that characterizes the wetland's vegetative health (Conrad et al., 2022). Different ranges of the cumulative score correspond to the qualitative description of the wetland's quality, with the poorest quality wetlands having a combined total of between 0 and 5 and the most pristine reference conditions occurring in scores between 41 and 50 (Puz et al., 2021).

Our methodology for calculating IBI values was identical to the previous year's (Team 2021-2022) procedure. We determined IBI by splitting each wetland management unit into an entire site, WMDE, and FES section, and delineating a quadrat as WMDE and FES by whether or not they were above or below 1 cm of water present. The first two years of Student Teams (2019-2020), 2020-2021) evaluated WMDE / FES conditions on a vegetative zone basis rather than by individual quadrats. Instead of examining each quadrat's water depth, they took the average depth of an entire vegetative zone and then characterized that zone as either WMDE or FES. This meant that rather than determining the vegetative and hydrologic conditions of the quadrat based on water depth, the first two team's methods more closely aligned with the standard employed by the Great Lakes Coastal Wetland Monitoring Program. This entails delineating the quadrat's status as WMDE or FES based on the natural gradient of habitat that occurs in traditional wetlands, with water depth decreasing consistently with distance from shore

(GLCWMP, 2018). These conditions do not exist in the SNWR wetland management units, as the restoration did not follow natural shore gradients in regard to depth.

We believed this resulted in a higher degree of generalization and assimilation of individual quadrats into incorrect hydrologic condition categories. Therefore we chose to categorize WMDE/FES at a standardized depth at quadrat level to provide a more refined illustration of changes in vegetation with water levels and to better delineate wetland zones (Conrad et al., 2022). As a result of these differences in categorization regarding WMDE and FES zones that help to comprise IBI, comparisons between the IBI data from 2021/2022 and 2019/2020 are not as useful. However, with the completion of 2022 data analysis, comparisons can be drawn from analyses between the 2019 and 2020, as well as the 2021 and 2022 field seasons.

RESULTS

Vegetation Structure at SNWR

Throughout the week of August 1st, 2022, we collected 190 quadrat samples of vegetation assemblages in the 4 SNWR wetland management units (MN, MS, P1A, MC). We found 86 species among the 4 wetland management units, with 7 being invasive, and the remaining 79 being native or non-invasive. We did not observe any rare species during the week-long vegetative survey, but this does not indicate rare species absence, as Conrad et al. 2022 identified *Barbarea orthoceras* during their field survey. Maankiki Center had the greatest number of species at 55, while MS had the least at 32 (Figure 20). This pattern remained consistent for native species alone, as MC had the greatest number of native species at 51, while MS had the fewest at 26 (Figure 20). Interestingly, MS had the most invasive species of the 4 wetland management units, hosting 6 of the 7 identified invasives on the refuge (Figure 20). In terms of vegetative structure, both MN and MS had significantly more flooded emergent/submerged species (FE/S) (Figures 35 & 20, respectively), while the compositions in P1A and MC were majority wet meadow and dry emergent (WM/DE) species (Figure 20).

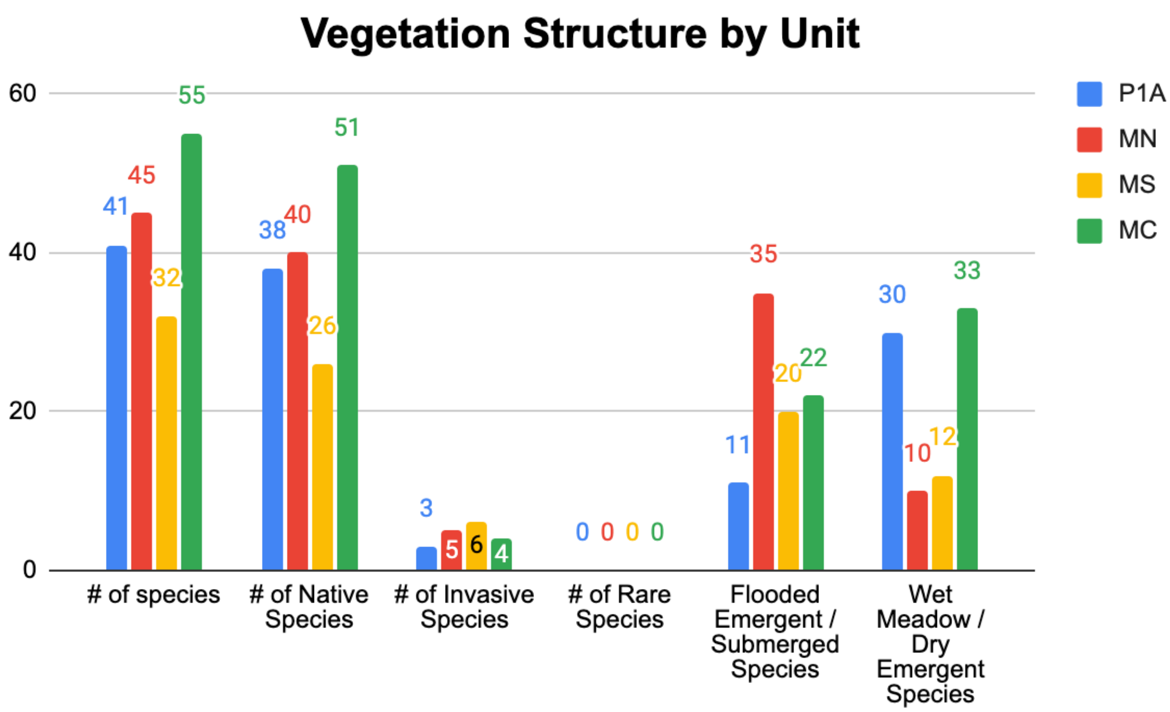


Figure 20. Vegetation composition based on flooded emergent, submerged and wet meadow, and dry emergent species per wetland management unit; alongside counts for total, native, invasive, and rare species per unit. Chart coloration per unit is consistent for all figures and tables in the vegetation analysis section, and total numeric counts are present above each bar in the histogram. Species counts represent only species with high confidence identification.

Importance Value Index & Identifying Influential Species at SNWR

The Importance Value Index (IVI) is a numeric score that quantifies the influence a particular vegetative species has on its surrounding community, based on frequency and abundance. We determined IVI for individual vegetation zones within each wetland management unit, calculating a unique value for each species in a particular zone to better understand the most abundant and influential species in each wetland management unit. This meant that species that appeared frequently across wetland management units and vegetation zones had several different scores depending on the unit and vegetation zone from which they were assessed. IVI scores ranged greatly, with a minimum of 0.89 (*Potamogeton crispus* in MS *Typha*) and a maximum of 119.13 (*Phalaris arundinacea* in MC *Phalaris*) (Table 8-11). Species most influential throughout both vegetation zones and ecological wetland management units included *Ceratophyllum demersum* and *Phalaris arundinacea,* both of which were present as a top 4 IVI value in 6 of the 15 vegetation zones (Tables 8-11). The highest IVI score for MS was *Acer* *saccharinum* (83.28), while the lowest ranking score was *Potamogeton nodosus* (16.43). The highest IVI score for MN was *Phalaris arundinacea* (88.63), while the lowest ranking score was *Persicaria lapathifolia* (7.498). The highest IVI score for MC was *Phalaris arundinacea* (119.13), while the lowest ranking score was *Riccia fluitans* (9.05). The highest IVI score for P1A was *Nymphaea odorata* (88.75), while the lowest ranking score was *Spirodela polyrhiza* (9.74). However, while invasives often claimed the highest IVI in each complete wetland management unit, the dominant 4 species present in each unique unit/vegetation zone combination differed in every case (Tables 8-11).

Table 8. Importance Value Index (IVI) for the three dominant MN vegetation zones. The three MN vegetation zones and their corresponding influential species are listed, ranked by their important value index score and complimented by the hydrologic condition in which they were observed, the two options being either wet meadow / dry emergent (WM / DE) or flooded emergent / submerged (FE / S) vegetative structure. Each unit was divided into dominant vegetation zones based on primary species or habitat structure. For MN, there were three zones: *Phalaris*, dominated by *Phalaris arundinacea*; SAV or submerged aquatic vegetation, dominated by floating and submerged species like *Ceratophyllum demersum*; and *Typha*, dominated by *Typha angustifolia*.

Maankiki North			
Vegetation Zone	**Latin Name**	**IVI**	**Hydrologic Condition**
Phalaris	*Phalaris arundinacea*	88.63	WM / DE
	Cirsium arvense	19.41	WM / DE
	Abutilon theophrasti	14.82	WM / DE
	Persicaria lapathifolia	7.5	WM / DE
SAV	*Ceratophyllum demersum*	57.93	FE / S
	Potamogeton nodosus	39.89	FE / S
	Stuckenia pectinata	20.41	FE / S
	Myriophyllum spicatum	17.8	FE / S
Typha	*Typha angustifolia*	69.47	FE / S
	Populus deltoides	12.36	WM / DE
	Abutilon theophrasti	11.81	WM / DE
	Scirpus sylvaticus	9.96	WM / DE

Table 9. Importance Value Index (IVI) for the 5 dominant MS vegetation zones. The four MS vegetation zones and their corresponding influential species are listed, ranked by their important value index score and complimented by the hydrologic condition in which they were observed, the two options being either wet meadow / dry emergent (WM/DE) or flooded emergent / submerged (FE/S) vegetative structure. Each unit was divided into dominant vegetation zones based on primary species or habitat structure. For MS, there were four zones: Forest, dominated by *Acer saccharinum* and overstory species; SAV or submerged aquatic vegetation, dominated by floating and submerged species like *Ceratophyllum demersum*; Mixed Emergent, consisting of shallow, near-shore emergent species; and *Typha*, dominated by *Typha angustifolia*.

Maankiki South			
Vegetation Zone	**Latin Name**	**IVI**	**Hydrologic Condition**
Forest	*Acer saccharinum*	83.28	WM / DE
	Phalaris arundinacea	39.81	WM / DE
	Ulmus americana	39.03	WM / DE
	Lemna minor	38.62	WM / DE
SAV	*Najas minor*	29.88	FE / S
	Potamogeton nodosus	26.97	FE / S
	Ceratophyllum demersum	21.78	FE / S
	Utricularia vulgaris	19.63	FE / S
Typha	*Typha angustifolia*	43.18	FE / S
	Utricularia vulgaris	32.49	FE / S
	Riccia fluitans	25.87	FE / S
	Potamogeton nodosus	16.43	FE / S
Mixed Emergent	*Stuckenia pectinata*	39.91	FE / S
	Algae spp.	24.23	FE / S
	Potamogeton foliosus	21.75	FE / S
	Potamogeton nodosus	19.36	FE / S

Table 10. Importance Value Index (IVI) for the 5 dominant MC vegetation zones. The five MC vegetation zones and their corresponding influential species are listed, ranked by their important value index score and complimented by the hydrologic condition in which they were observed, the two options being either wet meadow / dry emergent (WM/DE) or flooded emergent / submerged (FE/S) vegetative structure. Each unit was divided into dominant vegetation zones based on the primary species or habitat structure. For MC, there were five zones: *Phalaris*, dominated by *Phalaris arundinacea*; SAV or submerged aquatic vegetation, dominated by floating and submerged species like *Ceratophyllum demersum*; Mixed Emergent, consisting of shallow, near-shore emergent species; *Salix*, dominated by *Salix exigua* and *Salix interior*; and *Typha*, dominated by *Typha angustifolia*.

Maankiki Center			
Vegetation Zone	**Latin Name**	**IVI**	**Hydrologic Condition**
Phalaris	*Phalaris arundinacea*	119.13	WM / DE
	Bidens cernua	23.02	WM / DE
	Medicago lupulina	9.25	WM / DE
	Persicaria pensylvanica	8.03	WM / DE
SAV	*Ceratophyllum demersum*	55.19	FE / S
	Potamogeton nodosus	44.18	FE / S
	Algae spp.	21.21	FE / S
	Elodea canadensis	21.2	FE / S
Typha	*Typha angustifolia*	56.29	FE / S
	Lemna minor	33.92	FE / S
	Spirodela polyrhiza	26.83	FE / S
	Riccia fluitans	9.05	FE / S
Mixed Emergent	*Phalaris arundinacea*	29.72	WM / DE
	Typha angustifolia	18.24	FE / S
	Ceratophyllum demersum	17.01	FE / S
	Bidens cernua	12.02	WM / DE
Salix	*Spirodela polyrhiza*	35.29	FE / S
	Salix interior	35.11	WM / DE
	Lemna minor	27.37	FE / S
	Ceratophyllum demersum	23.12	FE / S

Table 11. Importance Value Index (IVI) for the four dominant P1A vegetation zones. The four P1A vegetation zones and their corresponding influential species are listed, ranked by their important value index score and complimented by the hydrologic condition in which they were observed, the two options being either wet meadow / dry emergent or flooded emergent / submerged vegetative structure. Each unit was divided into dominant vegetation zones based on the primary species or habitat structure. For P1A, there were four zones: Mudflat, dominated by *Scirpus sylvaticus* and other emergent vegetation tolerant to saturated soil; *Nymphaea*, dominated by *Nymphaea odorata*; *Salix*, dominated by *Salix exigua & Salix interior*; and *Typha*, dominated by *Typha angustifolia*.

Pool 1A			
Vegetation Zone	**Latin Name**	**IVI**	**Hydrologic Condition**
Mudflat	*Scirpus sylvaticus*	31.78	WM / DE
	Nymphaea odorata	25.39	FE / S
	Abutilon theophrasti	19.06	WM / DE
	Alisma plantago-aquatica	16.39	WM / DE
Nymphaea	*Nymphaea odorata*	88.75	FE / S
	Ceratophyllum demersum	34.52	FE / S
	Spirodela polyrhiza	22.7	FE / S
	Lemna minor	16.44	FE / S
Typha	*Typha angustifolia*	66.28	FE / S
	Persicaria pensylvanica	16.72	WM / DE
	Phalaris arundinacea	15.2	WM / DE
	Spirodela polyrhiza	9.74	FE / S
Salix	*Salix interior*	60.77	WM / DE
	Phalaris arundinacea	28.55	WM / DE
	Lythrum salicaria	18.72	WM / DE
	Boehmeria cylindrica	17.47	WM / DE

Invasive Species Presence and Influence at SNWR

We determined IVI scores for invasive species in the sampled SNWR wetland management units to better understand the influence of invasives on local native plant communities (Table 12). Both *Phalaris arundinacea* and *Typha angustifolia* were found in all 4 wetland management units, and also accounted for the four highest IVI scores among all invasive species (119.13 MC *Phalaris* and 88.63 MN *Phalaris* for *Phalaris arundinacea*; 69.47 MN *Typha* and 66.28 P1A *Typha* for *Typha angustifolia*) (Table 12). These invasives had so much influence that entire vegetation zones were named after them, and uncoincidentally were the same zones where they had greatest influence. *Phalaris arundinacea* was found in 4 vegetation zones in P1A, MS, and MC, but in only 1 vegetation zone in MN. *Typha angustifolia* was found in 3 of the P1A and MC vegetation zones, while only appearing in 2 of the MS and MN vegetation zones. Only one invasive species, *Potamogeton crispus*, was found in a single wetland management unit (MS), with low IVI scores of 3.21 (MS SAV) and 0.89 (MS *Typha*). Maankiki South had the highest number of invasive species present (6) throughout its 4 vegetation zones, with *Lythrum salicaria* being the only invasive absent from its species list. At the other end of the spectrum, P1A had the fewest number of invasives (3) throughout its 4 vegetation zones, with only *Phalaris arundinacea, Lythrum salicaria,* and *Typha angustifolia* present. Invasive IVI scores ranged from 0.89 (*Potamogeton crispus* in MS *Typha*) to 119.13 (*Phalaris arundinacea* in MC *Phalaris*).

Table 12. Invasive species' Importance Value Index scores listed next to the corresponding SNWR wetland management unit and vegetation zone in which they were documented. IVI scores are listed in order for each of the seven invasive species; C*irsium arvense, Lythrum salicaria, Phalaris arundinacea, Butomus umbellatus, Myriophyllum spicatum, Potamogeton crispus, and Typha angustifolia*. Each unit was divided into dominant vegetation zones based on the primary species or habitat structure. These consisted of: Mudflat, dominated by *Scirpus sylvaticus* and other emergent vegetation tolerant to saturated soil; *Nymphaea*, dominated by *Nymphaea odorata*; *Salix*, dominated by *Salix exigua* and *Salix interior*; Forest (overstory and understory), dominated by *Acer saccharinum* and *Ulmus americana*; *Phalaris*, dominated by *Phalaris arundinacea*; SAV or submerged aquatic vegetation, dominated by floating and submerged species like *Ceratophyllum demersum*; ME or Mixed Emergent, consisting of shallow, near-shore emergent species; and *Typha*, dominated by *Typha angustifolia*.

Invasive IVIs			
Invasive Species	Vegetation Zone	Unit	IVI
Cirsium arvense	ME	MC	3.23
	Phalaris	MC	3.04
	Phalaris	MN	19.41
	Typha	MN	0.98
	Forest Understory	MS	12.47
Lythrum salicaria	Salix	MC	6.71
	Mudflat	P1A	3.58
	Nymphaea	P1A	1.91
	Salix	P1A	18.72
Phalaris arundinacea	Typha	P1A	15.2
	Salix	P1A	28.55
	Nymphaea	P1A	3.68
	Mudflat	P1A	2.24
	Typha	MS	7.18
	SAV	MS	7.82
	ME	MS	12.1
	Forest Understory	MS	39.81
	Phalaris	MN	88.63
	Typha	MC	8.72
	Salix	MC	8.74
	Phalaris	MC	119.13
	ME	MC	29.72
Butomus umbellatus	SAV	MS	1.58
	Phalaris	MN	2.48
Myriophyllum spicatum	SAV	MN	17.8
	Typha	MN	1.01
	SAV	MS	1.63
Potamogeton crispus	SAV	MS	3.21
	Typha	MS	0.89
Typha angustifolia	Typha	P1A	66.28
	Salix	P1A	1.72
	Mudflat	P1A	1.62
	Typha	MS	43.18

Typha angustifolia	ME	MS	4.57
	Typha	MN	69.47
	Phalaris	MN	6.83
	Typha	MC	56.29
	Phalaris	MC	2.99
	ME	MC	18.24

Nonmetric Multidimensional Scaling and Understanding Differences in Compositional Characteristics Amongst SNWR Wetland Management Units

In order to better understand how the environmental characteristics of different wetland management units and vegetation zones compared, we used Nonmetric multidimensional scaling (NMDS). We used NMDS as a visual aid in helping us to determine similarities and differences between observed vegetative communities at SNWR. Utilizing Bray-Curtis dissimilarity values, this ordination aims to plot multidimensional data on a traditional x/y axis graph, where the distance between individual data points indicates the overall similarity or dissimilarity between the data in question (Conrad et al., 2022). The farther apart two points appear on the NMDS plot, the less similar they are in terms of the data's overall composition. With NMDS plots, we evaluated similarities and differences among vegetation compositions for the 4 units and 7 vegetative zones, based on a variety of different characteristics. The composition of points seen in the graph below is determined based on plant species and hydrologic conditions, namely water depth (Conrad et al., 2022).

Plotting vegetation zones showed distinct grouping for SAV and *Typha*, indicative of significant similarity in vegetation composition. In terms of wetland management units, the only apparent clustering was for MS, where most samples showed significant similarity; the Forest zone, however, was notably different from the SAV, ME, and *Typha* zones. Finally, the largest distance between vegetation zones of the same type was for the *Salix* community, indicating that while these zones were distinguished by the same dominant species, their vegetative makeup caused by differing ecological wetland management units caused greater dissimilarity than other vegetation zones. Overall, however, it was apparent that two sites being in the same unit did not indicate significant correlation, rather it was the vegetation zones that caused distinct groupings.

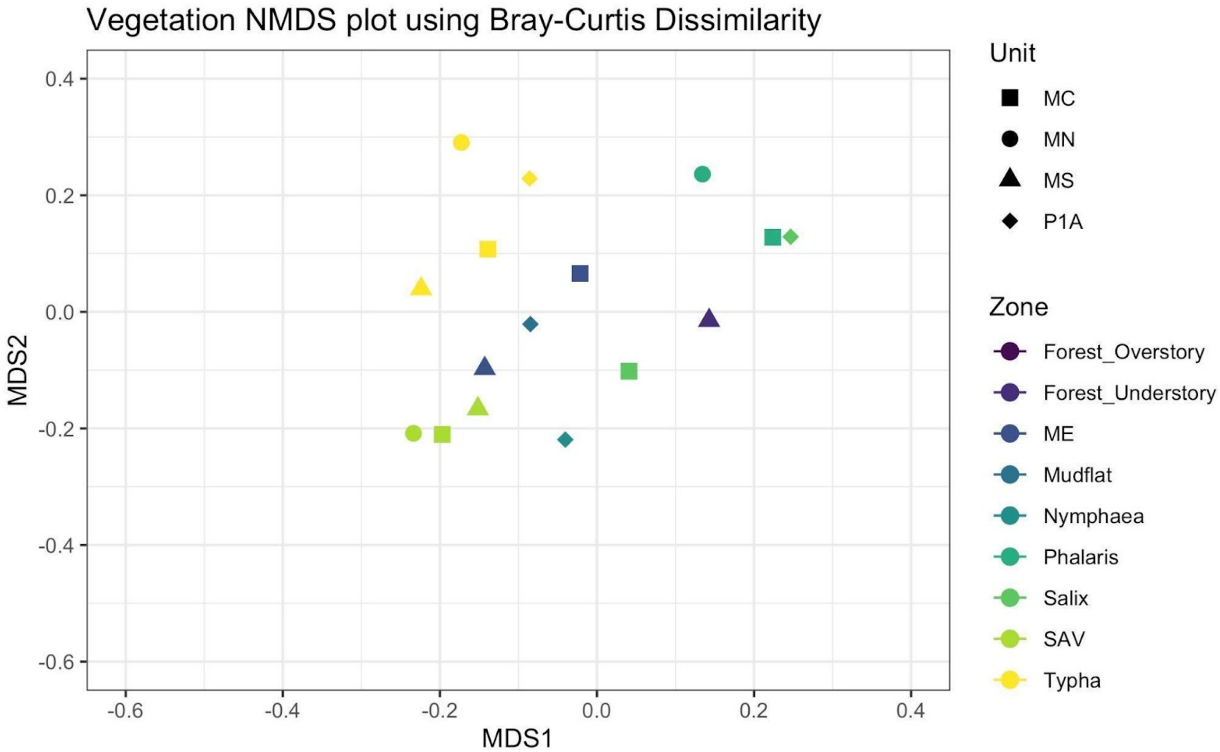


Figure 21. NMDS plot displaying compositional similarity and differences amongst the different combinations of vegetation zones and wetland management units sampled during 2022. Legend refers to the differing wetland management units (MC, MN, MS, P1A) and vegetative zones (Forest, Mixed Emergent (ME), Mudflat, *Nymphaea*, *Phalaris*, *Salix*, SAV, *Typha*) present in observed samples. The combination of specific shape and color indicate the exact survey sample that is represented (i.e., a yellow square represents a *Typha* MC site). Vegetation zones are: Mudflat, dominated by *Scirpus sylvaticus* and other emergent vegetation tolerant to saturated soil; *Nymphaea*, dominated by *Nymphaea odorata*; Salix, dominated by *Salix exigua* and *Salix interior*; Forest (overstory and understory), dominated by *Acer saccharinum* and *Ulmus americana*; *Phalaris*, dominated by *Phalaris arundinacea*; SAV or submerged aquatic vegetation, dominated by *Ceratophyllum demersum* and other submerged or floating species; mixed emergent, consisting of shallow, near-shore emergent species; and *Typha*, dominated by *Typha angustifolia*. Stress value is 0.092, indicating that the model is portraying a "great to excellent" visual illustration of the variance between each unit/zone combination.

Floristic Quality Analysis and Evaluating the Health of SNWR's Vegetative Community

We explored species sensitivity, composition, and community health between our four wetland management units using Floristic Quality Analysis (FQA). We broke each unit's species list into seven individual physiognomic categories to better understand how community composition differed among the four wetland management units. Species were entered into the online Universal FQA Calculator tool (https://universalfqa.org/), and species were assigned to physiognomic categories within the Calculator (Forb, Grass, Rush, Sedge, Shrub, Vine, or Tree). Some species we observed could not be located in the FQA database, as it was developed for only vascular species, and this altered the total number of species and total number of natives that were documented in each unit.

Community composition in the wetland management units was dominated by forbs, with MC having the highest overall count of 34. Conversely, not a single rush was identified in the four wetland management units, making it the least prevalent of the 7 physiognomic categories; shrub and vine had totals of 3 and 2 species, respectively, within the 4 wetland management units.

Maankiki North had the highest diversity, with an average of approximately 10 native species and 14 total species per vegetative zone, while MS proved to be the least diverse with an average of only 5.25 native species and 7 total species per vegetative zone. MC and P1A fell in the middle of these two extremes, but were closer to MS; MC averaged 6.8 natives and 9 total species per vegetative zone, and P1A averaged 8.25 natives and 10 total species per zone.

While MN had the highest diversity, it scored the lowest for both total and native mean conservatism values, an overall grade for sensitivity to anthropogenic degradation with 0 being least sensitive and 10 being most. Maankiki North scores included a 2.6 total mean C and a 3.7 native mean C, while MS, on the other hand, had the highest native mean C score of 4. Overlooking MN, the other three wetland management units had total C scores of exactly 3.

With regards to native and total floristic quality index (FQI), values that indicate overall vegetative community health, there again was a shift in which unit held the highest score. MC led in both categories with scores of 22.7 and 20.1, respectively, where 1–19 is low quality, 20–35 is high quality, and greater than 35 is exceptional vegetative health. P1A followed closely behind with a native score of 21.3 and a total score of 19, while MN and MS were close together near the bottom of the FQI totals. MS scored the lowest in both native and total FQI with 18.3 and 15.9, while MN was slightly higher at 19.9 and 16.6, respectively.

Table 13. Vegetation structure/physiognomy, native and total species averages, mean native and total C scores, and native/total FQI scores comprehensively assessed utilizing FQA analysis**.** Native and total mean C are listed and were calculated on a scale of 0-10, where 0 indicates a completely degraded community and 10 indicates reference or pristine conditions. Total and native FQI, which standardizes C for differences in landscape size and species totals for regional inter-site comparisons, is listed for all wetland management units and is scored on a scale where 0-19 indicates low quality vegetative communities, 20-35 is moderate quality, and above 35 is exceptional quality.

Unit	MC	MN	MS	P1A
Forb	34	30	21	29
Grass	3	3	1	2
Rush	0	0	0	0
Sedge	5	3	2	6
Shrub	1	1	0	1
Vine	0	1	0	1
Tree	2	3	4	1
Native Species	34	29	21	33
Total Species	45	41	28	40
Native Mean C	3.9	3.7	4	3.7
Total Mean C	3	2.6	3	3
FQI Values Natives Only	22.7	19.9	18.3	21.3
Total FQI Values	20.1	16.6	15.9	19
Native FQI Descriptive Score	Moderate	Low/Moderate	Low	Moderate
Total FQI Descriptive Score	Moderate	Low	Low	Low

Table 14. Numeric FQI values and associated ratings for wetland management units sampled during 2019-2022. Both Native and Total FQI values are shown, in addition to the descriptive category the score falls under.

Unit	MC	MN	MS	P1A
2019 Total FQI Descriptive Score	No MC Veg Data	Low (11.2)	Moderate (23.5)	Moderate (21.6)
2019 Native FQI Descriptive Score	No MC Veg Data	Low (12.9)	Moderate (25)	Moderate (22.7)
2020 Total FQI Descriptive Score	No MC Veg Data	Low (13.9)	Low (11.5)	Low (16.4)
2020 Native FQI Descriptive Score	No MC Veg Data	Low (17)	Low (12.7)	Low (18.8)
2021 Total FQI Descriptive Score	Low (12.58)	Low (10.8)	Low (11.9)	Low (12.05)
2021 Native FQI Descriptive Score	Low (13.58)	Low (12.03)	Low (13.57)	Low (13.3)
2022 Total FQI Descriptive Score	Moderate (20.1)	Low (16.6)	Low (15.9)	Low (19)
2022 Native FQI Descriptive Score	Moderate (22.7)	Low/Moderate (19.9)	Low (18.3)	Moderate (21.3)

Index of Biotic Integrity and Examining SNWR's Proximity to Reference Conditions

We calculated a wetland vegetation Index of Biotic Integrity (IBI) value for each wetland management unit using a series of 10 vegetative metrics; each was assigned a numeric value, and these 10 values were summed to create a total numeric score that provided a qualitative description of the overall health of a particular wetland management unit based on its proximity to pre-colonial reference conditions. Each metric received a score of 0, 1, 3, or 5, with 0 being indicative of incredibly poor or degraded quality and 5 being considered reference or pristine conditions. The sum of the 10 numeric scores was rated using a scale of 0-50, with 0-5 being very low quality, 6-20 being low quality, 21-40 being medium quality, and 41-50 being exceptional quality. The combined numeric scores across all 4 wetland management units did not differ greatly; P1A scored highest at 21 and MC scored lowest at 11.

Our overall IBI scores were relatively consistent with those of 2019 and 2020. However, in 2021, the sampling team assessed overall vegetative reference quality as higher by an average of roughly 10 total points per wetland management unit. MC in 2022 was drastically different, scoring in the Low quality category with a score of 11, whereas in 2021, it tied for the highest overall IBI at 28 and was rated a “medium” level of vegetative health. The first year of hydrologic reconnection of MC was 2021, and thus comparisons with 2020 & 2019 data were not possible.

Table 15. We summed four IBI parameters to create the Total 2022 IBI score**.** The score calculation included 3 site categories per unit (ENTIRE, WMDE, & FES) and took into account submergent coverage, invasive cover, frequency, and C score at each site. The 10 metrics' numeric assignments were then summed to produce a cumulative numeric score that assigned a standardized descriptive ranking for each site (Very Low, Low, Medium, and High). Each of the 10 total metrics ranged from 0-5, with 5 being the highest ecological quality.

<table>
<tr><th colspan="6">2022 IBI Scores</th></tr>
<tr><th colspan="2">Unit</th><th>MC</th><th>MN</th><th>MS</th><th>P1A</th></tr>
<tr><td rowspan="4">Entire Site</td><td>Invasive Cover</td><td>1</td><td>1</td><td>3</td><td>1</td></tr>
<tr><td>Invasive Frequency</td><td>1</td><td>0</td><td>0</td><td>1</td></tr>
<tr><td>Number of Quadrats</td><td>55</td><td>35</td><td>55</td><td>45</td></tr>
<tr><td>C</td><td>1</td><td>1</td><td>1</td><td>3</td></tr>
<tr><td rowspan="4">Wet Meadow & Dry Emergent Zone</td><td>Invasive Cover</td><td>0</td><td>0</td><td>3</td><td>1</td></tr>
<tr><td>Invasive Frequency</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Number of Quadrats</td><td>26</td><td>25</td><td>19</td><td>28</td></tr>
<tr><td>C</td><td>0</td><td>1</td><td>3</td><td>3</td></tr>
<tr><td rowspan="4">Flooded Emergent & Submerged Zone</td><td>Invasive Cover</td><td>1</td><td>3</td><td>1</td><td>3</td></tr>
<tr><td>Invasive Frequency</td><td>1</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td>Number of Quadrats</td><td>29</td><td>10</td><td>36</td><td>17</td></tr>
<tr><td>C</td><td>3</td><td>3</td><td>1</td><td>3</td></tr>
<tr><td colspan="2">Submergent Coverage</td><td>3</td><td>3</td><td>3</td><td>3</td></tr>
<tr><td rowspan="2">Combined Standardized Score</td><td>Numeric Score</td><td>11</td><td>12</td><td>16</td><td>21</td></tr>
<tr><td>Descriptive Score</td><td>Low</td><td>Low</td><td>Low</td><td>Medium</td></tr>
</table>

Table 16. Four IBI parameters summed to create the Total 2021 IBI score**.** The score calculation included 3 site categories per unit (ENTIRE, WMDE, & FES) and took into account submergent coverage, invasive cover, frequency, and C score at each site. The 10 metrics' numeric assignments were then summed to produce a cumulative numeric score that assigned a standardized descriptive ranking for each site (Very Low, Low, Medium, and High). Each of the 10 total metrics ranged from 0-5, with 5 being the highest ecological quality. (Conrad et al. 2022)

2021 IBI Scores					
Unit		MC	MN	MS	P1A
Entire Site	Invasive Cover	1	1	3	3
	Invasive Frequency	3	3	3	3
	Number of Quadrats	55	35	45	43
	C	3	3	3	3
Wet Meadow & Dry Emergent Zone	Invasive Cover	1	0	3	3
	Invasive Frequency	3	3	3	3
	Number of Quadrats	1	15	14	17
	C	5	1	1	1
Flooded Emergent & Submerged Zone	Invasive Cover	1	1	3	3
	Invasive Frequency	3	3	3	3
	Number of Quadrats	54	20	31	26
	C	3	3	3	3
Submergent Coverage		5	5	3	3
Combined Standardized Score	Numeric Score	29	23	26	28
	Descriptive Score	Medium	Medium	Medium	Medium

Table 17. Four IBI parameters summed to create the Total 2020 IBI score**.** The score calculation included 3 site categories per unit (ENTIRE, WMDE, & FES) and took into account submergent coverage, invasive cover, frequency, and C score at each site. The 10 metrics' numeric assignments were then summed to produce a cumulative numeric score that assigned a standardized descriptive ranking for each site (Very Low, Low, Medium, and High). Each of the 10 total metrics ranged from 0-5, with 5 being the highest ecological quality (Dellick et al., 2021)

2020 IBI Scores					
Unit		MC	MN	MS	P1A
Entire Site	**Invasive Cover**	No MC Veg Data	0	3	3
	Invasive Frequency		0	0	3
	Number of Quadrats		35	45	55
	C		1	3	1
Wet Meadow & Dry Emergent Zone	**Invasive Cover**		0	No Veg Data	No Veg Data
	Invasive Frequency		0	No Veg Data	No Veg Data
	Number of Quadrats		No Veg Data	No Veg Data	No Veg Data
	C		0	No Veg Data	No Veg Data
Flooded Emergent & Submerged Zone	**Invasive Cover**		1	3	3
	Invasive Frequency		0	0	1
	Number of Quadrats		No Veg Data	No Veg Data	No Veg Data
	C		1	3	1
Submergent Coverage			5	5	5
Combined Standardized Score	**Numeric Score**		8	17	17
	Descriptive Score		Low	Low	Low

Table 18. Four IBI parameters summed to create the Total 2019 IBI score**.** The score calculation included 3 site categories per unit (ENTIRE, WMDE, & FES) and took into account submergent coverage, invasive cover, frequency, and C score at each site. The 10 metrics' numeric assignments were then summed to produce a cumulative numeric score that assigned a standardized descriptive ranking for each site (Very Low, Low, Medium, and High). Each of the 10 total metrics ranged from 0-5, with 5 being the highest ecological quality. (Lugten et al., 2020)

2019 IBI Scores					
Unit		MC	MN	MS	P1A
Entire Site	**Invasive Cover**	No MC Veg Data	1	1	1
	Invasive Frequency		0	0	1
	Number of Quadrats		No Veg Data	No Veg Data	No Veg Data
	C		1	3	3
Wet Meadow & Dry Emergent Zone	**Invasive Cover**		0	3	No Veg Data
	Invasive Frequency		0	1	No Veg Data
	Number of Quadrats		No Veg Data	No Veg Data	No Veg Data
	C		5	5	No Veg Data
Flooded Emergent & Submerged Zone	**Invasive Cover**		1	1	No Veg Data
	Invasive Frequency		0	0	No Veg Data
	Number of Quadrats		No Veg Data	No Veg Data	No Veg Data
	C		1	3	No Veg Data
Submergent Coverage			5	5	5
Combined Standardized Score	**Numeric Score**		14	22	20
	Descriptive Score		Low	Low	Medium

DISCUSSION

SNWR Vegetation and Waterfowl Habitat

As in most restored wetlands, the process of ecological restoration and management at SNWR primarily focuses on the encouragement of conditions that support native vegetative species and hinder the encroachment of invasives and non-natives (Conrad et al., 2022). At SNWR, this is done primarily via the systematic manipulation of hydrologic control structures, which are opened and closed based on regional precipitation, temperature, and local water levels (Heitmeyer et al., 2013). This decision-making process primarily targets production of high-quality habitat for waterfowl, who rely heavily on submerged and floating wetland vegetation as a food source (Cohen et al., 2020).

Species such as coontail (*Ceratophyllum demersum*) and pondweeds (*Potamogeton spp.*) are critical food sources for diving and dabbling waterfowl who utilize the refuge year-round and were some of the dominant species in our survey of submerged aquatic vegetation (Tables 8-11) (Cohen et al., 2020). *Ceratophyllum demersum* ranked first in IVI score for each SAV zone, with the exception of Maankiki South, where it scored the third highest IVI. It also appeared as the second most influential species in P1A's submerged *Nymphaea* zone. While P1A's IVIs failed to reflect that *Potamogeton nodosus* was having the same success that *Ceratophyllum demersum* did, it had similar influence and presence across each of the Maankiki wetland management units, scoring second in IVI for all three of the sampled SAV wetland management units. As P1A is the refuge's oldest wetland management unit, it is quite possible that the increased exposure to riverine silt deposits and heightened turbidity has made it difficult for *Potamogeton nodosus* to thrive, amidst competition from species like *Ceratophyllum demersum* and *Nymphaea odorata*, which are far more tolerant to nutrient enrichment, sedimentation, increased turbidity, and water level fluctuation (GLCWMP, 2018).

During the 2022 sampling season, P1A water depth was at its lowest during the 4 years of monitoring (2019-2022). Average depths in the *Nymphaea* vegetation zone in 2022 was 24.47cm, compared to 75cm, 84cm, and 39cm in 2019, 2020, and 2021, respectively (Conrad et al. 2022, Dellick et al. 2021, Lugten et al. 2020, Table 7). Sites in P1A characterized as SAV in sampling years 2019-2021 were characterized as Mudflat in 2022, and average depths in these areas varied from 4.1cm in 2022 to 56cm, 64cm, and 29cm centimeters in previous sample years (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). This dramatic decrease in water depth in P1A left *Potamogeton nodosus* vulnerable to unfavorable conditions; the species needs at least 15cm of standing water to survive, and it's likely that the significant drop in water levels over the past several years has made it difficult for *Potamogeton nodosus* to proliferate.

With the tremendous decrease in hydrologic volume, it was also apparent that the conditions in P1A dramatically worsened in terms of excess nutrients (Figures 15 & 16). Between 2021 and 2022, P1A saw an increase in Kjeldahl nitrogen and Phosphorus, unlike the other 3 wetland management units. While MS, MN, and MC largely saw their concentrations hold constant, P1A had its Kjeldahl nitrogen levels almost double from roughly 1.25mg/L to just around 2.35 mg/L (Figure 15). Similarly, Phosphorus concentrations nearly doubled, going from .23 mg/L to roughly .38 mg/L in 2022 (Figure 16). This drastic increase in bio-available Nitrogen and Phosphorus may have impacted the less tolerant *Potamogeton nodosus*, and forced it to be outcompeted by species like *Ceratophyllum demersum* and *Lemna minor* (4th in P1A's *Nymphaea* IVI) that are much hardier when it comes to increased nutrient loads.

Vegetation Indices of Biotic Integrity: 2022 Conditions and Regional Comparisons

Overall, 2022 Indices of Biotic Integrity give us significant insight into the health of the restored wetland management units, as well as the metrics that have seen the most change and variability throughout the 4 sampling seasons.

Pool 1A, the unit with the longest history of reconnection and the reference unit for the 4 years of ecological monitoring, measured in with the highest IBI, with a collective numeric score of 21 and an overall descriptive score of "moderate" ecological health (Table 15). Pool 1A also had the fewest invasives (3) out of any of the wetland management units, though it had the highest concentration of *Lythrum salicaria*, likely due to the fact that low 2022 water levels caused an increase in muddy, saturated soil conditions in P1A (hence the introduction of the Mudflat vegetation zone) that would have otherwise been flooded in previous years. This gave rise to habitat conducive to *L. salicaria*.

Maankiki South had the next highest IBI, with a numeric score of 16, at the upper range of the "low" quality rating (ranging from 6 to 20) (Table 15). Despite having 6 different invasive species present across the entire unit, MS earned second place status largely due to the infrequent presence of invasives; MS had the highest numeric score for invasive frequency. MS had the highest score for invasive cover, scoring a numeric value of 3 on the IBI range for overall invasive cover present in a unit. This was one of the select few metrics that was consistent from 2021. Scoring a "3" in the numeric category for the entirety of MS meant that there were invasives species found in less than 25% of the 55 total quadrats sampled, indicating that outside of the management of the abundant *Typha angustifolia* present in the unit, MS has had effective mitigation of invasive species.

Maankiki North was at the lower end of the range of our IBI analysis, with a scoring of 12 and a firm rating of "low" quality (Table 15). Despite having the second highest number of species

(45), MN also had the second highest number of invasives species (5), just behind that of MS (Figure 20). Of the 3 vegetation zones present in MS, two were dominated heavily by invasive species. Its *Phalaris* zone was dominated by *Phalaris arundinacea* with an IVI score of 88.63, the next highest score being a second invasive, *Cirsium arvense,* with a score of 19.41. Maankiki North's *Typha* zone was dominated by *Typha angustifolia* with an IVI of 69.47*,* with the next most influential species (*Populus deltoides*) scoring a 12.36.

Maankiki Center, the youngest wetland management unit in terms of hydrologic connectivity, rounded out the IBI scoring with the lowest numeric value of 11 and a definitively "low" rating (Table 15). Surprisingly, MC also had the highest number of total species (55), but this likely can be attributed to the fact it shares a lengthy border with each of the other 3 wetland management units, and its internal acreage (835.8 acres) is nearly twice that of the next largest unit (Table 7 and Figure 20). While the unit had only 4 of the 7 invasive species present, its vegetative communities were unfortunately dominated by fast-growing invasives, as we would expect for a wetland management unit that was so recently reconnected. Maankiki Center's *Phalaris* zone had the highest IVI score of any of the wetland management units, with *Phalaris arundinacea* registering at 119.13 IVI. As was expected, its *Typha* zone was dominated by *Typha angustifolia* with an IVI of 56.29, but more surprising was that its Mixed Emergent zone top two IVI scores were both *Phalaris arundinacea* and *Typha angustifolia*, measuring in at 29.72 and 18.24 respectively.

According to the Great Lakes Coastal Wetlands Monitoring Program's Coastal Decision Support Tool (Coastal Wetland Monitoring Program 2019), wetland sites along Saginaw Bay and in nearby inland wetlands similar to SNWR range from 1.5 to 3.1 on the simplified 0-15 vegetation IBI scale that considers only 3 metrics (invasive cover, invasive frequency, and C score) for the whole site (Figure 22). Saginaw Bay River, the site identified as closest to SNWR, scored at the bottom of this scale (1.5) and was rated as "degraded" for its descriptive score (Table 19). In comparison, SNWR's wetland management units in 2022 scored roughly 3.5, placing these near the top of the list of regional sites and rating as "moderately degraded" (Conrad et al., 2022). Overall, it was clear, based on regional comparisons, that health of vegetative communities at SNWR is relatively high despite the ongoing presence of anthropogenic stressors, agricultural runoff, and legacy industry that has burdened Saginaw and its surrounding areas for decades.

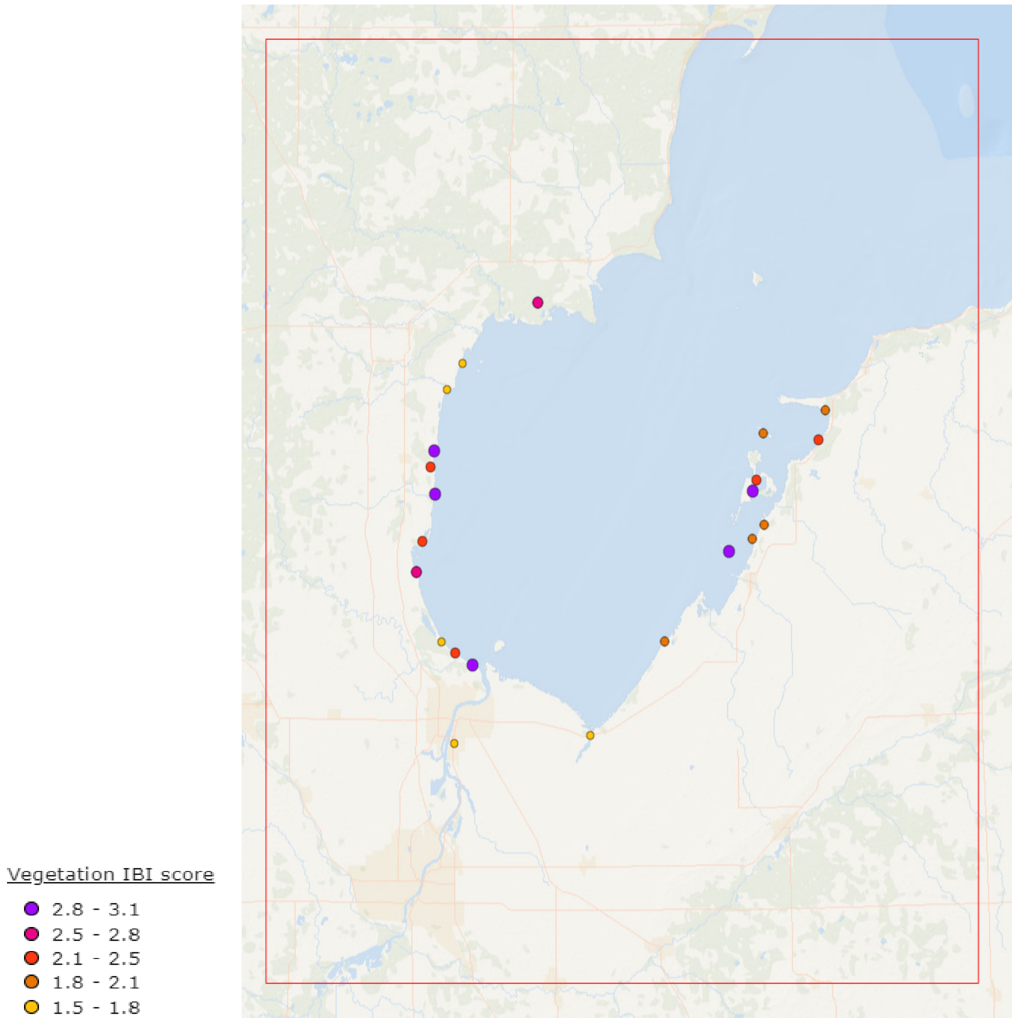


Figure 22. Map of the 22 wetlands neighboring SNWR. The wetlands are ranked from highest to lowest and their color corresponds to the range assignments in neighboring map. The most applicable measurement being Saginaw Bay River, lies at the bottom left of the range with an IBI value of 1.5.Vegetation IBI color value designation for the Coastal Wetland Decision Making Tool map that is listed above. Each colored dot corresponds to 1 of the 22 neighboring wetlands.

Table 19. The 22 wetland sites marked on the above CWDMST map shown in Figure 22. Saginaw Bay River, the most similar site, is at the bottom of the list.

Ranked Wetland Sites (22)

Rank #	Site ID	Site Name	Vegetation IBI score
1	522	Nayanguing Point Wildlife Area Wetland #1	3.1
2	760	East Saginaw Bay Coastal Wetland #10	3.1
3	485	Aplin Beach Wetland	3.0
4	520	East Saginaw Bay Coastal Wetland #9	2.8
5	523	Nayanguing Point Wildlife Area Wetland #3	2.8
6	499	West Saginaw Bay Wetland #1	2.7
7	521	Linwood Area Wetland #2	2.7
8	486	Lagoon Beach Wetland	2.4
9	494	Nayanguing Point Wildlife Area Wetland #2	2.4
10	493	Nayanguing Point Wildlife Area Wetland	2.3
11	508	East Saginaw Bay Coastal Wetland #15	2.3
12	461	Wildfowl Bay Wetland	2.2
13	515	East Saginaw Bay Coastal Wetland #5	2.1
14	460	Caseville Township Wetland	2.0
15	761	East Saginaw Bay Coastal Wetland #14	2.0
16	466	North Island Wetland	1.9
17	510	East Saginaw Bay Coastal Wetland #11	1.9
18	488	Tobico Lagoon Wetland	1.7
19	496	Nayanguing Point Wildlife Area Wetland #5	1.6
20	498	West Saginaw Bay Wetland	1.6
21	517	East Saginaw Bay Coastal Wetland #2	1.6
22	524	Saginaw Bay River	1.5

Index of Biotic Integrity: Discrepancies Between Sampling Year Calculations

Comparisons between 2021 and 2022 IBI highlighted some discrepancies in calculations between the 2021 sampling year and other sampling years. IBI scores for 2021 were on average 11.25 points higher than 2022 totals, and in the most extreme case (MC), there was a 17 point difference. While this immediately caused some concern about quality and accuracy of our collection and analysis, as well as fear of abrupt ecological degradation, further examination of the 2020 data illuminated the issues driving the higher 2021 scores. The IBI score increase from 2020 to 2021 (11.67 points) was nearly identical to the decrease in IBI from 2021 to 2022 (11.25 points) (Dellick et al., 2021 ; Conrad et al., 2022). This caused us to search for a distinct difference in either the sampling process or the IBI calculation that distinguished 2021 from the other years of data analysis.

We first examined the IBI score for MC, as this seemed unusually high in 2021. Maankiki Center was newly opened and first sampled in 2021; surprisingly, the 2021 IBI score for MC indicated that this newly reconnected wetland was in fact already the healthiest. MC was tied with P1A with the highest IBI numeric score of any of the four wetland management units (28 points); its submergent cover and C quotient each scored a perfect 5 for their respective categories, totaling 10 numeric points in only 2 categories (Conrad et al., 2022 ; Table 16). In comparison, the total IBI score of MC in 2022 was 11 (Table 15).

For the 2021 season, researchers were challenged in MC by a lack of sampling data for Wet Meadow and Dry Emergent zone; this comprises a third of the IBI score and heavily influences the overall score and rating. The IBI table for MC in 2021 showed only a single quadrat for this vegetation type, raising the possibility of bias due to limited sampling (Conrad et al., 2022 ; Table 16). In comparison, in MC in 2022, we sampled 26 quadrats representing the Wet Meadow / Dry Emergent type (Table 15).

The ecological and environmental conditions of a single 2021 quadrat created a massive discrepancy between numeric and descriptive scores for the unit. Looking at the 2021 calculation table for MC (Table 16 ; Conrad et al., 2022), we found that 10 points of 2021's numeric score came from the WM/DE type. In comparison, our 2022 calculation included 0 points from this type, and every metric in the WM/DE zone rated the lowest condition (Table 15).

Since criteria for the WM/DE type includes there being < 1 cm of water depth in a given quadrat, it could be that in 2021 there were very few MC quadrats on dry land. As a result, researchers may have been forced to utilize only the single quadrat to avoid having no data for this vegetation type. It also may be that there were errors in the reporting or analysis of water depth per quadrat; MC contains large *Salix* and *Phalaris* vegetation zones that typically are quite dry (i.e., < 1 cm of water depth). We suggest further examining data from 2021 to better understand what may have caused these discrepancies.

Further confirmation of a discrepancy comes from examination of the FQA for MC in 2021, particularly in regards to total FQI values. The IBI scores, which are representative of how close a vegetative community matches the reference conditions and plant assemblages of an undisturbed or undegraded natural area, should match closely with FQI values, which numerically describe the overall health of the vegetative community based on diversity, number of invasive species, and frequency of invasive occurrence. FQI values and ratings across the board for 2021 were lowest of any of the 4 sampling years, averaging 11.83 or "Low" total vegetative health, and the MC FQI score was 12.58 (Table 14 ; Conrad et al. 2022). On the other hand, average FQI scores for all 4 wetland management units in 2020 and 2022 were 13.9 and 17.9, respectively, with the 2022 MC value being 20.1, or of “Moderate” vegetative health (Table 14 ; Dellick et al. 2021). Overall, it’s highly unlikely that, during 2021, a unit could score so low on FQI while simultaneously scoring a high IBI score.

We also found discrepancies in 2021 data involving the invasive frequency calculation. Invasive frequency is calculated by dividing total number of quadrats with an invasive species present by the total number of quadrats in a sampling unit. A similar calculation provides invasive frequency for both FE/S and WM/DE vegetation zones. For 2021, every unit under each of the 3 categories (entire site, WM/DE, and FE/S) scored a 3 for invasive frequency (Table 16 ; Conrad et al., 2022). This means that invasives were present, but they were present in less than 25% of the quadrats that were sampled. Comparatively in 2022, invasive frequency was only given a “3” in one unit + zone combination (P1A FE/S) (Table 15). This was identical for 2020 (P1A entire site)(Dellick et al., 2021). In 2020, all but 1 zone’s invasive frequency measured in at a numeric score of 0, meaning that invasives were found in greater than 50% of the quadrats sampled (Table 17 ; Dellick et al., 2021). This was similar for 2022, with 7 of the 12 invasive frequency scores having a numeric total of “0” (Table 15).

This difference in year to year data, and the consistency with which 2021 varied from both 2020 and 2022, is indicative of two possibilities. The first is that the invasive species presence on the refuge decreased by 50% from 2020 to 2021, only to increase from 2021 to 2022 by 100%. This option is realistically infeasible, as that type of reduction and then subsequent resurgence would be almost impossible in such a short period of time. Furthermore, the amplified presence of European Frogbit (*Hydrocharis morsus-ranae*) on the refuge since it was first identified in the 2020 sampling year indicates that invasive presence on the refuge is currently growing, rather than receding (Dellick et al. 2021).

The second possibility is that there was a discrepancy in 2021’s sampling interpretation, meaning that the invasive frequency for the 2021 IBI was calculated or interpreted incorrectly. It is most likely that the latter option occurred, especially considering that so many of the vegetative zones are named after the dominant invasive that occurs there (*Typha* and *Phalaris*), which would in most cases cause the frequency of invasive appearance to at least surpass 25%, seeing as an invasive species is the most dominant and influential species found in that particular zone.

A final important note regarding this interannual discrepancy is the way in which *Phalaris arundinacea* was quantified in 2021. While *Phalaris* is technically native in Michigan, the aggressive nature in which it grows deems it necessary to quantify it as an invasive, which is accounted for in the FQA calculator utilize in our assessment. It is possible that the 2021 sampling team failed to interpret quadrats with *Phalaris* present as ones with a positive invasive presence, thus making any *Phalaris* quadrat appear more ecologically healthy (in terms of lacking invasives) than was otherwise the case.

Disturbance and Diversity at SNWR, Illustrated by FQA

Floristic quality analysis for our wetland management units, as well as the FQI scores and mean C values that they ultimately yielded, are indicative of recently connected and restored wetlands, with incredible potential for continued ecological resurgence. Both native and total FQI values for MN, MS, and P1A were highest since the first year of sampling at SNWR (2019), with MN scoring 19.9 (native) 16.6 (total), MS scoring 18.3 (native)15.9 (total), and long-term reference unit P1A scoring 21.3 (native) 19.0 (total) (Table 14). These FQI scores are mostly in the upper range of the "low" rating, which extends from 0-19. However, they were close to receiving "moderate" overall health ratings, and they show notable increases from 2020 and 2021 seasons.

FQI scores for the newest unit, MC, 2022 were vastly improved over those for 2021. Scores for 2022 were: 22.7 (native) and 20.1 (total), in comparison to 13.58 (native)and 12.58 (total) for 2021(Table 14; Conrad et al., 2022). This dramatic increase in floristic quality index from 2021 to 2022 in MC is exemplary of the stunning rate of colonization for native species in MC following only 2 years of hydrologic connectivity.

Maankiki North's FQA also illuminated interesting trends. MN had the second most invasives of the 4 wetland management units, while also having the most non-native species (12) (Figure 20). This statistic includes invasive (nuisance) species, as well as benign species that do not outcompete or harm native vegetation. This works to explain why, despite having been hydrologically reconnected more recently, MN has significantly lower overall FQI scores than MC.

Study Limitations

Despite thorough implementation of standard operating procedures for sampling of vegetation in the wetland management units, there were several limitations in the methods that hindered our analyses of the character and health of vegetative communities.

Pool 1A was intended to be the reference unit for this restoration study, due to its long-term connectivity with the Shiawassee River. While P1A proved to have the most healthy vegetative community based on its IBI rating and FQA, we found significant differences between it and the newly restored Maankiki units that made cross unit comparisons difficult. Two vegetative zones, Mudflat and *Nymphaea*, only occurred in P1A. This immediately made comparisons difficult between P1A and MS, MN, and MC, which are dominated largely by invasive *Typha angustifolia* and *Phalaris arundinacea* communities. The discrepancies between P1A and the Maankiki wetland management units was largely due to differing water levels, as the average depth of SAV wetland management units in the Maankikis, compared with those in the P1A Nymphaea unit (the only fully submerged vegetation zone in P1A,) was roughly 11cm greater (Table 7).

This meant that the vegetative composition and structure of P1A was extremely different from that of the other 3wetland management units (Figure 20). In fact, P1A had 3 times as many WM/DE species than it did FE/S species. Only one other unit, MC, had more wet meadow species than flooded emergent or submerged species, and this was only by a factor of 1.5x (22 FE/S species compared to 33 WM/DE) (Figure 20). This is likely due to P1A's lengthy open connection with the Shiawassee River, which was the first wetland management unit restored at SNWR (Conrad et al., 2022). The longer connection with Shiawassee has allowed for greater deposits of sediment and silt, which are suspended in high quantities in the turbid Shiawassee, to find their way to the bottom of P1A, limiting the unit's depth in comparison with the newly restored Maankiki wetland management units.

The other apparent limitation of our study was our lack of any rush species in our FQA. Rushes, which are often abundant and common in wetland environments, were not included in our FQA for the study wetland management units due to a gap in the FQA calculator database. As a result, non-native species like the flowering rush (*Juncus effusus*), which were found commonly in mixed emergent settings at SNWR, were not included in total FQI scores. Seeing as their non-native qualities almost certainly would have had a drastic impact on the total FQI quotients, this gap in the study is a minor confounding factor in terms accurately quantifying ecological health in our study wetland management units. As a result of these gaps in FQA data, 2022 FQI scores are biased towards showing a more ecologically healthy wetland than might otherwise exist.

Implications for Management

The largest takeaway from our vegetative analyses across the wetland management units was the need for increased management of invasive vegetation. While the top 4 IVI scores in each vegetation zone / unit combination yielded a different tiered collection of species, this did not necessarily reflect wetland management units being influenced by a wide variety of plant species. Despite the existing diversity of hydrologic conditions, habitats, and species across wetland management units, we saw familiar invasives having pervasive influence within almost every single wetland management unit and vegetative zone.

This may not be surprising, considering that 9 of the 16 vegetative zone/unit combinations had at least one invasive species in their top 4 IVI scores, and 3 had at least two in their top 4 (Tables 8-11). This is indicative of wetlands whose native species are under significant stress; species like *Typha angustifolia* and *Phalaris arundinacea* congregate in dense stands, consuming precious nutrients, sunlight, and outcompeting natives who are smaller and less hearty.

The upcoming removal of *Typha angustifolia* from SNWR wetland management units, beginning in late spring 2023, will undoubtedly prove helpful, as the plant has proliferated at SWNR. The anticipated removal and subsequent conversion of that biomass into ecologically productive biochar, as planned by SNWR Biologist Eric Dunton in conjunction with researchers at University of Loyola-Chicago, will clear valuable space for native emergent vegetation and has potential to give native species a chance to gain a foothold among the vast *Typha* stands that currently populate the refuge. After removal, submergence of the remaining *Typha* stalks has proved effective in eradicating the species in wetlands where it's become thoroughly established, and this technique would prove feasible utilizing current water control structures and may be able to reduce *Typha* populations in as little as 1 to 2 years' time (DiTomasso et al., 2013). This would provide significant relief for many of the native wetland species present at SNWR, and dramatically increase IBI and FQA metrics for the refuge as a whole, indicating a healthier, more ecologically robust vegetative system.

AQUATIC MACROINVERTEBRATE MONITORING

INTRODUCTION

Evaluating aquatic macroinvertebrates provides insight into both long-term wetland habitat quality, ecological community structure, and the availability of macroinvertebrates as a food source to the fish and waterfowl taxa that SNWR aims to protect. Managers at SNWR are interested in exploring changes in macroinvertebrate communities in the time since the restorative actions in MS, MC, MN, and P1A. In recent decades, assessment of aquatic macroinvertebrates has emerged as a standardized method of indicating wetland habitat quality due to varying levels of tolerance to environmental conditions across taxa (Bonancina et al., 2023). Such conditions include water quality variables, the presence of hydrophytes or general refugia, and anthropogenically driven disturbances. The presence of environmentally sensitive invertebrate taxa can indicate habitat suitability for other biotic organisms, such as fish and waterfowl (Burton et al., 1999; Cooper et al., 2007; Cooper et al., 2014; Uzarski et al., 2016).

Research Objectives

- *Compare changes in macroinvertebrate abundances over monitoring years 2019-2022*
- *Describe variations in macroinvertebrate communities and abundances by wetland management units, months, and vegetation zones*
- *Evaluate the influence of water quality parameters on macroinvertebrate abundance*
- *Evaluate the implications of restoration success at SNWR using four years of macroinvertebrate monitoring*

METHODS

Field Surveys and Lab Processing

The collection methods used in the 2022 macroinvertebrate sampling season followed those of Lugten et al. (2020). These methods, used throughout all four sampling seasons, have been adapted from Great Lakes CWMP (2019) sampling protocols to suit monitoring needs for

SNWR. We collected macroinvertebrates one day per month between May and August 2022, with sampling days spread roughly one month apart. Wetland management units of sampling were MS, MC, MN, and P1A, with exact locations in Figure 23.

We chose sampling site locations to be representative of the major vegetation zones present within a given unit, with each unit having three to four target vegetation zones. We identified the following vegetation zones across all wetland management units: submerged aquatic vegetation (SAV), mixed emergent vegetation (ME), *Nymphaea*, *Typha*, *Phalaris*, forest, open water, and River Bulrush. As the sampling season progressed and seasonal water levels changed, we selected alternative vegetation zones as representatives for the unit during that given month. The following changes were made due to the excessive drying of wetland management units: the *Phalaris* zone in MN dried before July sampling and was replaced with Submerged Aquatic Vegetation. The *Typha* zone in P1A could not be sampled in August and was replaced with *Nymphaea*.

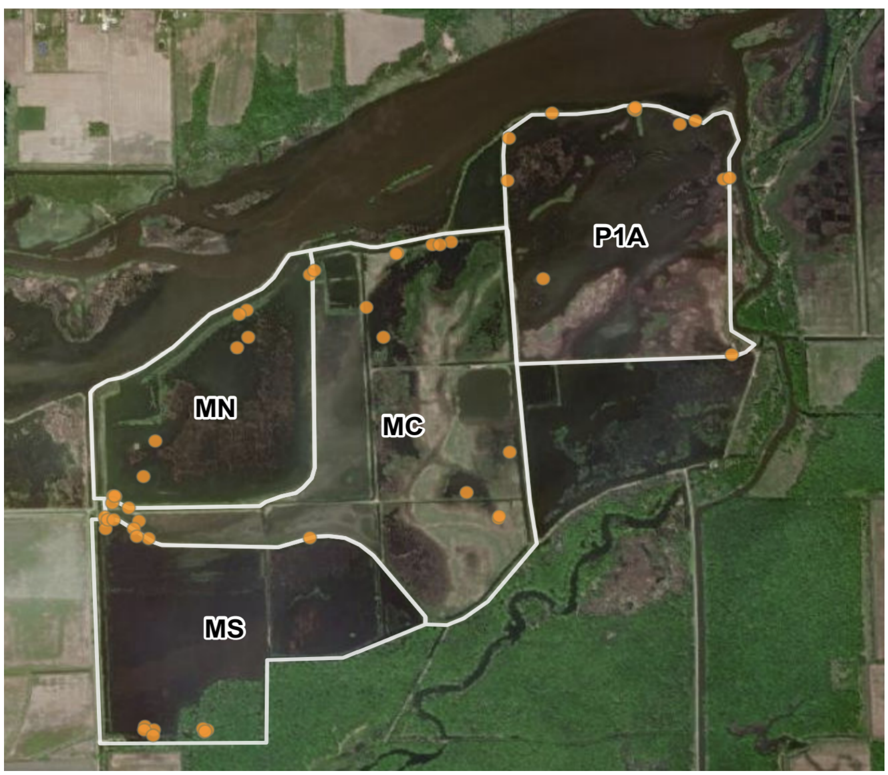


Figure 23. Sites Sampling site locations of macroinvertebrates within SNWR. White lines define unit borders, with each unit labeled, and orange circles are individual sites. The team prioritized sampling in a similar section of a vegetation zone each month when possible.

Within a vegetation zone site, the sampling area was selected randomly, tossing a 1m quadrant blindly to prevent sampling biases. Each sampling area was relatively near-shore and at a wadable depth (≤100 cm). Water quality measurements were taken in triplicate using YSI Sonde prior to sampling disturbance to ensure accurate readings. We recorded water depth within each quadrat and visually estimated percent vegetation cover. We used 0.5mm D-frame dip nets to collect macroinvertebrates within the quadrat. First, the substrate and vegetation were agitated; then nine sweeps of the net were made, vertical, horizontal, and throughout the entire water column. Team members transferred all net contents to a gridded enamel tray (25cm x 30cm x 5cm deep, with 5x5cm grid lines drawn on the inside bottom of the trays) (CWMP 2019). We repeated the collection process with two additional quadrat locations within a particular sampling site for a total of three quadrats per vegetation zone site.

Within the tray, readily visible individuals were collected regardless of grid location. Each team member selected a random grid square within the tray and thoroughly dissected the sample using forceps and pipettes until all available specimens were collected, then repeated at another grid square. The tray collection process for each site was carried out for a combined effort of 30 minutes, spread equally across participating team members (e.g., three people searching for 10 minutes, or two people searching for 15 minutes). With this collection method, not all grid squares are searched. Instead, the search effort is standardized by time. Our team stored macroinvertebrates in jars containing 70% ethanol (EtOH), labeled with a unique ID of unit name, site number, and date (e.g., MS 01 05232022).

Using a dissecting microscope, macroinvertebrate individuals were sorted and identified to either genus or the Lowest Operational Taxonomic Unit (LOTU). Identification was carried out using dichotomous keys by Merritt and Cummins (2008), Hilsenhoff (1975), and Thorp and Covich (2016), with additional identification assistance from experts when needed. Macroinvertebrate identification standards, including LOTU recommendations, were derived from the identification protocols of CWMP.

Data Management

Survey123, a customizable ArcGIS (ESRI) tool for survey response collection, was used to facilitate data recording in-situ. Each sampling site, consisting of three replicates, was documented within one ‘survey’. Parameters recorded in the survey include depth, vegetation percentage estimation, temperature (C), pH, turbidity (total dissolved/suspended solids), specific conductance (µS/cm), and dissolved oxygen (mg/L). When a survey is 'submitted,' ArcGIS Online automatically uploads survey data to a database housed within ArcGIS Online.

Macroinvertebrate identification data was recorded and organized within Survey123. One survey corresponds to each jar, which contains three replicates from a single site. Identification parameters recorded in the survey include order, family, tribe, subfamily, genus, and species, as well as the LOTU, count of individuals, and confidence in identification. Certain identification wetland management units, such as tribe, subfamily, genus, or species, were not required for all taxonomic groups and thus were recorded as "N/A."

Once all sampling site information was obtained, at the end of the monitoring season, data were extracted from ArcGIS Online and reviewed by our U.S. Fish and Wildlife partners for potential errors. After the completion of macroinvertebrate identification, team members completed the first review of data recorded for entry errors. Our partners conducted an additional review to verify if identifications were logical and probable for our geographic region, as well as checking for rare or invasive genera.

Data Analysis

Comparing Spatial and Temporal Variation in Abundances Using Analysis of Variance (ANOVA)

An Analysis of Variance test (ANOVA) was implemented to evaluate the response of CPUE by different wetland management units, months of sampling, and vegetation zones. A Shapiro-Wilk test of normality was performed on CPUE data to determine data distribution. After ANOVA, a Tukey HSD (multiple comparisons of means) posthoc test was used to compare differences in means within a parameter (wetland management unit, month of sampling, vegetation type). All tests were carried out in RStudio using packages "car" and "dplyr."

Comparing Spatial and Temporal Variation in Community Composition Using Permutation Analysis of Variance (PERMANOVA)

A Permutation Analysis of Variance (PERMANOVA) test was implemented to evaluate the influence of month, vegetation zone, and unit on macroinvertebrate community composition by calculating variance through distance matrices. Additionally, Non-Metric Multidimensional Scaling (NMDS) visualizations of distances were created for each influencer using Bray-Curtis dissimilarity and a 3-dimensional solution. Tests were carried out in RStudio using packages "vegan" and "tidyverse" for calculations and "gridExtra" and "ggplot2" for generating visualizations.

Evaluating the Influence of Water Quality Using Linear Regression

A Linear regression was implemented to explore the linear relationship between isolated water quality parameters (e.g., dissolved oxygen) and the CPUE. To account for non-normal variation in the response data, we used a generalized linear model (Family = "Gamma", Link = "Log"). Additionally, two outlier points were identified using Principal Components Analysis (PCA) and removed prior to analyses. For all macroinvertebrate data, the CPUE was calculated as the number of individuals collected at each site, as the unit of effort was a single sampling event. This test was carried out in RStudio using packages "car," "dplyr," and "lmtest."

Estimating Habitat Quality Using an Index of Biotic Integrity (IBI)

The Index of Biotic Integrity (IBI) was used to determine the level of anthropogenically driven disturbance to wetland habitats. The IBI chosen for our research was developed by Burton et al. 1999 (validation by Uzarski et al. 2004) for use by the CWMP and employed by the previous three student monitoring groups to characterize the biological quality of the wetland management units. This index's scoring divides sites into vegetation zones, then sums totals of zone scores to assign the overall wetland condition (i.e., degraded, moderately degraded, mildly impacted, or reference condition). For our purposes, only one vegetation zone included in the index was applicable in our wetland management units and therefore did not require summing zones. *Typha* is the only common vegetation zone between all four wetland management units as SNWR and the scoring index. While two wetland management units have wet meadow vegetation comparable to the IBI scoring zone, not enough sites exist to create a meaningful comparison across all wetland management units. IBI scoring calculations were carried out in Microsoft Excel.

RESULTS

Variation in Abundance and Diversity of Aquatic Macroinvertebrates

In the 2022 sampling season, we collected the fewest macroinvertebrate individuals but had similar representation in orders, families, and genera as in previous sampling seasons. Our team collected 4,377 individuals representing 16 orders, 45 families, and 81 genera of aquatic macroinvertebrates. The total family richness and genera richness of each unit varied, with the lowest richness in MN (29 and 38, respectively) and the highest richness in MS (35 and 49, respectively) (Table 20). In the 2021 field monitoring season, Conrad et al. (2022) collected 5,842 individuals representing 14 orders, 47 families, and 71 genera. In the 2020 sampling season, Dellick et al. (2021) collected 7,763 individuals representing 14 orders, 50 families, and 100 genera. Lastly, in the 2019 sampling season, Lugten et al. (2020) collected 7,587 individuals representing 43 families but did not identify beyond the family level.

Table 20. Total family and genera of aquatic invertebrates found in Shiawassee National Wildlife Refuge study wetland management units (MC: Maankiki Center; MS: Maankiki South; MN: Maankiki North; P1A: Pool 1A;) during each sampling season, 2019-2022. The 2019 sampling team only identified individuals to the family taxonomic level. MC was added as a sampling unit in the 2021 season after restoration completion.

	Families				Genera		
Unit	**2019**	**2020**	**2021**	**2022**	**2020**	**2021**	**2022**
MC	N/A	N/A	35	33	N/A	45	47
MS	41	37	27	35	52	33	49
MN	32	34	37	29	53	40	38
P1A	36	39	22	34	58	41	41

Across all sampling years (2019-2022), the nine most abundant families based on CPUE were quite similar but occurred in different rank orders (Table 21). Over the past three sampling seasons, *Hyalellidae* has been the most abundant macroinvertebrate, and it was second most abundant in the first sampling season (2019). The most significant difference across years was the abundance of *Hydracarina/Hydrachnidae*, as this taxon was abundant in the 2019 through 2021 seasons but not the 2022 season. Asellidae appeared in the top nine families only in the 2022 season.

Table 21. The nine most abundant aquatic invertebrate families for each sampling season ranked by catch per unit effort (CPUE). This list was limited to nine families due to the steep decline in CPUE thereafter. *Chironomidae* and *Hyalellidae* remained in the top 4 most abundant for all sampling seasons (2019-2022). Note that *Hydracarina* in the 2021 sampling season is the higher order for the family *Hydrachnidae* in the 2019 and 2020 sampling seasons but represents the same LOTU.

2019		2020		2021		2022	
FAMILY	**CPUE**	**FAMILY**	**CPUE**	**FAMILY**	**CPUE**	**FAMILY**	**CPUE**
Caenidae	28	Hyalellidae	67.8	Hyalellidae	21.2	Hyalellidae	20.5
Hyalellidae	21.9	Coenagrionidae	18	Chironomidae	13.6	Corixidae	12.2
Chironomidae	17	Caenidae	14.4	Caenidae	12.7	Chironomidae	7.6
Coenagrionidae	10.8	Chironomidae	5.7	Coenagrionidae	12	Coenagrionidae	5.5
Corixidae	6.1	Hydrachnidae	5.7	Corixidae	8.4	Caenidae	5.3
Pleidae	3.1	Physidae	5.4	Physidae	4.8	Physidae	3.2
Physidae	2.8	Libellulidae	4.8	Hydracarina	3	Pleidae	3.1
Hydrachnidae	2.8	Corixidae	4.7	Pleidae	2.8	Belostomatidae	3
Belostomatidae	2.4	Pleidae	3.8	Aeshnidae	1.3	Asellidae	2.4

The five most abundant families within each wetland management unit differed (Table 22). The only common family across all four wetland management units was *Hyalellidae,* which had the highest CPUE. However, MN had a significantly higher CPUE of *Hyalellidae* than P1A (30.1 and 11.2, respectively), while MS and MC had similar CPUE of *Hyalellidae* (19.6 and 22, respectively). Wetland management units MC and MN had the most similar families regarding catch abundance, with MC containing more *Corixidae* and MN containing more *Chironomidae*. P1A was the only unit where *Belostomatidae*, *Pleidae*, and *Asellidae* had representation in the top five families.

Table 22. The five most abundant families of aquatic invertebrates within each wetland management unit ranked by catch per unit effort (CPUE). This list was limited to five families due to the steep decline in CPUE thereafter. Across all wetland management units, *Hyalellidae* have the highest CPUE. MN and MC have the most similar family compositions in abundance. Abundant families in the three newly restored wetland management units differed from those in the reference unit, P1A. Note that *Corixidae* is the family and the LOTU in MS and MN and represents individuals of the *Corixidae* family with underdeveloped morphological structures, making them impossible to identify beyond the family level.

MAANKIKI SOUTH		
Family	**LOTU**	**CPUE**
Hyalellidae	Hyalella	19.6
Chironomidae	Pseudochironomini/Chironomini	11.4
Corixidae	Corixidae (immature)	7.4
Caenidae	Caenis	6.9
Physidae	Physa	5
MAANKIKI CENTER		
Family	**LOTU**	**CPUE**
Hyalellidae	Hyalella	22
Chironomidae	Pseudochironomini/Chironomini	5.9
Corixidae	Trichocorixa	5.3
Caenidae	Caenis	4.3
Coenagrionidae	Enallagma	3.1
MAANKIKI NORTH		
Family	**LOTU**	**CPUE**
Hyalellidae	Hyalella	30.1
Corixidae	Corixidae (immature)	12.3
Corixidae	Trichocorixa	6.7
Caenidae	Caenis	6
Coenagrionidae	Enallagma	3.3
POOL 1 A		
Family	**LOTU**	**CPUE**
Hyalellidae	Hyalella	11.2
Pleidae	Neoplea	10.6
Asellidae	Caecidotea	6.8
Physidae	Physa	6.7
Belostomatidae	Belostoma	5.7

Influence of Unit on Abundance and Community Composition of Aquatic Macroinvertebrates

The mean CPUE of macroinvertebrates did not vary significantly across wetland management units ($p = 0.46$; Table 23). Residuals of this test displayed normal distribution, and a Levene test found that the populations within each unit were of equal variance (homoscedastic). All wetland management units had similar median values, but MS had a slightly larger quartile range and whisker range (Figure 24). Both MS and MN had one outlier point (>1.5x upper whisker) with CPUEs 221 and 222, respectively (Figure 24). In MN, the family of most significant contribution to the outlier was *Hyalellidae,* with 121 individuals making up over half of the total macroinvertebrates collected at the site. In MS, the families *Hyalellidae* and *Chironomidae* were the most significant contributors to the outlier, with 46 and 47 individuals, respectively. However, the outlier point of MN had only 13 unique genera, while the outlier of MS had 25 unique genera, almost double the richness. These findings are reflected in the top five most abundant families, with *Hyalellidae* having the highest CPUE in MN, and *Hyalellidae* and *Chironomidae* having the highest CPUE in MS (Table 22).

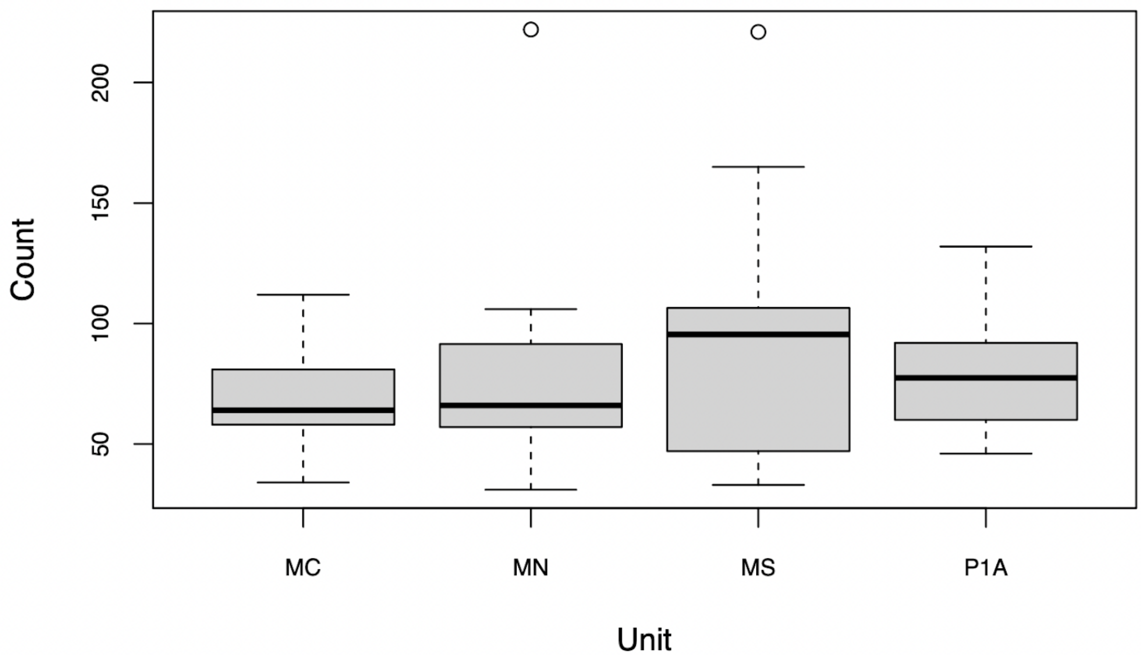


Figure 24. Catch per unit effort (CPUE) of aquatic invertebrates by Shiawassee National Wildlife Refuge wetland management unit. Units are Maankiki Center (MC), Maankiki North (MN), Maankiki South (MS), and Pool 1A (P1A). The unit MS has the most extensive whiskers range and largest median value. Units MN and MS each have one outlier point (>1.5x upper whisker) of a similar value.

To assess the influence of wetland management unit, we visualized variance in composition by calculating Bray-Curtis Dissimilarity from LOTU relative abundance per sampling site. From the results of our PERMANOVA, we found that the wetland management unit significantly contributed to the community composition of macroinvertebrates (*p = 0.002*; Table 23), with unit explaining 10.5% of the variation between samples. The Non-Metric Multidimensional Scaling (NMDS) plot showed ellipses of MS, MC, and MN overlapping considerably, indicating relatively similar macroinvertebrate communities (Figure 25). P1A showed some ellipses overlap in the ordination space, however, displayed some distinction from the other three wetland management units, with an increase in MDS1 and decrease in MDS2.

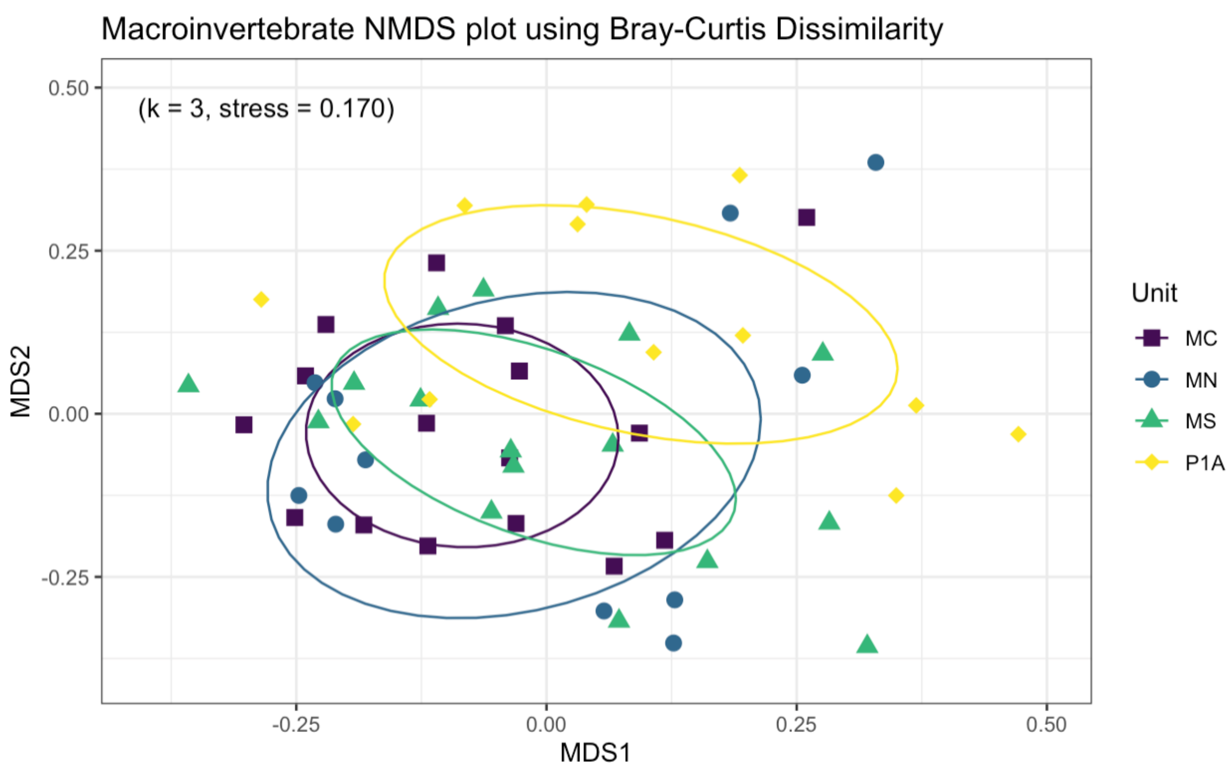


Figure 25. NMDS plot using Bray-Curtis Dissimilarity of aquatic macroinvertebrates per wetland management unit. Points represent sampled wetland management units MS, P1A, MC, and MN. Ellipses represent a 50% confidence interval around sampling points. P1A shows the highest variability across MDS1 and the lowest similarity with the other wetland management units; MN shows the highest variability across MDS2.

Previous sampling teams 2019-2021 found similar variations in macroinvertebrate community composition (Conrad et al., 2022; Dellick et al., 2021; Lugten et al., 2020). The 2019 and 2020 seasons observed the highest community dissimilarity between wetland management units MS and MN. However, those sampling years occurred before the MC hydrological reconnection was completed and therefore did not conduct macroinvertebrate sampling in that unit. The results of 2021 describing the influence of wetland management units better align with our team's findings. In 2021 and 2022, the sampling years that included MC, the most significant similarities in community composition were observed between MN, MC, and MS.

Influence of Month on Abundance and Community Composition of Macroinvertebrates

Mean CPUE of aquatic macroinvertebrates varied significantly across months ($p = 0.0327$; Figure 26; Table 23). Residuals of this test had normal distribution, and a Levene test found that the population of each month was of equal variance (homoscedastic). Average CPUE increased from May to June, then decreased June to July, and decreased again from July to August (Figure 26). The month of June yielded the highest average CPUE (avg. 105 per catch) and had a mean CPUE significantly different from the mean CPUE of May (avg. 61 per catch), with the difference being 43.55 individuals ($p = 0.0169$; Figure 26; Table 23). May, July, and August had similar upper and lower whiskers, while June had the most extensive whisker range. The outlier sites in MS and MN with large CPUEs occurred in June (Figure 26). The month of June, however, did not have any points greater than the upper whiskers of the boxplot.

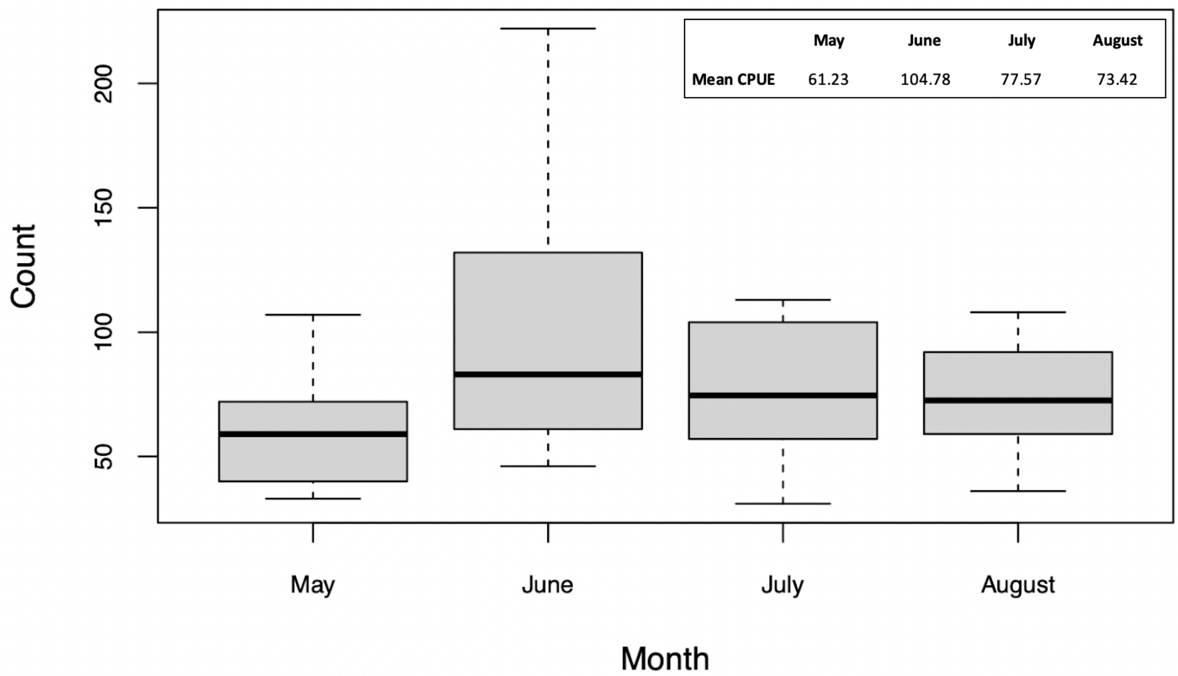


Figure 26. Catch per unit effort (CPUE) of aquatic invertebrates by collection month. June has the most extensive whisker range and the largest median and mean CPUEs of the sampling months. May has the lowest mean and median CPUEs.

Applying a PERMANOVA, we found that sampling month helped explain variability in macroinvertebrate community composition ($p>0.001$; Figure 27), explaining 14.5%. The NMDS graphic shows ellipses overlap of June, July, and August but little overlap with May (Figure 27). May had the most significant dissimilarity regarding community composition from June, July, and August. The months of August-July-June spanned linearly from negative to positive in MDS1. June showed the most significant variability in the MDS2 ordination space. May had high variability in MDS1 but variability equal to the other months in MDS2 (Figure 27).

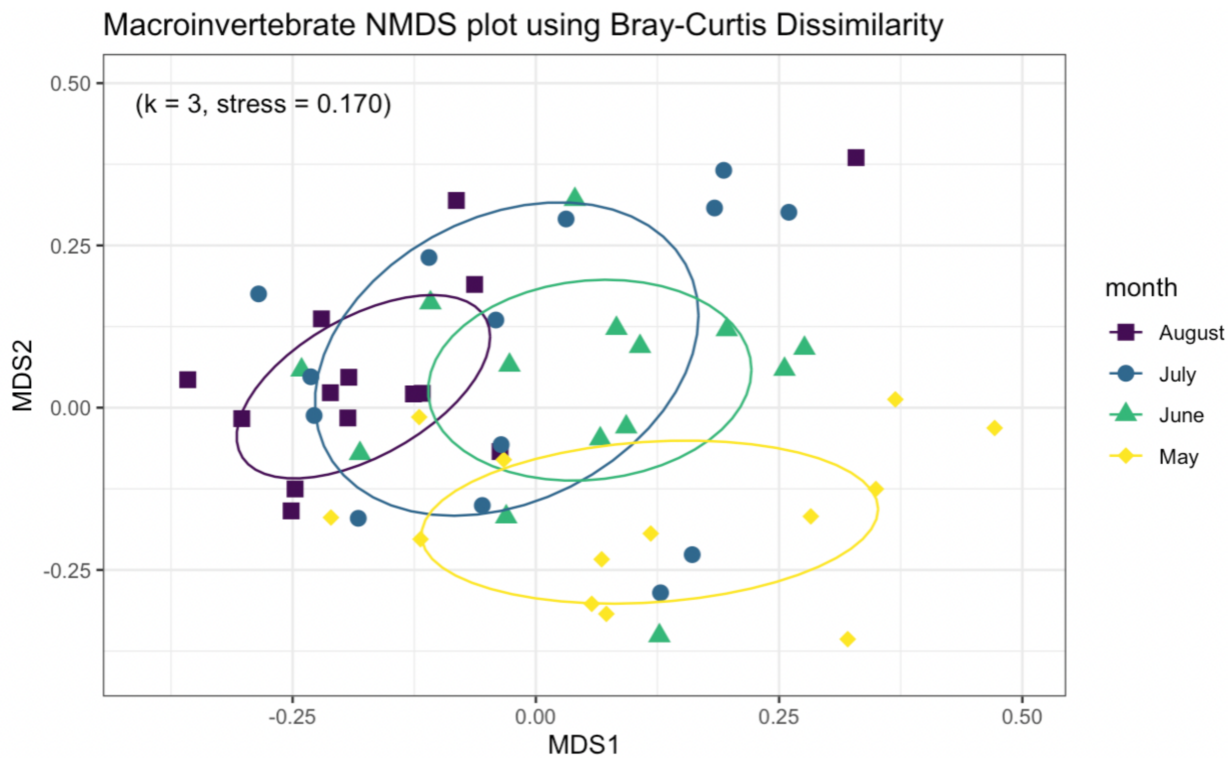


Figure 27. NMDS plot using Bray-Curtis Dissimilarity of aquatic macroinvertebrates per sampling month. Points represent sampling months May, June, July, and August 2022. Ellipses represent a 50% confidence interval of points for each month. May is distinct from the following months and showed the most extensive variability across MDS1. July shows the most considerable variability across MDS2.

Previous sampling teams, 2019-2021, unlike the 2022 season, did not exhibit significant differences in macroinvertebrate abundance relative to the month of sampling (Conrad et al., 2022; Dellick et al., 2021; Lugten et al., 2020). The 2019 team found no significant differences in community composition but noted that May and June had the most variation across sites. The 2020 team found slight variation across months but attributed this to reduced sampling efforts. Lastly, similar to the 2022 season, the 2021 team found significant variation in community composition across months, attributing the highest variability across sites to June. Additionally, the 2021 team identified one outlier site with an abundance of *Hyalellidae* in July.

Influence of Vegetation Zone on Abundance and Community Composition of Aquatic Macroinvertebrates

Mean CPUE of aquatic invertebrates did not vary significantly by vegetation zone ($p = 0.272$; Table 23). Residuals of this test had normal distribution, and a Levene test indicates that the populations of each vegetation zone had an equal variation (homoscedastic). Median CPUE of each vegetation zone displayed some variance, with Mixed Emergent and *Nymphaea* zones having the highest median value and Forest zones having the lowest (Figure 28). Three of the vegetation zones - Mixed Emergent, Submerged Aquatic Vegetation (SAV), and *Typha* - have outlier points (Figure 28). The Mixed Emergent and SAV outliers correspond to those from MS and MN, respectively, collected in June 2022 (Figure 26). The outlier point in the *Typha* zone was collected in July in MN, with the most abundant macroinvertebrates being *Trichocorixia* (genus within *Corixidae*) and immature *Corixidae* individuals. *Corixidae* (immature individuals) and *Trichocorixia* are the second and third-highest catch LOTUs in MN (Table 22).

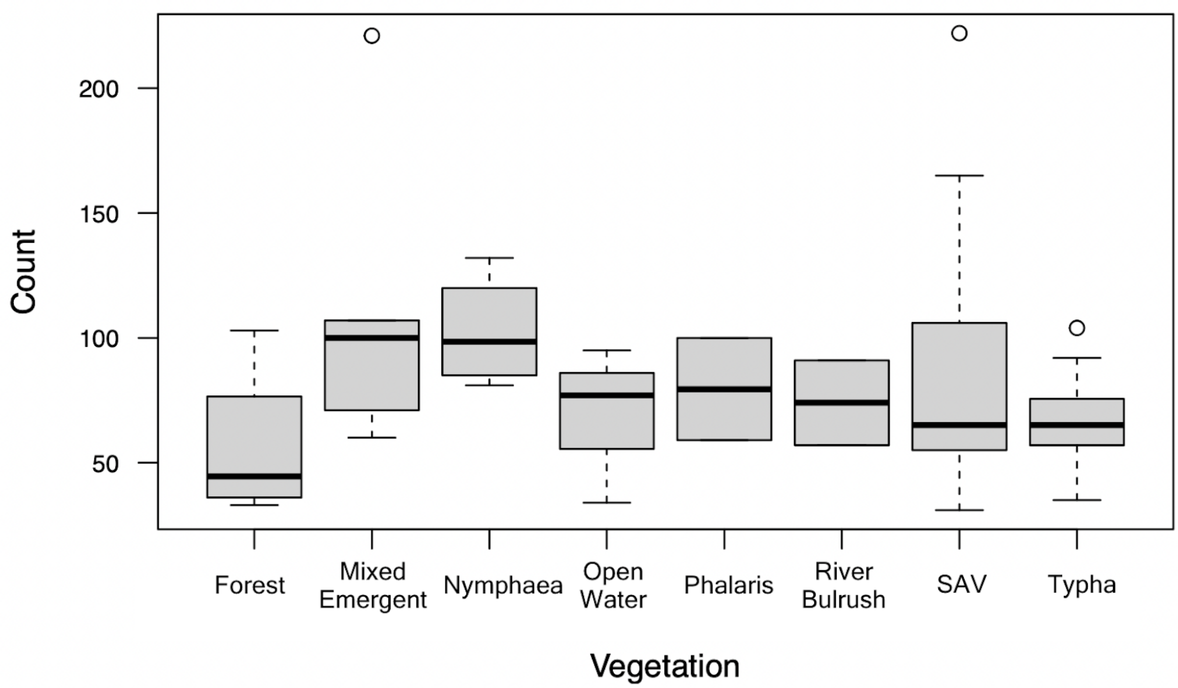


Figure 28. Catch per unit effort (CPUE) of aquatic invertebrates per sampled vegetation zone. The zone with the most extensive range was submerged aquatic vegetation (SAV). Zones with the highest median value are Mixed Emergent and *Nymphaea*, while Forest has the lowest median.

From the results of the PERMANOVA, we conclude that vegetation does not significantly influence community composition of macroinvertebrates (Table 23). The NMDS plot shows a considerable overlap of sites in all vegetation zones in the negative MDS1 ordination space (Figure 29). The distance between sites is greater in the positive MDS1 and MDS2 space, indicating increasing dissimilarity across sites. Several points representing *Typha*-SAV and *Typha-Nymphaea* zones overlap, meaning similar community assemblages at these sites. Overall, *Typha* and SAV sites appeared to have the most significant variability in the ordination space in MDS1 and MDS2. Corresponding wetland management units (MS, MN, MC, P1A) to vegetation zone sites do not have clear spatial trends in the ordination space.

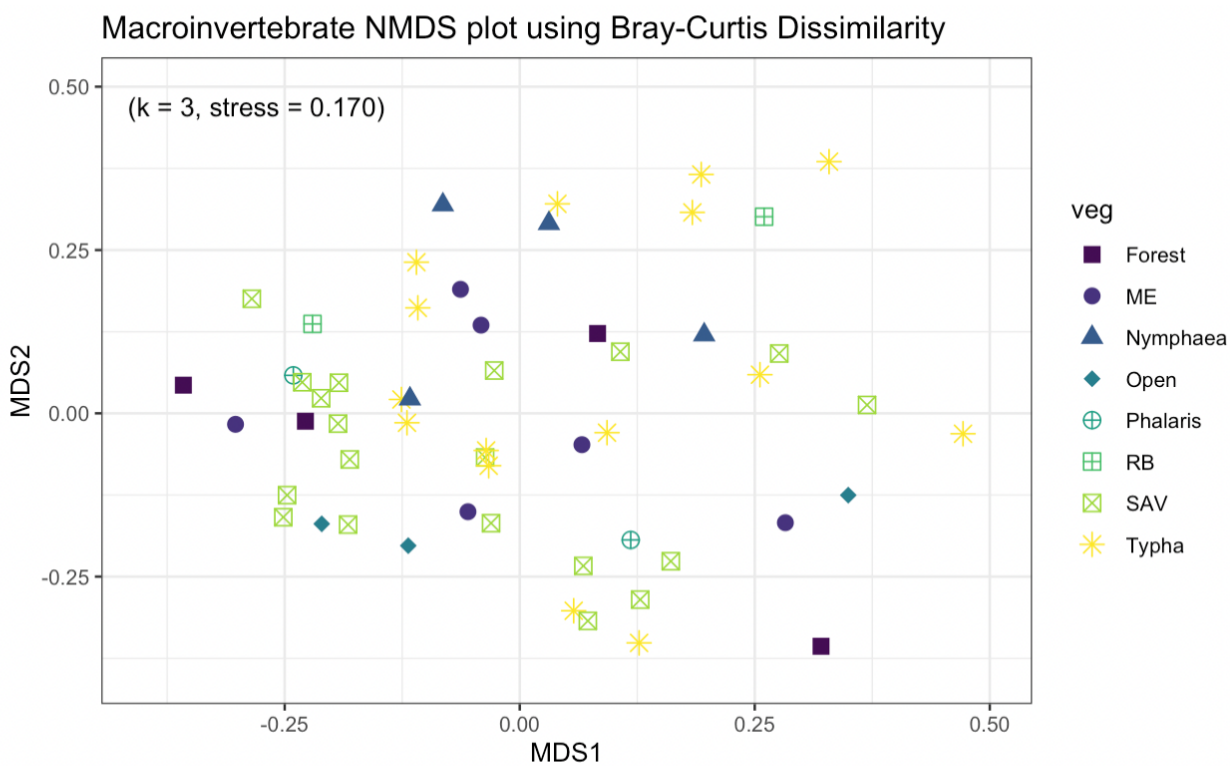


Figure 29. NMDS plot using Bray-Curtis Dissimilarity of aquatic macroinvertebrates per sampling vegetation zone. Points represent sample sites of a particular vegetation zone (point color and shape). Forest sites in MS and SAV sites across all wetland management units display the highest variability in MDS1. *Typha* zones across all wetland management units display the highest variability in MDS2. Certain vegetation zones (*Phalaris,* River Bulrush*,* and Mixed Emergent) could not plot as ellipses, as there were too few sampling locations (min = 4), so ellipses were omitted.

Previous sampling teams from years 2019-2021 found a mixed influence of vegetation on macroinvertebrate abundance and community composition (Conrad et al., 2022; Dellick et al., 2021; Lugten et al., 2020). The 2019 sampling team found that vegetation significantly contributed to abundance and community composition, with Forested zones having the lowest CPUE and Mixed Emergent and SAV zones having the most similar macroinvertebrate communities. The 2020 sampling team found similar results as the 2019 team, with Mixed Emergent and SAV zones having high similarity and Forested zones being dissimilar in community composition. However, the 2020 team could only sample Forest, Mixed Emergent, and SAV zones. During that season, abundance did not have a significant variation across vegetation zones. Lastly, the 2021 season, like the 2022 season, found no abundance or community composition differences across vegetation types. Like 2019 and 2020, the 2021 team observed that forested zones appeared most dissimilar in composition.

Table 23. Significance results of the Permutational Multivariate Analysis of Variance (PERMANOVA) and Analysis of Variance (ANOVA) tests. Bolded results signify statistical significance (P-Value <0.05).

	Permutational Multivariate ANOVA (PERMANOVA)		Analysis of Variance (ANOVA)	
	Effect Size	**P-Value**	**F-Statistic**	**P-Value**
Vegetation Zone	15.527	0.077	1.298	0.272
Month	**14.518**	**0.001***	**3.43**	**0.0237***
Unit	**10.511**	**0.002**	0.876	0.46

Influence of Water Quality on Macroinvertebrate Abundance

A generalized linear regression model (GLM) was employed to determine the influence of water quality on macroinvertebrate abundances. The water quality parameters included temperature (°C), pH, turbidity (total dissolved/suspended solids), specific conductance (µS/cm), and dissolved oxygen (mg/L). The GLM family chosen for this data was the Gamma distribution with the "log" link, due to data values being continuous and non-integer. To determine the importance of outlier points (Figures 24 and 28), we performed a principal component analysis (PCA) using a correlation matrix of water quality variables recorded at each site. We removed two sites from June with significantly higher macroinvertebrate catches (MS02-06/22/2022 &

MN01-06/22/2022), both with particularly large counts of *Hyalellidae*. From the PCA results, these sites showed little correlation to water quality variables that could explain the difference in total catch.

From the results of the GLM, we concluded that temperature has a significant relationship with the number of individuals caught per site ($p = 0.0295$; Table 24). For every 1°C increase in temperature, the number of macroinvertebrates captured increases by 3.36%. The remaining water quality variables did not have significant relationships with macroinvertebrate CPUE.

The results of the previous sampling teams, 2019-2021, were inconsistent with our findings for the 2022 season (Conrad et al., 2022; Dellick et al., 2021; Lugten et al., 2020). The 2019 sampling team found no significant relationship between macroinvertebrate CPUE and water quality variables. Their results, however, are less reliable compared to the following years. This is because the team implemented an ANOVA test as opposed to a linear regression, which does not indicate relationships between variables but differences in means across groups. The 2020 team found a significant relationship between CPUE with both temperature and conductivity. Additionally, this team observed significance in temperature, conductivity, pH, and turbidity in relation to the number of genera caught per sampling event. Lastly, the 2021 sampling team found a significant relationship between pH and CPUE. The team considered this result questionable, though, as high multicollinearity between dissolved oxygen and pH potentially altered their results.

Table 24. Significance results of the linear regression analysis using a generalized linear model. Temperature has a significant relationship with the number of macroinvertebrates caught per site.

Linear Regression		
	Coefficient	**P-Value**
Dissolved Oxygen	-1.50E-03	0.9377
Turbidity	-3.44E-03	0.4127
pH	-0.114	0.3787
Conductivity	1.69E-04	0.6309
Temperature	**0.0336**	**0.0295***

Index of Biotic Integrity (IBI) Using Aquatic Macroinvertebrates

To interpret wetland quality, we used an Index of Biotic Integrity (IBI) developed by Burton et al. (1999), which was developed for Great Lakes coastal wetlands and used by the previous monitoring teams (2019-2021 seasons). In this index, ratings correspond to levels of degradation due to anthropogenic disturbance, and scoring involves the abundance and richness of particular aquatic macroinvertebrate taxa per vegetation type. The rating levels, in order of lowest to highest ratings, are as follows: "Degraded" (11-19), "Moderately Degraded" (>19-38), "Mildly Impacted" (>38-57), and "Reference Conditions" (>57-65). In this context, the term "degraded" refers to the extent of apparent anthropogenic disturbance, and "reference conditions" are intact wetlands with little to no disturbance.

For 2022, all sampled wetland management units in Shiawassee National Wildlife Refuge were rated as "Mildly Impacted" wetlands (Table 25). MC, while the most recently completed restoration, earned a similar score to the wetland management units hydrologically connected earlier. MS earned the highest IBI score, close to rating as 'Reference Conditions' or a 'pristine' wetland (Burton et al., 1999). Previous sampling teams 2019 and 2020, found similar rating results, minus MC, which was still in the process of restoration. In 2021, the team found that MS, MN, and P1A were "Moderately Degraded." MC was "Mildly Impacted," the same rating as the 2022 sampling year (Table 25). This difference is likely due to vegetation zone sites included to calculate the score as opposed to actual changes in unit condition.

Table 25. Index of Biotic Integrity for wetland aquatic invertebrates across all sampling years. Data for MC were collected only in the 2021 and 2022 sampling seasons. The 2021 sampling team found decreased IBI ratings in MS, MN, and P1A. All four wetland management units are rated “Mildly Impacted” in the 2022 season.

Macroinvertebrate IBI Across Years				
UNIT	**2019**	**2020**	**2021**	**2022**
MS	Mildly Impacted	Mildly Impacted	Moderately Degraded	Mildly Impacted
MC	N/A	N/A	Mildly Impacted	Mildly Impacted
MN	Mildly Impacted	Mildly Impacted	Moderately Degraded	Mildly Impacted
P1A	Mildly Impacted	Mildly Impacted	Moderately Degraded	Mildly Impacted

DISCUSSION

Overall Macroinvertebrate Composition

Within SNWR, the newly restored wetland management units, as well as one reference unit, have similar aquatic macroinvertebrate assemblages to previous sampling years (2019-2021) and other Great Lakes coastal wetlands. Across all wetland management units, 79% of all macroinvertebrates collected in the 2022 season represented the taxonomic families: *Hyalellidae, Corixidae, Chironomidae, Coenagrionidae, Caenidae, Physidae, Pleidae, Belostomatidae,* and *Asellidae* (Table 21). The most abundant family within all wetland management units was *Hyalellidae*, making up about 26% of all macroinvertebrates. Compared with findings of previous SNWR sampling teams (Lugten et al. 2020, Dellick et al. 2021, and Conrad et al. 2022; representing 2019, 2020, and 2021 sampling seasons, respectively), the top nine families remained generally consistent. The top nine families comprised 86% of the 2019 catch, 79% of the 2020 catch, and 80% of the 2021 catch. Notably, the family *Asellidae* appeared in the top nine for the first time in the 2022 sampling season, replacing *Hydracarina*.

Our description of macroinvertebrate assemblages in SNWR's estuarine wetlands is comparable to those found in literature, particularly for Great Lakes coastal and restored temperate wetlands. Marchetti et al. (2010) evaluated recently restored, seasonally inundated wetlands in California, USA. The five most common taxa they found were crustacea*, Chironomidae, Corixidae,* and *Physidae,* which align with our results. Cooper et al. (2007) found similar assemblages in drowned river-mouth wetlands located on Lake Michigan. Their most abundant taxa were Amphipoda (*Gammaridae* and *Hyalellidae*), Isopoda (*Asellidae*), and chironomids (*Orthocladiinae* and *Chironomini*). Further sampling of Great Lakes coastal wetlands in the nearby Saginaw Bay area by Cooper et al. (2014) showed a high abundance of insects and a low abundance of gastropods and crustaceans. They found that the ten most abundant taxa contributed to 61% of all macroinvertebrates caught, similar to results of SNWR's four sampling years.

Influence of Wetland Unit on Aquatic Macroinvertebrates

We found that the wetland management unit of sampling did not influence the abundance of macroinvertebrates caught, but did significantly influence differences in community composition. The composition in P1A was distinct from the Maankiki Marsh units. Likewise, the 2021 sampling team found the composition of P1A to be different from the Maankiki Marsh units (Conrad et al., 2022). We speculate that "time passed since restoration" and "degree of hydrologic connection” may contribute to variation in composition among wetland management units. The restoration and hydrological reconnection of P1A to the Shiawassee River was completed in 1959, about 60 years ahead of the Maankiki Marsh restorations. Additionally, of the four target wetland management units, P1A has the most direct connection to the river. Maankiki Marsh units MS, MN, and MC, which restoration construction began in 2016, underwent their first complete inundation in the spring of 2017, spring of 2018, and fall of 2019, respectively. For that reason, managers at SNWR consider P1A a reference for the potential restoration outcome across Maankiki Marsh.

Prior to inclusion of MC as a sampling unit, the 2019 and 2020 teams found significant dissimilarity between MS and MN. This difference in composition could result from the distance between wetland management units and the dispersal method of aquatic macroinvertebrates, as MS and MN are not directly adjacent but connect only through the Maankiki Marsh Distribution Basin (MDB). MDB, a small intermediate unit, connects MS, MC, and MN through water control structures that allow the movement of water, fish, invertebrates, and vegetation. Additionally, MC contains the only water control structure connecting P1A to the Maankiki Marsh units. Conrad et al. (2022) (2021 sampling season) found that dispersal by 'crawlers and swimmers' characterized the nine most abundant families in MN, MS, and P1A. In contrast, 'flyers' were dominant in MC. They theorized that taxa with high dispersal ability, such as adult odonates, began occupying the unit immediately after restoration completion, and colonization of MC is ongoing. In the 2022 season, in MC, we found increased CPUE of “top-five families” that disperse as 'crawlers and swimmers' such as *Hyalellidae* and *Corixidae*.

In wetlands that have undergone restorative actions, macroinvertebrate populations continue developing for decades (Marchetti et al., 2010; Schummer et al., 2012). Schummer et al. (2012) investigated differences in aquatic macroinvertebrate communities between 'natural' and recently restored (i.e., dredged for vegetation removal) wetlands. They concluded that, within one to three years of the high-disturbance restorative actions, invertebrate communities were identical to wetlands of low anthropogenic disturbance. Furthermore, recently dredged wetlands saw higher abundances of invertebrates than their 'natural' counterparts. While differences in abundance may be attributable to increased nutrient release through restoration, the swift recolonization of macroinvertebrates is encouraging. Similarly, a study by Marchetti et al. (2010) evaluated changes in the composition of restored wetlands based on time since restoration completion. They found that, within the first ten years, rapid development of macroinvertebrate assemblages occurs. Then, after about 20 years, changes in assemblage settle to the point where they have a similar composition to undisturbed or 'natural' wetlands. In the short term, they attributed aquatic macroinvertebrate composition to variations in: landscape, environmental conditions, and dispersal ability. While abundance did not vary significantly across wetland management units at SNWR, variations in micro-topography, floral structure, and seasonal inundation levels may all contribute to the observed composition differences. Therefore, changes in macroinvertebrate composition may be expected to continue through the next decade, and we recommend further sampling at regular intervals to determine the impact of "time since restoration."

Influence of Month on Aquatic Macroinvertebrates

We found that the month of sampling had a significant impact on both abundance and community composition of invertebrates. The average catch of macroinvertebrate individuals per month increased from 61 in May to 105 in June. Then, average catch decreased to 78 in July, then to 73 in August. This trend in average CPUE corresponded with the seasonal trend in water temperature over the sampling period. The highest recorded water temperatures occurred in late June, with a maximum temperature of 36° C and an average temperature of 28°C. Meanwhile, May had an average water temperature of 20°C and a maximum water temperature of 24.5°C, the lowest of all months. When comparing community composition, May was significantly distinct from the following months. From this, we may presume that the seasonal shift from spring to summer, which triggers transition in the life-cycle stage, occurs in May-June at SNWR.

In support of these findings, through our water quality analysis we found water temperature to have a significant statistical relationship with the abundance of macroinvertebrates caught per site. However, the remaining water quality variables (dissolved oxygen, turbidity, conductivity, and pH) did not impact macroinvertebrate abundance. Previous studies (years 2019-2021) found varying relationships between water quality parameters and macroinvertebrate catch (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). Inconsistencies across years could be due to differences in analysis methods, limited sampling events, or non-normal data distribution.

Temperature, directly and indirectly, influences macroinvertebrate organisms through the impact on specific water quality parameters (e.g., oxygen and gas solubility, conductivity, and pH), nutrient cycling, and productivity (Bonancina et al., 2023). Each species has a range of optimal temperatures that maximizes productivity and metabolic efficiency, with the overall tolerance range described using a performance-temperature curve (Huey & Stevenson, 1979). In a review by Bonancina et al. (2023), temperature influences many physiological processes of aquatic macroinvertebrates, including: metabolic processes (e.g., osmoregulation ability, growth rate and body size, or size at emergence), phenological processes (e.g., total time of development, or time of emergence), fitness (e.g., fecundity), behavior (e.g., migration, predation, or feeding), and ecological trends (e.g., richness, density, or distribution).

Bonancina et al. (2023) found that relationships between certain metabolic processes of macroinvertebrates and temperature were reasonably consistent, as optimal temperature ranges guide most processes. Higher water temperatures decrease the total time of development, and the time and length of emergence, but require more significant food intake to maintain the increase in metabolic rate. Species richness generally increases with temperature, but diversity in species composition depends more on daily and seasonal temperature fluctuations. Density and distribution of macroinvertebrates in a given habitat depend on the thermal niche. In addition to temperature, we found similarities in seasonal trends between macroinvertebrate abundance and water nutrient concentrations, total phosphorus (TP) and total Kjeldahl nitrogen (TKN). Concentrations of both TP and TKN follow the trend of abundance, increasing from May to June, then steadily decreasing through July and August (Water Quality Monitoring, this report). Changes in nutrient concentrations during this season are not uncommon, nor are the effects on macroinvertebrate abundance a surprise. Seasonal increases in temperature lend to increases in degradation of plant matter and primary productivity in the system, both of which contribute positively to macroinvertebrate growth (Schummer et al., 2012; Schultz et al., 2020; Bonancina et al., 2023). While we did find significant differences in macroinvertebrates across months, our study was limited to measuring community composition and relative abundance as opposed to specific effects of temperature. Thus, we cannot speculate on the impact of temperature on biological processes.

Our abundance results, however, coincide with a study by MacKenzie & Kaster (2004) evaluating patterns of macroinvertebrate emergence in Lake Michigan coastal wetlands in Wisconsin. They found the dominant emergers were *Chironomidae*, other dipterans (*Dolichopodidae* and *Ephydridae*), ephemeropterans (*Siphlonuridae*), and odonates (*Coenagrionidae*). Similarly, the dominant emergers at SNWR are *Chironomidae*, odonates (*Coenagrionidae*), and ephemeropterans (*Caenidae*). MacKenzie & Kaster (2004) found that emergence in their study system was later than anticipated, peaking in late spring (early May to end of June) and late summer (end of July to end of September), with the fall being the more significant peak. They attribute this variation in anticipated emergence to, in part, peaks in temperature. Within our study, sampling occurred primarily in near-shore and edge habitats, likely a preferred location for emergence. Additionally, our data displayed peaks of the highest genera richness in June and August. We recommend further monitoring efforts to better understand patterns of emergence and the optimal temperature ranges at SNWR.

Influence of Vegetation Zone on Macroinvertebrates

We found that the vegetation zone of sampling did not have a significant impact on the abundance or composition of aquatic macroinvertebrates. Our sampling in vegetation zones was not even, as certain zones (e.g., *Phalaris*, River Bulrush, *Nymphaea*, Forest) did not occur in all wetland management units. We prioritized sampling in *Typha* zones as it was present in all wetland management units and to accommodate the IBI chosen for our habitat quality analysis. Despite inconsistencies in significance of vegetation zones across sampling years, all teams (2019-2022) observed that Forest was most dissimilar in macroinvertebrate community composition (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). MS was the only unit where we sampled Forest vegetation, as only limited patches of palustrine forested wetland exist within the four target wetland management units (U.S. Fish & Wildlife Service, 2018). We observed similar variation in community composition within other vegetation zones having limited (< 5) sampling sites (vegetation mentioned above; Figure 29). However, with so few sites, we cannot draw reasonable conclusions from our data.

While our research teams (2019-2022) did not observe significant differences in macroinvertebrate abundance or community composition across vegetation zones, studies evaluating the invasion of monotypic *Typha* patches in emergent wetlands have found negative impacts on macroinvertebrate abundance (Schummer et al., 2012; Lawrence et al., 2016; Schummer et al., 2021). *Typha* invasions prove problematic to wetland flora and fauna (Schummer et al., 2012; Schummer et al., 2021). Dense patches of *Typha* sequester large nutrient loads and block sunlight from penetrating water and soil, thereby decreasing temperature and limiting D.O. in water (Schummer et al., 2012; Lawrence et al., 2016; Schummer et al., 2021). As a result, lower temperatures decrease primary productivity and limit the growth of primary-consumer macroinvertebrates (Lawrence et al., 2016; Bonacina et al., 2023).

Lawrence et al. (2016) found that invasions of *Typha* negatively impact the diversity of vegetation in Lake Huron coastal wetlands, thereby decreasing structural complexity and available niches within these habitats. Macroinvertebrates rely on submerged aquatic plants with highly dissected leaves for feeding and refugia from predators, which *Typha* does not provide. Additionally, Schummer et al. (2021) found that increases in diversity and cover of submergent vegetation positively impact the density of macroinvertebrates in managed wetlands. Abundant vegetation and greater mean water depth create additional niche spaces throughout the water column and increase habitat capacity for colonization.

Typha (*angustifolia*) vegetation zones dominate MS, MN, and P1A, but have little representation in MC (Figure 18, Vegetation Monitoring). We did not find MC to have considerable differences in macroinvertebrate abundance or composition from the other Maankiki Marsh units. However, it had more assemblage variability across sites within the unit. We did not find reasonable evidence to support differences in vegetation zones but recommend continued monitoring of these wetland management units, especially within the first decade of restoration. To counter the negative impact of *Typha* on macroinvertebrates and other vegetation, removal processes such as dredging (Schummer et al., 2012) have been implemented with promising initial results. Removal of monotypic *Typha* patches increases edge nesting habitat for marsh birds (Schultz et al., 2020; Schummer et al., 2012) such as Pied-Billed Grebe and American Bittern, both of which reside within SNWR (U.S. Fish & Wildlife Service, 2018). However, we recommend further monitoring macroinvertebrate abundance, marsh-nesting bird trends, and fish communities before considering wide-scale *Typha* removal.

Trophic Role of Aquatic Macroinvertebrates

In palustrine marshes, aquatic macroinvertebrates are secondary producers, consuming primarily detritus and algae, acting as a link to higher trophic levels by releasing stored organic matter (MacKenzie & Kaster, 2004; Bonancina et al., 2023). Through trophic links across ecosystems, energy, and organic matter are continuously being transported, either through the migration of emerging insects or consumption by migratory consumers (MacKenzie & Kaster, 2004).

Within localized food webs, evasion of predators may vary by the availability of niches and refugia (Baxter et al., 2005; Lawrence et al., 2016). Certain life cycle phases, such as emergence, may allow for a greater risk of predator detection. Fishes, birds, and other predators that primarily reside near the preferred invertebrate emerging habitat, such as emergent vegetation at wetland edges, see greater prey abundance during peak emergence periods. Seasonal changes in the behavior of macroinvertebrate, like migration, invokes changes in predator behaviors (MacKenzie & Kaster, 2004; Baxter et al., 2005). A study of emergence patterns by Mackenzie & Kaster (2004) found that, in particular migratory waterfowl, nesting periods in late spring, and migration periods in fall coincided with times of emergence of chironomids in that system.

Within all of the wetland management units we sampled, amphipoda (*Hyalellidae*) was the most abundant taxa captured at SNWR (Table 21). We identified two statistical outlier points in MN and MS, both in June, where the abundance of Hyalellidae was considerably large compared to total site catches (MS02-06/22/2022 and MN01-06/22/2022). Additional studies of Great Lakes coastal wetlands show that large abundances of amphipods are common (Cooper et al., 2007; Cooper et al., 2014; Lawrence et al., 2016), particularly in *Typha* zones (Burton et al., 1999). Amphipods are a preferred food source for fish and wetland bird species, including juvenile fishes such as Yellow Perch (*Perca flavenscens*) (Parke et al., 2009), and juveniles and brooding adult birds such as Lesser Scaup (*Aythya affinis*) (Gurney et al., 2017) and Blue-Winged Teal (*Anas discors*) (Schultz et al., 2020). Interestingly, while chironomids are another prominent food source for juvenile and adult fishes, this family was abundant in only MS and MC.

We found that all fish species with the highest CPUEs consume macroinvertebrates as at least a portion of their diet (see Fish Monitoring Table 26, this report). Additionally, many fish species found at SNWR utilize the wetland management units as spawning grounds (U.S. Fish & Wildlife Service, 2018). A study by Diller et al. (2022) evaluated seasonal changes in the abundance of wetland fishes, spring through fall. They found that Great Lakes coastal wetlands are critical for carrying out spawning and feeding and providing quality habitats for nursing. Furthermore, diet changes with age. Fishes greater than one year old switched from a diet of primarily zooplankton to amphipods and other larger invertebrates. To put into perspective the importance of wetland macroinvertebrates in a larger trophic lens, O'Reilly et al. (2023) found that juvenile Yellow Perch consumed more resources from wetlands than near-shore areas in

Lake Michigan. In a general sense, comparable migratory fishes rely on wetland invertebrate prey as an energy resource to maintain populations in larger water bodies (rivers and lakes), creating trophic links across regional communities. We hypothesize that abundant macroinvertebrate populations at SNWR are integral to supporting resident fishes within the refuge and migratory fishes contributing to Great Lakes communities.

While this project did not conduct bird surveys as part of our biomonitoring efforts, we acknowledge the importance of macroinvertebrates in the diets of many wetland bird species. Reducing the abundance of common macroinvertebrate food sources may significantly decrease waterfowl productivity (Prince et al., 1992). For migratory waterfowl species that brood at SNWR, the high availability of macroinvertebrate food sources is essential to the growth and success of young ducklings, particularly during the summer months (Prince et al., 1992; Gurney et al., 2017; Schummer et al., 2021). The most highly preferred invertebrates include insects (*Chironomidae*, odonates, trichopteras), gastropods, branchiopods (*Daphniidae*), and amphipods (*Hyalellidae*, *Gammaridae*) (Gurney et al., 2017; Schultz et al., 2020). We found these invertebrate taxa to be abundant within the restored wetland management units at SNWR. In alignment with the mission of SNWR, acting as refugia to migratory birds and waterfowl, it is necessary to continue promoting robust populations of critical food sources and aquatic macroinvertebrates. We recommend that managers at SNWR consider the influence of food-web and trophic interactions on species composition within SNWR, and the impact of these wetlands on the greater Saginaw Bay watershed.

Transitioning to the terrestrial phase of life is not where the aquatic food web ends for emerging insects. A review by Baxter et al. (2005) determined that, in stream ecosystems, riparian vegetation zones are closely linked with the aquatic food web. Terrestrial invertebrates (semi-aquatic and entirely terrestrial) are major contributors to aquatic food webs by falling into streams or water bodies, making up substantial portions of freshwater fish diets. Contrarily, semi-aquatic macroinvertebrates, post-emergence, disperse throughout riparian zones and into neighboring habitats. In these ecosystems, invertebrates are consumed primarily by mammals, birds, lizards, and spiders. As the summer season progresses, rates of invertebrate input into the terrestrial trophic system rise with seasonal emergence. In response to seasonal variations in abundance, and similar to the findings of MacKenzie & Kaster (2004), consumers will migrate to a habitat where prey abundance is high, including the movement of birds and fish toward riparian zones. While many studies have examined stream riparian zones, few have followed the trophic link wetland-sourced macroinvertebrates create between aquatic and neighboring terrestrial food webs.

SNWR is home to robust populations of insectivores, including aquatic (e.g., Sunfish, Largemouth Bass) and terrestrial (aerial feeders such as Barn Swallow, foragers such as Lesser Scaup) (U.S. Fish & Wildlife Service, 2018). The wetland management units at SNWR are not stream ecosystems, but emergent marshes where tall, abundant vegetation coverage throughout may compensate for the low ratio of riparian edge to inundated wetland area in non-forested wetland management units. The emergence of high-dispersing invertebrates from wetland management units at SNWR likely substantially contributes to terrestrial insectivore diets. However, further monitoring may be required to reveal the extent to which edge habitat provides input of terrestrial invertebrates to aquatic consumers.

Index of Biotic Integrity

Applying the IBI for aquatic macroinvertebrates in Great Lakes coastal wetlands, we found that all four SNWR wetland management units rated as "Mildly Impacted," which is the second highest rating in this index (behind only "Reference Conditions") (Table 25). This rating has been relatively consistent across all sampling years (2019-2022), with a mild variation. The 2022 and 2021 sampling seasons used only *Typha* vegetation zones, the intended method of calculating the score (Burton et al., 1999). However, the 2021 sampling team's calculated the IBI using both live and dead *Typha* zones, as the *Typha* did not reappear until midway through the season. The dead *Typh*a zones proved to have lower richness and abundance of macroinvertebrates, leading to lower ratings of MS, MN, and P1A (Conrad et al., 2022). The 2020 and 2019 sampling teams, in order to obtain a robust sampling size, combined *Typha* and wet meadow zone sites to calculate IBI ratings. This method scored additional wet meadow sites as *Typha* sites (Lugten et al., 2020; Dellick et al., 2021). Numerical scores were not included because of the range discrepancy, as the vegetation zones used to calculate ratings were inconsistent.

Researchers have shown that monotypic *Typha* patches negatively impact macroinvertebrate abundances and diversity (Schummer et al., 2012; Lawrence et al., 2016; Schummer et al., 2021). While all SNWR sampling teams found live *Typha* to have no impact on macroinvertebrates, extensive patches of dead *Typha* were found to have a negative effect on richness and abundance (Conrad et al., 2022). Structurally, dead *Typha* fails to offer productive habitat due to significantly reduced emergent height and limited contribution of ecosystem services (e.g., the contribution of dissolved oxygen) compared to live *Typha* vegetation.

The most recent restorations (Maankiki Marsh) were completed between 2016 and 2019, and within three years of restoration, aquatic macroinvertebrates in all Maankiki Marsh units rated similarly to P1A (the reference unit). Marchetti et a. (2010) and Schummer et al. (2012) likewise found considerable resiliency of aquatic macroinvertebrate communities within several years of high-impact wetland restorations. While the community composition of P1A was significantly different from the Maankiki Marsh units, we may expect to see a convergence of assemblages over the coming years as the colonization of macroinvertebrates with low-dispersal ability continues. This finding of rapid recolonization is promising for unrestored wetland management units in SNWR and other anthropogenically disturbed coastal wetlands in the Laurentian Great Lakes region.

While the IBI used throughout this project has yielded consistent ratings of wetland management units, managers at SNWR should consider the development of a localized IBI for long-term monitoring. Conrad et al. (2022), the 2021 monitoring team, suggests that factors such as nuances in vegetation, extensive waterfowl usage, hydrogeomorphology, and consistent anthropogenic controls prevent wetland management units at SNWR from being directly comparable to unmanaged Great Lakes coastal wetlands. By contrast, Burton et al. (1999) developed their IBI by evaluating coastal wetlands with consistent wave action, *Scirpus* vegetation zones, and "Reference Conditions" from pristine, low-disturbance wetlands. Characteristics that would influence niche availability and colonization ability of macroinvertebrates in such coastal wetlands (e.g., high wave action) are unlike the circumstances of SNWR. Additionally, in order to implement this IBI, ratings for each unit were limited to *Typha*, as it was the only abundant vegetation zone at the refuge common to Burton et al. (1999). Restriction to a single zone may not capture important changes in macroinvertebrate communities across other, non-monotypic vegetation zones (such as SAV).
These distinctions may produce ratings lower than deserved for wetlands facing unique stressors, inaccurately representing the extent of restoration successes. Therefore, we recommend an aquatic macroinvertebrate IBI which considers the unique conditions of SNWR, such as managed wetlands, conversions from legacy agricultural land, and the abundant vegetation zones.

Implications for Management

In order to fully grasp the complexity of aquatic macroinvertebrate communities and their trophic role at SNWR, we recommend managers continue monitoring efforts in the newly-restored wetland management units. Across the first four years of monitoring (2019-2022), student teams have found macroinvertebrate communities to be relatively consistent (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). However, four years of observations does not properly capture shifts in communities occurring over decades, such as those occurring in P1A. We expect that as time passes, macroinvertebrate communities in P1A and the Maankiki Marsh units will become more similar due to the increased hydrological connectivity between units. Complete homogenization of communities is not likely though, as nuances within wetland habitats, such as vegetation zones and depth, contribute to variations in communities. Further monitoring to map such changes in communities may prove useful in future restorations focused on hydrological connections between units, acting as a guide for potential outcomes at SNWR. Detailed community mapping could allow managers to predict stages of colonization by “time since restoration.”

Across the four years of biomonitoring, the IBI ratings for all wetlands have remained relatively consistent (aside from MC where only two years of IBI ratings were calculated). The decline in the 2021 season rating likely does not represent a decline in habitat quality, but a difference in calculation procedures (Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). The swift invertebrate recolonization and relatively high IBI rating (“Mildly Impacted”) of the Maankiki Marsh units is remarkable given the historic conditions of these wetlands. Even still, these wetland management units may not reach the highest IBI rating “Pristine Conditions” given the unique history as converted farmland and present hydrological regime as diked wetlands. Comparing SNWR to "pristine" wetlands with limited anthropogenic disturbance diminishes the profound biotic successes achieved at the refuge. Given the circumstance of the IBI developed by Burton et al. (1999), the highest rating may not necessarily be the ideal target goal. Without the inclusion of additional vegetation zones, as different vegetation types support various taxa, the accuracy of the IBI ratings will be uncertain. SNWR fosters a multitude of rich vegetation zones, many of which are not represented in these calculations. Further, literature suggests that *Typha*, the only vegetation type used to calculate IBI, negatively impacts diversity and abundance in macroinvertebrate communities (Schummer et al., 2012; Lawrence et al., 2016; Schummer et al., 2021). We recommend that managers prioritize assessing aquatic macroinvertebrate communities within multiple vegetation types to better characterize the impact of restoration.

Lastly, conditions that are favorable to aquatic macroinvertebrates benefit other species within SNWR. Promoting and maintaining habitat heterogeneity allows for greater niche space for macroinvertebrates, as well as the many species of fishes and birds that rely on macroinvertebrates as a food source. In a similar fashion, habitat conditions that challenge macroinvertebrate colonization, such as large patches of monotypic *Typha*, do not provide adequate habitat for other species. Post-monitoring research teams have not yet determined a clear relationship between the prominent, dense *Typha* zones and species diversity at SNWR. Thus, we strongly urge refuge managers to thoroughly investigate the significance of vegetation structure in shaping ecosystem-wide biodiversity.

FISH MONITORING

INTRODUCTION

Shiawassee National Wildlife Refuge wetlands are a unique hybrid of Great Lakes coastal and river floodplain wetlands that provide fish with crucial, seasonal habitats for foraging, spawning, and refuge. These two wetland identities share characteristics of high productivity and variable habitats that accommodate lake, river, and resident wetland fish species (Jude & Pappas, 1992; Arthington et al., 2004; Diller et al., 2021). Water control structures direct water flow between the Shiawassee River and the wetland management units influencing water chemistry, seasonal vegetation zones, invertebrate colonization, and fish passage (Uzarski et al., 2005; Kowalski et al., 2014). We must characterize how these factors impact fish community composition both for basic understanding of fish assemblages and ecology and also as these fishes provide an abundant food source for SNWR birds and other predators. Anthropogenic interference can also degrade these crucial habitats, impacting fish diversity (Trebitz et al., 2009). This relationship means that fish community structures can indicate degree of anthropogenic disturbance and overall environmental health, which help inform management decisions (Uzarski et al., 2017; Cooper et al., 2018). To assess post-restoration fish composition, relative abundance, and ecosystem health, we sampled the fish communities across the SNWR wetlands over several months in 2022.

Research objectives

- *Characterize fish populations in each wetland management unit across the summer months*
- *Evaluate statistically, whether species assemblages varied by: unit, season, or vegetation*
- *Assess the health of the wetlands based on IBI calculations*
- *Determine what sampling effort is needed to assess wetland fish communities most accurately*
- *Evaluate annual variation in nutrient levels at each monitoring location between 2019 and 2022*

METHODS

General Sampling

To characterize fish abundance across various habitats at SNWR, we used fyke netting and electrofishing to sample fish during the 2022 field season. In total, we sampled 108 fyke nets throughout May, June, July, August, and October and 31 electrofishing sites throughout June, July, and August (Fig 30).

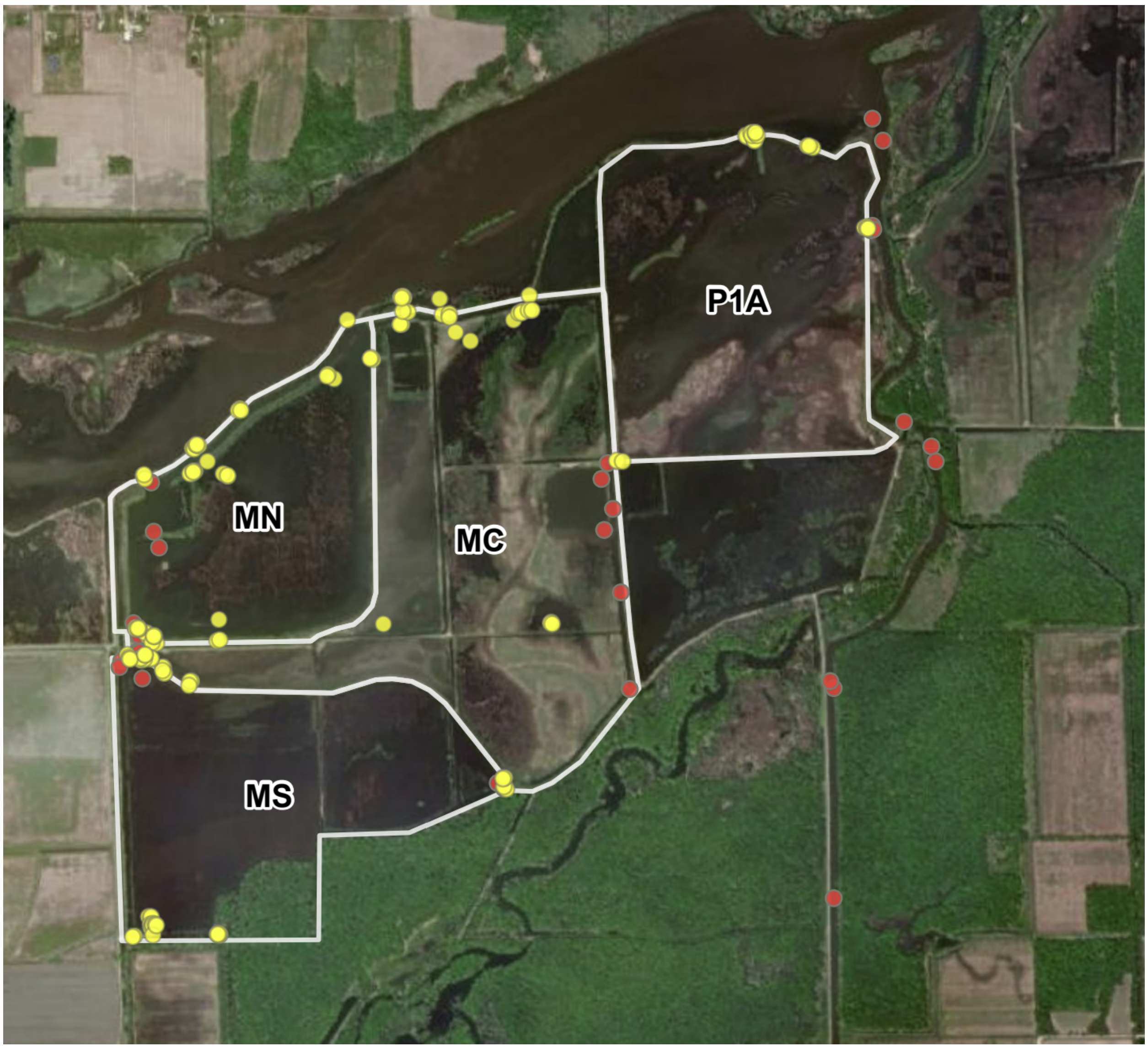


Fig 30. Fyke net (yellow) and electrofishing (red) sites across the Shiawassee National Wildlife Refuge from the 2022 field season. In total, we sampled 108 fyke nets throughout May, June, July, August, and October and 31 electrofishing sites throughout June, July, and August.

Fyke Netting

We followed fyke net sampling protocols documented by Lugten et al. (2020) for monthly sampling of fish assemblages within the wetland management units; these protocols were previously used during 2019, 2020, and 2021 field seasons (Dellick et al., 2021; Conrad et al., 2022). Fyke nets have three parts: lead, wings, and fyke trap (Figure 31). When fish encounter wings or lead, they follow this toward the fyke trap. Together, the lead and wings guide fish into the fyke trap, comprised of a series of cod rings. Each cod ring is a netted funnel with a hole in the center through which fish can easily swim in one direction but is difficult to navigate in the opposite direction. The net funnels fish towards the cod end of the net, which is securely tied to a conduit, a metal rod used to support the net, and can be untied to collect the fish. Each of our nets had either a large or small frame, and a large or small mesh, resulting in four types of nets. We determined which frame size to use based on the water depth (small = depth under 60 cm, large = depth over 60 cm) to ensure that the cod rings would be submerged entirely, allowing for fish passage. Mesh size has been shown not to affect fish catch, but does affect fish size caught. To avoid bias in fish size, mesh size was randomly selected, with each type of net equally represented across sites (Lugten et al., 2020).

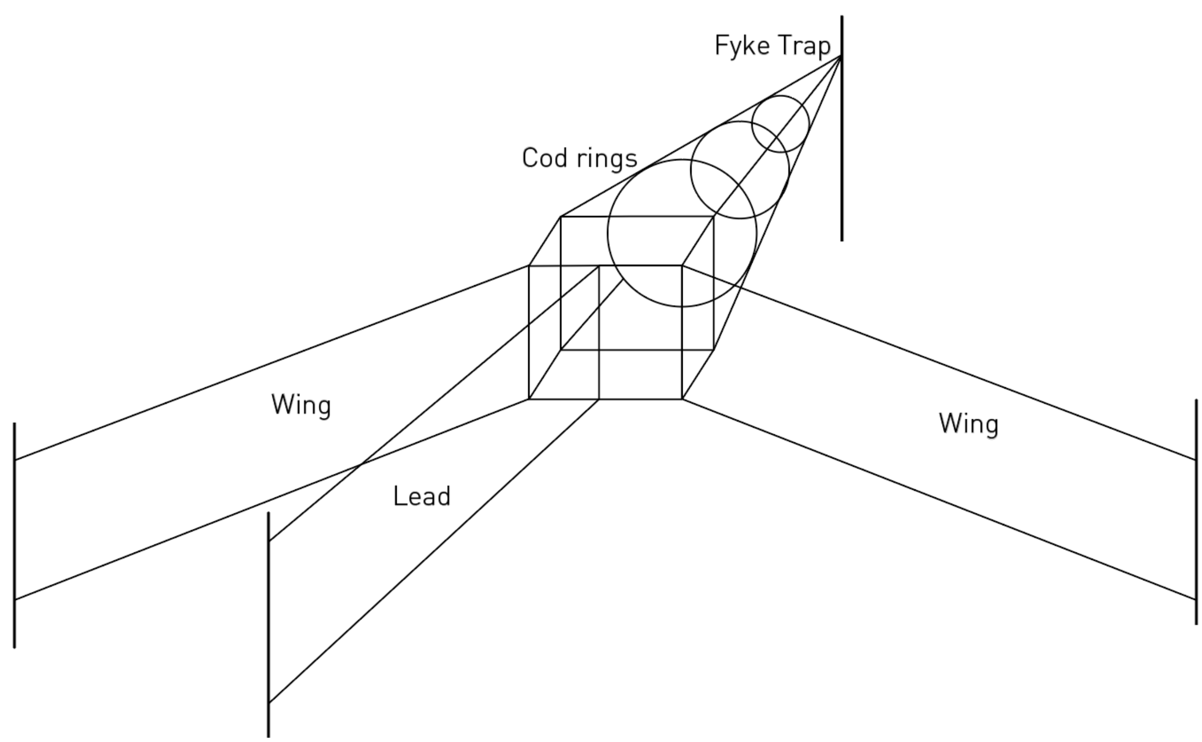


Fig 31. Fyke nets have three parts: the lead, the wings, and the fyke trap. When a fish encounters the lead, they follow it toward the cod end. Together, the lead and wings guide fish into the fyke trap, comprised of a series of cod rings. Each cod ring is a netted funnel with a hole in the center

through which fish can easily swim through but then become trapped. The cod end of the net where fish are funneled is securely tied and can be untied to collect the fish.

We determined fyke net placement by accessibility (wading depth), dominant vegetation type, and the likelihood of fish movement. Workable wading depth was considered to be greater than 30 cm and less than 100 cm, so sites were generally near shore. We set nets in six dominant vegetation types (Open Water, Submerged Aquatic Vegetation, *Typha*, Mixed Emergent, Forest, and River Bulrush) to characterize the full breadth of the fish community found at SNWR (Uzarski et al., 2017). Because fyke nets rely on fish behavior, we positioned nets across transitional habitats, considering that fish tend to move between open water and vegetated areas to escape predation, forage, and spawn (Munsch et al., 2016). Within a given area, exact net placement was then decided at random. While facing the wetland, one researcher divided it into some number of equally spaced sections. Another researcher chose a number within that given range to determine the direction. The first researcher provided a range of numbers to choose from and then multiplied the chosen number by either 3 or 5. The result determined the number of steps taken in the selected direction, where we set the net.

We set nets for two consecutive 24-hour periods, considering each a separate sample. After 24 hours, fish were collected from the cod end, identified to species, and counted. We measured length in cm of the first 30 randomly selected individuals of each species. If there were more than 30 individuals, the extra were counted but not measured. All non-invasive fish were released back into the wetland management unit after being recorded. At net set and pull, we recorded site information (location, date, water quality, water depth, net characteristics, and additional comments). When pulling a net, we assigned a compromised-net score from 0 to 4. Compromised nets might have had large holes under the water, cod rings that were not submerged, or a cod end that had detached from the stake or come untied. In abundance calculations, we did not include nets with a compromised level above 1. During the 2022 season (May-October), 19 out of 108 nets were considered compromised (18%). Many of these instances were a result of animal tampering or water levels changing overnight. There were a few nets that were compromised by implementation error, but those processes within our control such as securely fastening the cod end of the net were adjusted to avoid data loss.

Electrofishing

To sample fish assemblages in deeper sections of the wetlands that were not accessible by wading, we used boat electrofishing. A federally certified Electrofishing Crew Chief led all electrofishing activities. We used a Smith-Root Light-Duty E-Cat catamaran. The catamaran consisted of a metal frame attached to two inflatable pontoons and two anodes that could be extended off the front of the boat. Our one netter would deliver electricity through the anodes to the water by pressing a foot pedal at the front of the catamaran. As a safety measure, the electricity would be cut off from the source when the netter released the pedal. A second researcher rowed the catamaran. Other duties on the catamaran included keeping the anodes clear of plant debris, making sure fish did not escape the cooler, and watching for new fish. Site selection was determined by adequate water conductivity, water depth (deeper than waders), and the absence of obstructions. Weather conditions such as heavy wind or rain also had to be considered. We completed 31 sampling events across June, July, and August.

Triplicate water quality readings and water depth in centimeters were recorded at the beginning of each electrofishing session. All site information (water quality, vegetation zone, unit, and site number) was recorded using Survey123 (ESRI Inc., 2022). Each transect was sampled for 10 minutes, only including the time the foot pedal was actively pressed. The netter attempted to catch any fish that surfaced from the shocking. Any fish that was seen and could be easily identified but not captured was also recorded. After a transect, we recorded the transect length (in seconds) and electrical settings (voltage amps, DC pulse per second, and percent power). Captured fish were brought back to land to be identified and measured. Only the first 30 randomly selected fish of a species were measured; any extra were counted but not measured. After measuring the fish, we recorded the count and length of each fish (up to 30 of each species, then only the count was recorded) into Survey123 for each sampling site. The fish were then released back into the wetland management unit.

Data Management

Our team managed data collection of fish by first carefully placing all caught fish in a large bucket or cooler in a secure location. Next, each fish was measured, identified, and entered into Survey123. If a single species exceeded 30 at a site, only the first 30, of that species randomly selected, would be measured and the rest would be added to the total count. At each site and day, we completed a new survey to account for the fish measured. In addition to managing the fish, we also noted the water quality (pH, turbidity, conductivity, dissolved oxygen, and temperature), vegetation type, and water height in the survey. These surveys provided the information needed to quantify the ecological site, QAQC the data, and run statistical analysis on the data. Data was QAQCd by our partners at USFWS and USGS to check for any unlikely data points or user input mistakes before undergoing any statistical analysis.

Data Analysis

We followed the data analysis and statistical tests used by the previous field teams in 2019, 2020, and 2021(Lugten et al., 2020; Dellick et al., 2021; Conrad et al., 2022). To begin to characterize fish abundance, we first calculated basic summary statistics including number of species caught, total fish caught, and number of compromised fyke nets. The lengths of the fish were used to determine the mature and juvenile populations present. We also examined average length and percent juveniles of the most abundant species for each wetland management unit. Large numbers of juveniles signify reproduction occurring SWNR and seasonal use of the wetlands by transitory fish. Conversely, high numbers of large, mature fish may signify a dominantly resident population of fish.

Species Accumulation Curve

To test sampling efficacy, we created species accumulation curves (SACs) for each unit. These curves assess whether the number of sites sampled at each wetland management unit was sufficient to characterize the entire fish community. Data matrices were created in Microsoft Access using the CrossTab query function. These matrices allowed us to plot curves in R studio using modified code from previous field teams, which used the packages of "vegan", "permute", and "lattice". For each unit, we plotted the number of species against the number of sites giving us five SACs. An asymptotic curve represented that sampling efforts were sufficient.

Catch Per Unit Effort (CPUE)

We calculated two forms of CPUE for fyke net samples using Microsoft Access. The ANOVAs and Linear Regression used site CPUE which we calculated by dividing the total number of fish collected at a site divided by the total number of nets set at the site. The PERMANOVA used species CPUE which we calculated by dividing the total number of a species at a site by the total number of nets set.

ANOVAs and Linear Regression

We ran three ANOVAs to examine the relationships between site CPUE and month, unit, and vegetation type; and three linear regressions to analyze the impacts of water quality on site CPUE. We determined that our data were right-skewed due to outliers, so we applied a log transformation to the site CPUE, to normalize the distribution and avoid excluding data points. This particular transformation was chosen because it can be performed on zero values. All tests were run in R studio using code adapted from previous teams to accommodate the 2022 data. The R code for season 2022 can be accessed in Appendix I.

Non-metric Multidimensional Scaling (NMDS) & PERMANOVA

We used Non-metric Multidimensional Scaling (NMDS) to analyze differences in community composition across wetland management units, within and across months, and vegetation zones. Tests were run in R Studio, and code was adapted from previous field teams to fit 2021 datasets. Our code utilized the R packages "vegan" and "tidyverse".

Indices of Biotic Integrity (IBI)

We calculated Indices of Biotic Integrity (IBI) to assess the health of the wetlands and anthropogenic impact through the lens of fish CPUE. Calculations followed CWMP protocol and vegetation metrics described in Cooper et al. (2018). However, only two of our sampled vegetation types, *Typha* spp. and SAV, were consistent with those described in Cooper et al. (2018). The previous year's team included sites from both SAV and *Nymphaea* vegetation zones in their SAV IBI calculations and sites from *Typha, Phalaris, Salix*, and Forest vegetation zones in their *Typha* IBI calculations (Conrad et al., 2022). Our IBI calculations differed from Conrad et al. 2022 because we did not have *Nymphaea* vegetation sites for our SAV IBI and only used *Typha* vegetation zones in our *Typha* IBI calculations. All IBI calculations were calculated using Microsoft Excel.

RESULTS

Fyke Netting

Species Composition and Abundance

Throughout the 2022 field season (May-October), we collected data on fish species composition and abundance in SNWR wetlands from 108 fyke net efforts. We set nets in five wetland management units: Maankiki South (MS), Maankiki Center (MC), Maankiki North (MN), Pool 1A (P1A), and Shiawassee River (SHR). We caught 6037 fish of 38 different species from uncompromised fyke nets.

While some of the most abundant species were similar across SNWR, there was much diversity in fish populations among wetland management units (Table

Table 26. CPUE of the top four most-abundant species for each unit. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), and SHR (Shiawassee River). Bluegill was among the most abundant species in all units except Pool 1A. YOY Sunfish was among the most abundant fish in all units except Maankiki Center.

Unit	Species	Average Length (cm)	CPUE
MC	Bluegill	14.56	26.00
	Largemouth Bass	3.8	11.31
	Black Crappie	11.85	8.25
	Pumpkinseed	13.94	7.69
MN	YOY Sunfish	3.91	18.25
	Bluegill	9.04	10.55
	Black Bullhead	16.39	2.95
	Bowfin	25.49	2.60
MS	YOY Sunfish	3.69	4.14
	Bowfin	13.92	2.45
	Bluegill	6.22	1.55
	Black Crappie	11.13	1.05
P1A	Goldfish	3.87	19.60
	Banded Killifish	3.72	6.20
	YOY Sunfish	2.91	1.40
	Common Shiner	3.4	0.70
SHR	YOY Sunfish	3.49	84.53
	Yellow Perch	7.06	55.00
	Emerald Shiner	10.66	18.16
	Bluegill	19.99	7.32

26). Bluegill was among the most abundant species in all wetland management units except P1A. The most abundant species in Maankiki Center were Bluegill, Largemouth Bass, and Black Crappie. The most abundant species in Maankiki North were YOY Sunfish, Bluegill, Black Bullhead, and Bowfin. The most abundant species in MS were YOY Sunfish, Bowfin, Bluegill, and Black Crappie. The most abundant species in P1A were Goldfish, Banded Killifish, YOY Sunfish, and Common Shiner. The most abundant species at SHR sites were YOY Sunfish, Yellow Perch, Emerald Shiner, and Bluegill. The following species were each unique to a particular unit. We only caught Common Shiner in P1A. We only caught Central Mudminnow and Tadpole Madtom in Maankiki South. The Shiawassee River sites had the greatest number of unique species, including: Bigmouth Buffalo, Blacknose Shiner, Johnny Darter, Logperch, Pirate Perch, Redhorse, and White Bass.

Identifying the juvenile fish proportions provides insight into the reproduction occurring at SWNR and what services the wetlands provide for resident and seasonal fish. There was diversity between the number and species of juveniles caught between wetland management units. Juvenile lengths were considered to be less than length at maturity in centimeters as follows: Black Bullhead - 16, Bluegill - 7.5, Yellow Perch - 8, Black Crappie - 25, Largemouth Bass - 20, Pumpkinseed - 5, Bowfin - 40, Banded Killifish - 6, Common Shiner - 7.4, and Emerald Shiner - 4.2 (Schneider, 1915; Nelson, 1974; Portt et al., 1988; Daniels, 1993; Chippett, 2003; Dellick et al., 2021). YOY Sunfish was among the most abundant fish in all wetland management units except Maankiki Center, indicating that a large number of the fish at SNWR are juvenile sunfish. Black Crappie and Largemouth Bass caught in MC were predominantly juveniles, while Bluegill and Pumpkinseed were majority mature (Fig 32). MC serves as spawning habitat for Black Crappie and Largemouth Bass. The majority of fish caught in MN were mature individuals, except the YOY Sunfish which are inherently immature (Fig 33). MN may be only suitable for Sunfish spawning. Bluegill and Bowfin caught in MS were majority juveniles, whereas most Black Crappie caught in MS were mature (Fig 34). High juvenile populations of Bluegill and Bowfin in MS indicate that these species reproduce in this wetland management unit. Catches of the most abundant species in P1A were composed of either mostly, or entirely, juveniles indicating that those species are reproducing there (Fig 35). Most (77%) of the Yellow Perch caught in SHR were juveniles, while 85% of Bluegill in SHR were adults (Fig 36). Emerald Shiners were more evenly distributed, with adults making up 56% of individuals caught.

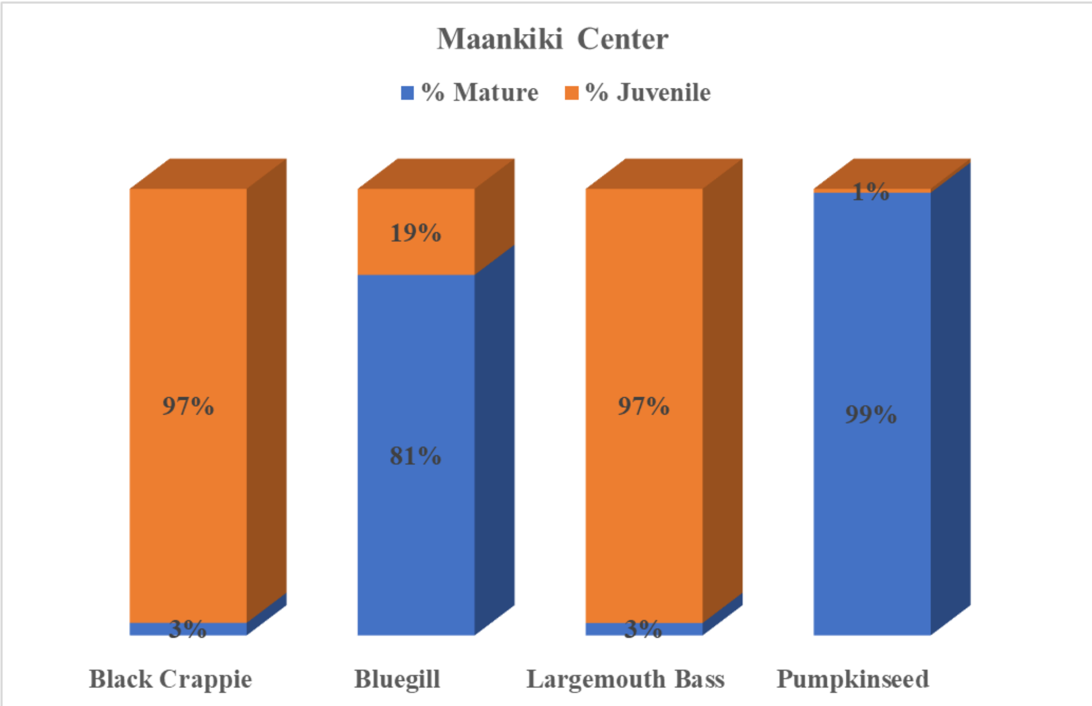


Figure 32. Age distribution of most abundant species in Maankiki Center. Black Crappie and Largemouth Bass caught in MC were predominantly juveniles. Bluegill and Pumpkinseed were majority mature.

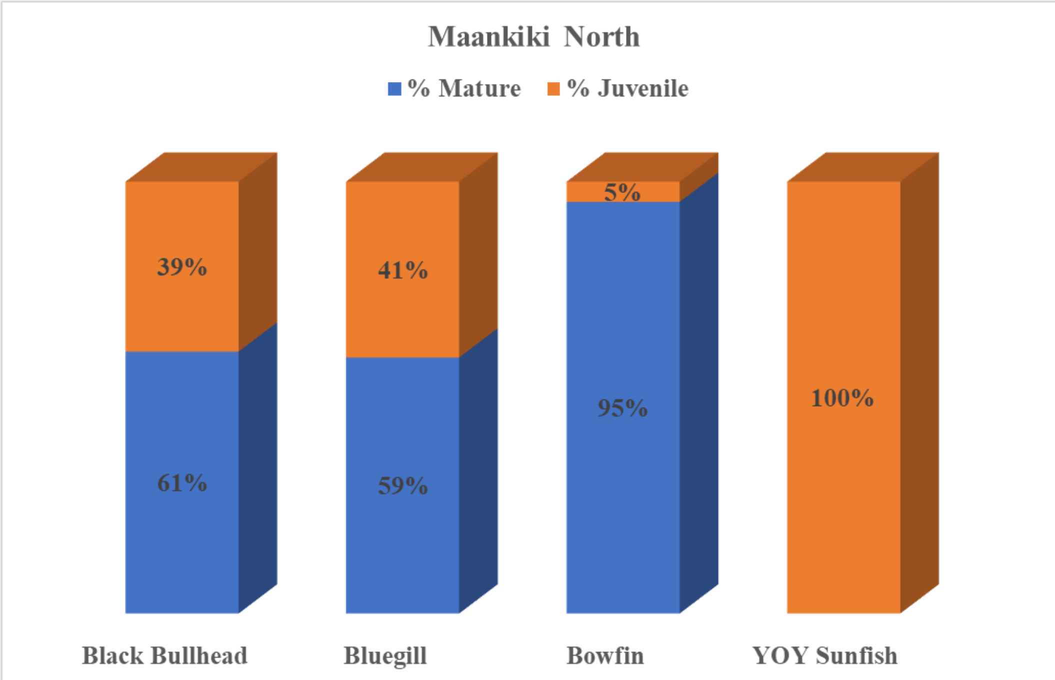


Figure 33. Age distribution of most abundant species in Maankiki North. The majority of fish caught in MN were mature individuals, except the YOY Sunfish which are inherently immature.

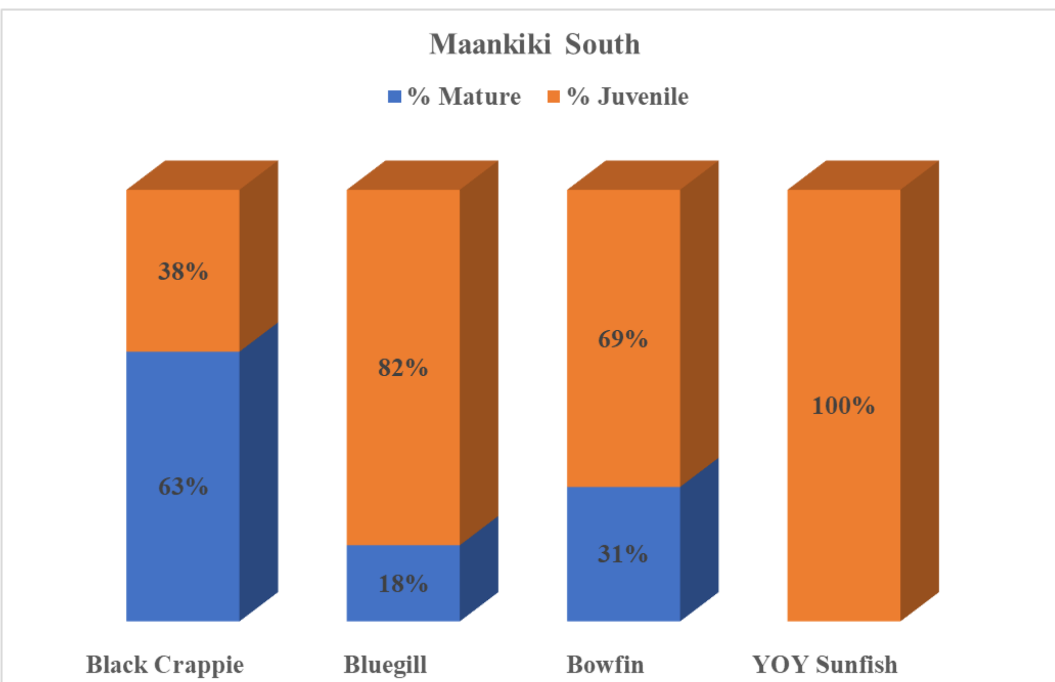


Figure 34. Age distribution of most abundant species in Maankiki South. Bluegill and Bowfin caught in MS were majority juveniles. Most of the Black Crappie in MS were mature.

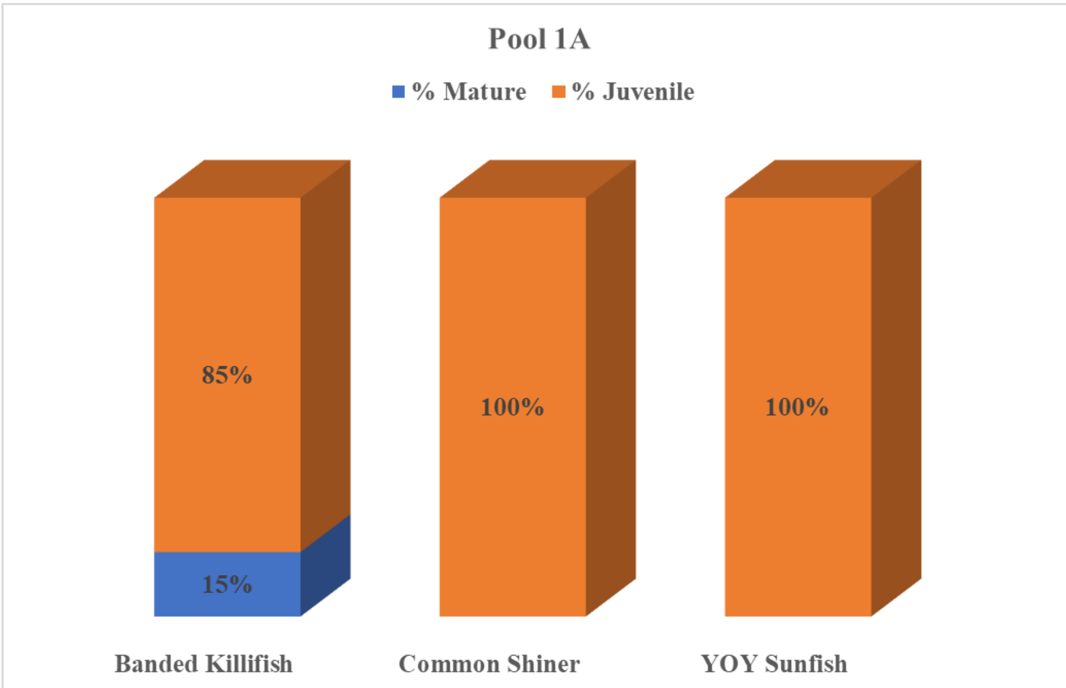


Figure 35. Age distribution of most abundant species in Pool 1A. All of the most abundant species sampled in P1A were either all or mostly juveniles.

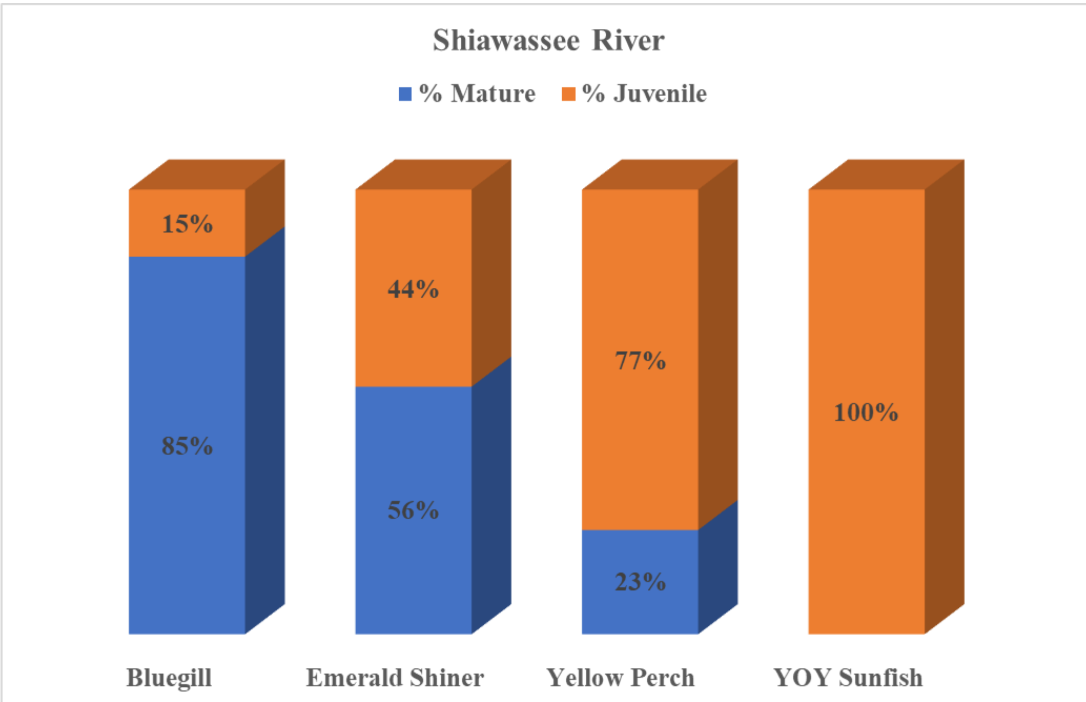


Figure 36. Age distribution of most abundant species in Shiawassee River. Most (77%) of the Yellow Perch caught in SHR were juveniles. 85% of Bluegill in SHR were adults. Emerald Shiner were more evenly distributed, with adults making up 56% of individuals caught.

Species Accumulation Curves

Species accumulation curves (SACs) showed that 2022 fyke net sampling efforts were sufficient to characterize fish species richness at SNWR (Figures 37 and 38). We sampled the following number of sites in each unit: MN - 22, MC - 21, MS - 27, SHR - 21, and P1A - 11. Comparing species accumulation curves across years, we conclude that rates of species accumulation slow and begin to asymptote at about 20 fyke net sites per unit. Sampling in P1A was challenging during the 2022 season due to low water levels limiting open waters where we could set nets. Due to the low number of sites, the curve for Pool 1A in 2022 was clearly incomplete.

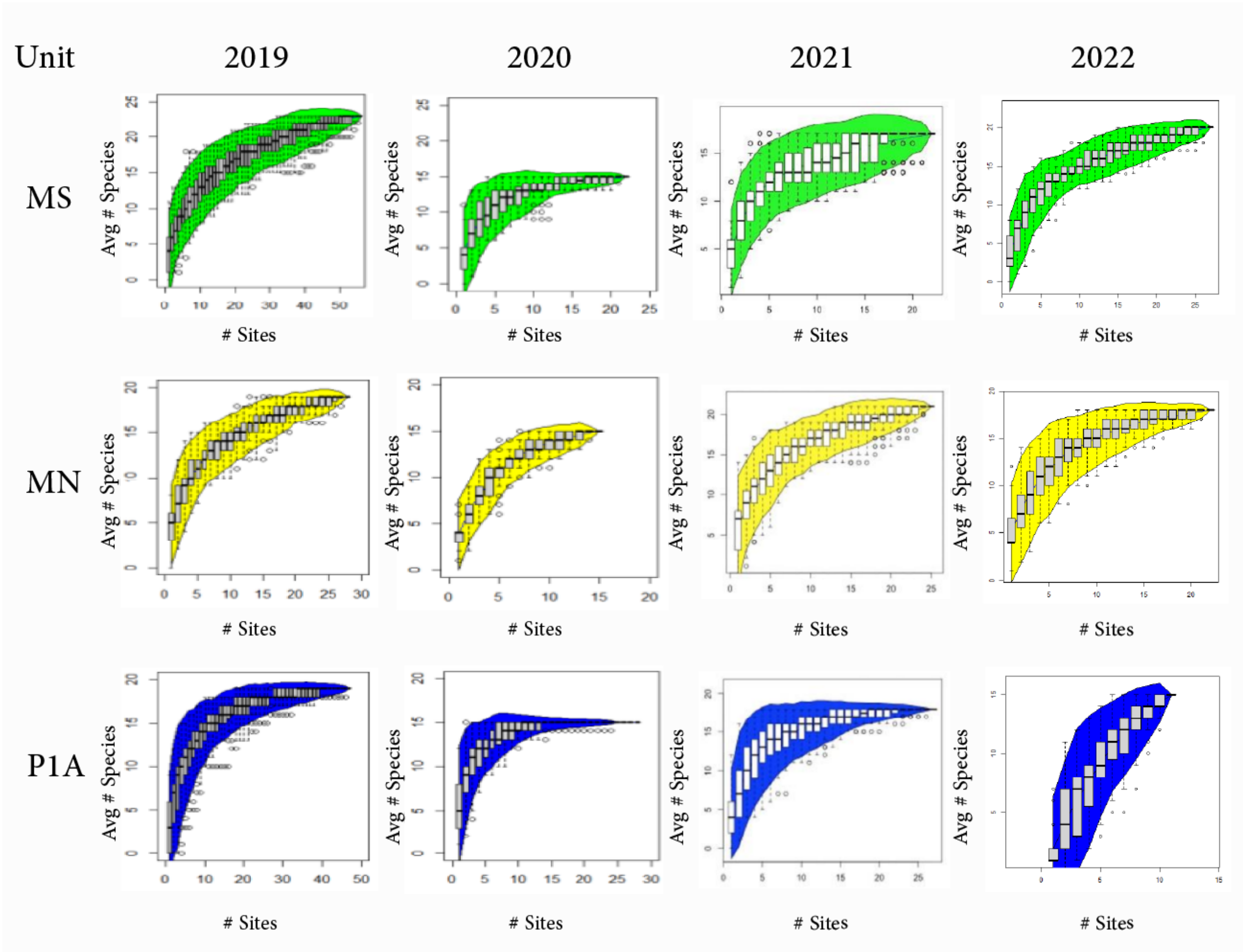


Figure 37. Species accumulation curves for three wetland management units (MS, MN, P1A) over the past four years of sampling at SNWR. The species curves begin to reach an asymptote at approximately 20 sites. The limited sampling in Pool 1A during 2022 did not fully characterize the species present.

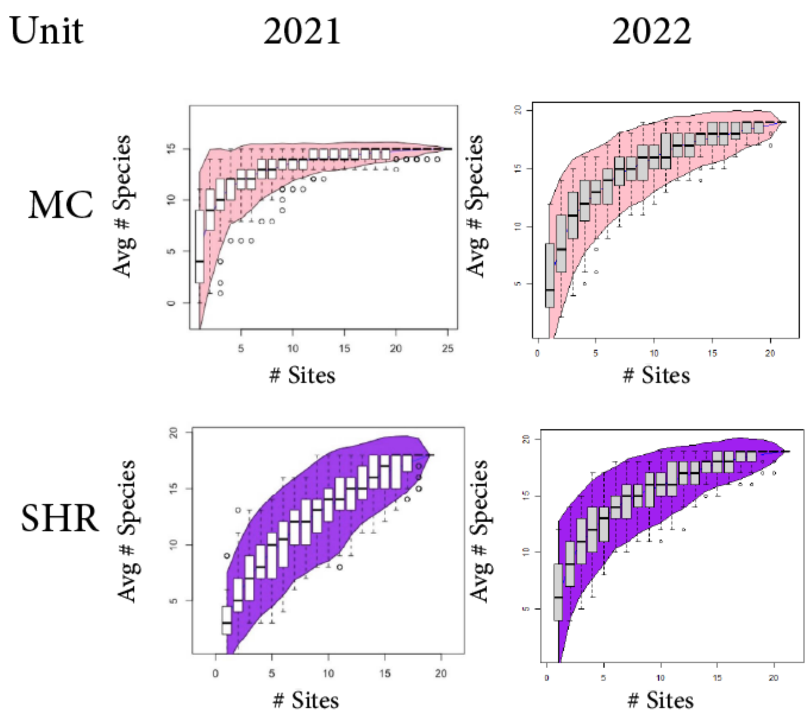


Figure 38. Species accumulation curves for two wetland management units (MC, SHR) over the past two years of sampling at SNWR. The species curves begin to reach an asymptote at approximately 20 sites.

Factors Influencing Species Composition and Abundance

To examine the effects of unit, month, and vegetation on the number of fish caught and community composition, we ran ANOVAs and PERMANOVAs, respectively. In NMDS plots, ellipses denote 50% variation within grouping variables explained, where sufficient data were available.

Influence of Unit

When comparing the wetland management units, MS and P1A had slightly lower abundances, while SHR and MC, the newest wetland management unit, had the highest site CPUEs (Figure 39). Statistical tests showed that unit was a significant factor explaining number of fish caught and community composition. The ANOVA indicated that wetland management unit had a significant effect ($p = 0.015$) on total number of fish at a site. In this test, SHR had a significantly higher logCPUE than Maankiki South. P1A had a lower logCPUE than MS, but fewer samples may have limited the statistical test from indicating significance. We also ran an ANOVA without the Shiawassee River sites, and wetland management unit was still significant ($p = 0.0256$). Maankiki South logCPUE was significantly lower than Maankiki Center. The PERMANOVA indicated that unit was also a significant factor in explaining variance in community composition with ($p = 0.003$) and without ($p = 0.002$) Shiawassee River sites (Figure 35). Each unit's assemblage of fish species was distinct. Unit did not significantly impact fish abundance or community composition in previous years, but sampling in MC and SHR only began in 2021.

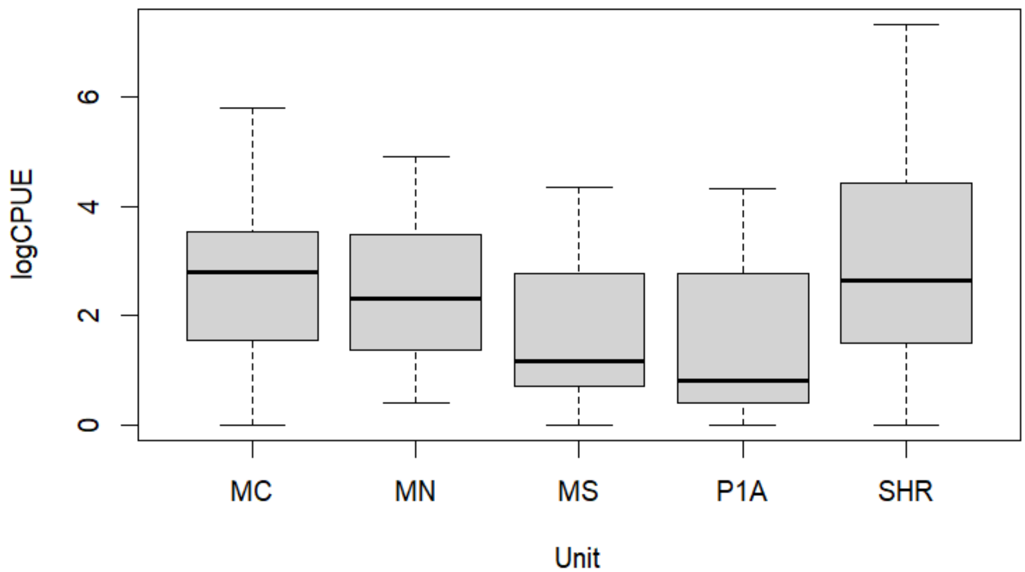


Figure 39. Log transformed Site CPUE as a function of unit. Abbreviations refer to wetland management units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), and SHR (Shiawassee River). The ANOVA indicates that the wetland management unit does have a significant effect ($p = 0.015$) on the total number of fish at a site. In this test, SHR was different from Maankiki South. We also ran an ANOVA without the Shiawassee River sites, and wetland management unit was still significant ($p = 0.0256$).

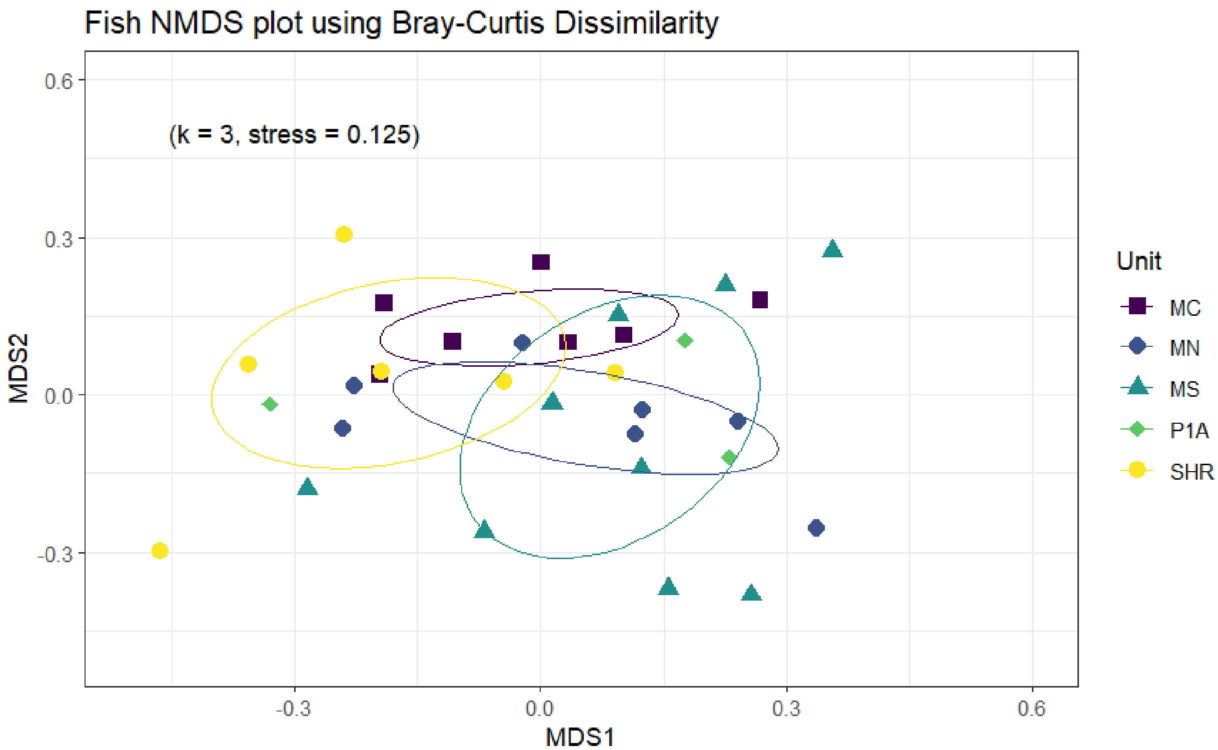


Figure 40. Fish Fyke NMDS for fish communities by wetland management unit. Ellipses denote 50% variation within grouping variables explained. The PERMANOVA indicated that unit was a significant factor in explaining variance in community composition with (*p = 0.003*) and without (*p= 0.002*) Shiawassee River sites.

Influence of Month

Fish abundance peaked in July and declined through late summer and fall (Figure 41). Statistical tests supported that month significantly affected the total number of individual fish (ANOVA, $p = 3.96e\text{-}5$) and fish community composition (PERMANOVA, $p = 0.032$). For composition, July was statistically different from May, June, and October; while August differed from May (Figure 37). Month was not a significant factor in abundance or composition in previous years, and there is no clear trend of fish abundance peaking in July in the past.

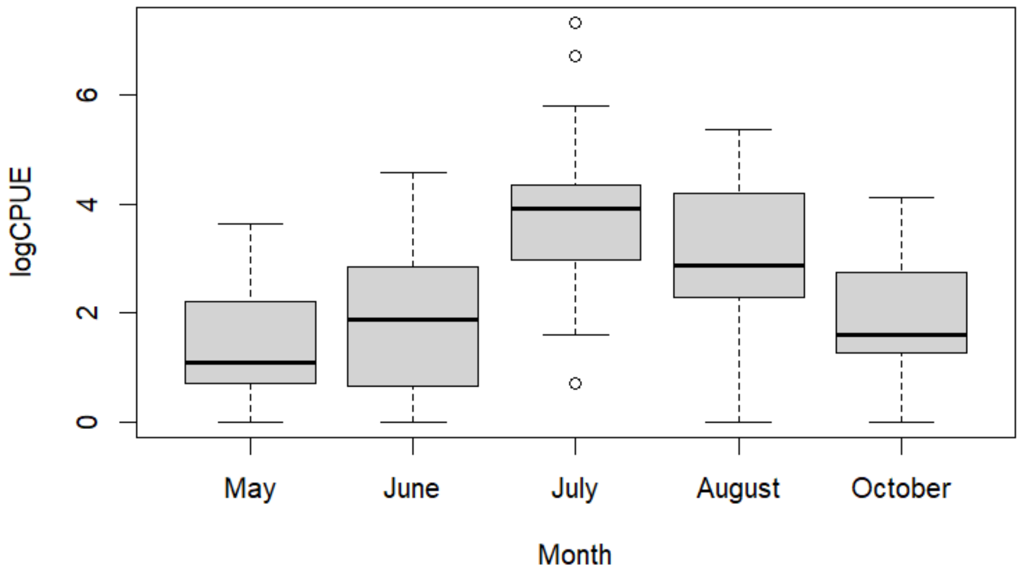


Figure 41. Log transformed Site CPUE as a function of month. Our tests show that month was a significant factor in the total number of individuals (p = *3.96e-05*) and the community composition (p = *0.032*). July differed from May, June, and October, while August differed from May.

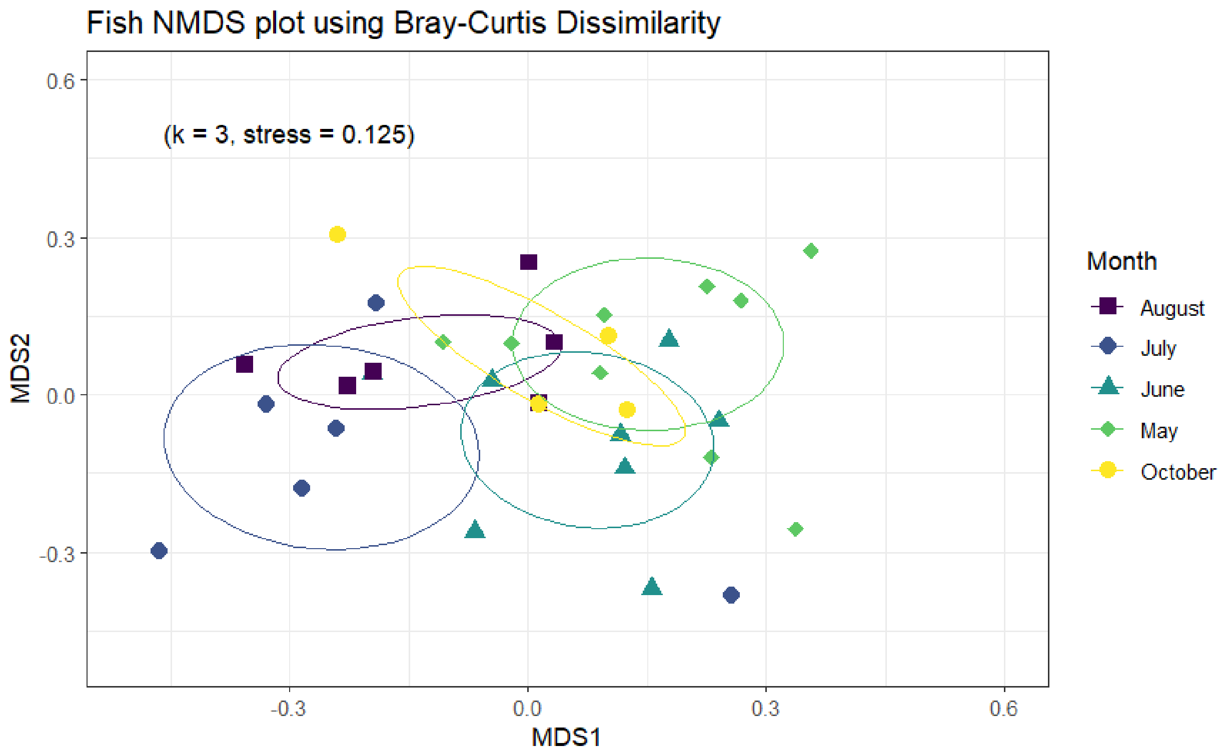


Figure 42. Fish Fyke NMDS for fish communities by wetland month. Ellipses denote 50% variation within grouping variables explained. The PERMANOVA indicated that month was a significant factor in explaining variance in community composition ($p = 0.032$).

Influence of Vegetation Type

Total abundance varied between and within vegetation types, with the most variability in SAV, but no statistical significance was found (Figures 43 and 44). Forest and *Typha* sites had the lowest abundance. The ANOVA and PERMANOVA tests, respectively, showed that vegetation type was not a significant factor explaining the total number of fish at a site ($p = 0.0797$) nor the species composition ($p = 0.074$). Across the years, there has been great variability in which vegetation types were associated with higher CPUEs. In 2019, mixed emergent had a higher CPUE than *Typha* and *Salix*, which we did not sample in 2022. In 2020, SAV had higher CPUEs than *Typha* and *Phalaris*. In 2021, the forest sites had extremely high CPUE when including outliers, contrasting with the low abundance at forest sites in 2022.

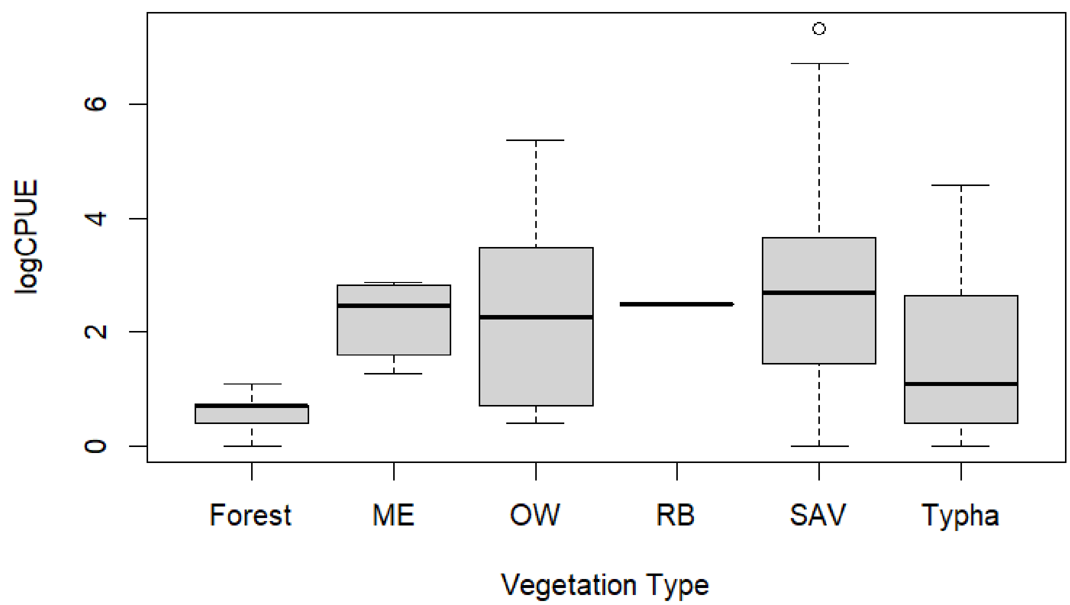


Figure 43. Log transformed Site CPUE as a function of dominant vegetation type. Abbreviations refer to vegetation types: ME (Mixed Emergent), OW (Open Water), RB (River Bulrush), SAV (Submerged Aquatic Vegetation). Vegetation did not have a significant effect on site CPUE ($p = 0.0797$).

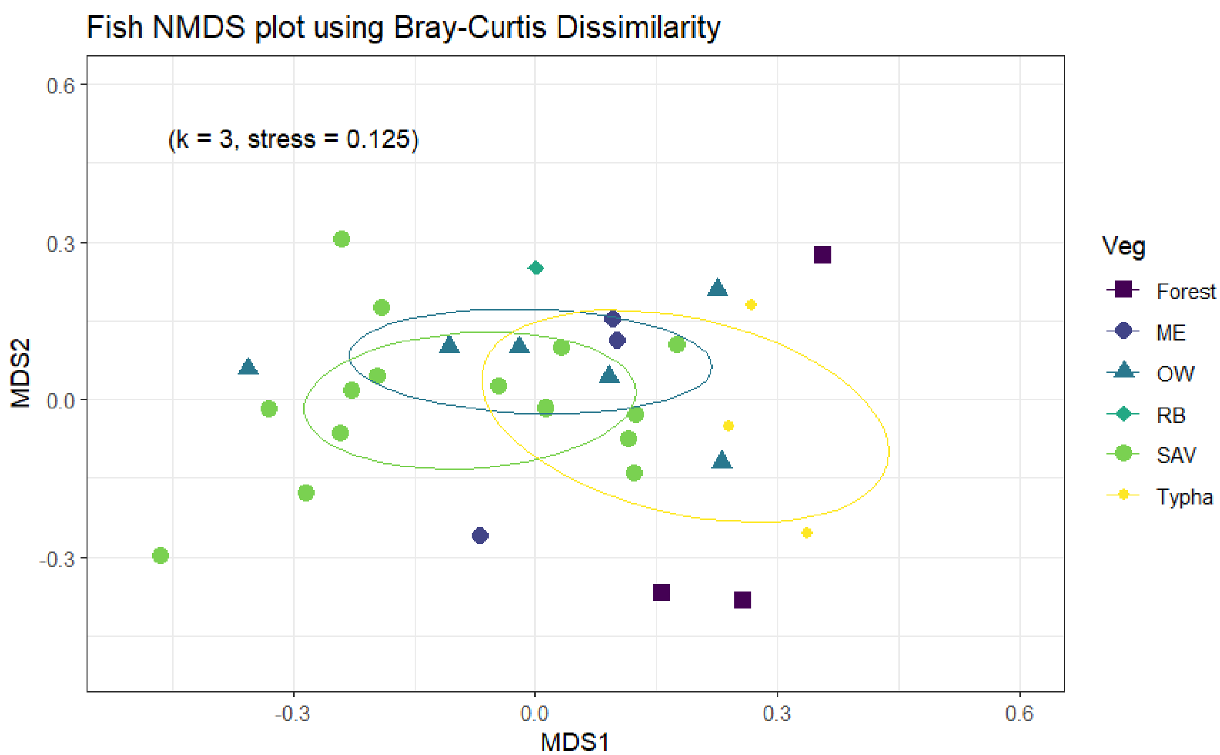


Figure 44. Fish Fyke NMDS for fish communities by wetland vegetation. Ellipses denote 50% variation within grouping variables explained. The PERMANOVA indicated that vegetation type was not a significant factor in explaining variance in community composition ($p = 0.074$).

Influence of Water Quality

We ran single linear regressions to evaluate the relationships between fish CPUE and five water quality variables. Dissolved oxygen had a slightly negative correlation with site CPUE. Temperature and conductivity were positively correlated with CPUE. Of the five parameters tested, temperature ($p = 0.0180$) and conductivity ($p = 0.0064$) were both significant (Table 27). Conductivity was also a significant predictor ($p = 0.00381$) for site CPUE in 2021, but temperature was not. The 2019 and 2020 teams performed different water quality analyses, so a comparison was not possible.

Table 27. Linear model results for water quality parameters on fyke netting site CPUE. Bold indicates significance (p<0.05). Conductivity was significant in 2021 as well.

Water Quality Parameter	P value
Temperature (C)	**0.018**
Dissolved oxygen (mg/L)	0.0867
pH	0.506
Conductivity (µS/cm)	**0.0064**
Turbidity (FNU)	0.520

Indices of Biotic Integrity (IBI)

To assess anthropogenic impact on wetland management unit habitats, we calculated Indices of Biotic Integrity (IBI) for fishes within two of the vegetation types sampled *(Typha* and SAV) and compared our scores to previous sampling years (Table 28). Following the methods of Cooper et al. (2018), we gave each unit an overall IBI score from 0 to 100. A higher score denotes higher quality, less degraded habitat. Scores designated narrative ratings of degradation: degraded (<36), moderately degraded (36-45), moderately impacted (>45-50), mildly impacted (>50-60), and reference quality (>60).

Site ratings from 2022 ranged from degraded to moderately impacted. Compared to last year, scores declined for P1A SAV and MN *Typha*, both rated as degraded. However, it is essential to note that vegetation types included in these calculations did differ from 2021. The MN SAV score remained at 36.36, rated as moderately degraded. All other scores increased from 2021 to 2022. SAV scores, except for P1A, fell in the moderately degraded rating. The MC *Typha* score fell in the moderately impacted rating.

Table 28. Unit IBI Scores across years in SAV and *Typha*. Numbers represent an IBI score between 0 to 100, with lower scores indicating poorer habitat quality. We denoted ratings of degradation by color. All other "N/A" indicates that no samples of that vegetation type exist for that unit. Abbreviations refer to wetland management units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Compared to last year, scores declined for P1A SAV and MN *Typha,* both rated as degraded. The MN SAV score remained the same at 36.36 (Moderately degraded). All other unit scores increased from 2021 to 2022, with SAV rated moderately degraded and the MC *Typha* moderately impacted.

	Vegetation Type: SAV				Vegetation Type: Typha			
Unit	2019	2020	2021	2022	2019	2020	2021	2022
MS	54.54	22.73	27.27	40.9	55	40	45	N/A
MN	N/A	45.45	36.36	36.36	40	40	40	25
P1A	22.72	40.91	36.36	27.27	35	40	40	N/A
MC	N/A	N/A	31.82	36.36	N/A	N/A	40	50
SHR	N/A	N/A	31.81	40.9	N/A	N/A	N/A	N/A

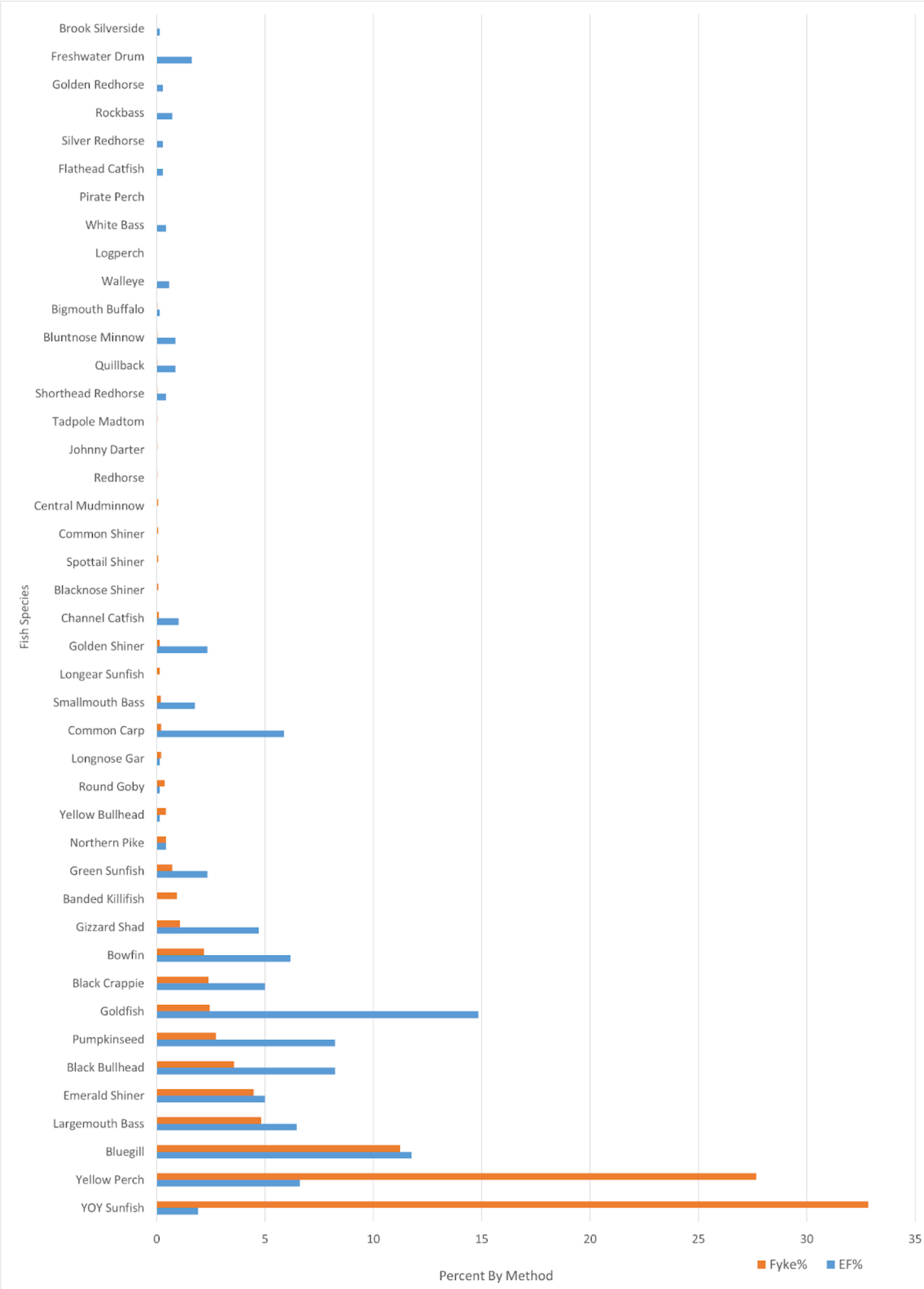
Legend
Mildly Impacted
Moderately Impacted
Moderately Degraded
Degraded

Electrofishing

Species Composition and Abundance

Throughout the field sampling season (June- August), we used electrofishing to collect data on fish species composition at 31 sites across our four study wetland management units, plus two riverine units. We sampled wetland management units MS, MC, MN, SPD, and SHR; and caught a total of 3,830 fish, representing 31 species.

Electrofishing was intended to sample waters too deep to access using fyke nets. Therefore, species caught fyke netting may differ. To measure the number of fish caught, we used Catch Per Unit Effort, with time spent electrofishing as effort. The CPUE for electrofishing was the total number of individual fish captured per time of active electrofishing (Figure 45). The fyke netting served as a baseline for the sampling parameters. We electrofished to answer the question of whether electrofishing provides a different species diversity by sampling than fyke nets. We ranked electrofishing samples against fyke net samples to determine the different distribution in species caught (Figure 45). River samples and wetland samples were separated within fyke netting and electrofishing to understand if sampling in the river and electrofishing add additional information to the study (Figure 46). The Shiawassee River is also a source population that feeds into the wetlands and serves as a baseline for the fish we would ideally find in the wetland management units.



Brook Silverside
Freshwater Drum
Golden Redhorse
Rockbass
Silver Redhorse
Flathead Catfish
Pirate Perch
White Bass
Logperch
Walleye
Bigmouth Buffalo
Bluntnose Minnow
Quillback
Shorthead Redhorse
Tadpole Madtom
Johnny Darter
Redhorse
Central Mudminnow
Common Shiner
Spottail Shiner
Blacknose Shiner
Channel Catfish
Golden Shiner
Longear Sunfish
Smallmouth Bass
Common Carp
Longnose Gar
Round Goby
Yellow Bullhead
Northern Pike
Green Sunfish
Banded Killifish
Gizzard Shad
Bowfin
Black Crappie
Goldfish
Pumpkinseed
Black Bullhead
Emerald Shiner
Largemouth Bass
Bluegill
Yellow Perch
YOY Sunfish
Fish Species
0
5
10
15
20
25
30
35
Percent By Method
Fyke%
EF%

Figure 45. Fish community composition, sampled by fyke netting vs. electrofishing. Data are percentage species caught of the total gear catch. Species are ordered, bottom to top, by decreasing catch by fyke net. Both gears caught a variety of fish species, but there is a different distribution in electrofishing compared to fyke netting, indicating the importance of both methods.

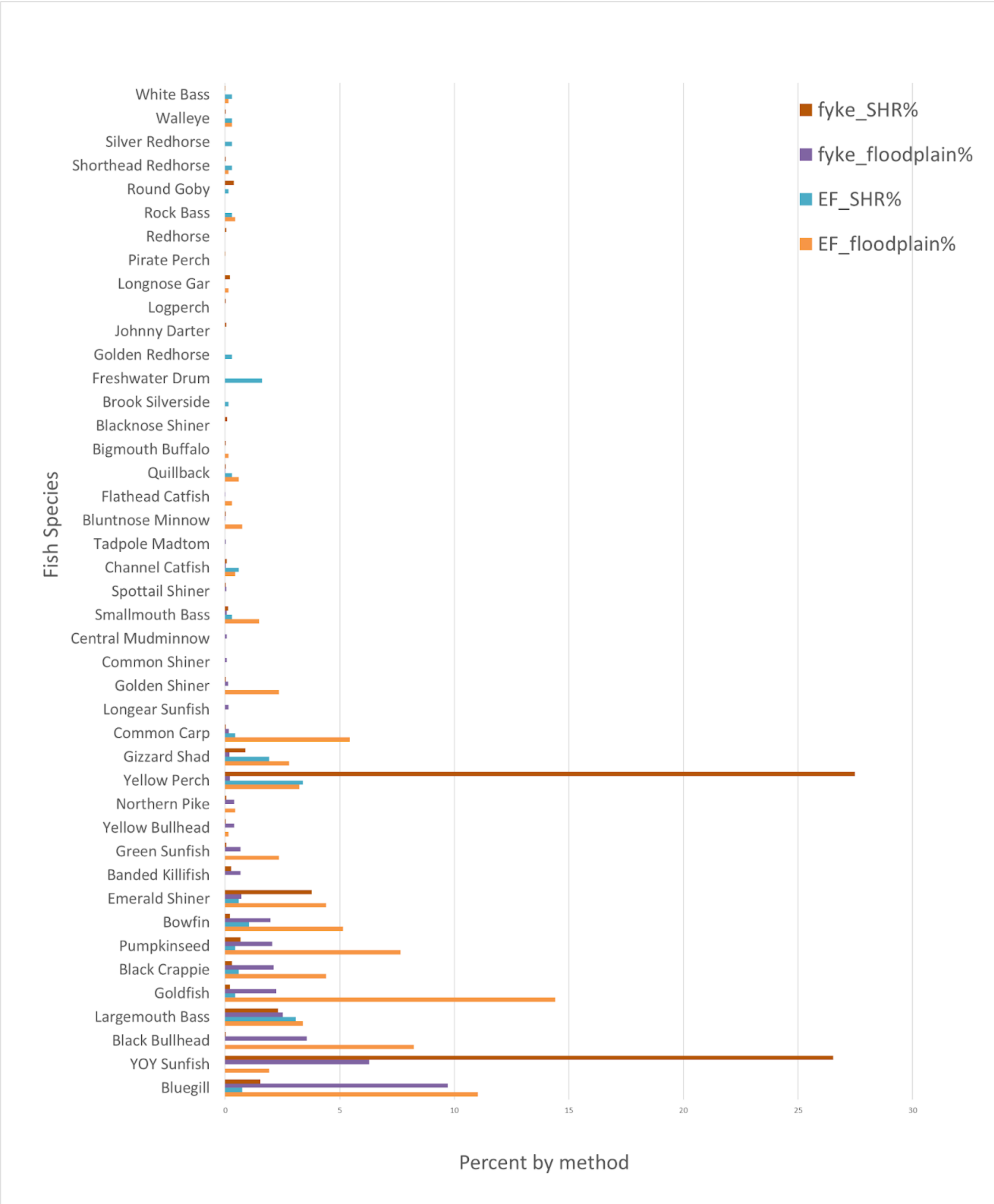

fyke_SHR%
fyke_floodplain%
EF_SHR%
EF_floodplain%
Fish Species
White Bass
Walleye
Silver Redhorse
Shorthead Redhorse
Round Goby
Rock Bass
Redhorse
Pirate Perch
Longnose Gar
Logperch
Johnny Darter
Golden Redhorse
Freshwater Drum
Brook Silverside
Blacknose Shiner
Bigmouth Buffalo
Quillback
Flathead Catfish
Bluntnose Minnow
Tadpole Madtom
Channel Catfish
Spottail Shiner
Smallmouth Bass
Central Mudminnow
Common Shiner
Golden Shiner
Longear Sunfish
Common Carp
Gizzard Shad
Yellow Perch
Northern Pike
Yellow Bullhead
Green Sunfish
Banded Killifish
Emerald Shiner
Bowfin
Pumpkinseed
Black Crappie
Goldfish
Largemouth Bass
Black Bullhead
YOY Sunfish
Bluegill
0
5
10
15
20
25
30
Percent by method

Figure 46. Fish community composition, sampled by fyke netting vs. electrofishing, in either the floodplain wetland management units or in the Shiawassee River. Data are the percentage of species caught of the total gear catch. Species are ordered, bottom to top, by decreasing catch by fyke net in the floodplain wetland management units. The distribution shows a wider range of species caught in the Shiawassee River and illustrates the importance of combining the two sampling methods to understand species composition. Electrofishing helps us to understand the species composition of the river better.

Influence of Unit on Fish Abundance

Mean CPUE varied across wetland management units. Using ANOVA, we found there was a significant difference between CPUE across wetland management units ($p = 0.031$). SHR served as our control wetland management unit because it has a long history of open connectivity with the Shiwassee Floodplains as it was connected in 1958 (Conrad et al., 2022). However, MC's mean CPUE was the highest, even greater than the mean CPUE for SHR or SPD. Maankiki North's mean CPUE was similar to SHR, and the mean CPUE for MS was lowest at 1 (Figure 42).

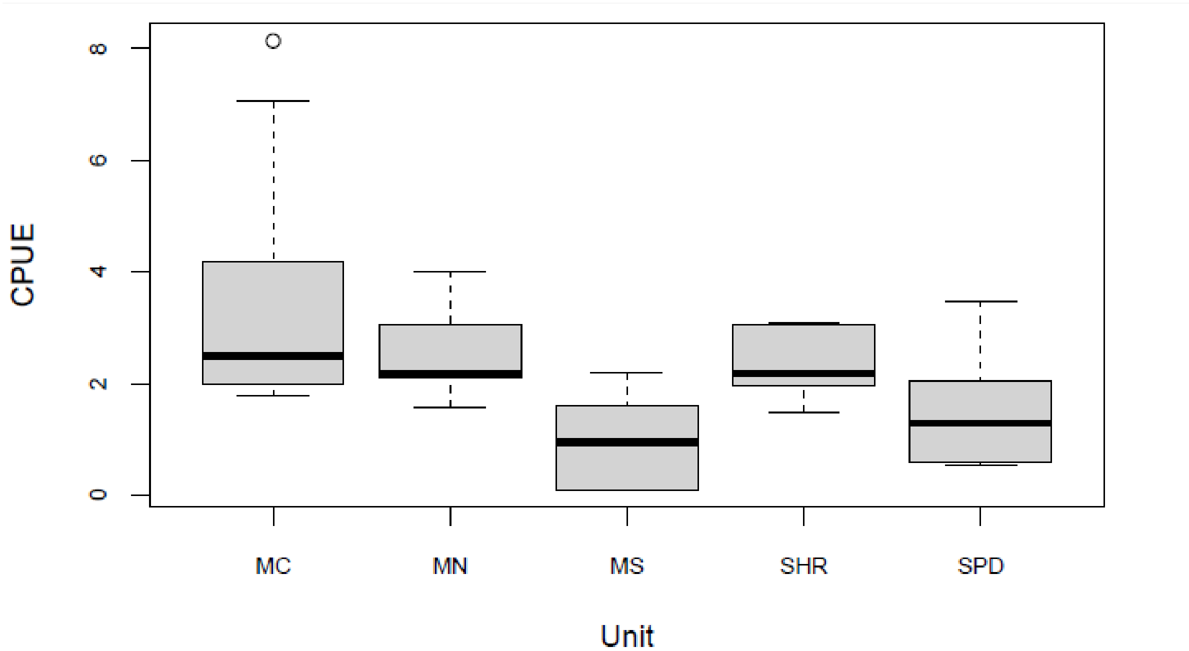


Figure 47. Electrofishing catch per unit effort (CPUE) of fish per wetland management units within the SNWR. Box and whisker plots show variation between units of fish caught. MC was the only unit with an outlier and with the largest whisker range. MS had the lowest mean CPUE.

Influence of Month on Fish Abundance

Mean CPUE of fish did not vary significantly across months ($p > 0.0923$)(Figure 48). Residuals were normally distributed, and a Levene test indicated equal variance. The Shapiro- Wilks test indicated normality for the CPUE data. June had the highest CPUE average at 3 individuals per ten minutes. June and August both had outlier points outside the box and whisker plot range. July was the only month without any outliers. However, none of these months supported a finding of a significant difference in mean CPUE among months ($p = 0.147$).

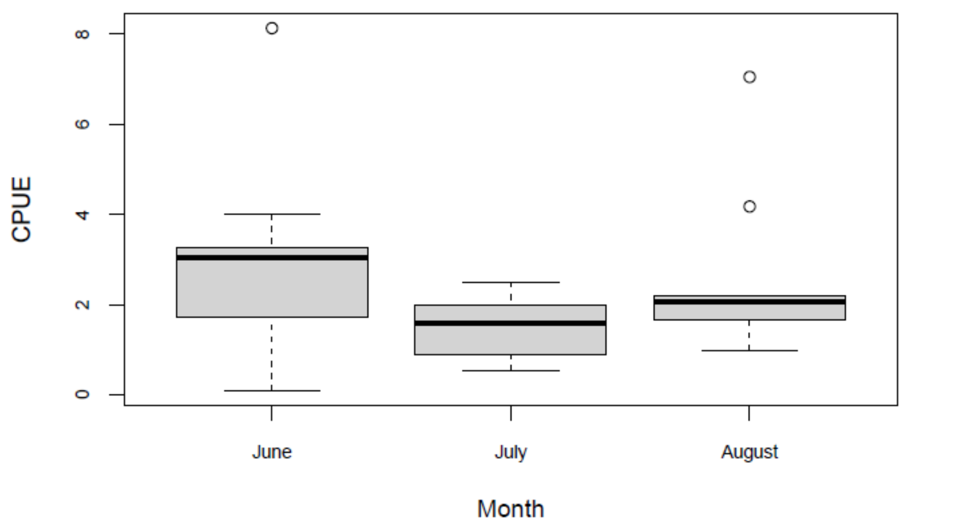


Figure 48. Mean Catch Per Unit Effort (CPUE) of fish during sampling months at SNWR. This box and whisker plot the average abundance of fish caught across the months sampling at the SNWR. In the month of June, there is one outlier with a CPUE of 8, and in August, there are two outliers with a CPUE of 4 and a CPUE of 7.

Applying PERMANOVA to examine fish species composition, however, told a different story (Figure 49). When we looked at the interaction between month and unit with the Non-metric Multidimensional Scaling (NMDS), we observed significant patterns in both wetland management units and months. There is a large spread across MDS2 axis for each month (June, July, and August) and each unit (MS, MN, SPD), indicating variation in fish community composition across months and wetland management units. Along the MDS3 axis, June and August were more widely distributed than July, but all showed great spread with a variety of unit types. Most of the distance spread is in the positive region of MDS2 and is widely distributed across MDS. Additionally, the p-value was 0.001, which is extremely significant. When wetland management units are compared with the months ($p = 0.023$) they are also significant. The largest distribution was for Maankiki South in July and August, which spans most of the distance of MDS2. This means there was more similarity between these wetland management units and months which translates to a variance in species of fish being caught across these wetland management units and months. The Shiawassee River, however, had little correlation with month, as most points were clustered together in the negative region of MDS2 and MDS3, indicating little variation in fish species caught in SHR between months, and distinction between these and fish species caught at the other sites. Maankiki Center is also largely clustered in the MDS2 positive region, indicating little variation between fish species across the MC site, and some distinction of this site.

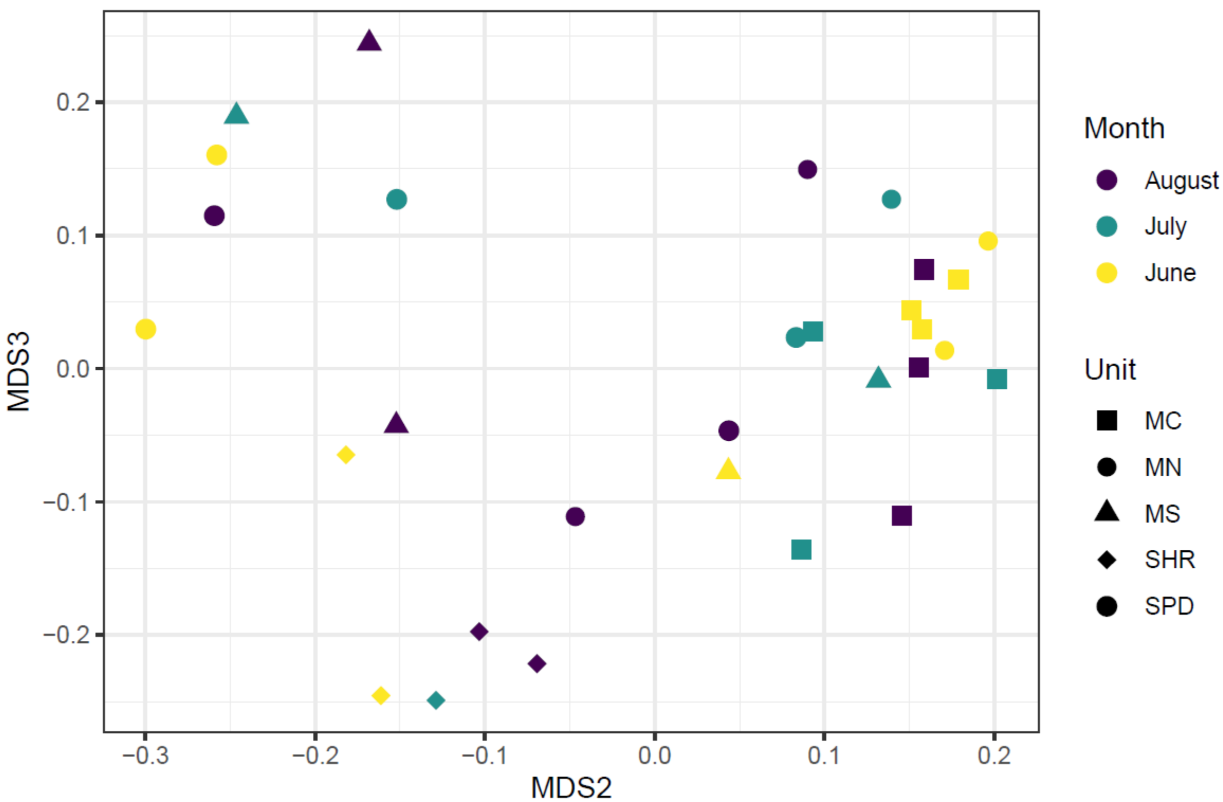


Figure 49. The stress level is relatively low (0.1243) therefore, this is the best fit for visulizing the data**.** Electrofishing NDMS Distribution Across Month and Unit. There is a large spread of MS across MDS2 and MDS3 throughout June, July, and August from -0.25 to 0.15. Stress level is 0.1243 which is relatively low. Other wetland management units and months also have a strong correlation, like MN and SPD, that range the entire spread and MDS2 and a large portion of MDS3. Overall there is less spread along MDS3 than MDS2. Wetland management units SHR

and MC are tightly clustered, showing the distinction of fish species for these wetland management units.

Influence of Vegetation Type on Fish Abundance

We performed electrofishing in two vegetation zones: Open Water and Submerged Aquatic Vegetation (SAV)(Figure 50). The ANOVA showed that vegetation type did not significantly influence CPUE ($p = 0.309$). Neither vegetation type supported a significantly higher number of fish in our electrofishing samples.

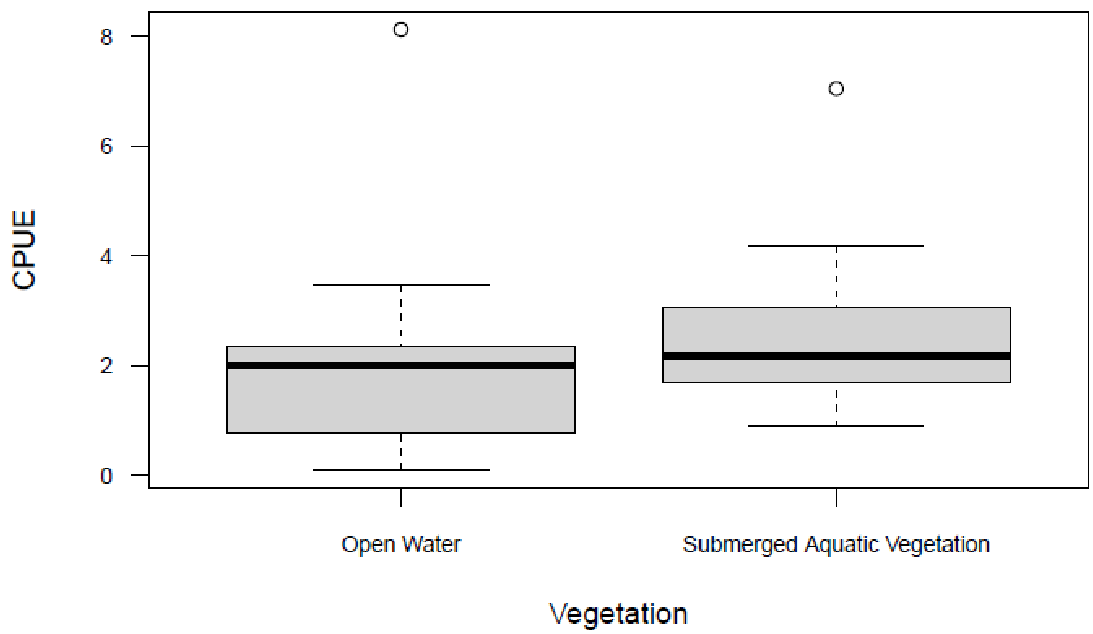


Figure 50. Mean Catch per Unit Effort (CPUE) by Vegetation type sampled. Open Water or SAV vegetation zones did not have significantly different electrofishing CPUEs. Both vegetation types had outliers in the higher CPUE range of 7-8.

Using PERMANOVA, we found that vegetation type did not influence community composition of fish sampled ($p = 0.753$) between wetland management units. There was inconsistent spread of vegetation types across all quadrants, which is an indication that there is not significant variation in fish species per vegetation zone because there is no pattern ($p = 0.071$) (Figure 51).

The Bray - Curtis NDMS plot for vegetation provides a visualization of the distribution of vegetation and wetland management units. The PERMANOVA shows no significant trends or patterns of spread, which means there is no correlation between the two. With the x-axis being MDS1 and the y-axis being MDS 3, there is a large amount of spread, meaning sites are dissimilar (Figure 51). This is also supported by the PERMANOVA results showing no significant difference between community composition based on vegetation type.

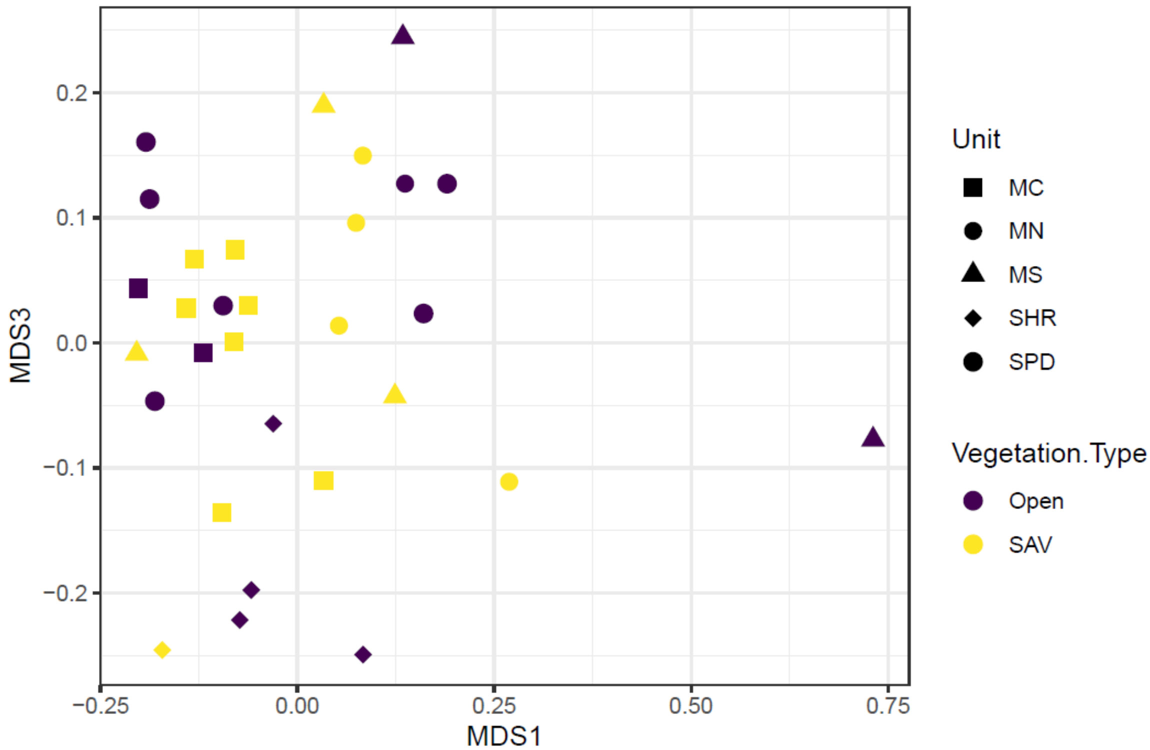


Figure 51. The stress level is relatively low (0.1243) therefore, this is the best fit for visulizing the data**.** Electrofishing NDMS Distribution across vegetation zone and Unit. The points represent a certain sampling unit (shape), and the sampling vegetation zone (color). The Spaulding drain sites and open vegetation showed the highest variability in MDS1. .The Maankiki Center (MC) sites and submerged aquatic vegetation show the highest variability in MDS3. Not all ellipses were able to be drawn due to the limited number of points. Maankiki South's open water site is an outlier sampling point.

There was greater distance of vegetation and unit variation between MDS axis 1 and 3. MN and MS both had a large spread over the y-axis MDS3. The SAV is more correlated with MS, MC, and MN(Figure 51). There is noticeable grouping of the MC sampling sites and the SHR sampling sites. MC SAV and open water are mostly grouped together with the exception of two MC SAV outliers from this grouping. SHR sites are grouped together at the bottom of the graph regardless of the vegetation type. There is a lack of grouping in other sites like MS, MN, and SPD. There is one MS open water site that is an outlier and is not grouped with any of the other sampling sites.

Influence of Water Quality on CPUE

To determine the influence of water quality on fish communities, we applied a generalized linear regression model (GLM) (Figure 52). If you were to look at the multiple linear regression, then none of the water quality values are significant *($p > 0.05$),* which is why the GLM is important. Independent variables were: temperature (C), pH (standard units), turbidity (FNU), conductivity (µS/cm), and dissolved oxygen mg/L (DO). With the individual linear regressions, the temperature was the only significant water quality parameter influencing electrofishing CPUE ($p = 0.01862$). The p-values for the other parameters were: dissolved oxygen, $p = 0.8280$; pH, $p = 0.567$; turbidity, $p = 0.225$; and conductivity, $p = 0.23517$.

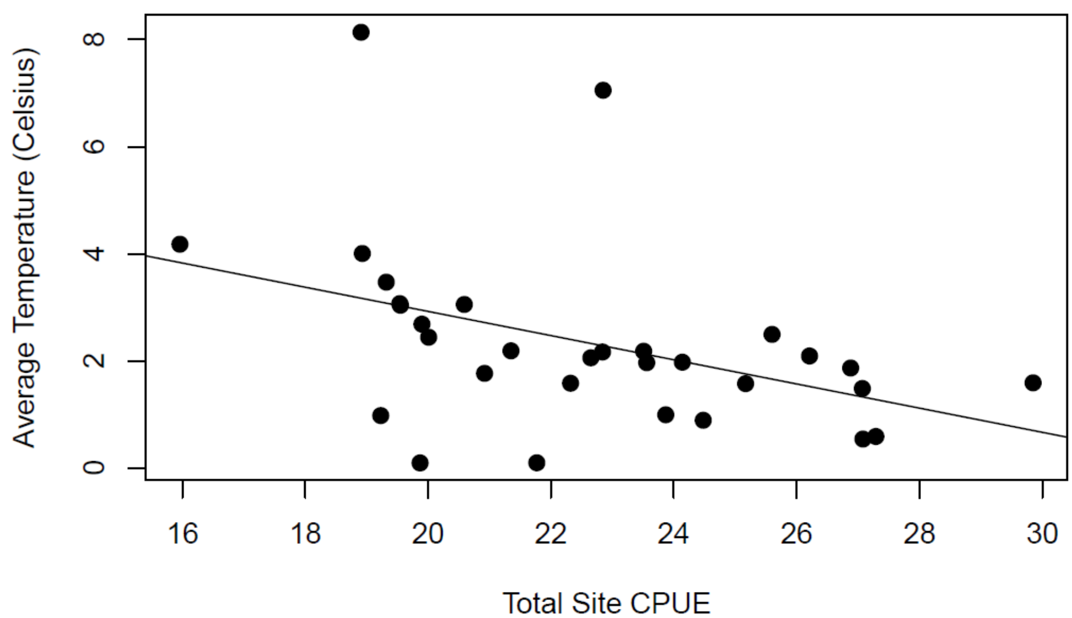


Figure 52. Average temperature distribution of CPUE across all sites on a log scale. Data points show a negative relationship; as temperature decreases, total site CPUE increases. The p-value is 0.01862, and the R^2 value is 0.1765. Even though the R^2 is relatively small, it is large enough to determine that it is a good fit.

DISCUSSION

Species Composition and Abundance

Connectivity of SNWR to the surrounding river and bay network acts as a dynamic local filter on fish species' movements in and out of the wetlands. Connectivity filters on multiple spatial and temporal scales to determine wetland-specific pools of species as well as species abundance (Tonn, 1990; Bouvier et al., 2009). At the continental scale, the diversity of the Laurentian Great Lakes species pool has been filtered by its glacial history. Regional filters on fish species found in Saginaw Bay and the Saginaw River watershed further constrain the SNWR species pool. Locally, the legacy uses of SNWR act as a temporal filter, and water-control structures that connect SNWR to the surrounding watershed directly dictate the types and numbers of species found. Major episodic flooding events, which have been natural and anthropogenic, also allow for dispersal of species into the wetlands and between wetland management units.

Along with the complex hydrologic position of being both floodplain and coastal wetlands, the management of SNWR wetlands makes them inherently a unique type of wetland in the region. Among Great Lakes Coastal Wetlands (GLCW), complex riverine wetlands tend to have more fine-scale heterogeneity of fish assemblages within the wetland (Trebitz et al. 2009). The variability in community composition between wetland management units at SNWR is consistent with the fine-scale heterogeneity of other complex riverine GLCW. We observed several unique riverine species within our wetland management units. Of the restored wetland management units (i.e., not SHR), the most recently reconnected unit, MC, had the highest total abundance of fishes in fyke net data. MC is the largest of the three wetland management units in Maankiki Marsh, but area has not been found to have a great impact on fish richness and abundance in GLCW (Bouvier et al., 2009). The fyke net NMDS plot for unit (Figure 39) suggests that the fish assemblage in MC is more similar to that of SHR, than to the other management units within the wetland. This may reflect the unit's being in early stages of colonization after reconnection and species are competing to establish in this previously inaccessible habitat (Pander et al., 2015).

Considering the length of time that the wetland management units have been restored and the species pool of the Great Lakes region, the number of fish found at SNWR indicates a healthy wetland ecosystem. Generally, coastal and floodplain wetlands recover rapidly after hydrologic reconnection (Brockmeyer et al., 1996; Pander et al., 2015). Using fyke nets we caught 9209 individual fish of 31 species within the wetlands and 7 additional species in the Shiawassee River. Compared to fyke netting, electrofishing found unique species (e.g., Golden Redhorse, and Freshwater Drum) and revealed higher abundances of some species such as Black Bullhead, Pumpkinseed, and Bowfin (Figure 45). The species diversity and relative abundances represented by electrofishing were useful in characterizing the overall picture of fish abundance at SNWR (Figure 46).

While vegetation does spatially structure the wetland management units, neither method of fish sampling (fyke net and electrofishing) suggested that vegetation had a significant influence on the number or diversity of fish species. We attribute this to the fact that vegetation at SNWR is patchy and fish are mobile. It is plausible that fish would frequently move between vegetation types, depending on changing needs. This would also contribute to the heterogeneity of fish assemblages within the wetland management units.

Abiotic Influence on Fish Abundance

The peak of fish abundance during July that we see in the fyke netting data supports the idea that fish use wetlands seasonally. While there are permanent wetland residents, many species use GLCW seasonally (Diller et al., 2022). During the spring and summer, many species move into wetlands for spawning and age-0 fish find refuge in the various vegetation types. When water levels rise in the spring and fall, the shallow wetland management units get an influx of relatively cool river water. Fish lengths reveal the presence of many juvenile fish, indicating a healthy ecosystem that supports fish spawning and reproduction. Most wetland management units had a large population of juvenile fish, except for MN where dominant fish species were represented by more mature individuals. High number of juveniles further supports the seasonal use of wetlands by fish.

Month and temperature are closely correlated and both factors play an important role in the total fish abundance and fish community assemblage at SNWR. Physiologically, fish species have specific temperature tolerances, which can limit habitat availability. During 2022, the highest temperature that we measured was 35°C in June, which may have resulted in avoidance or mortality for less tolerant fish. As we experienced in P1A, elevated temperatures can increase evapotranspiration and lower water levels, sometimes even to the point of drying. In 2022, we experienced the lowest water depths in P1A over the course of this study, at least 15 cm lower than in past years. Such disturbances can affect movement and survival of fish.

Temperature also impacts abiotic factors such as water level, DO, and conductivity; that further influence fish abundance and assemblages. Temperature and conductivity have a positively correlated relationship, while temperature and DO have an inverse relationship (Barron & Ashton, 2005). Physiological processes that impact survival, growth, and reproduction of fish species can be limited by tolerance levels of low DO. Great Lakes Coastal Wetlands generally have low DO because of extreme biological productivity, small water volume, and low water turnover rate (Arthington et al., 2004; Diller et al., 2021). Low oxygen levels can be exacerbated by high temperatures, limiting habitat for more sensitive species during the warmest summer months. While DO was significantly different between months, wetland management units, and vegetation zones; DO levels did not significantly impact fish abundance or community composition because species that reside in the wetlands are more tolerant. For tolerant fish like those found in wetlands, the window between sublethal and lethal DO concentrations is narrow (Tang et al., 2020). According to Tang et al. (2020), about 94% of the species found at SNWR are considered tolerant or mesotolerant to low DO levels. As discussed previously, MS had the lowest CPUE and experienced extremely low DO levels below 1 mg/L DO. Despite conditions, we still caught 21 species of fishes in MS.

Black Bullhead and Bluegill are both warm-water fish species and their abundance may be positively correlated, playing an important role in structuring fish community composition. In past monitoring, Black Bullhead were more abundant, whereas in 2022 Bluegill and other sunfish were predominant. It seems DO levels did not get low enough, but temperatures may have been high enough to cause mortality or avoidance of Black Bullhead. Black Bullhead are mesotolerant to DO levels (lethal, 1.98 mg/L), while Bluegill can be more tolerant (lethal, 1.06 mg/L) to extreme DO levels (Tang et al., 2020). Bluegill can tolerate up to 33°C, while Black Bullhead have a higher tolerance up to 35°C, which was our highest temperature reading. Optimal temperatures for both species range from 24-30°C (Wismer & Christie, 1987). A study in Wisconsin showed that when Black Bullhead were removed from lakes, Bluegill declined, while game fish (Walleye, Yellow Perch, and Black Crappie) increased (Sikora et al. 2021). While the relationship is still unclear, it appears that Bullhead and Bluegill are closely associated.

Role of Trophic Interactions in Community Composition

Fish abundance and community composition indicate complex trophic interactions and high-quality ecosystem services. Richness at multiple trophic levels indicates higher-quality ecosystem services than richness at any one trophic level (Soliveres et al., 2016). Fish play an important role in the larger trophic web at SNWR. They feed on macroinvertebrates and also provide food sources for birds and piscivorous fish. Many fish feed on amphipods, a high-quality prey source, which were very abundant across all wetland management units (reference invert table). The presence of multiple species of piscivorous fish like Northern Pike, Largemouth Bass, and Black Crappie indicates a healthy population of prey fish.

Predation by piscivorous birds and fishes can influence the fish community composition we sampled. If bird abundance or communities changed from year to year, this may impact what species of fish were predated upon more frequently. It is possible that fish were more vulnerable to predation for some years due to conditions like shallower water, like in P1A. For instance, in 2021 researchers caught a high number of juvenile Black Bullheads. Counterintuitively, Black Bullhead CPUE decreased in 2022. In general, juvenile fish have rather low survivorship, often as a result of predation. It may be possible that lower water levels resulted in higher predation on juvenile fish, including Black Bullhead. Future research could help to understand the interactions between bird and fish abundance and composition.

We found a large number of juvenile fish, including Yellow Perch, which are important game fish. Yellow Perch utilize many prey sources throughout ontogenetic shifts, spanning from zooplanktivory to insectivory to piscivory (Parke et al., 2009). Most of the Yellow Perch we found were caught in the Shiawassee River. Their abundance at SNWR could represent a trophic connection of juvenile perch using wetland resources and transferring energy to nearby Shiawassee and Saginaw rivers, and Saginaw Bay, emphasizing the importance of hydrologic connectivity (O'Reilly et al., 2023).

Impact of Connectivity on Community Composition

Separation and connectivity play a key role in the wetland management units' fish composition and ecology at SNWR. Island Biogeography Theory (IBT) states that ecological environments are separated by a physical barrier and species abundance is determined by how close islands are to the mainland, proximity to other islands, and water levels (Angeler and Alvarez- Cobelas, 2005). In the case of SNWR, wetland management units act as islands and the Shiawassee River as the mainland, guiding us to consider the distance and connectivity between the control structures and the Shiawassee River.

Each of the Maankiki units has unique fish populations despite being connected through the distribution basin to each other and the Shiawassee River. Connection of the Shiawassee River to the wetlands allows fish to swim into the restored floodplain management units for a variety of life history events and physiological regulation. For instance, accessibility to the floodplains is an important factor in thermoregulation; if the fish can navigate easily in and out of the floodplain, then they are able to search out stable conditions and avoid stress from extreme temperatures. The separation by dikes also contributes to the distinct fish communities found in each wetland management unit. While units at SNWR are effectively separated, there is enough connectivity to facilitate some movement between populations. Wetland management units are not closed systems because they are connected to the Shiawassee River and each other, which allows fish movement to preferable ecological wetland management units.

There are two routes of dispersal that would allow fish movements between wetland management units. The primary route is the water control structures allowing or preventing fish from moving through the gates, between wetland management units and the Shiawassee River (Conrad et al., 2022). The two water-control structures can be opened or closed periodically depending on the weather and seasonal conditions. For example, during the summer of 2022, water control structures were closed to protect the wetlands after a chemical spill upstream. Closing them also allows the wetland management units to retain water when they are at risk of drying out. The other mechanism for fish dispersal throughout the wetland management units is when the refuge experiences major floods. This happens roughly once every ten years when waters overtop the barrier dikes, and all wetland management units become connected, allowing for potentially broad fish dispersal.

Study Limitations

Throughout the field season, we had success in sampling by fyke netting and electrofishing, but there were some complications that may have limited the study. Challenges included weather conditions and some human sampling errors.

Low water levels in P1A limited sampling with both fyke netting and electrofishing. There were times when we were forced to use a small frame net or were completely unable to set a net due to low water levels. We did no electrofishing in P1A because there simply was not enough water to support the catamaran.

Several limitations arose with fyke netting. Our goal was to sample at least 20 sites per wetland management unit. Due to low water levels and impeding circumstances, we were unable to meet this goal for all wetland management units. Based on the four seasons' species accumulation curves, in the future, we recommend sampling a minimum of 20 sites with a goal of 25 sites in all wetland management units to obtain a full species assessment. Because we did not obtain a minimum of 20 sites in all wetland management units during the sampling season, we cannot be confident that the entire assemblage of fish species was revealed. Another issue that arose was the significant percentage (%) of compromised nets whose data were unusable. One common cause of compromise was catching a snapping turtle in the net. The snapping turtle likely ate many of the fish and sometimes created a hole through which fish could escape. Other instances of compromise included when a net was untied from a conduit or a hole was discovered in the net. Other times we faced issues where water was too high, like in SHR, which made setting and tying fyke nets and transferring fish very difficult.

There are a variety of study limitations that specifically pertain to electrofishing. Unfortunately, during our first month in the field, we experienced a Covid-19 outbreak and were unable to electrofish for the month of May. Because of this setback, we were only able to sample a total of three times throughout the summer instead of the standard four (Conrad et al., 2022). Our sampling may be misleading in the number of fish that were actually caught because the number of people shocking and netting fish changed. For our first electrofishing sampling in June, we only had one individual who was allowed to shock and net the fish. This was due to a misunderstanding about how many people could be on the catamaran at the same time. We later learned for our sampling in July and August that we could have a total of three people on the boat (one rower and two fish netters). Other natural conditions affected our ability to sample. For instance, if it was raining, we were unable to electrofish for safety reasons. There were a total of two days where our sampling was disturbed or cut short because of rain (June 6th and July 20th). Other natural conditions, such as wind and low water levels, created difficulties with electrofishing. The wind made it very difficult to maneuver, so we had to go at a slower pace but also reaching the shocked fish was more of a challenge (July 19th & 20th).

Implications for Future Restoration

Comparing across the four years of the study, while IBI scores have generally seen a positive trend, we suggest continued sampling to account for fluctuation. Based on our findings, some areas of the refuge are improving in habitat quality, and others are decreasing. Fish IBI scores for 2022 ranged from 'moderately impacted' to 'degraded' depending on the unit. IBI scores of 2022 shifted both upwards and downwards from previous year's findings (table 3X). Many IBI scores went from degraded in 2021 to moderately degraded in 2022 or from moderately degraded to moderately impacted; showing positive improvement. We suggest that restoration conditions within SNWR be looked at in the terms of conditions in surrounding wetlands. The coastal wetland fish IBI that we used was based on metrics that included wetlands from all five Laurentian Great Lakes, including pristine Northerly sites, and did not include any diked wetlands (Cooper et al., 2018). If SNWR wetland management units were compared to other more similar wetlands, they might rate relatively well.

Many of the fish sampled were juveniles in the wetland management units and the Shiawassee River (Figures 32 - 36). This suggests that the floodplain wetland management units serve as important breeding habitats for many fish species. However, if the wetland is not adequately connected to other water systems, the regional metapopulations and metacommunities will suffer (Bouvier et al., 2009). There are major areas within the Great Lakes region that suffer from a lack of connected wetlands and tributaries. Bouvier et al. (2009) explained that area does not have a great impact on fish richness and abundance, but connectivity of wetlands does. Connecting wetlands to greater tributaries allows for dispersal of juveniles into metapopulations within the greater region.

It is possible that the hydrologic complexity of SNWR wetlands results in such heterogenous fish distribution that vegetation-based assessment can't be an accurate predictor of wetland health.

Fish-based assessments of wetland health, especially in hydrologically complex and heterogenous wetlands like those at SNWR, may not need to rely on vegetation types (Trebitz et al., 2009). In our findings, the vegetation type did not have a significant impact on fish distribution, and we believe vegetation has minimal impacts on fish populations. However, the combined ecological communities of fish are only part of a much bigger story of overall wetland health.

CONCLUSIONS AND RECOMMENDATIONS

In consideration of the four parameters of biomonitoring evaluated within this study, we found distinct trends across seasons and the four years of sampling. Overall trends show similarity across parameters, illustrating how changes in the abiotic conditions impact trophic interactions, ultimately determining the type of biotic communities present on the refuge. Water quality, vegetation, aquatic macroinvertebrates, and fish all indicate the state of restoration, but to examine the overall ecosystem health, we must consider their ecological linkages. Looking at these interactions allows for the most complete view of how SNWR's wetlands have responded to hydrologic reconnection.

Over the four years of sampling, 2019-2022, there were consistent trends in water quality conditions that in turn, impacted all other sampling parameters. Conductivity was consistently higher in SHR and P1A than in the Maankiki Marsh units, and MS consistently had the lowest dissolved oxygen levels. Our data helps illustrate that connectivity status has clear impacts on the abiotic conditions and explains in part the difference in biotic communities between wetland management units. The differences in aquatic macroinvertebrates and fish community assemblages between SHR and P1A and the Maankiki Marsh units are in part, due to the varying levels of influence from the Shiawassee River. P1A has maintained hydrologic connection to the river for over 60 years, whereas the Maankiki Marsh units have been connected within the past 8 years. Furthermore, Maankiki Marsh does not connect to the Shiawassee River directly but instead relies on hydrologic movement facilitated through P1A.

Our 2022 data suggests that the reconnected wetland management units have improved in overall ecological health and integrity over the four years post-restoration. Notably, plant communities in the 2022 sampling season had the highest recorded FQI and Mean C since 2019. Furthermore, when evaluating aquatic macroinvertebrate communities, all four wetland management units were rated as "mildly impacted," indicating rapid recolonization of aquatic macroinvertebrates within a short period of time. In general, fish IBI scores have also improved over the sampling years.

Vegetation data tells a more complicated story with outliers and inconsistent trends over the years. Specifically, in the year 2021, there was a significantly higher IBI score of 11.25 and 11.67 compared to 2022 and 2020, respectively. However, we did not find that the outlying year of 2021 vegetation sampling had significant impacts on fish and invertebrate populations because there were no consistent trends or patterns that supported this.

In addition to the yearly trends, seasonal impact is also an important factor in determining species composition and floodplain conditions. Seasonal fluctuation in water quality parameters affects temperature, dissolved oxygen, and nutrient levels, all of which influence habitat quality and species abundance. As water temperatures warm from spring to summer, aquatic macroinvertebrate abundances increase, followed by a decline in abundance from mid to late summer. Bottom-up shifts in populations of secondary producers directly affect the behaviors of higher-trophic consumers. Seasonal trends in fish populations align with trends in macroinvertebrate abundance, a significant food source for many fishes, with both reaching peak abundance in July of 2022.

The evaluation of compounding interactions, as opposed to individual, between vegetation, invertebrates, and fish holistically paint a more complete picture of overall wetland health. For example, connectivity can allow restored wetlands to rapidly accumulate diverse invertebrate populations via dispersion (Marchetti et al., 2010). DO is one of the most vital determinants of aquatic ecosystem health. Low levels of DO limit sensitive taxa making the preservation of high DO refugia critical for maintaining diverse populations at the species level. Managing water control structures and controlling for invasive species influences the plant communities on the refuge, which play a role in determining macroinvertebrate and fish communities. Many of these organisms are in turn, consumed by higher trophic-level birds and reptiles that inhabit the refuge in large numbers. Managing floodplain wetland management units at SNWR for waterfowl by providing productive habitats and food sources for them impacts overall communities. Specifically, CD (*Ceratophyllum demersum*) was the highest IVI in all but 1 SAV site (MS), where it ranked 3rd. PN (*Potamogeton nodossus*), another critical food source for waterfowl, was ranked second in IVI in all 3 SAV veg zones. Macroinvertebrates create trophic links between vegetation and secondary consumers (fish, birds) and fulfill the role of secondary producers/primary consumers. They depend on vegetation stability and are essential for supporting the entire food web. Additionally, migration by birds, fish, and emerging invertebrates links SNWR to broader ecological communities, including those of Lake Huron. The complex trophic interactions and richness at multiple levels facilitate high-quality ecosystem services.

Pool 1A, our reference site, expectedly the highest vegetation IBI score (21), which supports the theory that the longer and better the connection is between wetland management units and the Shiawassee River, the better the overall health will be of that unit. Vegetation IBI scores at SNWR were comparable to the uppermost scores in the region, demonstrating the ability of this restoration effort to be used as a restoration model in wetland restoration of post-agricultural / legacy industrial sites throughout the Great Lakes region. P1A Macroinvertebrate species composition was different than Maankiki Units, likely due to its long history of reconnection and the dispersal ability of macroinvertebrates. The evidence of rapid recolonization by macroinvertebrates emphasizes the importance of wetlands and unit connectivity, especially with them being a key player in the food web. Fish communities have also been recorded to have rapid recovery after wetland restoration (Brockmeyer et al., 1996; Pander et al., 2015). In the context of the Great Lakes Region, SNWR has high fish abundance and species richness in a short amount of time after restoration which is encouraging for future restoration projects and overall wetland health and restoration. Based on the Island Biogeography Theory, the better connected the wetland management units, the better the overall ecosystem will be (Bouvier et al., 2009). While recovery of fish and macroinvertebrate species is relatively rapid, the more recently restored units, like MC, differ in community composition. Our data shows that wetland management units continue to change over time and vary in habitat factors that benefit different species and communities. This variation is crucial to sustaining the overall health and balance of the entire ecosystem.

The past four years of data have illuminated important trends that characterize the success of restoration efforts at SNWR, and allowed us to make recommendations for future management. In order to improve overall diversity of invertebrates and fish, removing patches of monotypic vegetation, such as *Typha*, may be beneficial because diversity in vegetation assemblages creates more available niche spaces throughout the water column to be colonized by aquatic species. Furthermore, focus on the removal, mitigation, and prevention of invasive establishments on the refuge will likely benefit native species and diversity. The preservation and protection of the heterogeneous habitats provide important habitat including areas of higher DO which provide sanctuary for more sensitive taxa.

Increasing the depth of data collection in a few selected areas would reduce uncertainty and allow managers to characterize ecological conditions better. Specific to fyke netting, we recommend a minimum of 25 sites per unit in order to best characterize fish abundance and community assemblages. Broader-scale food web implications would also be interesting to explore. Food-web mapping of species found at the refuge could be useful to further characterize the role of SNWR in the greater Saginaw Bay watershed and Great Lakes area. Many of our conclusions drawn about the trophic interactions are based on primary and secondary producers and consumers and do not directly draw on data from higher level consumers. Collecting data tying the populations of waterfowl and other higher trophic level consumers to those of invertebrates and fish may lead to valuable insight supporting refuge goals.

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APPENDICES

Appendix I: R Code

Water Quality

SNWR2023_WQ_Correlation: https://rpubs.com/acurwin/1030080
SNWR2023_WQ_ANOVA: https://rpubs.com/acurwin/1030087
SNWR2023_Nutrient_Regression_Analysis: https://rpubs.com/acurwin/1030095

Vegetation

SNWR2023_Veg.Permanova.Ordination: https://rpubs.com/eweaves/1030127

Aquatic Macroinvertebrates

InvertLR: https://rpubs.com/mbholm/SNWR2023_InvertLR
InvertANOVA: https://rpubs.com/mbholm/SNWR2023_InvertANOVA
InvertNMDS&PERMANOVA:
https://rpubs.com/mbholm/SNWR2023_InvertNMDS_PERMANOVA

Fish - Fyke Netting

fykeLR: http://rpubs.com/mfroeba/1025343
fykeANOVA: https://rpubs.com/mfroeba/1026059
fykeSAC: http://rpubs.com/mfroeba/1026064
fykeNMDS&PERMANOVA: http://rpubs.com/mfroeba/1026070

Fish - Electrofishing

EFLR: https://rpubs.com/HaydenZav/1030197
EFANOVA: https://rpubs.com/HaydenZav/1030145
EFNMDS/ PERMANOVA: https://rpubs.com/HaydenZav/1030135

Fish - Gill Netting

GillnetANOVA: https://rpubs.com/mbholm/UMSEAS-SNWR2023GillnetANOVA

GillnetPERMANOVA/NMDS:
https://rpubs.com/mbholm/UMSEAS-SNWR2023_GillPERMANOVA

Appendix II: ARIS

INTRODUCTION

Over the past four years, there have been teams of graduate students from the University of Michigan to assess the post-restoration progress of the Shiawassee National Wildlife Refuge. Within this process, the Sonar Imagining tool ARIS was also incorporated into the project (Dellick J, 2020). The ARIS tool can recognize fish swimming in and out of specific wetland pools, and so can provide constant surveillance of fish movements It uses sound waves to detect fish swimming in murky or deep water that would not be visible to the human eye or a light-based camera. Two Sonar Cameras have been run in ice-free conditions over the past three years. Many image frames have been hand-labeled in the past two years to identify "what a fish is" to train the Machine Learning Model (MLM). There have been initial versions of MLM that have already been developed and tested.

However, last year's data analysis team found an issue with the MLM. So they were not done because there was too much error with the MLM identifying individual fish. There proved to be high false positive and false negative rates for labeling individual fish. Schools of fish remained relatively accurate with a rate of 90% and 91% precision rate. In contrast, individual fish percussions rate was estimated at 57% and 45% (Sharp, 2021).

Therefore, the 2022 project team's team goal was to analyze the past years' data that was collected in 2021. And supply the USGS researchers with an adequate sample of hand-labeled images. These hand-labeled images would be used in training and evaluating the model. We worked to analyze images to help correct the error in identifying fish and assist in the machine-learning model.

METHODS

Image Labeling

Initial Machine Learning Model (MLM) development was based on a series of still sonar images that were hand-labeled by graduate students from the University of Michigan. The ARIS machine was stationed in unit P1A in front of the opening connecting it to the Shiawassee River. The data was collected in a series of video recordings by the United State Geographical Survey (USGS) Great Lakes Science Center (GLSC). Days and time blocks were chosen randomly, and the result was four hours of footage, randomly chosen per month. From the ARIS footage, two days for every month of recording were randomly chosen from the 2021 data. The first day of sonar images was selected from the first half of the month, and the second day was selected from the second half of the month. However, if the footage was unavailable for half the month or less, then random days within the sampled timeframe were chosen. If there was only footage for two days of the month, those two days were selected for their footage. After days were chosen, two one-hour footage segments were randomly selected from each day.

After the data was selected, it then had to be analyzed, but several steps were taken prior so that this could be done properly. Here we partnered with the University of Michigan ARC team, which processed and compared the labeled images using a supplementary comparison script. The ARIS files were converted into MP4 format using a conversion script to extract individual frames. Following MP4 formatting, a subsampling script was used to extract half of the frames from the set, about every other one. The resulting set of image frames were representative samples across the entire ARIS deployment for that year. The last step was further subsampling by selecting 100 consecutive frames from the middle portion of the random hour blocks set earlier. This was neither from the first 25% nor the last 25%, but somewhere in-between. Supplemental frame selection was made to ensure an adequate number of frames that include a school of fish. In total, 4,000 total frames were selected initially.

Our project team then worked on labeling the images as either a fish or a school using bounding boxes. When a frame was reviewed, the labeler would create a tight box around an individual fish or school of fish; this box is known as a bounding box. Each fish visible was labeled either as a ‘fish’ or as a ‘school’ (a group of similar fish swimming in the same direction). For Quality Assurance Quality Control (QAQC), each image frame was examined by two laborers, Labeler A and Labeler B. Two different labelers reviewed the same image frames, but their annotations did not always match, so a comparison script was used to calculate labeling accuracy. Meghan Daily wrote the comparison script to systematically compare each of the two labelers bounding boxes. The comparison script compares every bounding box of a labeled fish (or school) for a set of 100 frames from each labeler and then overlays them. The comparison script does this by calculating the overlapping area and non-over overlapping area for each bounding box from the two different labelers[1]. The overlap in bounding boxes or lack thereof is calculated to determine if there is a match of at least 85% to assess[2] labeling accuracy. If the two labeled frames of the same image, marked by different laborers, were less than an 85% match, then the images were reviewed by a third-party expert from the USGS GLSC. Responsibilities for labeling were described in a master Google spreadsheet.

Differentiating between individual fish, groups of fish, and schools of fish proved challenging. A school of fish was to be labeled separately then individuals or groups of individuals so that the MLM could differentiate between large schools of fish swimming by and clumped or single individuals. In the 2021 report, the MLM struggled with counting individual fish. Therefore, the goal was to label more individual fish because the MLM did not recognize them as accurately as the schools of fish. It is still necessary to have schools labeled for a base level and to ensure they are still being recognized.

For the Machine Learning Model, we formulated a set of rules to distinguish between a school of fish and a group of individual fish. We felt that a school of fish in the context of the MLM is “a large congregation (... generally greater than 20 individuals) is a school if the morphological features of fish overlap or blend resulting in a heavily blurred object making it impossible to

[1] Criteria given by Militello 2023
[2] Criteria given to us by Bozimowski 2022

distinguish and label individuals"[3]. If this is not the case, then the following criteria and rules were used to define a school: 1) Fish must be present in a large congregation. 2) A majority of the fish are swimming in the same direction. 3) The perceived size of the fish is the same within the congregation. If the initial definition of a school is not met but does meet all three subsequent decision rules, it was categorized as a school. If not, it was considered to be "grouped individuals" that were individually labeled and had separate bounding boxes[2]. Grouped individuals can be of different size classes and may or may not be different species of fish, but they happen to be found near each other in the image frame. Such fish were annotated as "fish."

After we labeled the images, J. Militello and A. Bozimoski used the supplementary comparison script to calculate an accurate percentage of overlap of labeled image frames to use them to train MLM. The labeling took place with five different graduate students doing quality control and keeping track of the analyzed image frames. This data is now accessible to leading scientists. Through this process, we learned that with more labelers, there is higher variability but you have higher quality control. The answer is statistics with variability. This analysis process set up the stage for variable statistical studies.

[3] Criteria on ARIS training methods by Bozimowski and Militello 2022

ARIS Work Cited

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