UNIVERSITY OF MICHIGAN, SCHOOL FOR ENVIRONMENT AND SUSTAINABILITY

Fixing a Flat:

The Third Year of Post-Restoration Monitoring at Shiawassee National Wildlife Refuge

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

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EXECUTIVE SUMMARY

The Shiawassee floodplain is a hybrid floodplain located in mid-Michigan and is home to our study site, the Shiawassee National Wildlife Refuge (SNWR). The refuge was established in 1953 to designate an area for migratory birds to seek refuge during migration. This location is topographically quite flat and is the convergence of four different rivers, thus making it very prone to flooding. These factors drove many historic farmers to dike the tributaries of these four rivers to prevent their crop fields from flooding with water. Historic diking caused SNWR to be disconnected from the Shiawassee River. Through the Great Lakes Restoration Initiative, SNWR received funding to start a restoration project to reconnect these two features.

A team was formed to study the effects of restoring this wetland and reconnecting it to the river. This team includes the United States Fish and Wildlife Service (recipients of funding and managers of this refuge), the United States Geological Survey (federal researchers with expertise in this area), and a master's team from the University of Michigan (the person power to drive this study). 2021 marks the third year of consecutive monitoring for this study and is crucial for understanding 1) the multiple life cycles of taxa present at the refuge, 2) successional changes over monitored years; while 3) establishing that funding for this project has been effectively used, and 4) providing future monitoring teams with robust data to better predict the future of SNWR. The team set out to learn how abiotic and biotic elements change across refuge wetland units of Maankiki South (MS), Maankiki North (MN), Maankiki Center (MC), Pool 1A (P1A), and Shiawassee River (SHR) and sampling months, describe the relationships between these elements, and to describe changes that have occurred across the three years of monitoring. The elements sampled included water quality, vegetation, aquatic macroinvertebrates, and fishes.

Water Quality: We describe each water quality variable sampled (temperature, dissolved oxygen, conductivity, turbidity, pH, and nitrogen and phosphorous levels) and provide statistical analyses of each variable across units, seasons, and in comparison to 2019 and 2020 data.

- We found that refuge wetland units experienced harsh conditions of high average temperatures and low dissolved oxygen levels in the summer season, but refugia zones for aquatic organisms when there were unfavorable conditions elsewhere also existed since water quality conditions varied across different sampling sites.

- We found that summer conditions in 2021 lasted longer than 2020, and conductivity was consistently higher in all wetland units compared to the 2019 and 2020 sampling seasons.
- We found the frequency and impact of seiche event on our wetland units were much smaller than our reference coastal wetland (ie. Pool2B next to Lake Erie), which indicated the dissimilarity in hydrology between these two study systems.
- Due to the lack of high turbidity data, we were unable to predict nutrients level based on turbidity data.
- Future recommendations include sampling nutrients later in the season to capture the effects of more frequent seiche events and creating a more uniform schedule for the opening and closing of water control structures.

Vegetation: We describe the plant species located and identified throughout the refuge units, and the statistical analyses and indices used to determine quality of habitat, compared to the 2019 and 2020 sampling seasons across three previously sampled wetland units and one new wetland unit.

- We found that IBI scores increased to medium across all units compared to the 2019 and 2020 sampling seasons.
- We found that MC had the highest FQA and IBI scores even though it is the most recent connection to the Shiawassee. MC also had the highest number of invasive species compared to MS, MN, and P1A.
- We found that dissimilarity indices were influenced by water depth, which we attribute to changes in nutrient cycling, seed dispersal, and seed bank viability.
- Future recommendations include conducting a water quality assessment when collecting vegetation samples to better understand the effects of water quality on vegetation communities.

Macroinvertebrates: We describe the abundance, composition, and quality of aquatic macroinvertebrate communities across the three previously studied units and one new location within SNWR, and compare these findings to those of the 2019 and 2020 sampling seasons. We also analyzed these data in their relation to water quality, vegetation, location, and season.

- We collected 60 samples, similar to previous years, but we collected thousands fewer individuals, and 5-10 fewer genera and families than 2019 and 2020, representing a strong decline in macroinvertebrate richness and abundance which we attribute to a historic 2020 flood.
- We determined that SNWR could be identified as a ‘mid-fetch’ or low fetch wetland based on distributions of macroinvert communities.
- We found that IBI in MN, MS, and P1A was moderately degraded, a step down from 2020 scores, but MC had a first time score of mildly impacted.
- We found significant differences between units and months on CPUE, but not vegetation zone.

- Future recommendations include using floodplain wetland statistical tools for analysis, fuzzy coding analyses, and sampling more evenly across vegetation zones.

Fish: We describe the abundance, composition, and quality of fish communities throughout three previously studied units and two new locations within SNWR, and compare these findings to those of the 2019 and 2020 sampling seasons. We also analyzed these data in their relation to water quality, vegetation, location, and season.

- We conducted fish sampling through multiple frame and mesh size fyke nets, and electrofishing.
- We found more species than 2020 and similar amounts to 2019, though unique species were found in all years. Most unique species in 2021 came from the Shiawassee River.
- We found that community composition across all units were homogeneous, which we attribute to harsh conditions only being tolerable to a few wetland adapted species.
- We found the same abundant species as both 2019 and 2020, with the most abundant species varying by unit but with Black Bullhead, Bowfin, Pumpkinseed, Bluegill, and YOY sunfish being some of the most abundant species across all units.
- Future recommendations include continued sampling to build robust datasets, water depth analysis, and tracking of fish between the Shiawassee River and SNWR.

Our research has implications for ecosystem variability and succession, as we found both increases and decreases in habitat quality, abundant species, and community compositions. This third year of sampling has shown evidence of succession following a large disturbance and provides insight into how restored wetlands vary over multiple years.

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“Secretary of the Interior Douglas McKay today announced that the Migratory

Bird Conservation Commission, of which he is chairman, has approved the purchase of 9,185 acres and the lease of 1,783 acres of land in 12 national wildlife
refuge units administered by the Fish and Wildlife Service in 10 States.

A new refuge, designated as the Shiawassee National Wildlife Refuge in Saginaw County, Michigan, was authorized by Commission action and the initial purchase of 2,246 acres of land for this unit was approved. This refuge, which has the support of the Michigan United Conservation Clubs and the Saginaw Valley Regional Commission, will adjoin a unit which the State of Michigan is purchasing for management as a public shooting area."

Department of the Interior
May 29, 1953

INTRODUCTION

The Shiawassee National Wildlife Refuge (SNWR) is an ecologically and historically dynamic wetland system, providing crucial habitats for many resident and migratory species as well as valuable ecosystem services to nearby communities. SNWR is located in Saginaw, Michigan and is nested within the Shiawassee Flats, central floodplain and freshwater estuary within the larger Saginaw River system (Figure 1). The SNWR drainage area spans approximately 6,060 square miles, which equates to approximately 10.6% of Michigan's water storage (U.S. Fish & Wildlife Service 1999). The major rivers of the watershed include the Tittabawassee, Cass, Flint, and Shiawassee which meet within a shared lowland floodplain, much of which is SNWR. These rivers then run downstream through the Saginaw Bay and then Lake Huron. The flat topography of the watershed allows for seiche events originating in Lake Huron to travel upstream the Saginaw River and influence SNWR hydrology, even though the refuge is 20 miles inland (U.S. Fish & Wildlife Service 2018). These uniquely coastal events result in SNWR being understood as an inland freshwater estuary. SNWR is located along Michigan's climate-driven tension zone between hardwood and coniferous forests, making it an important stopover for migratory birds both as they enter and leave the boreal ecosystems of Canada and the arctic (Audubon 2016). Because SNWR was initially created as migratory bird habitat, the services it provides to migratory fishes and other wildlife are understudied (Jude and Pappas 1992; Langer et al. 2018). Cultural ecosystem services enjoyed at SNWR include birdwatching, hunting, fishing, and hiking. Currently, recreational hunters buy deer tags for use on the refuge through a lottery system run by the Michigan DNR (U.S. Fish & Wildlife Service 2018). Fishing is common in the surrounding rivers of SNWR and includes the Cass, Tittabawassee, and Flint rivers. Licensing dues for hunting and fishing are reinvested in refuge conservation efforts (Gable 2018). Another important ecosystem service the SNWR provides that affects the surrounding human population is its water holding capacity. SNWR demonstrated its flood mitigation capability during the historic 2020 Midland flood and left approximately 10,000 acres of the refuge inundated with 10 feet of water, likely sparing surrounding property (Ducks Unlimited 2020).

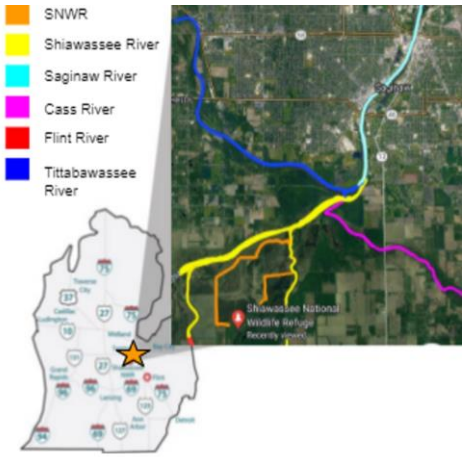


Figure 1. Inset map of Shiawassee National Wildlife Refuge's location near Saginaw, Michigan

The SNWR was created to designate and preserve migratory bird habitat, but did not initially address the health of its aquatic ecosystem. Historic land use alteration left the floodplains in the Shiawassee Flats disconnected from their

rivers, unable to provide hydrologic and habitat services. In the early 1800s, prior to European settlement, the Shiawassee Flats was a hybrid riverine floodplain and coastal wetland comprising approximately 50,000 acres of hardwood swamp, 25,000 acres of emergent marsh and shrub swamp, and several hundred acres of wet prairie (MNFI 2022). The federal Swamp Lands Act of 1850 granted Michigan approval to drain and convert wetlands for agricultural purposes, a process known then as “wetland reclamation” (Dahl and Allord 1996). This legislation reflected the tone of the Federal Government at the time, where drainage and alteration of wetlands was promoted (Dahl and Allord 1996). Saginaw County, Michigan reflected this trend with 96% of all wetlands in the area were destroyed since 1830 (Buchanan et al. 2013). Michigan was also a top lumber producer of white pine (*Pinus albus*) in 1850 (Schrouder et al. 2009; Ziegler 2010), leading to clear cutting forests across the state. Developers cleared large expanses of land for agriculture and constructed systemic ditches and dikes to mitigate overbank waterflow and prevent cropland flooding (Heitmeyer et al. 2013). Disconnecting tributaries from the main river led to water distribution being spread out across more land; however, the price for these actions reduced floodplain water storage, excess discharge sent to Saginaw Bay, and the gradual drying of wetlands (Zedler 2003; Schrouder et al. 2009). In 1953, under the authority of the Migratory Bird Conservation Commission (DOI), 2,246 acres outside of Saginaw were designated for migratory bird habitat and named the SNWR (Department of the Interior 1953). This was to be one of multiple designated refuges which protect crucial wetland habitat for migratory birds, using this chain of refuges for travel and rest. The initial acreage was only the beginning of conservation efforts in the Shiawassee flats, with continued acreage being added over the course of the next 70 years.

So that the SNWR ecosystem can best serve to provide ecosystem services to people and ecosystem function to wildlife, a long-term restoration plan was created for the refuge. In 2011, the United States Fish and Wildlife Service (USFWS) received 1.5 million dollars in funding from the Great Lakes Restoration Initiative (GLRI) to reconnect 994 acres of former farmland at SNWR to the Shiawassee River (U.S. Fish & Wildlife Service 2018). Goals for the SNWR restoration project are to restore hydrologic connection to historic river channels, the hybrid floodplains, and coastal wetlands (HMP 2018 Appendix). This project allows refuge managers to assist seasonal fish migrations in and out of the refuge through a series of gates. The restoration project consists of four phases, each one focused on the hydrologic reconnection of floodplain wetland units to the Shiawassee River and to each other (U.S. Fish & Wildlife Service 2018). Phases directly relevant to unit monitoring have been completed (Phases I and II), as there are additional phases for refuge restoration outside the scope of our monitoring. Refuge management restored the first three wetland units by 2018 with 2019 being the first operational year; the fourth unit, Maankiki Center, was flooded in 2020 (U.S. Fish & Wildlife Service 2018). Initial post-restoration

monitoring occurred in 2019 and 2020, with 2021 as the third consecutive year. Monitoring these new wetlands is pivotal as it is unknown how these wetlands will aid the functionality of the encompassing floodplains and Saginaw Bay. Restoration monitoring is conducted through a partnership with the School of Environment and Sustainability (SEAS) at the University of Michigan, where a team of 5 master's students conduct annual monitoring of SNWR's changing biotic community and abiotic conditions.

Post-restoration monitoring for the previous two years does not provide enough information for refuge managers to make future management decisions or to ensure funding for the remainder of the monitoring. Data collected from previous years has been helpful for depicting initial short-term changes after restoration but does not convey the entire story of compounding changes taking place over a longer period after restoration. Each sequential year of monitoring allows for a better understanding of succession and multiple life cycles the biota undergo to fully establish themselves within wetland units. This third year of post restoration monitoring is crucial to reinforce that funding for this study has been effective and that data collected in 2021 will help guide successive studies. Refuge managers realize both societal and ecological needs must be met when choosing whether to open or close specific wetland gates, and the timing and duration of the open/closed gate can have lasting effects on future management. We are building upon previously established goals and methods created by the partnerships among the USFWS, United States Geological Survey (USGS), and the SEAS master's project team.

We conducted the third consecutive monitoring year during 2021, with the objectives to:

- Characterize conditions within the 3 restored units and 1 long-connected reference unit for 2021 monitoring:
    - ***Abiotic conditions–water quality***
    - ***Biotic communities–vegetation, macroinvertebrate and fish assemblages.***

- Describe variation in abiotic and biotic elements among units and among months for 2021 monitoring.
- Explore correlative relationships among the abiotic and biotic elements measured in 2021 monitoring.
- Describe temporal patterns within and across the sampled units across 2019-2021 monitoring.
- Provide recommendations to USFWS and USGS for future monitoring and in support of future refuge management decisions.

Study System

The refuge is split into 36 named management units based on hydrologic criteria or management goals and the ability to provide clear management objectives (U.S. Fish & Wildlife Service 2018). Refuge wetland units are separated by earthen dikes, with many having water control structures, allowing refuge managers to control the hydrologic connectivity of, and conditions within, the units. Water control structures warrant the movement of water between units, the Shiawassee River, and the Spaulding Drain when opened, or restrict the movement of water when closed. In addition to varying hydrological connectivity, differences in microtopography and biology result in ecologically distinct features within each unit. Refuge managers must take into account these differences in order to achieve conservation and management goals (U.S. Fish & Wildlife Service 2018).

Monitoring work at SNWR spans four wetland units and two riverine locations, with objectives to sample water quality, vegetation, invertebrates, and fish. Monitored wetland units include Pool 1A (P1A), Maankiki North (MN), Maankiki South (MS), and Maankiki Center (MC), monitored riverine locations include Spaulding Drain (SPD) and Shiawassee River (SHR) (Figure 2). Refuge managers connected P1A to SPD, and subsequently the SHR in 1958, making it the longest-tenured river-connected unit in the SNWR; it serves as a long-term control site for the study of hydrologic connection. MN, MS, and MC hydrologic reconnection construction began in 2016, but were first fully flooded in 2017, 2018 and 2020 respectively, thus previous monitoring only covered MS, MN, and P1A. The three Maankiki units are connected to the SHR via a single distribution basin, from which water flows into the units depending on which unit-specific control structures are open or closed (U.S. Fish & Wildlife Service 2018). P1A also has control structures that connect to MC and SPD.

Two new sampling locations of SHR and SPD were added, in addition to the Phase I sampling locations (MC, MN, MS, P1A) because they serve to supply newly reconnected wetland units with fish and act as a reference as to what potential species may migrate into the units. Additional information on each wetland unit is listed in Appendix I.

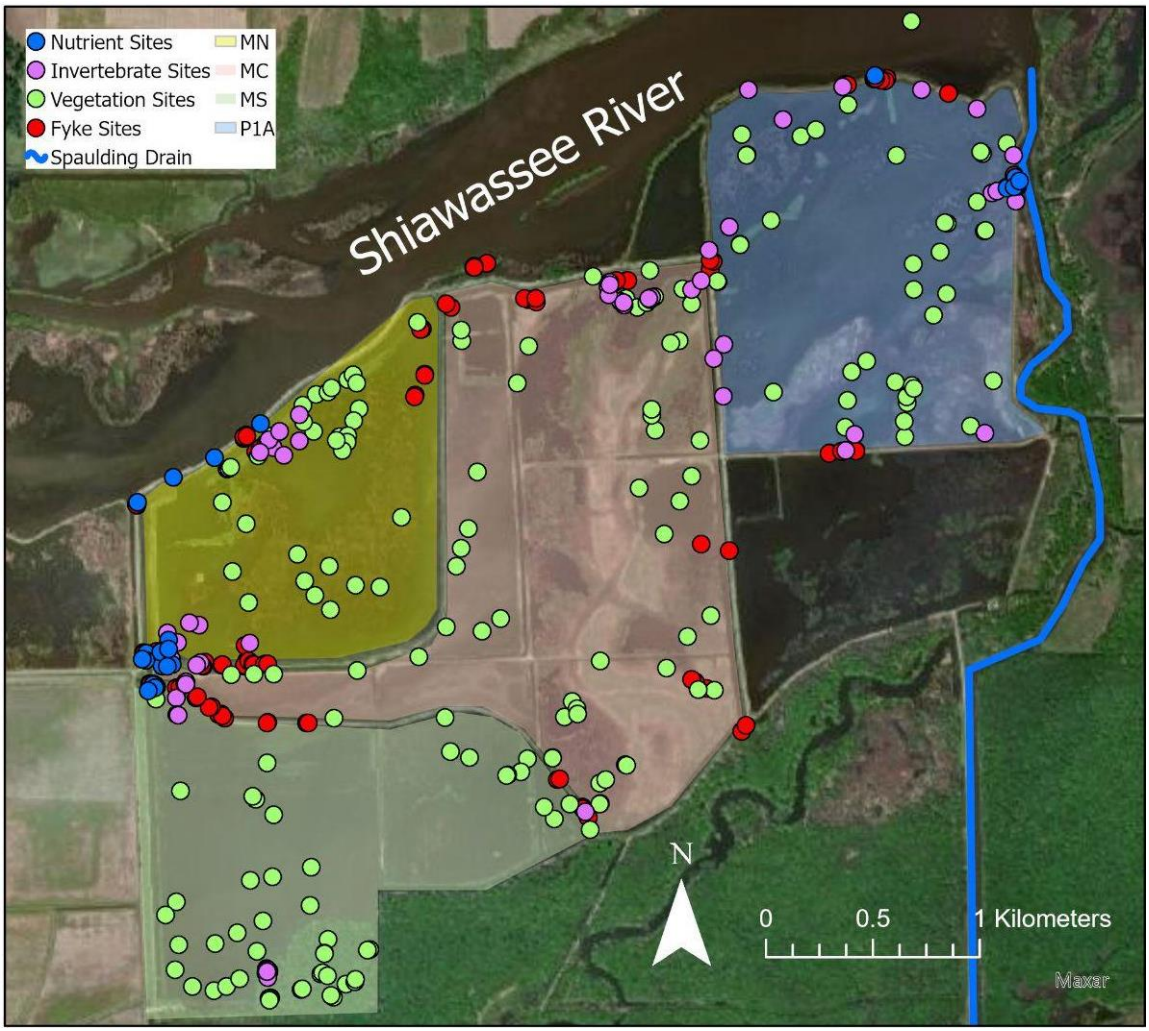


Figure 2. Wetland unit locations and sampling sites at SNWR. Water nutrient, invertebrate, vegetation, and fish fyke sampling sites across the monitored pools at SNWR and in the Shiawassee River and Spaulding Drain. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

Methods shared across the monitoring efforts

Our methods reflected previous sampling efforts in 2019 and 2020 (Lugten et al. 2020, Dellick et al. 2021). SNWR sampling was based on research conducted at Crane Creek coastal wetlands in Oak Harbor, Ohio (Baustian et al. 2018). Studies at Crane Creek were based on methods from the Great Lakes Coastal Wetland Monitoring Program (Uzarski et al. 2016). The sampling schedule for 2021 summer monitoring spanned May to August, with: weekly water quality sampling, biweekly macroinvertebrate sampling and fish fyke netting, monthly nutrient sampling, and vegetation sampling spanning one week in August. Spring sampling only spanned one day in late April, whereas fall sampling took place from October to November on a monthly basis with only fyke nets being utilized, each sampling event spanned one weekend (Table 1).

When appropriate, we applied similar field sampling and data analysis methods across the four sampling categories–water quality, vegetation, macroinvertebrates, and fish–to produce the most cohesive results. Some equipment was reused across categories such as depth sticks, sondes, and quadrats. We used a depth stick and multiparameter sonde for collection of hydrologic data. For macroinvertebrate and vegetation monitoring, we used four, one-meter PVC pipes fitted together as $1m^2$ to represent the sampling area, which we refer to as a "quadrat."

Table 1. Sampling schedule from April to November 2021. This includes schedules for water quality (light blue), nutrients (orange), macroinvertebrates (purple), fish (blue), and vegetation (green). Note that the second row is the week of the year, and that some weeks may span across two different months.

Month	April				May				June				July				August				September				October				November			
Week of the year				17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Water Quality																																
Nutrient																																
Macroinvertbrate																																
Fish																																
Vegetation																																

Data Management

Data collected for the federal government must adhere to standards for accuracy, completeness, and accessibility. As such, we worked with our federal clients to employ data management practices which incorporate Quality Assurance and Quality Control **(**QAQC) procedures throughout the project lifecycle (i.e., data collection, data entry, and data analysis). We created Metadata to assist data interpretation by future users, regulate QAQC checks of collected data, and provide detailed comments where necessary.

We used the ArcGIS Online data collection platform, Survey123, to collect and store data, as well as Microsoft Access and Excel to organize and manipulate vegetation, macroinvertebrate, and fish monitoring data. We input all the original collected data into Survey123 forms on iPads or personal cell phones outfitted with the app (e.g., when iPads were not available) and uploaded the data to USFWS Survey123 accounts, then our USFWS and USGS partners reviewed them for user- or computer-generated errors and sent us the Excel data produced from Survey123. We performed a second check for any errors in the produced Excel data (e.g., missing data or entry errors.) by calculating the IQR (Interquartile Range) for numerical variables. We identified data points out of IQR as outliers, then investigated each of them: if the outlier was produced by an entry error, we removed it; or if it was a true outlier, we assessed it later in statistical analysis. Lastly, we manipulated data in Microsoft Access and Excel so that they were appropriate for statistical tests.

We employed two different data management methods for water quality datasets. Water quality sampling included *in situ* water quality sampling via the YSI EXO3 sonde (See methods in page 10), and nutrient sampling using grab samples for lab nutrient analysis (See methods in page 11). Observations collected via the sonde used the data management method described above, while for associated water nutrient samples, we received data in Excel spreadsheets from the Heidelberg University Water Quality lab, then stored them on USFWS and USGS servers. We managed them the same as above using Microsoft Access and Excel, in preparation for statistical analysis.

The QA procedures for data initially recorded on physical data sheets were different from data initially recorded on Survey123 as stated above. For electrofishing data, we first entered data on waterproof datasheets designed for the process, then transcribed the data into Excel spreadsheets (See methods in page 110). To QA these data, one member of our team cross checked all electronic entries to the original waterproof datasheets. If any inconsistencies were identified, a different reviewer from our team would make a final check of those inconsistencies, then we determined a final value. For macroinvertebrate identification data, we used paper sheets, Survey123 electronic datasheets,

Microsoft Excel and Access to manage data (See methods in page 71). Each researcher first entered species identifications and invertebrate counts into a waterproof notebook. Upon completion of all identifications and counts, researchers then transcribed their data into Survey123 separately. We then downloaded these data to Excel, exchanged notebooks among team members, and reviewed all entries for inconsistencies. The reviewer then specified any errors in the original notebook and returned the notebook to the original researcher, where the researcher checked and corrected data where applicable. Once this initial QA was completed, we performed additional QC checks (i.e., IQR as above) to prepare data for statistical tests.

Cross-year Data Management

We also closely collaborated with our clients from USGS and USFWS to standardize the data collection methods, metadata techniques, cross-year data in format, and report writing structure, so that future student teams can take over project work from us more smoothly. Meanwhile, we tried to summarize the differences in cross-year data and created a log for it, recording things such as how research questions and sampling methods have evolved across years, so that not only our team, but also future researchers, can develop independent thinking and research ideas with ease based on these stories behind the scenes. Since this project is still considered in its early developing stage and faces annual change of its primary research team, we argued that this effort is particularly worthy in order to provide enough supplement information for the future researchers who may use the monitoring data for their own cross-year data analysis.

WATER QUALITY MONITORING

INTRODUCTION

Managers designed SNWR to restore wildlife habitats for waterbirds and other wildlife. In order to measure the success of these efforts, a water quality monitoring program was created to quantify the hydrologic and chemical processes in the refuge and how these change with restoration. We employed and refined a water quality protocol designed for SNWR to determine how water quality parameters are responding to restoration efforts.

Water quality data help us understand the overall health of wetland ecosystems and habitat conditions for various flora and fauna (Weaver & Fuller 2007), and provides insights into how biotic communities respond to abiotic factors. In 2021 we measured temperature (ºC), pH, conductivity (µS/cm), turbidity (FNU), and dissolved oxygen (DO) (mg/L), consistent with water quality protocols used in 2019 and 2020 (Lugten et.al. 2020; Dellick et al. 2021). To support cross-year comparisons, we replicated many of the research objectives and sampling units from 2019 and 2020. However, in 2021 we had the opportunity to increase sampling to additional units and adjoining rivers, and months. In doing so, we encountered a greater variety of vegetation zones than previous years.

Phosphorus and nitrogen are both limiting nutrients for vegetation and are common fertilizers in agricultural settings, and excess nutrient runoff is a source of pollution in aquatic habitats. Wetlands have the potential to retain excessive nutrients, but quantifying nutrient concentrations at large capacities is difficult because analyses are costly (Baustian et al. 2018). However, Baustian et al. (2018) found that it was possible to estimate TP by using proxy turbidity data at a broader temporal and spatial scale. We look to build upon this research and create a similar TP-turbidity curve for SNWR to inform managers about nutrient retention in monitored units. In 2021 we measured total phosphorus (TP) (mg/L) and total Kjeldahl nitrogen (TKN) (mg/L), consistent with the water nutrient sampling method used in 2020 (Dellick et al. 2021).

We developed the following research objectives to guide our water quality and nutrient sampling and data analysis for the 2021 monitoring season:

RESEARCH OBJECTIVES

- *Characterize water quality parameters among monitored locations, months, and vegetation zones.*
- *Determine correlations between different water quality parameters.*
- *Determine whether there are significant differences in water quality parameters across months, monitored locations, and vegetation zones.*
- *Determine how high turbidity events affect phosphorus and nitrogen levels in the wetland units, control structures, and surrounding rivers, and whether we can make reliable predictions for nutrient concentrations based on turbidity data.*
- *Determine how nutrient levels in each monitored location change through years 2019-2021.*

METHODS

Multi-Parameter Sonde Sampling

As Lugten et al. (2020) found that distance from water control structures did not significantly affect water quality, we followed Delick et al. (2021) in using a random sampling method for collecting water quality data, and deployed it in conjunction with fish and macroinvertebrate sampling to analyze the relationships of these biotic communities and abiotic factors, as well as to maximize the efficiency of data collection (Dellick et al. 2021). To collect daytime water quality data in the management units and river channels, we used a YSI EXO 3 handheld multiparameter sonde to collect in situ measurements of temperature (ºC), dissolved oxygen (DO) (mg/L), turbidity (total dissolved/suspended solids (mg/L)), pH (std units), and conductance (µS/cm). So as to minimize sediment disturbance, a single researcher took these parameters in triplicate halfway down the water column before anyone else entered the area. Using the depth stick, we then measured water depth in the same location. We also identified sampling unit, date, coordinates, and vegetation zones. These data were input into ArcGIS Survey 123 then automatically uploaded to ArcGIS cloud storage. The water quality measurements were taken in association with each nutrient, macroinvertebrate and fish sample site in the monitored units and river channels.

Nutrient Sampling

We followed methods from Dellick et al. (2021) in collecting samples for TP and TKN monthly from P1A, MS, MN, and MC, but we added sampling sites within SPD and SHR and at individual water control structures. Sampling efforts in April, October, and November were also added. We did not sample in September because our intention was to capture seasonal changes for summer and fall, and we felt that, as September is a transition month, it would not add much new information. To measure the relationship between TP and turbidity, we attempted to collect more nutrient samples during high turbidity events, specifically during or after storms, floods, and seiche events (Baustian et al. 2018).

DATA ANALYSES

During summer 2021 we gathered sonde data on 5 water quality parameters from 302 different sites and conducted analysis on the mean of triplicate measurements from each site using the R Studio. We referred to R code from the Dellick et al. (2021) UM-Shiawassee Master's Project team and modified it to carry out our analyses. We investigated the effect of month, monitored location, and vegetation zone on the variation in water quality using ANOVAs and Tukey HSD test to detect where differences were, and visually categorized these differences using boxplots. We also tested the assumptions of normality and equal variance for ANOVA. We investigated correlation relationships between different water quality metrics using the Dellick et al (2021) data. We compared 2019-2021 water quality data by using monthly average values. The sampling frequency and timing was basically consistent among 2019, 2020 and 2021 (except during COVID), though the specific day of the year when we sampled for water quality ,or a specific taxa, may not have been the same (Table 2 and Table 3).

We created a turbidity-nitrogen regression model and a turbidity-phosphorous regression model using nutrient sample data from 2019-2021 to assess whether SNWR managers can use turbidity as a proxy for TP and TKN (Baustian et al. 2018). In our analysis, we identified 2 data points which have low turbidity value but extremely high TP value from the turbidity-phosphorous regression plot and removed their relevant data rows from the data frame for all nutrient analysis. We strived to minimize removal of such outstanding data points because all data points contain information, still the removal of these data helped improve fit in regression plots. We made regression curves using Excel to visually present the relationship between nutrients and turbidity.

We calculated the annual average nutrient data for each monitored location from 2019 to 2021, and visualized these using Excel line graphs, in order to examine temporal patterns in nutrient levels.

Table 2. Location and time for water quality sampling from 2019 to 2021. Highlighted cells indicate presence of sampling effort. Number of unique sampling observations in each unit and month were entered into relevant cells from the year 2021. Abbreviations refer to wetland units and adjacent waterbodies: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), Shiawassee River (SHR), and Spaulding Drain (SPD).

	Unit						Month								Total sample size
	SHR	SPD	P1A	MN	MS	MC	4	5	6	7	8	9	10	11	
2019															
2020															
2021	37	4	66	66	60	69	1	46	88	64	55		24	24	302

Table 3. Water quality samples by vegetation zone and year. Green highlights indicate a vegetation zone where water quality was sampled. Number of samples within each vegetation zone is available only for 2021 and is indicated by the number in the corresponding cell. Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: open water, no vegetation in relatively deep water; submerged aquatic vegetation (SAV); mixed emergent vegetation (MEV),; channel, open flowing water with no vegetation; dead *Typha*, dead *Typha* spp; *Nymphaea*, *Nymphaea odorata*, RB, *Schoenoplectus tabernaemontani*; Smartweed, *Persicaria amphibia*; forest, *Acer saccharum*; Cottonwood, *Populus deltoides*; *Salix*, *Salix nigra* and/or *Salix exigua*; Shore, denoting any vegetation located on shore adjacent to water; and *Phalaris*, *Phalaris arundinacea.*

	Vegetation													
	open water	Channel	SAV	Dead *Typha*	*Typha*	MEV	*Nymphaea*	RB	Smartweed	Forest	Cottonwood	*Salix*	Shore	*Phalaris*
2019														
2020														
2021			129	1	61	12	16	13	2	22	3	3	12	4

RESULTS

ANOVA summary

There was no significant difference detected in water temperature across wetland units by applying ANOVA, which was also the case for 2019 and 2020 (Lugten et al. 2020; Dellick et al. 2021). We did observe significant differences between water quality metrics across months, vegetation zones, and wetland units, with the only exception of insignificant water temperature variation by units (Table 4).

Table 4. One-way ANOVA result table. Each wetland water quality parameter is dependent variable; Month (April-Nov), Vegetation Zone (Submerged Aquatic Vegetation, *Typha*, *Nymphaea*, shore, forest, *Phalaris*, smartweed, river bulrush, Mixed Emergent Vegetation, cottonwood, *Salix*), and Unit (MC, MN, MS, P1A, SHR, SPD) are independent variables. Significance code: 0 " *** ", 0.01 " ** ", 0.01 " * ", 0.05 " . " P-value less than 0.05 was highlighted in bold. F-value in an ANOVA was calculated as “variation between sample means / variation within the samples”, numbers in parenthesis are “between groups df”, “within groups df”, respectively.

Water Quality Parameter	Variation by Month		Variation by Vegetation Zone		Variation by Unit	
	P-value	F value	P-value	F value	P-value	F value
Temperature (C)	**<2e-16 *** **	F(6, 295) = 378.9	**7.72e-05 *** **	F(11, 266) = 3.652	0.351	F(5, 296) = 1.118
DO (mg/L)	**3.04e-06 *** **	F(6, 295) = 6.296	**7.11e-09 *** **	F(11, 266) = 6.077	**6e-06 *** **	F(5, 296) = 6.719
pH	**5.59e-05 *** **	F(6, 295) = 5.079	**1.45e-11 *** **	F(11, 266) = 7.713	**3.34e-13 *** **	F(5, 296) = 15.09
Conductivity (uS/cm)	**<2e-16 *** **	F(6, 295) = 49.37	**1.59e-08 *** **	F(11, 266) = 5.867	**2.75e-12 *** **	F(5, 296) = 13.99
Turbidity (FNU)	**0.000493 *** **	F(6, 293) = 4.163	**0.000292 *** **	F(11, 264) = 3.299	**0.0344 * **	F(5, 294) = 2.443

Variation by month

We did not find a difference in any water quality metrics between the summer months, but when we include spring or fall data, the seasonal pattern at SNWR immediately becomes apparent (Figure 3). Since we only had one sample effort in April, which was on the last day of the month, April data were retained in the boxplot mainly as supplementary information. We found water temperature was mostly high during May through August, then quickly dropped from October to November (Figure 3a), so these two months had significantly lower water temperatures than the others (Appendix II). Water temperature in May and June was sometimes lower than 20 °C, while in July and August it ranged from 20 °C to 25 °C for most of the time; in October and November, water temperatures fell to 18 °C and 7 °C respectively (Figure 3a). We found a significant decrease in DO from June onwards, followed by a significant increase in November, reaching a level similar to or even higher than the spring months; the variation in DO could be huge from May to August, ranging from 1 mg/L to 17 mg/L (Figure 3b). Conductivity constantly dropped from May to November (Figure 3c). Tukey HSD test showed that conductivity in May was significantly higher than June, then remained non-significantly changed from July to October, followed by a significant decrease in November (Appendix II). The general changing trend of pH was very similar to DO, higher from May to June, then lower from June to October, then higher again in November. This correlation between DO and pH was also demonstrated in the correlation analysis (Table 5). PH varied widely within the individual months of June, July and August, ranging from around 7.25 to 10 (Figure 3d). Tukey HSD test showed that pH was significantly lower in October than in June and July (Appendix II). For turbidity, it was lower than 20 FNU for the majority of our sampling time across May to November; most of our outstandingly high turbidity data were collected in June and November (Figure 3e). July was significantly lower than May, June, and November in turbidity (Appendix II).

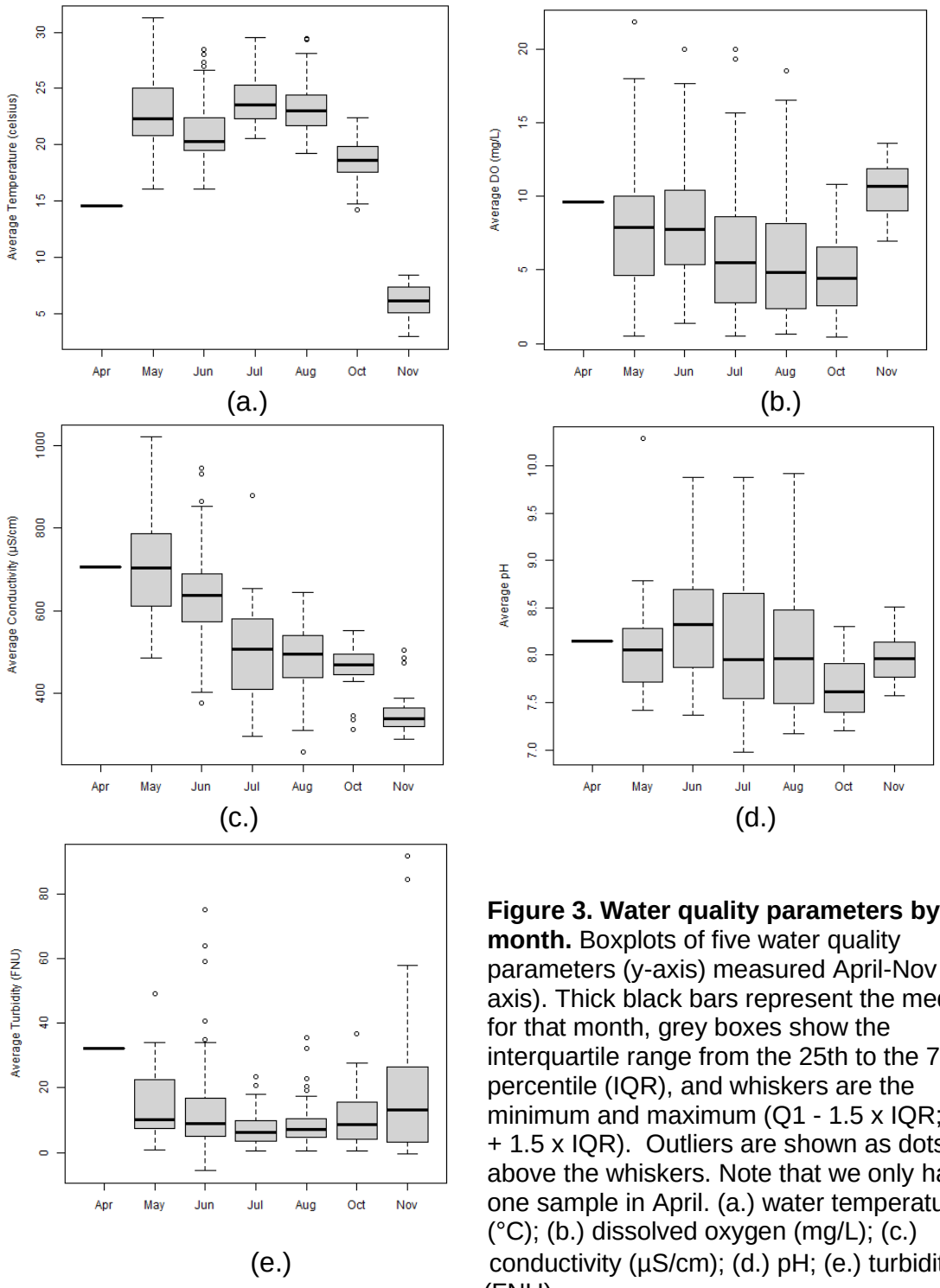
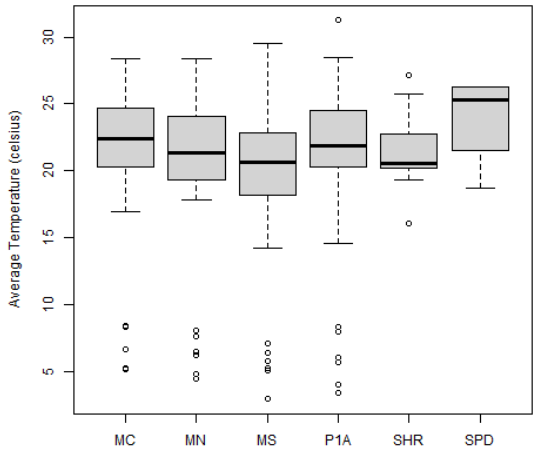


Figure 3. Water quality parameters by month. Boxplots of five water quality parameters (y-axis) measured April-Nov (x-axis). Thick black bars represent the median for that month, grey boxes show the interquartile range from the 25th to the 75th percentile (IQR), and whiskers are the minimum and maximum (Q1 - 1.5 x IQR; Q3 + 1.5 x IQR). Outliers are shown as dots above the whiskers. Note that we only had one sample in April. (a.) water temperature (°C); (b.) dissolved oxygen (mg/L); (c.) conductivity (µS/cm); (d.) pH; (e.) turbidity (FNU)

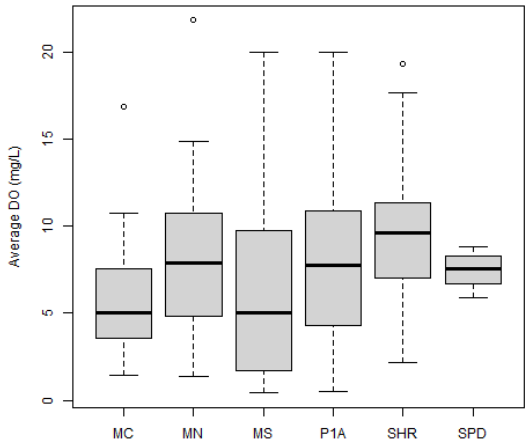
.

Variation by monitored locations

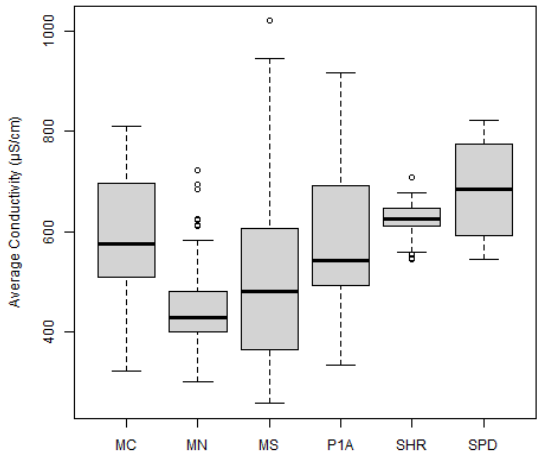
Turbidity and temperature did not change much across monitored wetland units and river sites; while DO, conductivity and pH were relatively more different across units and sites (Figure 4). Water temperature was similar across wetland units and river sites (Figure 4a.), which was also confirmed by ANOVA and Tukey HSD tests (Table 4 and Appendix III). MS had lower DO levels than other units, and MC had the second lowest DO values, although not significantly lower than MN and SHR (Figure 4b.); the corresponding Tukey HSD test showed that MN and MS had significantly lower conductivity than most other units (Appendix III); MC had lower pH than most other units, while MN was the only wetland unit that had no significantly different pH value between SPD and SHR; though there was significant difference in turbidity across units, the magnitude of variation was neglectable. When compared to MN and MC, we found a relatively large variation in temperature, DO and conductivity in MS and P1A (Figure 4a.b.c). MN had the largest variation in pH among all the sampling units, followed by P1A and MS, while MC had the smallest pH variation among all the wetland pool units (Figure 4d). Although the difference between minimum and maximum turbidity data in each wetland pool unit was very close, P1A had significantly more high turbidity outlier data points than the others (Figure 4e.).



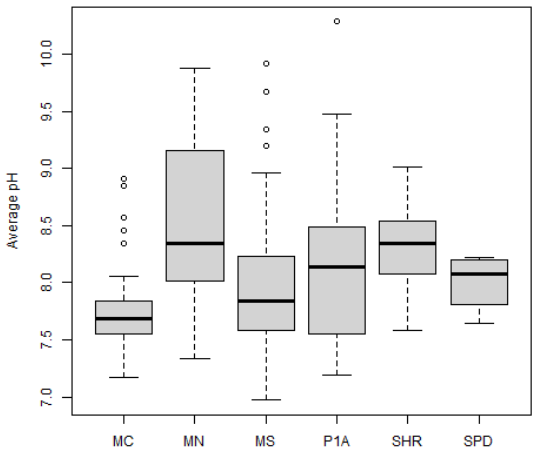
(a)



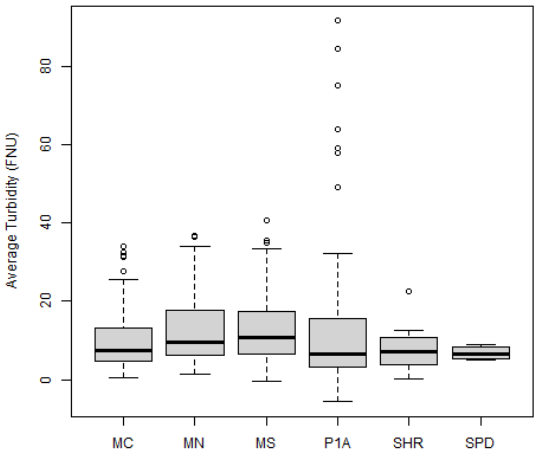
(b)



(c)



(d)



(e)

Figure 4. Water quality parameter by monitored locations. Boxplots of five water quality parameters (y-axis). Abbreviations refer to wetland units and river locations: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), Shiawassee River (SHR), and Spaulding Drain (SPD). Thick black bars represent the median values, grey boxes show the interquartile range from the 25th to the 75th percentile (IQR), and whiskers are the minimum and maximum (Q1 - 1.5 x IQR; Q3 + 1.5 x IQR). Outliers are shown as dots above the whiskers. (a.) water temperature (°C); (b.) dissolved oxygen (mg/L); (c.) conductivity (µS/cm); (d.) pH; (e.) turbidity (FNU).

Variation by Vegetation Zone

Water quality varied by vegetation zones in the 2021 sampling season. Water temperature in the forest zone was significantly lower than *Nymphaea*, shore, river bulrush, and *Phalaris* (Figure 5a, Appendix IV.1). We also found DO in the forest zone was significantly lower than SAV, *Nymphaea, Typha*, shore and *Phalaris*; DO in shore zone was significantly higher than river bulrush and mixed emergent vegetation; and SAV was significantly higher than river bulrush (Figure 5b, Appendix IV.2). We found conductivity in the SAV zone was significantly lower than *Nymphaea*, river bulrush, and forest zones; forest was significantly higher than *Typha* and shore zones (Figure 5c, Appendix IV.3). We found that the pH in the SAV zone was significantly higher than river bulrush, mixed emergent vegetation, and forest zones; forest was significantly lower than *Nymphaea*, *Typha*, and shore zones(Figure 5d, Appendix IV.4). We found that turbidity in the shore zone was significantly higher than SAV, *Typha*, river bulrush, and forest zones (Figure 5e, Appendix IV.5). Regarding the magnitude of data variation within each single unit, if only considering the difference between the first and third quartile data: SAV, *Typha* and shore had relatively large variation in DO, pH and turbidity (Figure 5b.d.e.); while shore also had relatively large variation in temperature (Figure 5a).

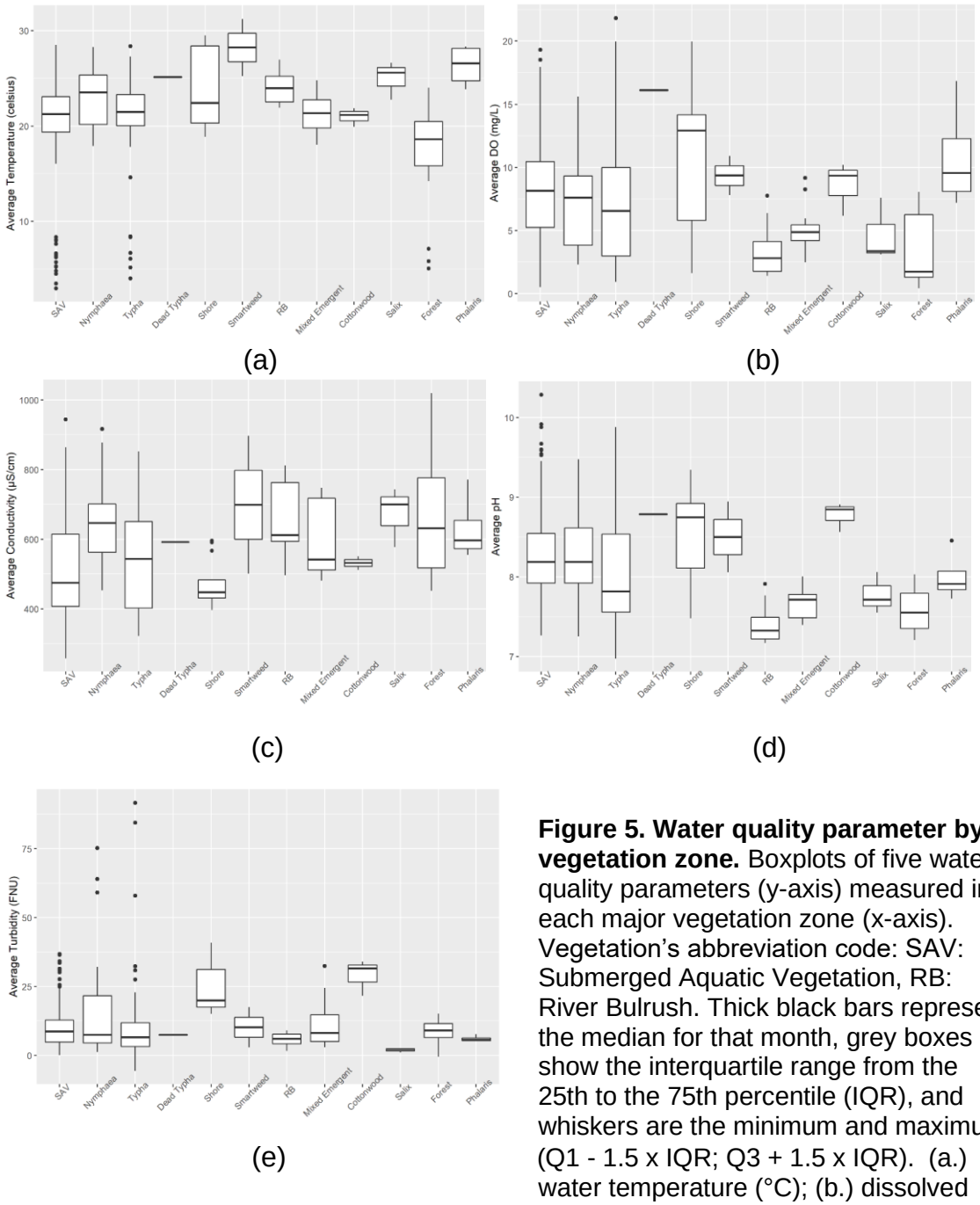


Figure 5. Water quality parameter by vegetation zone. Boxplots of five water quality parameters (y-axis) measured in each major vegetation zone (x-axis). Vegetation's abbreviation code: SAV: Submerged Aquatic Vegetation, RB: River Bulrush. Thick black bars represent the median for that month, grey boxes show the interquartile range from the 25th to the 75th percentile (IQR), and whiskers are the minimum and maximum (Q1 - 1.5 x IQR; Q3 + 1.5 x IQR). (a.) water temperature (°C); (b.) dissolved oxygen (mg/L); (c.) conductivity (µS/cm); (d.) pH; (e.) turbidity (FNU).

Correlation Analysis

Using a Pearson correlation analysis, we identified significant correlations between nine pairs of variables. We observed a strong correlation between pH and DO (r= 0.71, p<0.000), a moderate correlation between conductivity and temperature (r= 0.4, p<0.000), and weak correlations between conductivity and pH (r= -0.25, p<0.000), and turbidity and temperature (r= -0.24, p<0.000) (Table 5, Figure 6). Though we did find significant correlation between pH and temperature, as well as water depth and temperature, DO, pH and turbidity, their correlation coefficients were too small (<0.2) to warrant consideration (Table 5, Figure 6).

Table 5. Pearson correlation coefficient table for averaged water quality parameters (N=276). Abbreviations refer to water quality parameters: average temperature (avg.Temp), average pH (avg.pH), average dissolved oxygen (avg.DO), average conductivity (avg.Cond), average turbidity (avg.Turb), and average depth (avg.Depth). Significance codes refer to statistical significance of a correlation between two parameters: 0 " *** ", 0.001 " ** ", 0.01 " * ", 0.05 " . " Statistically significant values are bolded (P < 0.05). Diagonal values represent a variable's correlation with itself, which is always 1; zero represents no correlation. Only half the table is reported, as the bottom half is the mirror image of the top.

	avg.Temp	avg.DO	avg.pH	avg.Cond	avg.Turb	avg.Depth
avg.Temp	1	-0.04	**0.16 ***	**0.4 *****	**-0.24 *****	**-0.12 .**
avg.DO		1	**0.71 *****	-0.11	0.1	**-0.14 .**
avg.pH			1	**-0.25 *****	0	**-0.17 ****
avg.Cond				1	-0.03	-0.08
avg.Turb					1	**-0.12 .**
avg.Depth						1

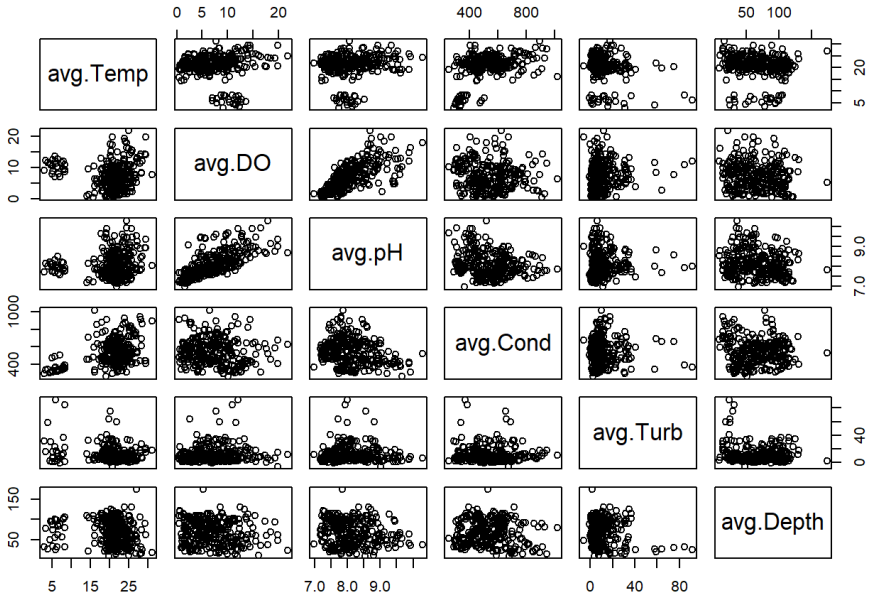


Figure 6. Scatter plot correlation matrix for five average water quality parameters. For all samples, N=276; left and right y-axes show units for the parameter labeled in the to right or left, respectively; top and bottom x-axes show units for the parameter labeled below or above, respectively. Linear, increasing scatter plots indicate a strong positive correlation. Abbreviations refer to water quality parameters: average temperature (avg.Temp), average dissolved oxygen (avg.DO), average conductivity (avg.Cond), average turbidity (avg.Turb), and average depth (avg.Depth).

Cross Comparison of 2019-2021 data

In 2021 we obtained data from November for the first time, and it seems the overall seasonal pattern in average water quality parameters was consistent from 2019 to 2021. In 2021 we observed a peak in average water temperature of around 25°C in June, followed by a drop to the lowest observed average water temperature of around 7°C by November (Figure 7). Although data were not available for every month across all years, the general seasonal variation pattern in average temperature showed a late spring or early summer peak followed by a decline. Growing season temperature patterns varied among years with an earlier increase and later decrease in 2021, compared to other years- this resulted in an extended summer season. By contrast, 2020 had a much shorter summer season but more extreme summer temperatures. DO was less consistent across years. In 2021, we found that average DO declined steadily from April to October, and increased dramatically in November resulting in a higher measurement compared to the April results, a 5.5 mg/L increase (Figure 8); this change was likely due to weather conditions. In 2019 and 2020, conductivity remained relatively constant, but in 2021 it dropped slowly and monotonically from April to November (Figure 9). However, in any month where all three years were sampled, conductivity in 2021 exceeded previous years' measurements by around 200 µS/cm. Where pH data were available across all years, we found negligible change in average monthly pH before June, then a steady decline from June to October; our additional pH data from November 2021 showed a fall increase similar to that seen for average DO (Figure 10). Finally, we observed a sharp drop in spring turbidity, followed by negligible fluctuations from June to October (Figure 11).

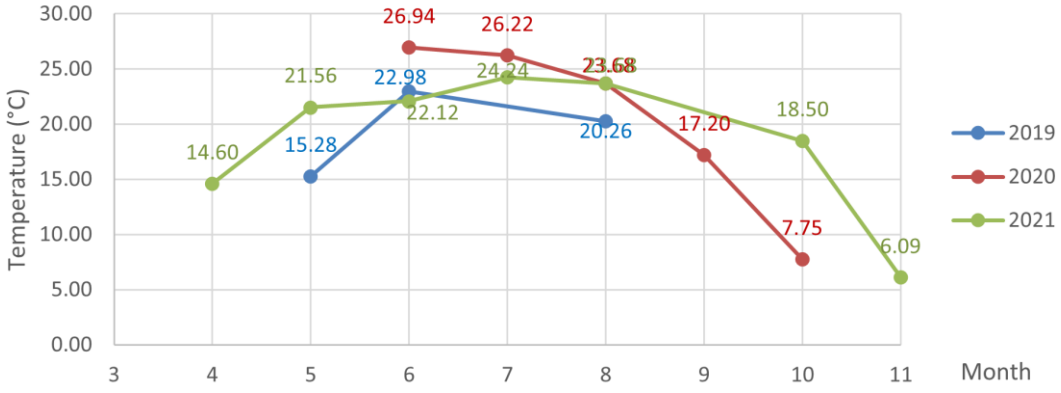


Figure 7. Average water temperature for sampling months 2019-2021. Calendar months (3-11, Mar-Nov) are numbered on the x-axis, and temperature (°C) on the y-axis. Monthly average is labeled above the corresponding month. Years are identified by color: blue (2019), red (2020), and green (2021). Data is only available for three months in 2019, and five months in 2020.

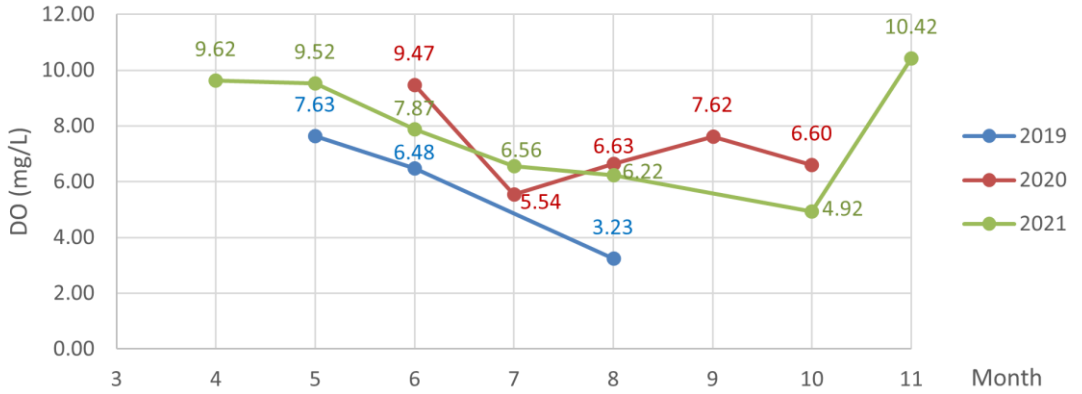


Figure 8. Dissolved oxygen for sampling months 2019-2021. Calendar months (3-11, Mar-Nov) are numbered on the x-axis, and dissolved oxygen (DO, mg/L) on the y-axis. Monthly average is labeled above the corresponding month. Years are identified by color: blue (2019), red (2020), and green (2021). Data is only available for three months in 2019, and five months in 2020. Note that dissolved oxygen increases with declining temperature, so the November value shows a natural increase.

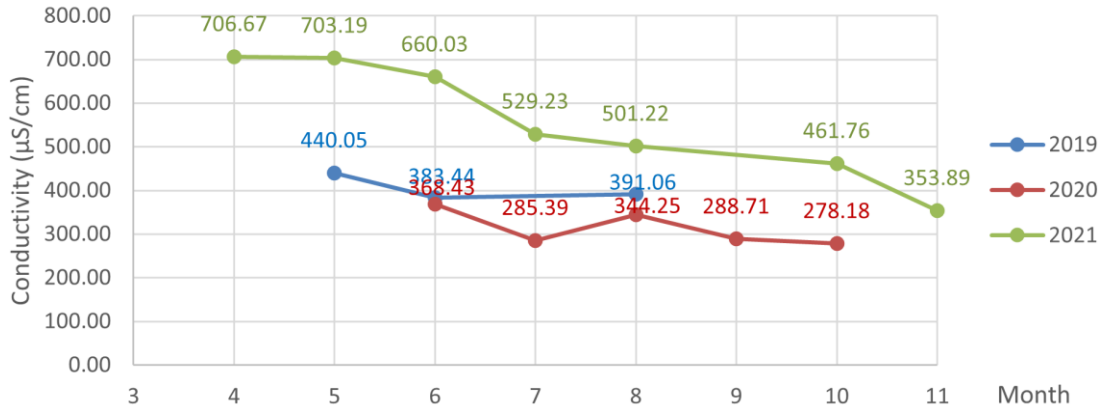


Figure 9. Average conductivity for sampling months 2019-2021. Calendar months (3-11, Mar-Nov) are numbered on the x-axis, and conductivity (µS/cm) on the y-axis. Monthly average is labeled above the corresponding month. Years are identified by color: blue (2019), red (2020), and green (2021). Data is only available for three months in 2019, and five months in 2020. Note that conductivity monotonically declines in 2021.

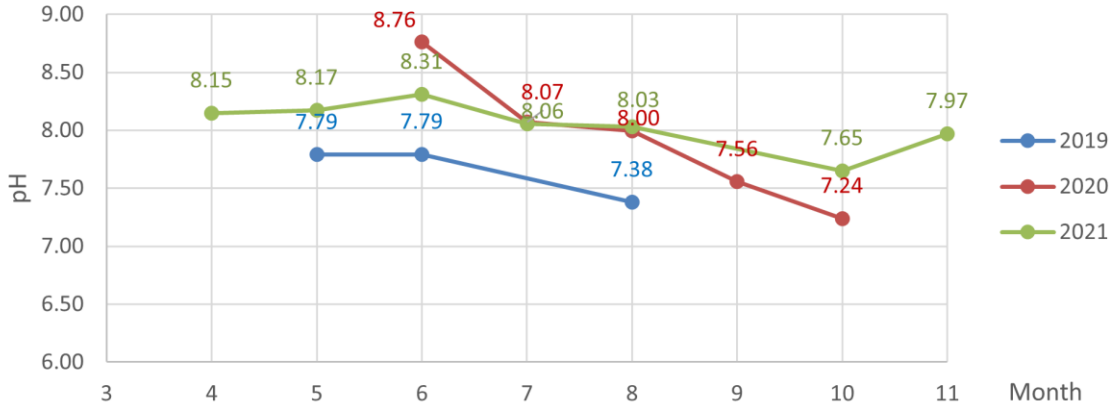


Figure 10. Average pH for all sampling months 2019-2021. Calendar months (April-Nov) are numbered on the x-axis, and pH on the y-axis. Monthly average is labeled above the corresponding month. Years are identified by color: blue (2019), red (2020), and green (2021). Data is only available for three months in 2019, and five months in 2020.

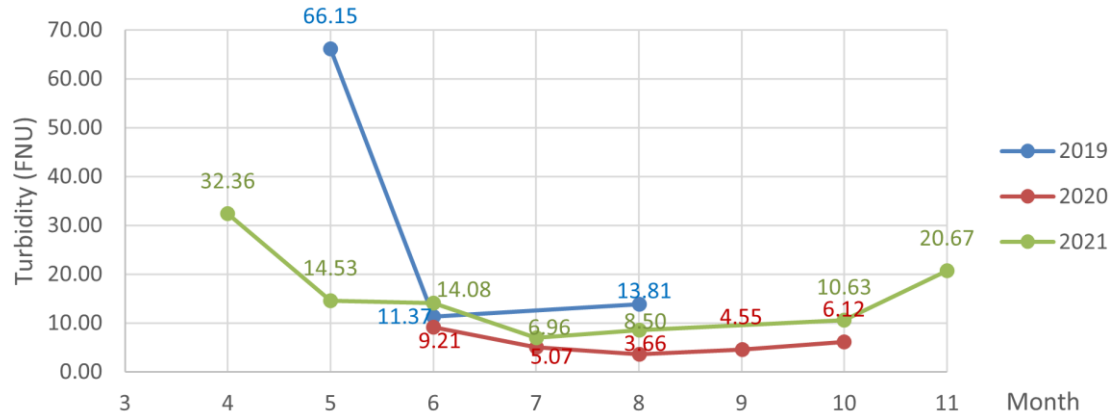


Figure 11. Average turbidity for all sampling months, 2019-2021. Calendar months (3-11, Mar-Nov) are numbered on the x-axis, and turbidity (Formazin Nephelometric Unit, FNU) on the y-axis. Monthly average is labeled above the corresponding month. Years are identified by color: blue (2019), red (2020), and green (2021). Data is only available for three months in 2019, and five months in 2020. Note that conductivity monotonically declines in 2021.

Linear Regression between Turbidity and Nutrients

The regression model cannot make predictions of TP and TKN concentration based on turbidity, with all four models having significance at 0.05 level, but show fairly low R-squared values (Table 6). However, R-squared and adjusted R-squared both nearly doubled after two distinctively high TP data points were removed (turbidity = 15.29 FNU, TP = 0.5313 mg/L, TKN = 1.222 mg/L; turbidity = 7.27, TP = 0.6127 mg/L, TKN = 2.795 mg/L) (Table 6, Figure 12, Figure 13). Additionally, assumptions of "Normality of residuals" and "Homogeneity of residuals variance" were violated in these models.

Table 6. Turbidity as a proxy for nutrient flux during storm events, 2019-2021. Four regression model summaries (linear model, lm; ordinary least squares, OLS): turbidity (Turb) as an explanatory variable for total phosphorus (TP), with and without outliers, and total Kjeldahl nitrogen (TKN), with and without outliers nutrient and turbidity data from 2019 to 2021 and its corresponding subset data without outstanding outliers. Significance code: 0 " *** ", 0.001 " ** ", 0.01 " * ", 0.05 " . "

Model	Data in Use	Model significance	R^2		Beta coefficient	
		(P value)	R^2	Adjusted R^2	Intercept	Turbidity
lm (OLS) TP & Turb	original data	0.01221*	0.09011	0.07653	0.1300255***	0.0020599*
	subset data without outlier	0.0005054***	0.171	0.1582	0.1147999***	0.0022409***
lm (OLS) TKN & Turb	original data	0.01458*	0.1094	0.09225	1.007352***	0.008622*
	subset data without outlier	0.001518**	0.1838	0.1675	0.95614***	0.00963**

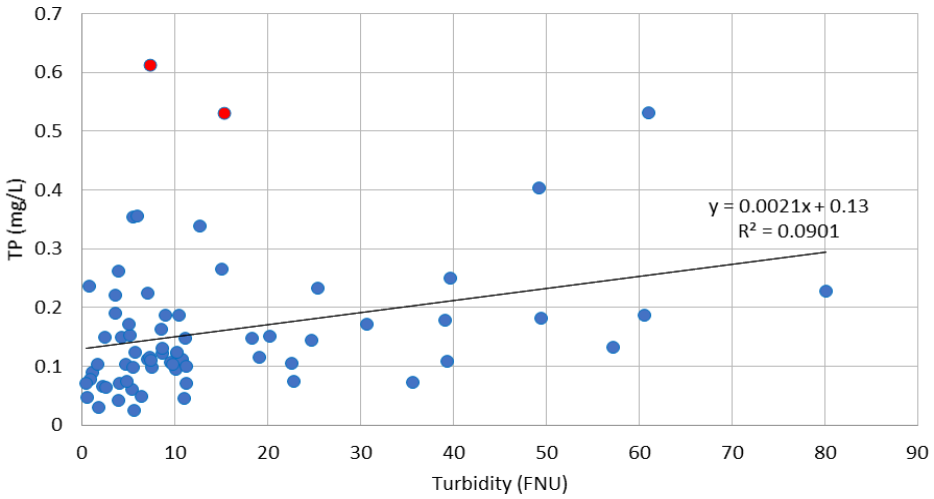


Figure 12. Turbidity as a proxy for total phosphorus, 2019-2021. A linear regression model of Turbidity (Formazin Nephelometric Unit, FNU, x-axis) as an explanatory variable of total phosphorus (TP, mg/L, y-axis), using all available data, including outliers shown as red points. Linear equation and R^2 (effect size = 90.1%) shown above. Sampling locations (not labeled) include all four wetland units, Shiawassee river, and Spaulding drain (MC, MN, MS, P1A, SHR, SPD) measured May-Aug, 2019-2021.

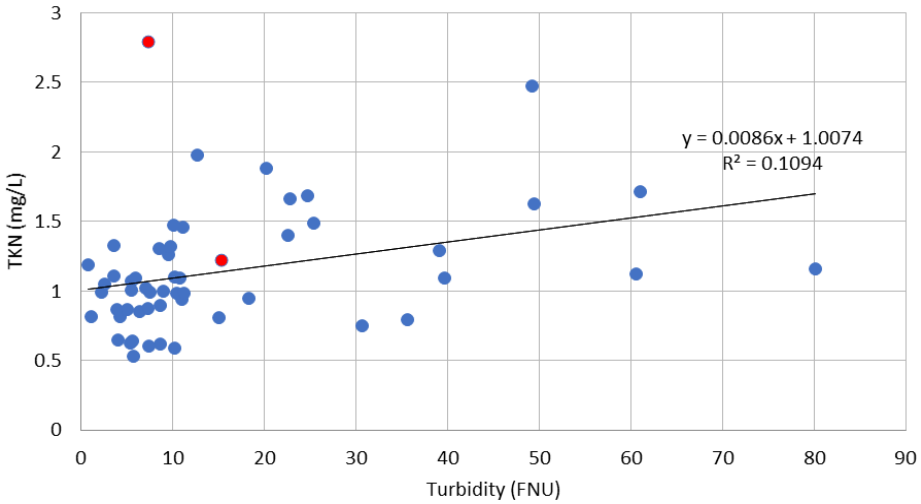


Figure 13. Turbidity as a proxy for total Kjeldahl nitrogen, 2019-2021. A linear regression model of Turbidity (Formazin Nephelometric Unit, FNU, x-axis) as an explanatory variable of total Kjeldahl nitrogen (TP, mg/L, y-axis), using all available data, including outliers shown as red points. Linear equation and R^2 (effect size = 90.1%) shown above. Sampling locations (not labeled) include all four wetland units, Shiawassee river, and Spaulding drain (MC, MN, MS, P1A, SHR, SPD) measured May-Aug, 2019-2021.

Temporal change of nutrients in different monitored locations

TP followed the same pattern of change across years in the majority of sampling locations, while TKN was less consistent. TP dropped in all monitored locations between 2019 and 2020, followed by an increase between 2020 and 2021 with the only exception of MS; the range of TP variation between 2019 and 2021 was 0.072 ~ 0.350 mg/L (Figure 14). TKN increased in MN, MS and SPD between 2019 and 2020, and decreased in these locations between 2020 and 2021; TKN decreased P1A and SHR between 2019 and 2020, and increased in these locations between 2020 and 2021; the range of TKN variation between 2019 and 2021 was 0.704 ~ 1.948 mg/L (Figure 15).

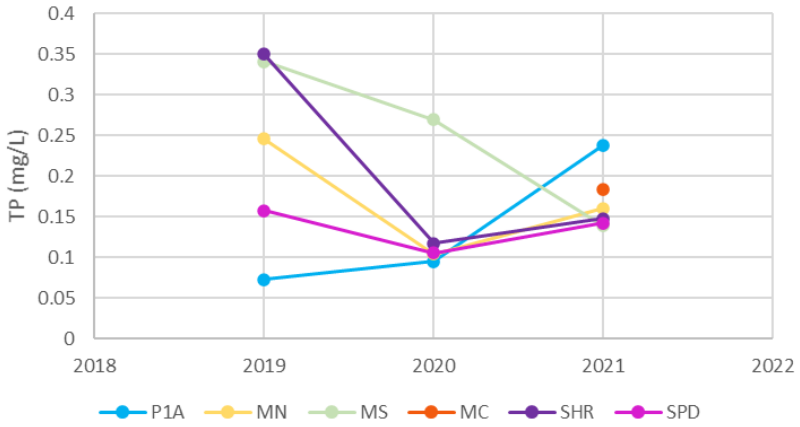


Figure 14. Average annual total phosphorus for all sampling locations, 2019-2021. Variation in total phosphorus (TP, mg/L) in each wetland unit and river location for years 2019-2021. Note that MC only has one sampling year (2021), so it is represented as a point. Unlike all other sampling locations, MS monotonically decreases.

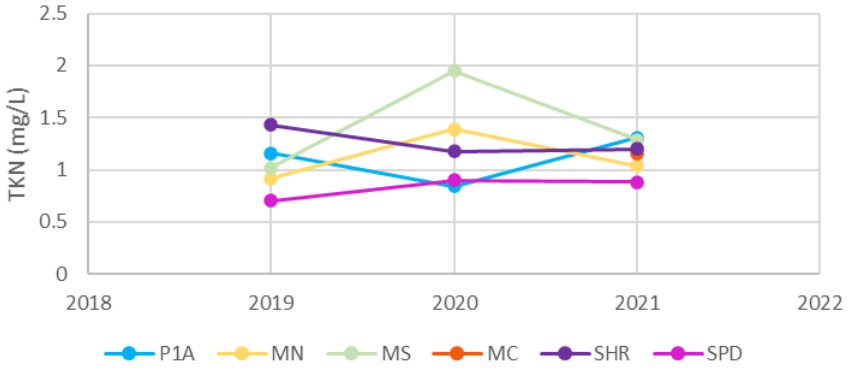


Figure 15. Average annual total Kjeldahl nitrogen for all sampling locations, 2019-2021. Variation in total phosphorus (TP, mg/L) in each wetland unit and river location for years 2019-2021. Note that MC only has one sampling year (2021), so it is represented as a point, obscured by MC.

DISCUSSION

What does this water condition mean to aquatic lives?

Our results indicated that SNWR wetland units were harsh environments for many aquatic organisms during summer months, June to August, but there were also potential refugia within wetland units to support the survival of some species. Daytime temperature during June and August remained as high as 23 ºC, with DO less than 5 mg/L for nearly half of the time; most fish species are distressed in such conditions, except for some very tolerant species such as sunfishes carp, and catfish (Edwards and Twomey 1982; Stuber et al. 1982; Volkoff and Rønnestad 2020; Sikora et al. 2022). Variation among wetland units did not significantly affect summer water temperatures, showing there were likely few temperature refugia in our wetlands for non-heat-tolerant species, at least for those locations where we sampled water quality. By contrast, DO values varied by wetland units, reflecting heterogeneity in the local environment. The low DO values in summer can be explained by either high temperature, stagnant water, or the coverage of plant species. Some plant species such as duckweed (*Lemna spp.*), hybrid and narrow-leaved cattail (*Typha × glauca* and *Typha angustifolia*) may spread across the water surface and reduce the contact area between water and air, eventually reducing the local DO level (Schrank and Lishawa 2019; Gomez et al. 2021). The relatively high DO values more frequently occurred in units MC and P1A rather than in MS and MN; and in SAV and shore vegetation zones rather than forest and river bulrush zones. Such heterogeneity can support survival of aquatic organisms in two potential ways, first is simply to provide the essential oxygen, second is to create shelter for some highly hypoxia adaptive species from predation by the less hypoxia-tolerant predators (Domenici et al. 2007). Theoretically, aquatic organisms can immigrate and emigrate freely among sub-habitats within these wetland units seeking their own habitable location (Gomez et al. 2021), but hypoxia dead zones may sometimes block emigration, thus trapping and killing the organisms that can’t escape. We observed fish kill phenomenon in P1A and hypothesized its reason as such a “hypoxia trap”. Conductivity, pH, and turbidity did not severely influence the survival of species. Our sampled conductivity was mostly below 800 µS/cm and pH was neither below 7 nor higher than 9 for the majority of our sampling efforts; these findings were within the normal ranges for the survival of most aquatic species (U.S. EPA 2022).

Some observed water quality phenomena were not expected, and we hypothesized as to the cause of these events. First, DO was most strongly correlated with pH (r=0.71) rather than temperature (r=0.16). DO is not affected by pH in the sense of a physical-chemical reaction, but can be indirectly associated with it. High temperatures in summer can increase solid solubility in water, while reducing oxygen solubility. Meanwhile, nutrients may more easily be

released into the water and speed up the process of decomposition of decaying organic materials, which further reduces DO in water (USGS 2018). This process may either release or absorb chemicals, which contributes to pH reduction, eventually causing a much stronger correlation relationship between pH and DO rather than temperature and DO. Second, we noticed that the conductivity was distinctively higher than the previous two years in every sampling month and with an approximately fixed difference. A possible reason for this is that we connected the wetland units with the rivers this year for a longer period of time, so that they received more ions from the catchment and runoff nearby. Conductivity clearly dropped from April to November, indicating the ions were constantly removed from water solution. These may be absorbed by vegetation or by soil particles and settle down to the substrate (Matagi et al. 1998).

Why were we unable to predict nutrients using turbidity?

We still cannot construct a significant regression model to predict water nutrients by using turbidity in our SNWR wetland units, as Baustian et al. (2018) did for Pool 2B adjacent to Lake Erie. Three specific reasons for this are the narrow range of variation in our turbidity data, the relatively weak seiche event in SNWR, and the low level of connectivity between SNWR wetland units and the rivers nearby. First and the most direct reason is that we did not collect much high turbidity data during our sampling season, simply because the turbidity was mostly low. Even if there is a strong correlation between turbidity and nutrients, our data that contained mostly low turbidity values may not be able to support a strong model. Across the sampling season in 2021, turbidity in SNWR was less than 40 FNU for both river and wetland units, and even the highest turbidity we sampled during the seiche event in November did not exceed 90 FNU. By contrast, seiche events in Pool2B usually lead to turbidity ranging from around 100 FNU to more than 1000 FNU (Baustian et al. 2018), which provided a much larger turbidity span for exploring the statistical relationship between turbidity and nutrients.

Such differences in turbidity between SNWR wetland units and Pool2B indicated that turbidity in these two wetland systems may respond to the same process (e.g., seiches) at very different magnitudes. One reason is probably due to the inequivalent volume of water adjacent to these two wetland systems. Pool2B sits right next to Lake Erie (only 1.5 km linear distance apart), so seiche events in Lake Erie can easily push large amounts of water into Pool2B and cause high turbidity. By contrast, SNWR wetland units sit in a flood plain right next to Shiawassee River which floods periodically (Heitmeyer, et al. 2013). Therefore, SNWR wetland units are more influenced by river dynamics than by Lake Huron which is around 33.5 km away in linear distance. The kinetic energy directly

brought by a large water body as a Great Lake is probably larger than by smaller water bodies such as a river, thus SNWR wetlands may experience a smaller velocity of influx flow and lower total suspended solids and turbidity than Pool2B.

Another reason for such differences in turbidity between our study units and Pool2B is their different proximity to the water source nearby. Most of our studied SNWR wetland units had less connectivity to their nearby rivers than Pool2B to its nearby lake, thus it was harder for our units to recruit sediments from the rivers and create high turbidity data. P1A was an interesting case; it was the only wetland unit that directly and continuously connected with the river and thus had accumulated a relatively thick layer of silt sediments since opening in 1959 (Heitmeyer et al. 2013, Figure 13B). Additionally, our top eight turbidity data points for 2021 sampling were all collected from P1A, which was not surprising since P1A was recognized as the most silty unit to walk through during our field work experience. Interpreting our turbidity data, field work experience, and a LiDAR topographic map of SNWR (Heitmeyer et al. 2013, Figure 13B), the second siltiest and highly turbid unit was probably MS, closely followed by MN, with MC considered as the least turbid unit. This ranking order matched well with their proximity to the river, since MS, MN and MC were all periodically connected to the river rather than continuously connected like P1A. All three Maankiki units (MS, MN, MC) were connected with the river by a “distribution basin” so they were considered to have a “secondary connection” with the river, and among these, MC even experienced a “tertiary connection” with the river, in which case the water had to flow from the west side through a 1000m long by 100m wide channel to reach the main body of MC, or traveled from the east side through P1A first to reach MC. Compared with MC, MS and MN can directly get river water flowing in through the distribution basin, therefore they more often had relatively higher turbidity than MC.

Assumptions and limitations

We assumed that our water quality and nutrient data can accurately represent the environment in their corresponding sampling months and units, which ignored the impact of individual, short-term events. For example, weather events such as rain and wind can increase DO and turbidity (US EPA 2016), which may bring in extreme outlier data and lead to inaccurate representation of the monthly value if we only conduct sampling on such days. This happened to our November data collection when we only sampled for three continuous days, where every single day was windy or rainy. In this case, we may have overlooked water quality on days that were calm, which could have falsely represented water quality for November. Some other factors can also influence the sampled water quality, but not to the extent that weather can. Since we conducted most of our water quality

sampling between 9 am and 3 pm, the impact of daytime water temperature variation was considered as neglectable. However, the real temperature situation in different layers of the water column may be more complex than we assumed (Jacobs et al. 2008). Our individual sampling efforts can also be influenced by very localized events. For example, turbidity value can increase due to sampler's movement or fish's movement, but these cases rarely or only occasionally happened, thus would not bring in considerable error to our data. Compared with water quality data, nutrient data were much more likely subject to an individual event's influence since we only had 4 to 6 sampling efforts in each month. Nutrient data were all conducted on the same day of that month, thus not able to represent the monthly nutrient regime very accurately. Therefore, we only did a preliminary comparison of the nutrient data across units and months, and we interpreted the result cautiously.

In some cases, sample size may limit our ability to interpret our data. We only collected 1 sample in the last day of April to represent April's environmental condition; 4 and 37 samples in SPD and SHR respectively, compared with around 65 samples in each of the wetland units; less than 5 samples for vegetation zones dead *Typha*, smartweed, cottonwood, *Salix*, and *Phalaris*. Interpretation involving these categories may not accurately reflect the true condition, thus should be treated cautiously. For the nutrient analysis, we have not yet gathered enough high turbidity data, which is one of the key reasons that our regression models were not significant. Therefore, there is still a chance that we can obtain a regression model for accurately predicting nutrient level in SNWR wetland units using turbidity data.

Implications for management and science

We suggest that future research focus more on the heterogeneity within each wetland unit and for future monitoring to conduct more sampling efforts in the spring season from the last frozen day to May. First, we already understand the average summer season conditions of each wetland unit through the three-year monitoring and have found large variation in DO within the units. As for the next step of our investigation, seeking out high DO refugia during summer may help us summarize the characteristics of such DO refuge spots and identify potential ones in our wetlands, eventually helping us better manage those less hypoxia tolerant species such as Largemouth Bass (French and Wahl 2018). Second, compared with the harsh summer season, spring season is much more favorable for many non-heat-tolerant fish species to live and spawn, thus we should conduct more frequent water quality monitoring in the spring time to understand the abiotic factors which may affect their foraging or reproduction. Additionally, since there is an annual recurring pattern in water quality, it is cost effective to

invest in spring sampling for at least a few years and collect some data for long-term use.

We suggest a more standardized schedule for operation of water control structures across years, and conducting further research on fish migration and reproduction patterns during spring. So far, we have found the seasonal water quality pattern to be fairly similar through the years, indicating that the seasonal changing patterns were basically predictive from the aquatic wildlife's perspective, which favored the colonization process of some species in certain spots. However, connection with river water can change the water quality in wetland units; for instance, we have found conductivity and TP were generally much higher in 2021 than 2020. This change or disturbance may or may not be expected by some less tolerant wetland species, depending on the magnitude of change. For example, if the river happens to be loaded with high nutrients such as TP and TKN during a certain period of time, connection with the river might lead to eutrophication in our units, thus influencing the manager's choice to whether or not connect the units to the river. We may need to further investigate the tolerance levels of SNWR aquatic species in their different life stages, so that we can better evaluate the ecological effect of wetland connection.

For the development of the turbidity-nutrient model, we suggest conducting more nutrient sampling in the fall season when seiche events frequently happen. Three-year monitoring has already shown us that turbidity in summer season was rather low for both rivers and wetland units, so if we want to keep exploring the relationship between turbidity and nutrient level in the SNWR wetland units, we suggest allocation of more effort in fall season targeting high turbidity data. Also, from a cost-benefit perspective, if we can prove that there is no significant difference in water nutrient levels across wetland units as well as across different water stratifications in the summer season, we may be able to reduce sampling efforts in summer when water is relatively stagnant within the units and exchange less with the rivers nearby, then translocate these efforts to fall season sampling.

Lastly, it is probably more appropriate to consider SNWR wetland units dynamic in the context of the river floodplain more so than Lake Huron. From the comparison between SNWR wetland units and Pool2B, we found there were significant differences between these two wetland systems regarding the magnitude of their response to seiche events. Therefore, although there is implicit link between Lake Huron's dynamic and upstream river influence, SNWR was still directly influenced by rivers more than the lake. More focus should be given to river dynamics when we try to synthesize information for analyzing the abiotic and biotic process in the SNWR units.

VEGETATION MONITORING

INTRODUCTION

Vegetation within SNWR wetland units was deemed an important variable to survey in the post restoration process due to: (1) its expression of wetland condition; and (2) it's impact on macroinvertebrates and fishes, as well as migratory waterfowl and waterbirds. The hydrologic reconnection of Maankiki Marsh allowed for water to inundate the restored wetland units giving rise to a habitat supportive to a potentially wide variety of aquatic plants. Previous monitoring (2019 and 2020) documented introduction and succession of many native and invasive species(Lugten et al. 2020; Dellick et al. 2021). Wetland plant communities are shaped by various factors including, but not limited to; topography, hydrology, water quality, seed banks, disturbance and management practices (Keddy and Reznicek 1986; Johnston and Brown 2013). Emergent and submerged vegetation across the refuge provide; food sources, spawning habitat, and seasonal refuge for macroinvertebrates, fishes, and migratory birds (Jude and Pappas 1992; De Szalay and Resh 2000; Wilcox et al. 2002).

The wellbeing of vegetation communities across the refuge is essential for supporting current biota. Also, successional changes in biodiversity can only be understood through continuous monitoring. A prosperous vegetation community bolsters counts and diversity macroinvertebrates and fishes; and similarly provides optimal habitat for migratory waterfowl and waterbird communities for which SNWR was created to protect and preserve. Annual monitoring is necessary as it captures fluctuations in dominant plants, variations in weather, and natural hazards across years. A great representation of this is how the historic 2020 Midland flood influenced the vegetation communities at SNWR. We developed the following research objectives to guide our vegetation sampling and data analysis for the 2021 monitoring season:

RESEARCH OBJECTIVES

- *Determine the emergent, submergent/floating species, groundcover, understory, and overstory species present.*
- *Determine the variation in structure, composition, and abundance of species between each vegetation zone in a unit.*
- *Determine the variation in structure, composition, and abundance of species across the vegetation zones for all units.*

- *Determine how vegetation varies across units- based on native, exotic, and invasive species present.*
- *Observe composition change among units and vegetation zones across years 2019-2021.*

METHODS

Vegetation Zone Delineation

To understand the vegetation zones present and determine sampling locations, we used images from Google Maps and modified these in ArcGIS to determine the number and distribution of sampling quadrats (Figure 16). A stratified sampling design was utilized to capture variation in vegetation zones within and across units. Vegetation zones were based on dominant vegetation (e.g. *Phalaris* sp. or *Typha* sp.) or plant structure (e.g. Submerged Aquatic Vegetation; SAV) (Lugten et al. 2020). The largest vegetation zone was deemed dominant in each unit and received a higher number of samples (15), while remaining vegetation received a lower number of samples (10). Distribution of each sample/quadrat in a vegetation zone was randomly determined using GIS. Spacing between sampling points was manually determined by our team and varied based on acreage of vegetation zone with 100 m spacing as the maximum and 10 m the minimum. Distances between sampling points differ and were inconsistent based on satellite-surveyed area coverage, maneuverability and *in situ* vegetation coverage. Vegetation zones with smaller coverage may have some spacings as small as 10 m.

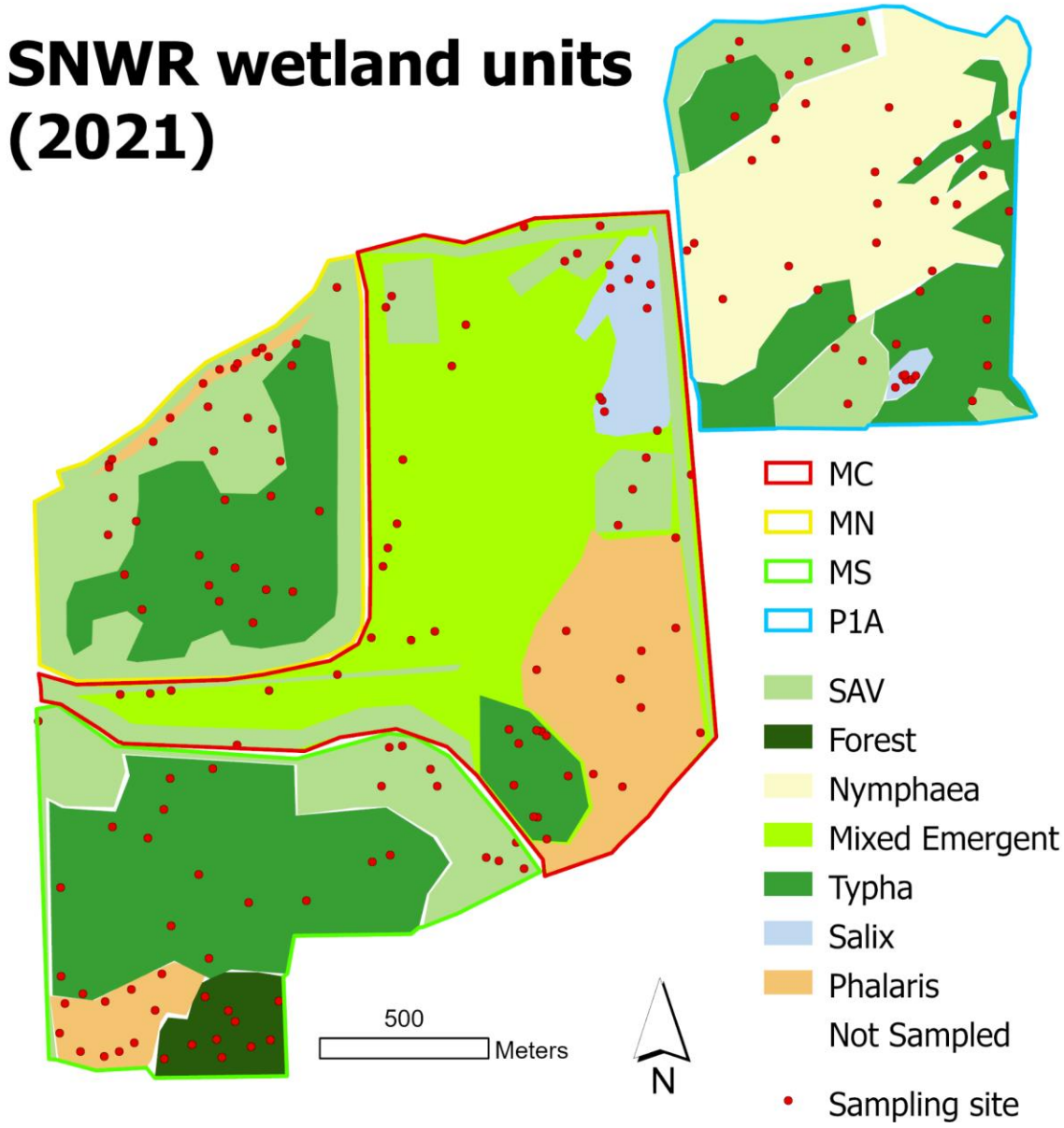


Figure 16. Overview of all vegetation zones, to better clarify dispersion of vegetation sampling points described in Figure 2. Vegetation zones include: Submerged Aquatic Vegetation (SAV), Forest, *Nymphaea*, Mixed Emergent, *Typha*, *Salix*, and *Phalaris*.Locations for each vegetation sample are depicted to better show dispersion of sampling sites across vegetation zones. Abbreviations refer to wetland units and river locations: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), Shiawassee River (SHR), and Spaulding Drain (SPD).

Table 7. Environmental conditions of wetland units. Description of unit vegetation zones and number of zones, vegetation zone area and total vegetation zone area, as well as average water depth for a given vegetation zone are shown.

Unit	Number of Vegetation Zones	Vegetation Zones	Vegetation Zone Area (Acres)	Average Depth (cm)	Total Vegetation Zone Area (Acres)
Maankiki Center	5	Mixed Emergent	465.4	39.87	856
		Phalaris	163.3	58.8	
		Salix	51.2	41.8	
		SAV	133.3	116.2	
		Typha	42.8	72.9	
Maankiki North	3	Phalaris	11.19	0	194.9
		SAV	67.17	56.1	
		Typha	116.54	29.2	
Maankiki South	4	Forest	26.43	0.9	315.34
		Phalaris	40.3	4.5	
		SAV	86.31	37.3	
		Typha	162.3	24.8	
Pool 1A	4	Nymphaea	290.2	39.31	557.6
		Salix	11.4	0	
		SAV	86.5	29.2	
		Typha	169.5	9.2	

Our surveying app this year ran into technical difficulties and deleted two sampling points from the dominant vegetation in Pool 1A (*Nymphaea*); thus the results will show only 13 sampling points for this vegetation zone.

Vegetation Survey

We sampled vegetation structure and composition in all four units–MC, MS, MN, and P1A–from August 9 to August 13 2021. We conducted vegetation sampling in August when many of the herbaceous wetland species were flowering, making their identification easier. We uploaded SNWR unit maps overlaid with random sampling points to ArcGIS Survey123, and navigated to sampling locations on foot. If team members were not able to get to a precise location, we would distance ourselves from other unsampled locations (by approximating 10 meters

traversed) and use a random number generator to try and collect the sample in question within the same vegetation zone as the planned sampling point. After arriving at the sampling point, a 1 m^2 quadrat was haphazardly tossed to determine where the vegetation sample was to be collected. Wherever the quadrat landed, we measured depth inside the quadrat to the nearest centimeter; if no standing water was available, a depth of 0 cm was recorded. All data were captured using the ArcGIS Survey 123 (ESRI Inc. 2021) app on an iPad, or a mobile phone (if the iPad battery died). Documented information included: GPS coordinates, site information, sample number, water depth, vegetation zone,sampled species, plant coverage, and time.

Plant identification, in the field, was determined by the team to the lowest taxonomic level possible, and plant coverage was assessed visually. We entered coverage of the quadrat as a percentage, this was determined through group averaging. Team members were first calibrated to each other's estimation abilities at the beginning of the sampling week. When sampling, team members silently guessed percent coverages for a specific species; after 10 seconds, estimations were announced and the average between numbers was documented (values typically differed by about 5%). We used guides created by previous student teams and the application *Picture This Plant Identifier* (Glority LLC) to aid in field identification. Additionally, SNWR biological technician Eliza Lugten assisted our plant identification and sampling. Unfamiliar plants were documented in surveys, however they were not identified through USGS expertise, as in previous years, due to mislabeling and inadequate storage preparation.

Our team identified aquatic quadrats from top to bottom of the water column, with floating species typically identified first before disturbing the water. Plants with smaller coverage were sampled next, then dominant species defining the vegetation zones, and submerged species were sampled last. Terrestrial quadrats were sampled from the ground to the top of vegetative growth- this was to account for smaller species with less coverage, before looking at larger species which take up more space and hide other species. Forest quadrats were sampled in a different manner, where the quadrat's extent was expanded to a 10m radius for overstory species present. If trees were considered understory, and were 4.6 m tall or less, the sampled vegetation only included what was captured in the quadrat. If trees were considered overstory, such as MS Forest and P1A *Salix* vegetation zones, and were greater than 4.6 m tall, the range surveyed included the center of the 1m quadrat and expanded to a radius of 10m (Figure 17).Radii were measured by diameter at breast height (DBH) and were visually defined using measuring tape.

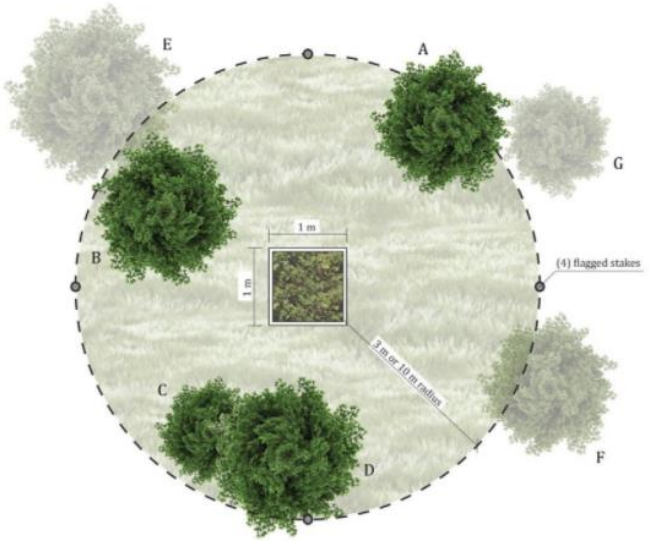


Figure 17. A representation of how a forest sample would be conducted. The small square in the center of the circle represents the quadrat. If trees were considered understory (4.6 m tall or less), the radius of the circle was 3m (similar to what is shown in the picture). With larger trees and a present overstory (4.6m tall or more), a larger radius of 10m was used.

DATA ANALYSES

Vegetation survey data were analyzed through a series of tests to address the objectives set for the 2021 sampling season. We calculated the following parameters to assess the integrity of the wetland: species dominance (Importance Value Index), differences between vegetation zones (Non-metric Multidimensional Scaling and Principal Component Analysis), condition of a vegetation community based on species present (Floristic Quality Index), and the impact and current health of a vegetation community with introduced invasives (Index of Biotic Integrity). We used these parameters to explore differences among units and through time.

Importance Value Index (IVI)

This index characterizes a species' dominance in a given area and takes into consideration any influence on other species. All sampled species for the 2021 monitoring were included in the IVI calculation to determine influence based on abundance and frequency. We calculated IVI using formulas from Curtis and McIntosh (Curtis 1959):

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

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

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

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

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

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

We used the NMDS Ordination of a Bray-Curtis Dissimilarity Index to plot relative differences between IVI's within and across vegetation zones and monitoring units in a 2-dimensional plane. Information was plotted using R Studio and was based on R code from both the 2020 and 2021 sampling teams using packages including "vegan","ggplot2",and "ape" (Dellick et al. 2021). Differences between plotted samples are depicted with longer distances while similarities are depicted with shorter distances and clusters of points. Axes for this plot are unconstrained and the significance of a point's placement is determined after the statistical test has been run. Interpreting results is dependent on groupings of points and evaluating sampling information which can depict abiotic and biotic factors contributing to the wetland vegetation structure and composition.

Principal Component Analysis (PCA)

The PCA test processes all similarities of IVI's for the sampled vegetation units and produces a simplified result, with minimal information loss. This test plots observations in multidimensional space, but does so using Euclidean rather than Bray-Curtis distances. A PCA reduces the dimensionality of large, multivariate data sets into new, synthetic variables- called eigen vectors- which can be plotted in 2-dimensional space while maintaining as much variation from the original dataset as possible. A correlation plot of the eigen vectors was created to visualize the similarities and differences among sampled data points.

Floristic Quality Analysis (FQA)

We conducted an FQA to assess all plant species in monitoring units at SNWR and the condition of these habitats. FQA's cover a wide variety of variables, but for this research, we will only be using two variables, the mean conservatism index (*C*) and floristic quality index (FQI). *C* measures a plant's specificity to a given habitat, while FQI measures the quality of the site based on the species present in comparison to regional scores (Coastal Wetland Monitoring Program 2019). *C* is calculated by assigning a coefficient of conservatism to each species based on its probability of inhabiting an unaltered landscape (i.e. pre-European settlement; Herman et al. 1997). Species coefficients range from 0-10, with 10 indicating a plant is restricted to areas without alterations and 0 indicating the plants most tolerant to landscape changes (Herman et al. 1997). The coefficients of conservatism for the entire community are then averaged to produce *C,* which predicts a plant's adaptability. *C* is then modified to produce the FQI score (Herman et al. 1997), which is determined based on weighted FQI scores for the geographical region.

We generated FQA values using an online calculator (Universal FQA Calculator) where all plant species and their respective coverages per sample were entered. The regional FQI used for this study was based on the 2014 Michigan assessment. FQI and *C* scores analyzed are for an entire monitored units and sub-wetland zones within a given units.The FQA assessment required some data to be reformatted as particular species of vascular plants, dead plants, and introduced plants to the region were not recognized due to the age of the regional FQI.

Index of Biotic Integrity (IBI)

We used the IBI to analyze wetland vegetation community health by quantifying the coverage and impact from invasive species as compared to reference conditions or pristine wetlands. To calculate IBI, the sampled area is differentiated into three different types: entire site, wet meadow/dry emergent (water level $\geq$ 1cm), and flooded emergent/submergent (water levels < 1cm) (Coastal Wetland Monitoring Program 2019; Dellick et al. 2021). Each wetland type is then assessed with three variables including: invasive coverage, invasive frequency, and mean conservatism index (*C*, calculated through FQA). These nine metrics are assessed in addition to a tenth metric which represents the quality of submerged and floating species based on nutrients, sediments, and turbidity (Coastal Wetland Monitoring Program 2019). A score from 0 to 5 is given to each of the ten metrics, with the quality of the wetland detailed as follows: 0 being 'Very Low', 1 being 'Low', 3 being ' Medium', and 5 being ' High'.

These scores are then combined to produce an overall standardized score from 0 to 50 representing the wetland's health. 'High' scores indicate a wetland closer to reference (i.e. "pristine") conditions and range from 41-50, ' Medium' wetlands range from 21-40, ' Low' wetlands range from 6-20, and 'Very Low' wetlands range from 0-5. Combined standardized scores are often paired with a descriptive score, transitioning these numeric results into worded results.

Previous sampling teams (2019 and 2020) averaged water depth across a given vegetation zone then categorized the wetland zone type (i.e. wet meadow/dry emergent, or flooded emergent/submergent) to determine IBI scores (Lugten et al. 2020; Dellick et al. 2021) .We analyzed each quadrat sample based on water depth per quadrat instead of averaging sample depth for a vegetation zone. This change was utilized to fully capture the gradient of water levels within a vegetation zone and to better distinguish edges of wetland zones. Submergent zones were calculated utilizing only submerged aquatic vegetation (SAV) and *Nymphaea* samples.

RESULTS

Overview

We collected 189 samples across the 4 units in the one week span of vegetation sampling at SNWR. There were 101 identified species present: 93 of the species were native, 8 species were invasive, and 1 was a rare species (*Barbarea orthoceras*) (Figure 18). The rare species was identified in the MS *Typha* zone. Overall, P1A had the most total species observed at 76 and MN had the fewest species observed at 30. MS had the highest number of native species at 52, whereas the highest number of invasive species in a unit was 6 and all units except MN had these invasives present. The most common vegetation structures at SNWR were emergent and understory, with the least common structure being overstory. We observed 12 unknown species, which would increase the overall plant total to 113 species.

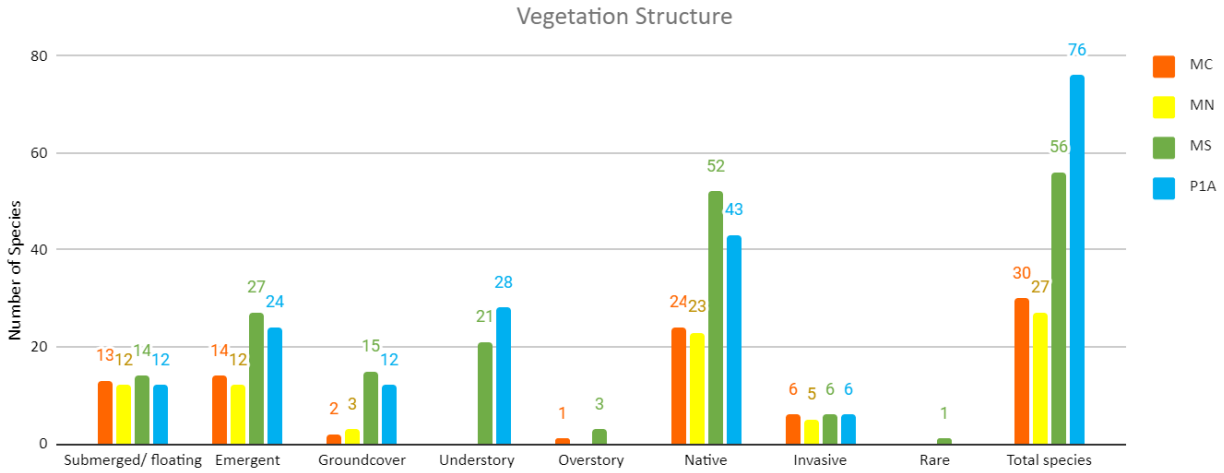


Figure 18. Summary vegetation structure and species type. Structures include submergent/floating, emergent, groundcover and overstory. Species type includes native, invasive, and rare. Number of species for each structure and type are located above each histogram bin, with colors of bin and number corresponding to wetland unit. Only species totals without unknowns are summarized. Number of species present ranged from 1 to 76.

Importance Value Index (IVI)

IVI scores range from 0-100, which reflect the influence a plant has on other competing plants where a higher score indicates more influence. The IVI scores reflect species dominance across each vegetation zone in the four sampled wetland units (Table 8, 9, 10, 11). The top four dominant species in each vegetation zone highlighted here show high values for frequency and abundance; ultimately defining the coverage of this dominant vegetation. Species which were dominant across vegetation zones and units were *Lemna minor* and *Ceratophylumn demersum. Lemna minor* was present in 7 of the 15 total vegetation zones, and 3 of the 4 sampling units. *Ceratophylum demersum* was present in 8 of the 15 total vegetation zones and all 4 of the sampled units.

Table 8. Importance Value Index (IVI) for the three dominant MN vegetation zones. Three vegetation zones in MN (*Phalaris*, SAV, *Typha*) and the most abundant species in each zone ranked by importance value and labeled with the species' vegetation structure. The highest ranking species for this unit was *Phalaris arundinacea* (97.42) and the lowest ranking species was *Ceratophyllum demersum* (19.74). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: open water, no vegetation in relatively deep water; submerged aquatic vegetation (SAV); *Typha* is *Typha sp.;* and *Phalaris* is *Phalaris arundinacea.*

MAANKIKI NORTH			
Vegetation Zone	**Latin Name**	**IVI**	**Structure**
Phalaris	*Phalaris arundinacea*	95.18	Emergent
	Poaceae	26.69	Emergent
	Cirsium arvense	18.51	Ground cover
	Echinocloa crusgalli	10.59	Emergent
SAV	*Ceratophyllum demersum*	45.74	Submerged/Floating
	Potamogeton natans	36.22	Emergent
	Elodea nuttallii	36.06	Submerged/Floating
	Myriophyllum spicatum	27.35	Submerged/Floating
Typha	*Typha angustifolia*	63.97	Emergent
	Utricularia vulgaris	29.39	Submerged/Floating
	Ceratophyllum demersum	21.26	Submerged/Floating
	Lemna trisulca	15.11	Submerged/Floating

Table 9. Importance Value Index (IVI) for the four dominant MS vegetation zones. Four vegetation zones in MS (Forest, *Phalaris*, SAV, *Typha*) and the most abundant species in each zone ranked by importance value and labeled with the species' vegetation structure. The highest ranking species for this unit was *Phalaris arundinacea* (95.18) and lowest ranking species was *Echinocloa crusgalli* (10.59). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); *Typha* is *Typha spp*; forest is *Acer saccharum*; *Phalaris* is *Phalaris arundinacea.*

MAANKIKI SOUTH			
Vegetation Zone	**Latin Name**	**IVI**	**Structure**
Forest	*Setaria pumila*	41.11	Emergent
	Lemna minor	29.53	Submerged/Floating
	Acer saccharinum	21.37	Overstory
	Boehmeria cylindrica	20.22	Emergent
Phalaris	*Setaria viridis*	53.49	Emergent
	Persicaria pensylvanica	22.14	Emergent
	Panicum spp.	17.84	Emergent
	Persicaria lapathifolia	17.45	Emergent
SAV	*Ceratophyllum demersum*	80.47	Submerged/Floating
	Lemna minor	29.48	Submerged/Floating
	Potamogeton nodosus	27.73	Emergent
	Najas	18.53	Submerged/Floating
Typha	*Typha angustifolia*	47.48	Emergent
	Ceratophyllum demersum	21.70	Submerged/Floating
	Utricularia vulgaris	17.51	Submerged/Floating
	Lemna minor	14.77	Submerged/Floating

Table 10. Importance Value Index (IVI) for the five dominant MC vegetation zones. Five vegetation zones in MS (Forest, *Phalaris*, SAV, *Typha*) and the most abundant species in each zone ranked by importance value and labeled with the species' vegetation structure. The highest ranking species for this unit was *Ceratophyllum demersum* (80.47) and lowest ranking species was *Lemna minor* (14.77). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); *Typha* is *Typha spp*; *Salix* is *Salix nigra* and/or *Salix exigua*; Shore is any littoral zone with or without any vegetation; and *Phalaris* is *Phalaris arundinacea.*

MAANKIKI CENTER			
Vegetation Zone	**Latin Name**	**IVI**	**Structure**
Mix	*Phalaris arundinacea*	57.11	Emergent
	Lemna minor	31.38	Submerged/Floating
	Spirodela polyrhiza	28.28	Submerged/Floating
	Algae spp.	20.81	Submerged/Floating
Phalaris	*Phalaris arundinacea*	97.42	Emergent
	Utricularia vulgaris	33.55	Submerged/Floating
	Spirodela polyrhiza	28.88	Submerged/Floating
	Lemna minor	28.10	Submerged/Floating
Salix	*Salix interior*	39.83	Emergent
	Lemna minor	26.81	Submerged/Floating
	Lemna trisulca	25.27	Submerged/Floating
	Utricularia vulgaris	24.86	Submerged/Floating
SAV	*Stuckenia pectinata*	36.99	Submerged/Floating
	Algae spp.	29.76	Submerged/Floating
	Potamogeton natans	26.72	Emergent
	Ceratophyllum demersum	19.74	Submerged/Floating
Typha	*Typha angustifolia*	50.82	Emergent
	Lemna minor	45.39	Submerged/Floating
	Riccia fluitans	39.91	Submerged/Floating
	Spirodela polyrhiza	29.57	Submerged/Floating

Table 11. Importance Value Index (IVI) for the five dominant P1A vegetation zones. Four vegetation zones in MS (*Nymphaea*, *Salix*, SAV, *Typha*) and the most abundant species in each zone ranked by importance value and labeled with the species' vegetation structure. The highest ranking species for this unit was *Salix interior* (67.74) and lowest ranking species was *Vitis riparia* (10.48). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*; *Salix* is *Salix nigra* and/or *Salix exigua*.

POOL 1A			
Vegetation Zone	**Latin Name**	**IVI**	**Structure**
Nymphaea	*Nymphaea odorata*	56.00	Emergent
	Ceratophyllum demersum	52.27	Submerged/Floating
	Spirodela polyrhiza	23.81	Submerged/Floating
	Stuckenia pectinata	15.05	Submerged/Floating
Salix	*Salix interior*	67.74	Understory
	Boehmeria cylindrica	25.14	Emergent
	Phalaris arundinacea	19.09	Emergent
	Vitis riparia	10.48	Groundcover
SAV	*Ceratophyllum demersum*	70.55	Submerged/Floating
	Nymphaea odorata	26.91	Emergent
	Spirodela polyrhiza	21.82	Submerged/Floating
	Stuckenia pectinata	19.04	Submerged/Floating
Typha	*Dead Typha*	37.28	Emergent
	Spirodela polyrhiza	30.89	Submerged/Floating
	Typha angustifolia	30.35	Emergent
	Ceratophyllum demersum	13.61	Submerged/Floating

Invasive species

Invasive species were assessed for their influence on native species (Table 12). Two invasive species *Phalaris arundinacea* and *Typha angustifolia* were found in all units. *Phalaris arundinacea* was predominantly found in MC and had the widest coverage across the vegetation zones. This species also had the two highest IVI scores of 97.42 and 95.18, within MC and MN *Phalaris* zones, respectively. *Typha angustifolia* was found in equal coverages of vegetation zones in MC and MS, but its highest IVI scores were 63.97 and 50.82. Invasive species that were only found in one vegetation zone included *Butomus umbellatus, Phragmities australis,* and *Typha x glauca*, with IVI scores of 3.8, 2.15, and 1.66 respectively. MC had the strongest presence of invasive species with multiple invasives found within the same vegetation zone. MC had the highest number of sampled vegetation units (5), but also had the highest variety of invasive species (5). MN had the lowest presence of invasive species with 4 species spanning three vegetation zones - there were only two occurrences of more than one invasive species being in the same vegetation zone.

Table 12. Invasive species' Importance Value Index. Seven invasive species zones (*Butomus umbellatus, Cirsium arvense, Lythrum salicaria, Myriophyllum spicatum, Phalaris arundinacea, Phragmites australis, Typha angustifolia*) from the wetland units and the most abundant species in each zone. The highest importance values are bolded. Scores ranged from 1.00 to 97.42 and encompassed 8 invasive species. Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*; *Salix* is *Salix nigra* and/or *Salix exigua*; Shore is any littoral zone with or without any vegetation; and *Phalaris* is *Phalaris arundinacea.*

Invasive Species	Vegitation Zone	Unit	IVI Score
Butomus umbellatus	Salix	MC	3.80
Cirsium arvense	Mixed Emergent Vegetation	MC	1.31
	Phalaris	MN	18.51
	Forest	MS	10.59
	Phalaris	MS	4.17
Lythrum salcaria	Mixed Emergent Vegetation	MC	1.31
	Salix	MC	6.20
	Typha	MS	1.00
	Phalaris	MS	3.69
	Salix	P1A	7.68
	Typha	P1A	4.13
Myriophyllum spicatum	Submerged Aquatic Vegetation	MN	27.35
	Typha	MN	2.87
	Nymphaea	P1A	1.27
Phalaris arundinacea	Mixed Emergent Vegetation	MC	57.11
	Phalaris	MC	97.42
	Salix	MC	16.25
	Submerged Aquatic Vegetation	MC	10.85
	Phalaris	MN	95.18
	Phalaris	MS	15.43
	Salix	P1A	19.09
	Typha	P1A	9.57
Phragmites australis	Phalaris	MS	2.15
Typha angustifolia	Mixed Emergent Vegetation	MC	6.11
	Typha	MC	50.82
	Typha	MN	63.97
	Phalaris	MS	9.73
	Typha	MS	47.48
	Typha	P1A	30.35
Typha x glauca	Typha	P1A	1.66

Nonmetric Multidimensional Scaling (NMDS)

This ordination technique plots multidimensional data using Bray-Curtis dissimilarity values and distance. The NMDS plot visualizes relative similarities and differences of observed vegetation community data (Figure 19).This plot is based on the vegetation composition for each zone, which can be based on a multitude of different factors. Clustered points indicate similarities of those communities while points with greater ordinal distance represent dissimilar communities. Point dispersal may be based on water depth and dominant vegetation zones. After an analysis of the point distributions across the x-axis, water depth seemed to be one of the explanatory factors. Averaged water depths for each of the vegetation zones revealed: *Phalaris* zones were the driest, *Salix* zones were the second driest, *Typha* zones were the third driest, and SAV zones were the wettest (Table 7). Note that these averages were taken from vegetation zones that had 2 or more samples, thus MS Forest, MC Mixed Emergent Vegetation, and P1A Nymphaea were not included in the calculation here. This trend is displayed on the x-axis with the left side having no standing water, and the right side having the most standing water depth. Zones that stood out in this trend were P1A *Salix* and MS Forest, as these samples had little to no standing water depth, and P1A *Nymphaea* which followed trends for all SAV zones. Three main clusters arose in this plot, *Typha* zones, SAV zones, and all of the MC vegetation points. *Typha* points across the four monitored units clustered together, this also occurred for SAV points. This indicates these vegetation zones are much more similar to each other, based on vegetation species composition, than to other vegetation zones within their monitoring pool. *Salix* points had the largest spacing of all vegetation zones indicating that, although these two zones have the same dominant vegetation, the units that they grew in are quite different. Another cluster was formed across units and vegetation zones and includes MC *Salix*, *Phalaris*, and Mixed Emergent Vegetation (MEV) as well as MS Forest points.

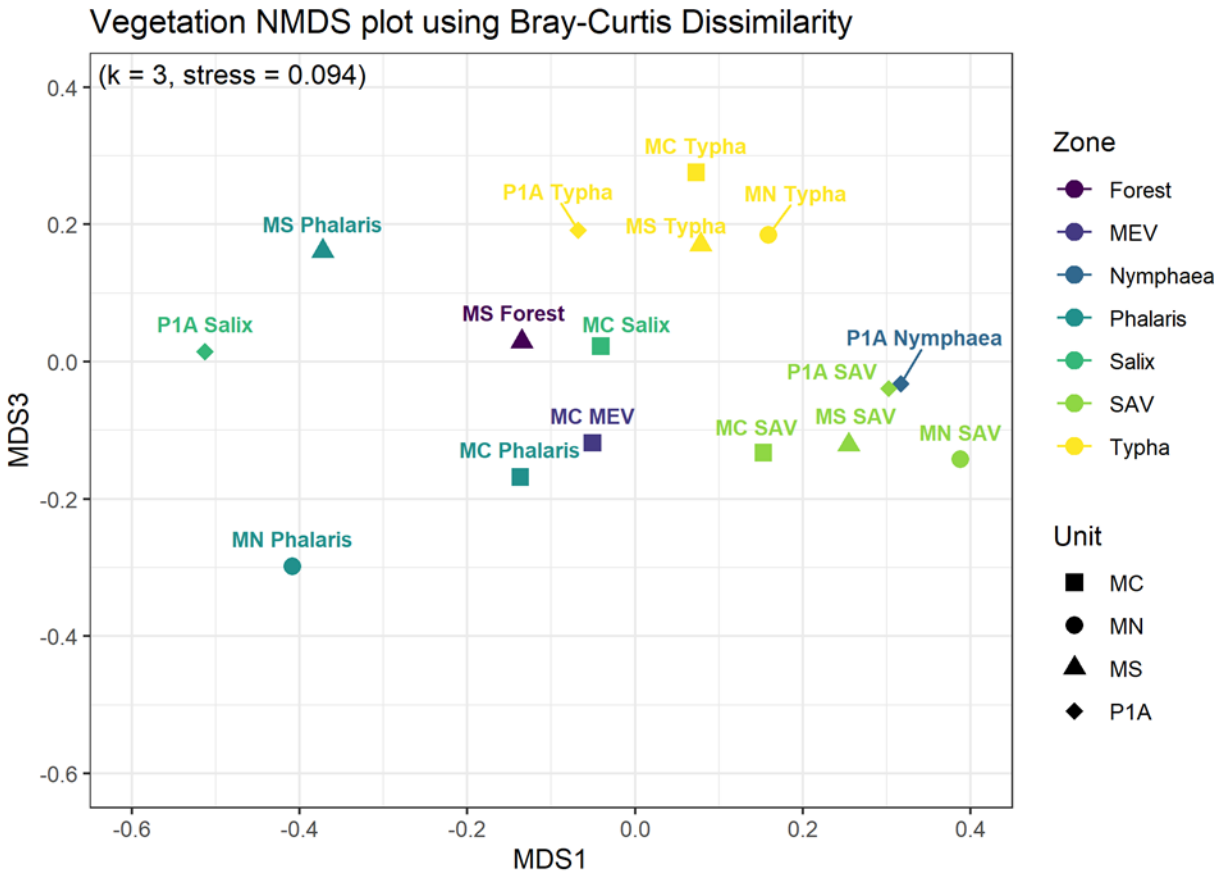


Figure 19. Plot of the 16 combinations of different vegetation zones and units sampled during the 2021 season. Abbreviations refer to wetland units and river locations: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); dead *Typha* is dead *Typha* sp; *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*; forest is *Acer saccharum*; *Salix* is *Salix nigra* and/or *Salix exigua*; and *Phalaris* is *Phalaris arundinacea.*

PCA

Our PCA test results represent similarity with clusters of names and differences with spacings between names. The first graph had three outliers, P1A *Salix*, MS *Phalaris*, and MS Forest that were distanced from the cluster of the remaining 13 combinations (Figure 20). MS Forest is the closest point to the cluster with about 3.0 units of distance on the y-axis. P1A *Salix* is the furthest point away from the main cluster at approximately 17.5 units on the x-axis. MS *Phalaris* is similarly distanced from the cluster at approximately 16 units on the y-axis. Upon further analysis, all these outliers had more than 1 sampling quadrat with no standing water, indicating that the cluster depicts vegetation zones with any standing water. Water depth averages can be observed in Table 7.

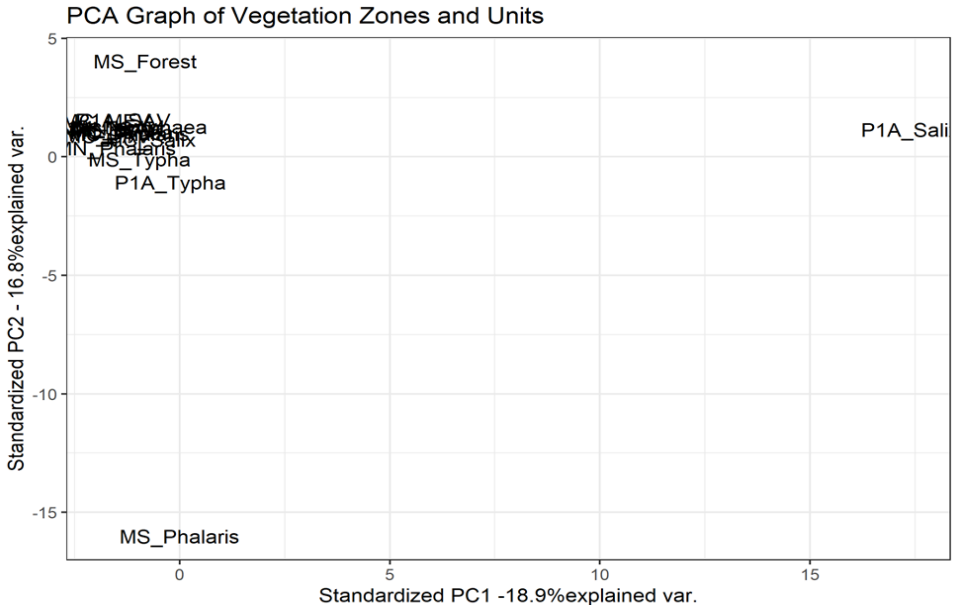


Figure 20. PCA graph showing all 16 vegetation zone and unit combinations. PCA ordination of all vegetation zones within each unit on two axes of variation, standardized principle component 2 (PC2) and standardized principle component 1 (PC1), with the percent of variation that each axis explains. Each “Unit_Vegetation zone” combination refers to the vegetation zone found with that unit. The first abbreviation refers to wetland unit: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). The second abbreviation or word refers to vegetation zones, which are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*; forest is *Acer saccharum*; *Salix* is *Salix nigra* and/or *Salix exigua*; *Phalaris* is *Phalaris arundinacea.*

We did a second PCA analysis to minimize the distance of outliers and better discern the placement of the 13 points within the main cluster. This analysis did not include MS Forest. This analysis more clearly defined similarities between points in the cluster and generated a new combination of outliers (Figure 21). MS *Phalaris* is again an outlier, but now along the x-axis, and has the highest distance at approximately 15 units. MN *Phalaris* has the second highest distance at 11.5 units on the y-axis. P1A *Typha* and MC MEV are on opposite sides of the cluster and show differences compared to each other. Similar to the first PCA analysis, this second analysis also highlights that these new outliers also represent relatively dry plots. Upon review, the two new outliers for this plot (MN *Phalaris* and P1A *Typha*) had dry quadrats for almost half of the total sample. Both plots show that *Phalaris* zones for MN and MS were dry, whereas the *Phalaris* zone for MC was wet. All samples for MC *Phalaris* had more than 1cm of standing water. P1A showed different outliers between the two graphs, whereas the second graph shows P1A *Typha* closer to the cluster of vegetation samples. This can be attributed to all samples of P1A *Salix* having no standing water, and more than half of the samples for P1A *Typha* also having no standing water.

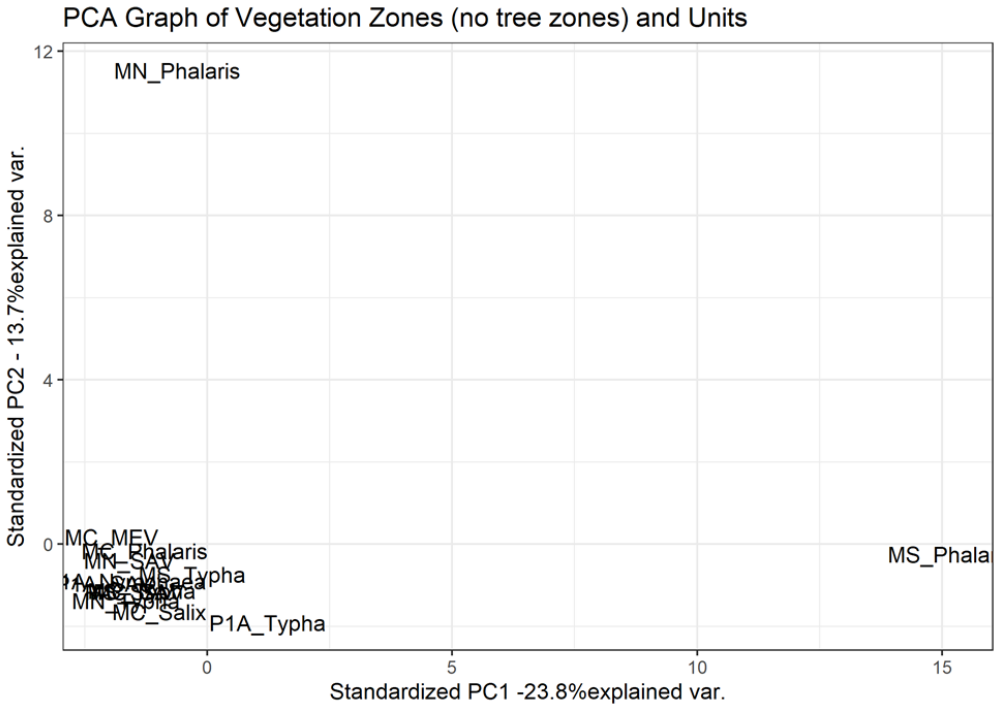


Figure 21. PCA graph with MS forest zone removed to better assess all vegetation zones within cluster. PCA ordination of all vegetation zones within each unit on two axes of variation, PC2 and PC1, explaining 37.5% of the variation in vegetation zones by unit. Each “unit_vegetation zone” combination refers to the vegetation zone found within that unit. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); channel is open flowing water with little to no vegetation; *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*; *Salix* is *Salix nigra* and/or *Salix exigua*; *Phalaris* is *Phalaris arundinacea.*

FQA

Our FQA results highlighted wetland units with higher counts of disturbance-intolerant species (Table 14). To better understand the diversity of species across all units in the FQA results, vegetation structure was differentiated into categories to visualize tallies of all species. Categories were based on broad classifications in the Universal FQA Calculator (Universal FQA Calculator): forbs, grasses, rushes, sedges, shrubs, vines, and trees.

The category with highest number of species was forbs (37) and the lowest was rushes(1) (Table 14). The unit with the most diversity was MS with an average of 16 different species across 4 vegetation zones. Of those, an average of 14 species were native. P1A also averaged 14 native species, with 15 total species across its 4 vegetation zones. MN and MC averaged 11 total species each , MN hosted 10 native species on average and MC hosted 9 native species on average.

Vegetation structure composition for each pool helps drive the mean *C* scores. MC had the highest *C* score of 3.92 with more species that are site specific, whereas MS had the lowest *C* score of 3 but a higher count of species that are not site specific. MN had the second highest *C* score of 3.16 and P1A had the third highest *C* score of 3.12.

Averaged FQI total scores and native species scores reflected the mean *C* score where MC had the highest scores of 13.58 (Native FQI) and 12.58 (Total FQI). MN had the lowest scores with 12.03 for native FQI and 10.8 total FQI, but had the second highest *C* score of 3.16

Table 14. Vegetation structure, native and total species averages, C scores, and native and total FQI values were assessed in the FQA analysis. Mean C possible range is 0-10 with 10 as the highest, and FQI possible range varies from 0-35 with >35 being exceptional. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Structures were defined from the FQA index (MDNR 2001).

Unit	Forb	Grass	Rush	Sedge	Shrub	Vine	Tree	Native Species (Avg.)	Total Species (Avg.)	Mean C with invasive Species	FQI Values Natives Only	Total FQI Values
MC	23	2	0	0	1	0	0	9	11	3.92	13.58	12.58
MN	21	1	1	2	0	0	0	10	11	3.16	12.03	10.8
MS	37	6	0	2	0	1	2	14	16	3	13.57	11.9
P1A	31	1	0	2	1	4	0	14	15	3.12	13.3	12.05

IBI

Each IBI metric is assigned a numeric score (0,1,3,5), which is then summed to produce the combined numeric score (0-50), this score is then assigned a corresponding combined descriptive score (Very Low, Low, Medium, High). The combined numeric scores were similar across all four units with the range spanning 5 points (Table 15). MC and P1A each had the highest IBI score of 28, while MN was the lowest (23). Each of the four units has a combined descriptive score of 'Medium' quality.

Compared to previous years, the integrity of the monitored units increased, from a 'Low' combined descriptive score to a 'Medium' score (Tables 15, 16, 17). The MC unit had one of the highest individual unit scores even though it was recently hydrologically connected.

Table 15. Four IBI parameters summed to create the Total IBI score. Sample areas include the entire wetland unit (Entire), Wet Meadow and Dry Emergent Zone, Flooded Emergent and Submergent Zone, and Submergent coverage. Each metric ranges from 1 to 5, with 5 being the highest rating. The metric 'C' represents the mean conservatism index . The Total IBI score is then compared to the IBI table to establish its Combined Standardized score which ranges from 'Very Low' (0) to 'High' (50). Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

	2021														
	Entire				Wet Meadow and Dry Emergent Zone				Flooded Emergent and Submergent Zone					Combined Standardized Score	
Unit	Invasive Cover	Invasive Frequency	Total Quadrats	C	Invasive Cover	Invasive Frequency	Quadrats in Wetland Zone	C	Invasive Cover	Invasive Frequency	Quadrats in Wetland Zone	C	Submergent Coverage	Numeric Score	Descriptive Score
MC	1	3	55	3	1	3	1	5	1	3	54	3	5	28	MEDIUM
MN	1	3	35	3	0	3	15	1	1	3	20	3	5	23	MEDIUM
MS	3	3	45	1	3	3	14	1	3	3	31	3	3	26	MEDIUM
P1A	3	3	43	3	3	3	17	1	3	3	26	3	3	28	MEDIUM

Table 16. IBI scores from 2020 sampling season. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Sample areas include the entire wetland unit (Entire), Wet Meadow and Dry Emergent Zone, Flooded Emergent and Submergent Zone, and Submergent coverage. Each metric ranges from 1 to 5, with 5 being the highest rating. The metric 'C' represents the mean conservatism index . The Total IBI score is then compared to the IBI table to establish its Combined Standardized score which ranges from 'Very Low' (0) to 'High' (50).

2020												
	Entire			Wet Meadow and Dry Emergent Zone			Flooded Emergent and Submergent Zone				Combined Standardized Score	
Unit	Invasive Cover	Invasive Frequency	C	Invasive Cover	Invasive Frequency	C	Invasive Cover	Invasive Frequency	C	Submergent Coverage	Combined Numeric Score	Combined Descriptive score
MN	0	0	1	0	0	0	1	0	1	5	8	LOW
MS	3	0	3				3	0	3	5	17	LOW
P1A	3	3	1				3	1	1	5	17	LOW

Table 17. IBI scores from 2019 sampling season. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Areas in grey were not sampled this year and include P1A Wet meadow/Dry emergent zone. Sample areas include the entire wetland unit (Entire), Wet Meadow and Dry Emergent Zone, Flooded Emergent and Submergent Zone, and Submergent coverage. Each metric ranges from 1 to 5, with 5 being the highest rating. The metric 'C' represents the mean conservatism index . The Total IBI score is then compared to the IBI table to establish its Combined Standardized score which ranges from 'Very Low' (0) to 'High' (50).

* note the Low/Medium score was given to P1A because it scored high in the Total IBI score compared to other low values. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

2019												
	Entire			Wet Meadow and Dry Emergent Zone			Flooded Emergent and Submergent Zone				Combined Standardized Score	
Unit	Invasive Cover	Invasive Frequency	Mean Conservatism Index (C)	Invasive Cover	Invasive Frequency	Mean Conservatism Index (C)	Invasive Cover	Invasive Frequency	Mean Conservatism Index (C)	Submergent Coverage	Combined Numeric Score	Combined Descriptive score
MN	1	0	1	0	0	5	1	0	1	5	14	LOW
MS	1	0	3	3	1	5	1	0	3	5	22	MEDIUM
P1A	1	1	3							5	20	LOW/MEDIUM

DISCUSSION

2021 was the initial sampling year for MC, and this unit had some of the highest and lowest values across our analyses of vegetation structure and composition. Adding this new unit to the analyses changed historic observations made for the vegetation community at SNWR.

Disturbance at SNWR as depicted by FQA

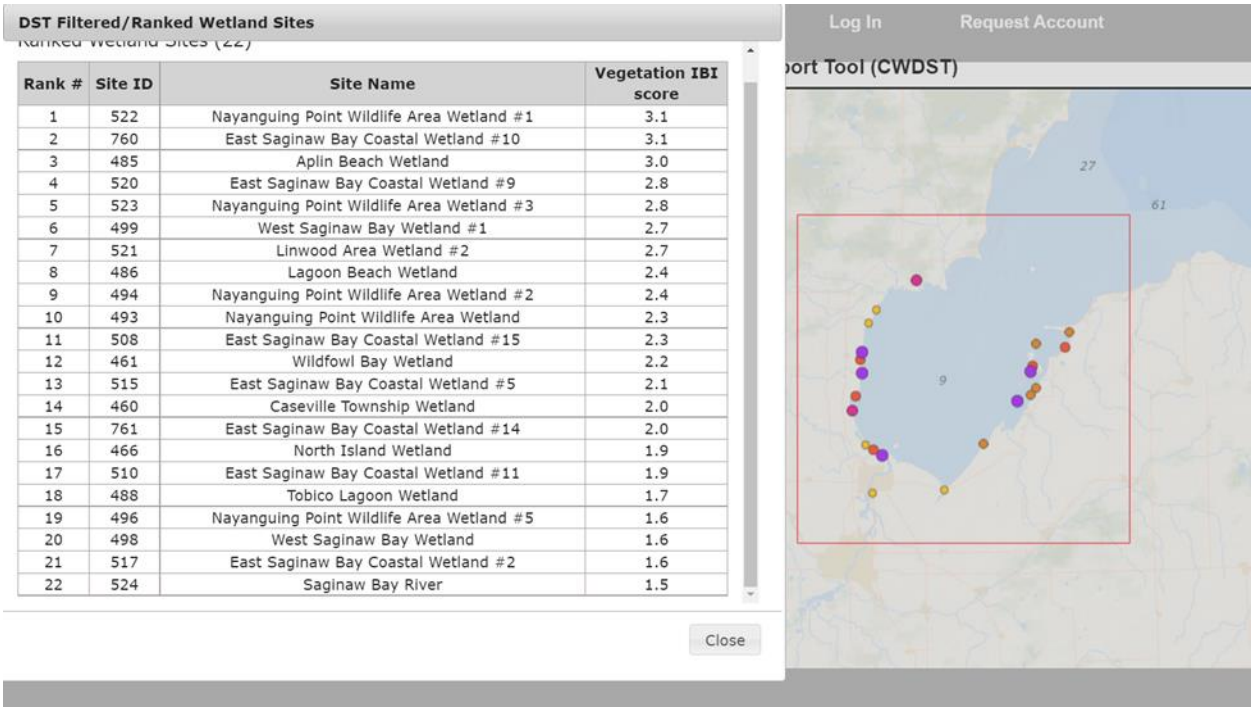
Wetland unit MC had the highest *C* score and native and total FQI scores, indicating that: (1) MC had recruited a relatively diverse vegetation assemblage within 1 year of hydrologic reconnection; and (2) MC had the least amount of disturbance compared to the remaining units. Interestingly, MC matched and surpassed P1A's results for this index, which has been hydrologically reconnected without disturbance for nearly 70 years, and thus serves as a reference unit for the restoration effort. P1A also housed plant species intolerant to change with both FQI scores second to MC, but had the third highest *C* score. This indicates that while many of the plants in P1A were tolerant of degraded habitat or disturbance, based on the *C* score, the overall site is capable of supporting plants intolerant of change. MS had the second highest native FQI score, and the third highest total FQI score, but the lowest *C* score. This indicates that the plants present are native species found within a disturbed site. MN had the lowest scores across both FQI scores and the third highest *C* score.

While our SNWR FQA scores were somewhat low, this is not unusual for similar wetlands in the region. FQA scores depict historic land alterations, as higher FQA scores indicate plants, and ultimately sites, with little disturbance whereas lower scores indicate significant disturbance. Compared to other wetlands throughout the state of Michigan, Saginaw county wetlands experienced 50% or more wetland loss and land alteration- there were 14 other counties that were subjected to the same circumstances (U.S. EPA 1996). Locationally, all 15 of these counties (including Saginaw county) are in the southern half of the Saginaw watershed (U.S. EPA 1996). FQA studies within the Saginaw Bay region had relatively low scores, due to prevalent human disturbance, and no locations had pristine conditions (Stanley et al. 2005). Plants with the highest scoring *C* values were found in prairies as well as southern Michigan bogs and fens, which are generally rare habitats (Herman et al. 1997). Typically, plants found in undeveloped lands had C values between 0 and 2, whereas 85% of the native species found had *C* values of 4 or higher (Herman et al. 1997). Trends of disturbance and lower *C* values were observed with the mean *C* values for SNWR ranging from 3 to 3.92.

SNWR's IBI scores and how they compare to other wetlands

Results for IBI were similar to the FQA analysis, yet they indicate minimal differences between the wetland units at SNWR. FQA indicated that present vegetation experienced landscape changes and supports species tolerant to disturbance, whereas IBI depicted overall abiotic and biotic degradation from landscape alteration and invasive species dominance. MC and P1A were tied for the highest combined numeric IBI score with 28, MS had the third highest combined numeric score at 26, and MN had the lowest combined numeric score at 23. Although the combined descriptive scores for all units in the IBI analysis had a medium rank, the range of the combined IBI numeric score spanned only 5 points; which indicates that the quality of wetland at MN is not much different than its counterparts.

Compared to surrounding Saginaw Bay coastal wetlands, SNWR scores were not far off. The Great Lakes Coastal Wetlands Monitoring Program (Coastal Wetland Monitoring Program 2019) reported that metric scores for the Saginaw Bay marsh were as follows: the entire site averaged 3.9, flooded emergent zones averaged 4.5, and wet meadow zones averaged 4.2. Across the 2019-2021 monitorings, the metric scores for SNWR were: an average of 2.5 for the entire site, wet meadow zones averaged 2.0, and flooded emergent averaged 3.0. Using the CWMP's Coastal Wetland Decision Support Tool (CWDST), we identified an inland wetland along the Saginaw River near Bay City. Of the coastal wetlands in Saginaw Bay, it had the lowest rating of 1.5 out of 5 of approximately 20 sites (Figure 22), the remaining scores ranged from 1.6 to 3.1. IBI from CWMP across the state of Michigan defines the vegetation communities in coastal wetlands along Saginaw Bay as 'moderately degraded' to 'degraded' (Figure 23).



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

Figure 22. Screenshot from the Coastal Wetland Decision Support Tool website (link) where the IBI scores were presented for 22 of the surrounding coastal wetlands of Saginaw Bay, near to SNWR. IBI scores ranged from 0.6-5.0, with each of the wetlands in the red box being ranked from highest to lowest quality. These scores are calculated based off of the entire surveyed site metric, which ranges from 0-5, with 5 as the highest, and are not a cumulative IBI score for each site, which is what most of the results are based on Table 15. The Saginaw River wetland was identified as the closest to SNWR (which was not sampled for this assessment) and had the lowest score of 1.5.

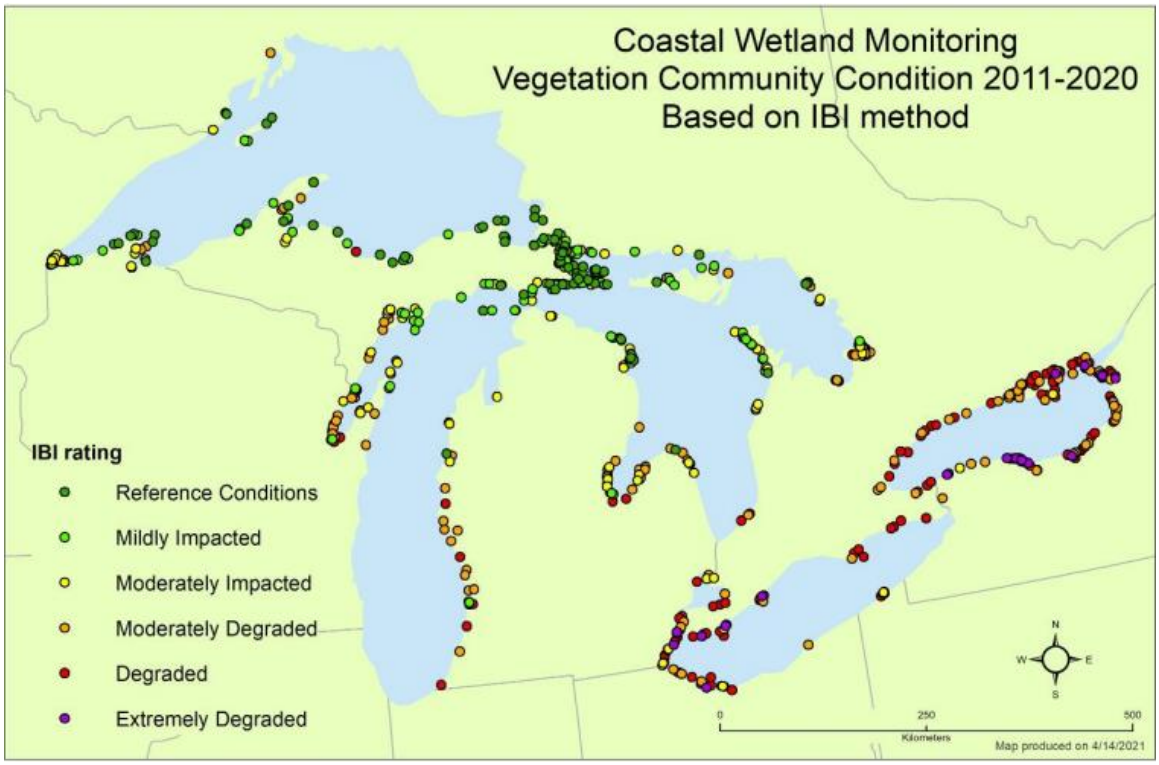


Figure 23. This image was taken from Uzarski et al. (2022) and describes all coastal wetland vegetation communities across the Great Lakes. Information was compiled from sampling efforts spanning 2011- 2020. With ‘Reference Conditions' being the highest quality of wetland, located mainly in the Upper Peninsula of Michigan, sites near industrialized areas show more degraded conditions. Saginaw Bay coastal wetlands ranged from moderately degraded to degraded (third and second lowest ranking, respectively).

Water depth impact on vegetation composition

Unit hydrology and microtopography, and resulting trends in water depth, can influence unit differences in: seed bank viability, seed dispersal, and resulting vegetation composition across SNWR (Lugten et al. 2020; Dellick et al. 2021). NMDS and PCA analyses highlighted the differences in water depths between vegetation zones.

Trends in the NMDS analysis showed a gradient of water depths observed from the left side of the x-axis to the right, with three notable clusters forming. All *Typha* zones clustered together, with SAV zones and the *Nymphaea* zone forming its own cluster. The differentiation can partially be accredited to water depth, as well as dominant species or those species present, possibly counting as another variable. Species composition may be able to describe why each unit point was at a certain location in a cluster or why the two clusters with uniform vegetation zones across units formed. Depth was not the only factor creating this plot, as MS Forest was a hidden outlier. MS Forest had an average water depth of 0.9 cm, yet was found close to MC *Salix* and MC *Phalaris* with water depths of 58.8 and 41.8, respectively. Depths for samples within the *Typha* cluster were lower than these values (Table 7). Similarly, the MS unit stood out in the PCA analysis with factors such as water depth and sedimentation likely contributing to these results. The first PCA for this site (Figure 20) indicated that MS Forest and *Phalaris* zones were very different from the remaining 13 units in the cluster- with the MS Forest point removed, only the *Phalaris* point remained as an outlier in the second PCA plot (Figure 21). These vegetation zones were outliers in the PCA because of the low average water depth (Table 7).Calculating the average water depths for each unit revealed that MS was the shallowest unit, and MC was the deepest unit, with P1A and MN as the second and third shallowest, respectively. This could explain the diversity of species and outcomes from the various analyses.

In managed diked wetlands, like SNWR, water levels can be manipulated to change vegetation communities, in order to establish habitats for waterfowl and waterbirds (Mitsch 1992; Mitsch and Gosselink 2015). A study conducted by Herrick et al. (2007) analyzed different wetland vegetation zones for species richness, based on seed banks and standing vegetation, in diked and undiked wetlands.Three of the seven sampled wetlands in this study were along Saginaw Bay. Herrick et al. (2007) found significantly higher species counts in diked wetland seed banks than undiked wetlands, in the following vegetation zones: sedge meadow/wet prairie, mudflat, shore, and floating/submerged. Analyzing the methods of this study (Curtis 1959), these zones can be compared to the vegetation zones used at SNWR with the floating/submerged zones representing SAV areas and mudflat representing areas in P1A that were not present in 2021 (Lugten et al. (2020) found mudflats in their study). On the other hand, emergent

aquatic was a sampling zone in Herrick et al.'s (2007) study which was similar to *Typha* zones at SNWR; this zone had a higher presence of invasives but was not significantly different than undiked wetlands. Submerged/floating zones had 1 invasive species per 1 m^2, and mudflats had the highest number of invasive species in this test with 12 species per 1m^2 (Herrick et al. 2007). Diked wetlands were considered to be "seed traps" which had high levels of organic matter and nutrients (Herrick et al. 2007). This type of wetland supports a wider variety of plant species, up to 20% by some analyses, due to the protection from open water with high velocities (Herrick et. al. 2007). Relating these findings to SNWR, conditions in MS promoted the highest number of total averaged species (Table 14). Presence of upland plants may have increased the diversity in MS as these plants were able to rapidly grow and disperse their seeds across the unit. Increased water level, such as SAV areas, could have limited species diversity due to many upland and emergent plants being intolerant of a greater depth. In one study, periods of high water on a Great Lake coastal wetlands deterred woody plants and terrestrial species from growing close to the water, and changed the studied area from an emergent zone to a submergent zone. This disturbance also killed some emergent species like *Typha* (Keddy and Reznicek 1986). Prevalent invasive species found in diked wetlands included *Lythrum salicaria* and *Typha spp.*; *Lythrum salicaria* had the highest densities in seed banks while *Typha* was found in large dense stands constricting seed bank diversity. Keddy and Reznicek (1986) concluded that this study noted that the lack of hydrological disturbance would allow for these species, which were also present at SNWR, to continue to flourish. Combined results from Herrick et al. (2007), Keddy and Reznicek (1986), and our 2021 analysis may depict what future vegetation community compositions look like for the diked wetlands of SNWR.

While sampling vegetation in P1A, the team experienced considerably high levels of silt in this unit (approximately 2 ft high from bottom) which resulted in a lower water depth. P1A had a larger surface area with shallower water, due to high silt levels, which facilitated a wider variety of aquatic macrophyte species over time, compared to other units. The vegetation structure in the community can be influenced from the varied depths across units (Figure 24). P1A had one of the lowest and most uniform depths of all of the units; this may be caused by the direct and prolonged connection to SPD for 70 years. Diked wetlands typically accumulate organic material and nutrients that would normally be washed out of the wetland during high flows (Albert and Minc 2004; Herrick and Wolf 2005). In comparison, MS and MN had deeper depths- an increasing depth gradient was observed across both units moving from the southern edge of MS to the northern edge of MN.

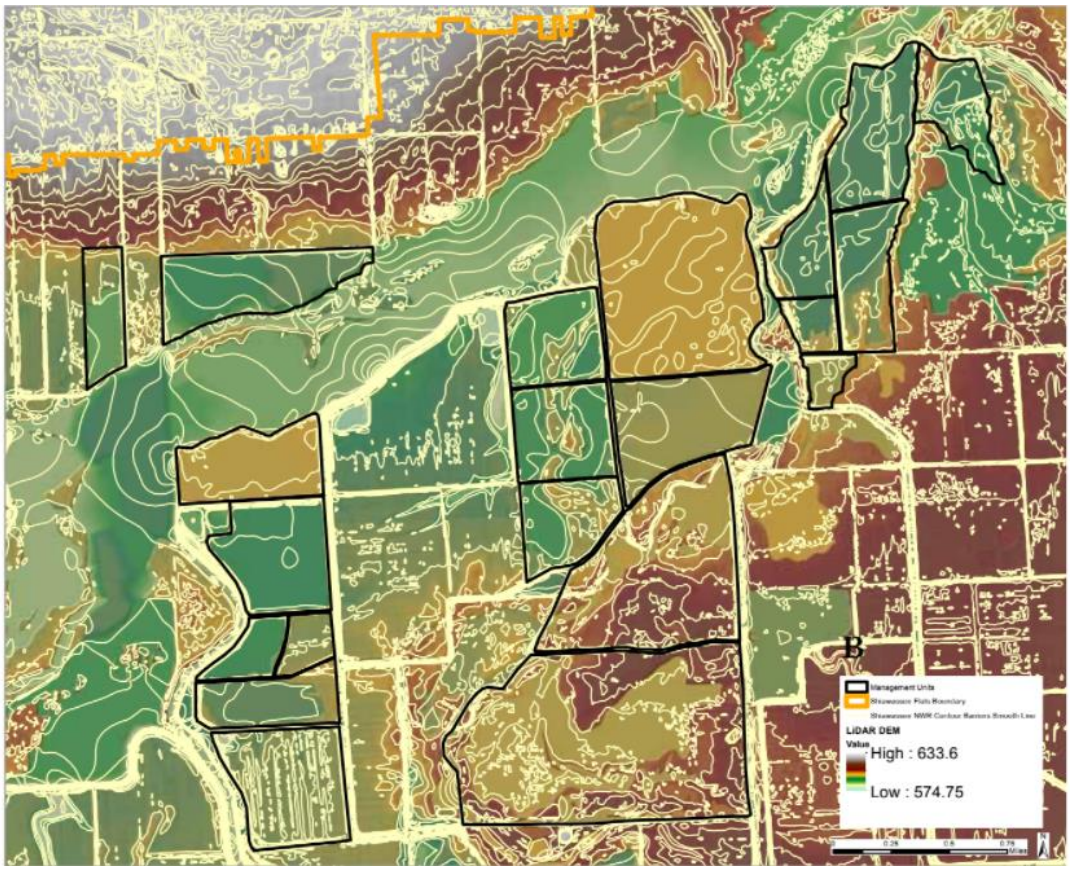


Figure 24. LiDAR topographic maps for Shiawassee National Wildlife Refuge (Heitmeyer et al. 2013, Figure 13B).

This change in gradient may be explained by 1) natural topography of SNWR and 2) slight alterations of topography to create a visitor road across the refuge. The northern edge of MN has the highest depth between these two units and has an established sightseeing route on a man-made dike. The depth for this area may have been trenched to create the dike, as referenced by Dellick et al.(2021). In comparison, MS has a higher elevation on the southern portion of this unit. This elevation and relatively flat area could possibly be attributed to historic land disturbances for restoration. The goals of the SNWR restoration project were to make heterogenous units and this difference in elevation facilitates with heterogeneity. Inundation and reconnection of these units began in 2017 for MN and 2018 for MS. Both units only have one water outlet, which is typically closed off from river connections as water level is often at a target height for this unit (Dellick et al. 2021). The 2021 sampling season was the driest for MS, as there was not enough snowmelt to contribute water for inundation compared to Lugten et al. (2020) and Dellick et al. (2021). MC had the highest depth of all the units but also had an elevated area across the center, this can be partially attributed to an artificially created low level barrier dike for the unit. This dike was created to

mitigate the fetch, or wave effects, from windy days. Some of the material to create this was taken from within the unit, thus creating greater depths. MC experiences a secondary hydrologic connection to either side of the river. This could result in sediments entering and exiting this unit at a slower rate.

Invasive Species impact on SNWR

Spread of invasive vegetation throughout SNWR can be attributed to anthropogenic stressors such as diking, sediment deposition, or nutrient availability (Herrick and Wolf 2005; Dybiec et al. 2020). Although P1A was similar to MC in several analyses, it was starkly different in the amount of invasives present. MC had the highest number of invasive species per vegetation zone whereas P1A had third highest number of invasives per vegetation zone.This result could be attributed to the fact that P1A is a well established wetland unit, and could be considered a late successional habitat, thus minimizing niche openings for invasive species in this unit. *Phalaris arundinacea* was the most prominent invasive species for MC and was found in 4 of the 5 vegetation zones for this unit. The highest IVI scores for this invasive species were 97.42 and 57.11. MS had the second highest number of invasive species and MN had the fewest species. Diked wetlands promote a significantly higher number of invasive plant species in seed banks and standing vegetation (Herrick and Wolf 2005). In the Herrick and Wolf (2005) study, the two most common invasive species found were *Lythrum salicaria* and *Typha spp*. t SNWR their highest IVI ratings were 7.68 and 68.97, respectively. *Lythrum salicaria* was found in 3 of the 4 units, whereas *Typha spp.* was found in all 4 units, these two invasive species had the second and third highest dispersal across SNWR (Table 12).

Hydrocharis morsus-ranae, also known as European Frogbit (EFB), was an invasive species also observed at SNWR in 2021 but similar to Dellick et al. (2021), was not captured in our vegetation survey. This species is concerning as it may impact wetland and recreational use of open water due to its ability to grow in dense mats, which can reduce light, nutrients, and dissolved oxygen in the water column, thereby harming other aquatic plants, as well as impeding recreational water traffic (Hansen et al. 2022). Cahill et al. (2021) found that, across 5 different sites throughout Michigan, the established populations of EFB were the most fecund in the Saginaw Bay area, and recommended that additional education and outreach is needed to mitigate its spread across the state. Dellick et al. (2021) mentioned that this species may have entered SNWR during the Midland flood of 2020. We observed this species during our aquatic macroinvertebrate and fish samplings.

Identifying the presence of certain invasive species, or in some cases native species, at SWNR can help determine impacts that may be occurring upstream or historic disturbances which may need to be addressed. Wetlands across Michigan have been subjected to anthropogenic stressors which have allowed invasive species to spread. Specific disturbances can give rise to certain invasive species observed in wetlands, with examples including: nutrient deposition giving rise to dense stands of *Typha spp.* which do not support other species, diking and dredging which disturb sediments and distribute *Phragmites australis* or *Lythrum salicaria,* and road density which promotes the creation of drainage ditches thus creating disturbed corridors for wetlands (Dybeic et al. 2020). Correlation between invasive species and various stressors is so strong, that the presence of an invasive species can be used as a rapid stand-alone assessment for disturbance as suggested by Trebitz and Taylor (2007). Comparatively, positive wetland health was attributed to the presence of *Carex* species, as well as others included in the Cypereaceae family (i.e., *Schoenoplectus* spp.) that are found in wet meadows (Dybeic et al. 2020). Carex spp, were found in two of our vegetation zones in small quantities (Appendix V) but did not contribute on as large of a scale as the Dybeic et al. (2020) study.

Cross-year Analysis

Assessing SNWR across three years of monitoring shows succession of the vegetation community. Overall trends across these years show decreased IBI and C scores for 2020 sampling, in comparison to 2019 and 2021 sampling, where 2021 had the lowest FQI values and 2019 had the highest values. Lugten et al. (2020) showed similar trends of MN scoring low for the FQA and IBI, while having the lowest occurrence of invasives. MS was the highest scoring site for FQA and IBI, in addition to having the highest amount of invasives. P1A scored between the other two units for this year. The lowest total IBI score for 2021 (23) was just above the highest score for 2019 (22) (Lugten et al. 2020). The FQA had a wider spread of values with 2019 *C* values ranging between 2.9 and 3.5, and total FQI between 23.5 and 11.2; the range of *C* values for 2021 was 3 – 3.92 and the range of total FQI values was 10.8-12.58 (Lugten et al. 2020). Invasive plants for 2019 totalled 13 species with MS having the most invasive coverage, and for 2021 totalled 8 species with MC having the most coverage (Lugten et al. 2020). The 2020 sampling season had different results compared to both our 2021 sampling season and the 2019 sampling season. IBI scores all resulted in ‘low’ rankings, with the highest score being 17. Both P1A and MS had this score (Dellick et al. 2021). FQA analysis for 2020 resulted in MS having the highest C score of 2.8 and the range of total FQI scores between 11.5-16.4 (Dellick et al. 2021). Invasive plants for the 2020 sampling team had 7 species with MN having the most invasive species coverage (Dellick et al. 2021). Before

sampling MC, MS had the highest results and spread of invasives across each of these tests. This may have been caused by the cross unit flow for MC distributing nutrients, seeds, and sediment.

Study Limitations

In situ sampling limitations included maneuverability and access, as well as sampling limitations. Sampling points were randomly scattered across each of the 4 sampled wetland units, but did not take into account how these locations would be accessed. Some samples required a different sampling spot than the initial intended spot because it could not be accessed due to 2021 sampling conditions. Density of plants for this growing season prevented access to some sampling points as well as water depth. Additionally, species that were portrayed in collected vegetation samples may not have been representative of all of the species present in SNWR. On several occasions, our team encountered European Frogbit–a highly invasive species–in all of the pools sampled, but our data do not show that this species was present. We do not know the number of species outside our sampled list. Uncatalogued species in the refuge may have been in areas that were not accessible or were simply in areas that were not sampled.

Information transfer and data analysis also encountered limitations. Unknown species were encountered during vegetation sampling but were not analyzed by USGS experts due to poor preservation; these specimens also could have contributed new species to the current array observed in 2021. Data entered from field sampling were recorded on ArcGIS Survey 123 and stored virtually through the ArcGIS website, transferring data from the website's storage to individual computers caused 2 sampling sites to be deleted from the *Nymphaea* zone samples. This disappearance could have removed species which were only observed in this vegetation zone as well as disproportionately altering data analysis results from a lowered number of sampling points from the dominant vegetation zone. Data analysis may have also been altered due to outdated sources, the FQA analysis utilized a regional species guide from 2014. The date of this assessment does not accurately capture native or invasive species that have entered SNWR or accept vascular plants. Inputting plant species for this analysis resulted in the removal of 12 different species entries from the cumulative species list; at least 5 were recognized species and not accepted.

Implications for Management and Science

We recommend continued vegetation monitoring to better cover and assess continued development of the vegetation community at SNWR based on Dellick et al. (2021) sampling procedures incorporated with updates from this report.

If possible, we suggest continued randomized sampling of vegetation communities using ArcGIS. This app allows users to easily measure a season's vegetation zones and manually determine the spacing of sampling points. This flexibility is exceedingly helpful as some in situ situations can hamper spacing. Additionally, some of the issues experienced through Survey 123 may have future surveyors considering paper documentation; this is so that information is not lost upon transferring onto the Survey 123 cloud from the field. In addition to the loss of 2 *Nymphaea* vegetation zone samples, there were naming conventions in Survey 123 which incorrectly identified one sample quadrat to other quadrats in that vegetation zone (i.e. *Nymphaea* sample 7 quadrat is placed with sample 8 quadrats and is incorrectly named " sample 8") as well as some of the unique identifications given to each sample being mixed up. With time, we were able to find and fix all the errors, but paper documentation of each sample would eliminate this concern.

To address the scale of sampling and potential species that were not surveyed this year, we recommend creating a species accumulation curve (SAC), similar to that used for fishes. This test would be able to predict the total number of species present at SNWR even if they were not captured in our samples. One example of this test being used for wetland vegetation analysis was Spieles (2005), a SAC was utilized to determine vegetation composition in mitigation wetland banks throughout the United States. This test will better assist monitoring teams in determining the proportion of species documented from the predicted total species, and what impact management actions will have on undocumented species. We also recommend creating a cumulative list of species found with annual updates of new species from each monitoring to better position future monitoring teams. This can track newly sampled species that are introduced or have been present as well as introductions of new invasive species. Providing this list to new teams and utilizing it each year can quickly compare species presence and absence. Additionally, we recommend an agreed upon naming and preservation process for unknown species. Communication for labeling unknown species was challenging as our team was divided in half to sample all sites. Naming each sample similarly as information is put into Survey 123 (i.e., Unit name,Vegetation zone, Sample number, Unknown number) would be able to differentiate species at a fast rate. Designating one team member at the beginning of vegetation sampling week to press each unknown species, would ensure that all unknowns are preserved adequately. If a plant species is too large

for pressing with books, coordinating with USFWS or USGS to use a plant press may also be an option.

Sampling for water chemistry and nutrients is recommended for vegetation sites where sonde sampling is applicable. This additional information can help managers better understand: 1) the microhabitats outside of typical fish and aquatic macroinvertebrate sampling locations which can determine unsampled refugia, and 2) nutrient and sediment dispersal for an entire unit. Water quality and nutrient sampling across the three years of post-restoration monitoring has primarily occurred along the edges of units where wading is feasible (Figure 2).This suggested sampling would be different than those conducted in other sections of this report as sites would be spread throughout wetland units and vegetation zones. Water quality has been concurrently sampled with macroinvertebrates and fish, these sites are located in areas that are easiest to maneuver due to the weight of equipment and time constraints. Nutrient and sediment dispersal across each wetland unit can better depict the abundance and types of vegetation species present. Invasive species typically spread faster from increased nutrient dispersal and sedimentation, since they quickly find open areas to grow and can outcompete natives (i.e., bare ground elevated from sedimentation) (Zedler and Kercher 2010). Invasive plants are able to effectively utilize phosphorus over native plants because of their growth rate and allocation efficiency in leaf area and shoot growth (Zhang et al. 2022). Determining the nutrient load of vegetation zones can delineate areas where invasive species spread is possible, which would be helpful for future management. This can influence plant communities, thus impacting species in higher trophic levels.

We also recommend accessing and interpreting EFB surveys of nearby bodies of water. The 2021 and 2020 sampling teams noted that this invasive species was present, yet were not able to capture it in vegetation surveys. Reviewing literature of EFB presence and density in bodies of water near SNWR may serve as some sort of monitoring for this concerning invasive species, even if its presence cannot be recorded in annual data sets.

A bathymetry survey is recommended to better understand the water depths across SNWR. This information can help predict the potential for vegetation communities across sampled wetland units. Water depth can also reveal sediment accumulation across units. Heitmeyer et al. (2013) detailed that P1A had a uniform and high elevation compared to the remaining units. Our team determined that this may be due to sediment accrual over 70 years from hydrological connection to SPD. This was evidenced as no other site had such a high level of silt throughout the unit (approximately 2 ft of silt). The northwestern corner of MN had the highest water depth along with the majority of MC. Analyzing the plant communities and results across these sites may suggest that a higher water depth for MC can bolster the variety and health of this unit.

Although P1A had some similar scores in the results, we posit this to its longstanding hydrologic connection and succession over multiple decades. These vegetation communities will ultimately influence macroinvertebrate and fish communities, which can impact the variety of birds at SNWR.

AQUATIC MACROINVERTEBRATE MONITORING

INTRODUCTION

Aquatic macroinvertebrate communities are important bioindicators, and an essential part of wetland communities, and SNWR managers wish to know how hydrologic reconnection of MS, MN, and MC will affect communities of these primary consumers. Moreover, numerous waterfowl such as mallards, American black ducks, and blue-winged teals are priority refuge resources that, along with other waterbirds, rely on invertebrates as food for at least part of their life cycle (Stafford et al. 2016; U.S. Fish & Wildlife Service 2018). Fishes common to SNWR, such as brown bullhead and common carp, also rely on and likely affect assemblages of aquatic invertebrates (Batzer 1998). Vegetation also plays a role in controlling invertebrate distributions and abundance, and abiotic factors like water depth, temperature, nutrient balance, and hydrology also exert influence (Cooper et al. 2014; Stafford et al. 2016). Given the complexity of these interactions and SNWR's need to determine the effects of reconnection on this essential food web component, we set out to monitor SNWR's invertebrate communities during the 2021 sampling season.

RESEARCH OBJECTIVES

- *Characterize aquatic macroinvertebrate richness and abundance, and variation among units, vegetation zones, and months.*
- *Determine variation of aquatic macroinvertebrates across years 2019-2021*
- *Explore the relationship between water quality parameters and aquatic macroinvertebrate communities and taxonomic diversity.*
- *Examine aquatic macroinvertebrates as bioindicators of restoration success.*

METHODS

Field Sampling

In recent decades ecologists have refined their use of aquatic invertebrate diversity in wetland health assessments of Saginaw Bay marshes (Cooper et al. 2014). Following an initial sampling season in 2019, Lugten et al. (2020) in collaboration with SNWR management established sampling protocols for the refuge. Delick et al. (2021) further refined the protocols during the 2020 field season, and we again implemented these with a few modifications.

We sampled for two days near the beginning of each month for June, July, and August; in May sampling took place across three days. Each sampling session took place across all four management units: MS, MN, MC, and P1A. Within each unit, we sampled all vegetation zones that were accessible by wading, and where standing water indicated the likelihood of aquatic biota. Due to changing water levels throughout the season, this resulted in sampling a variety of vegetation zones: *Typha, Nymphaea,* submerged aquatic vegetation (SAV), *Salix, Phalaris*, and flooded forest; all of which were represented in previous years' samples. We also sampled in three new zones: smartweed, river bulrush, and dead *Typha.*

In order to avoid unnecessary sediment disturbance, we used a sonde to capture triplicate water quality parameters before collecting invertebrates. We next conducted a blind toss of a 1m quadrat, and then estimated percent cover of vegetation and measured water depth within it.

We used a 0.5 mm mesh D-net to make nine sweeps in the water column within the quadrat's bounds, taking care to sample all vegetation, the water bottom, and open water. We then deposited the contents of the net into a white tray, this coloration aids in locating macroinvertebrates.We repeated this process three times and deposited all contents into one tray.

From the tray, we first collected highly visible individuals that might represent unique taxa, and then we selected a small section of the tray and collected exhaustively using pipettes or tweezers before moving to another section. We collected for a combined effort of thirty minutes across 4 researchers, and deposited all specimens into a vial containing 80% ethanol (ETOH) solution. Vials were labeled by unit name, sample number, and date sampled (e.g. MC 02 06072021). Later, we sorted and counted specimens using dissecting microscopes, and identified and labeled them according to keys in Merritt and Cummins (2008), with unknown specimens stored for later identification by experts.

We identified specimens using methods derived from the Great Lakes Coastal Wetland Monitoring Program (CWMP) and two taxonomic identification manuals (Hilsenhoff 1975; Thorp and Covich 2010). In 2019, Lugten et al. (2020) identified samples to family; in 2020, Dellick et al. (2021) identified to genus. We further refined identification techniques during 2021 by adopting the lowest operational taxonomic unit (LOTU) as the primary measure of taxonomic richness. Using this method, we completed identification to genus, but in rare cases where genus-level identification was beyond our expertise, we identified to family (various families), subfamily or tribe (Chironomids only), and in rare instances, to higher levels (Oligochaeta, Hydracarina), thus providing this highest level of taxonomic resolution possible (Burton et al. 1999). In two cases, we used a split identification where the distinction between two taxa was not possible (*Bezzia* or *Palpomea*, Pseudochironomini/ Chironomini). This method involves uncertainty where, for example, there may be overlap between specimens identifiable to family and those from the same family identifiable to genus. However, the method also allows us to account for any unknown genera without undercounting, and because most taxa are known, we deemed the level of uncertainty acceptable.

DATA ANALYSES

Catch per unit Effort (CPUE)

We used Microsoft Access to standardize macroinvertebrate catch per unit effort (CPUE) for LOTU, where effort was calculated as the total number of a given taxon divided by the number of months sampling occurred, the number of samples within a vegetation zone, or the number of units. We also calculated total CPUE by dividing taxon counts by the number of total sampling efforts, in this case 60.

Analysis of Variance (ANOVA)

We used the R software program in base R and the ‘cars’ and ‘dplyr’ packages for all statistical tests. We tested datasets for normality and equal variance, and then ran analyses of variance (ANOVA) on both LOTU CPUE, as well as summed counts of invertebrates per month, unit, or vegetation zone. We also examined multiple linear regressions of pH, turbidity, temperature, and conductivity as independent variables plotted against LOTU CPUE.

Permutational Analysis of Variance (PERMANOVA)

We also ran permutational analysis of variance (PERMANOVA) tests using the 'adonis' package and created nonmetric multidimensional scaling (NMDS) plots using the 'vegan' and 'ggplot2' package. PERMANOVA uses an F-test to measure the sum-of-squares within and between groups, and randomly permutes the community matrix based on our distance measures of months, units, and vegetation zones. For all PERMANOVA, we constructed NMDS plots using Bray-Curtis dissimilarity indices and Euclidean distances condensed to three dimensions (k=3). To aid visualization, we presented only one NMDS plot most representative of our results out of the three possible dimensional combinations, and used ellipses to assist interpretation of 95% confidence intervals for each vegetation zone, unit, and month. We presented an NMDS plot which best described the variation.

Index of Biotic Integrity (IBI)

To construct indices of biotic integrity (IBI) for macroinvertebrates, we used Microsoft Excel, and followed Standard Operating Procedures for the Coastal Wetland Monitoring Program (Coastal Wetland Monitoring Program 2019) as well as Burton et al. (1999). Burton et al. (1999) developed different scores depending on the vegetation zones present in the wetland. Specific scoring is available for *Typha* only, and it utilizes points for each indicator taxa which are then summed and given one of four qualitative assessments from "degraded" to "reference conditions" (i.e. pristine). Lugten et al. (2020) and Dellick et al. (2021) used different methods, although these were also derived from Burton et al. (1999), so while our results are reliable, comparison across years may not be.

Characterizations of Functional diversity and Successional Change

We reviewed the literature on macroinvertebrates and characterized SNWR richness and abundance on three parameters. First, we followed Burton et al. (2002) in their characterization of coastal wetland macroinvertebrate communities as high fetch, mid-fetch, and low fetch. We summed unit CPUE for those taxa that Burton et al. (2002) identified as strongly associated with each of those zones in order to identify fetch interactions with SNWR wetland units.

Second, Pollock et al. (2017) uses an island biogeography framework to sort samples into strong, weak, and nonflying taxa and quantify colonization rates of more distant, nearer, larger, and smaller wetland units. We followed Sarramejane et al (2020) in coding primary dispersal method from affinity traits (the likelihood of a dispersal method for each LOTU, scored from 0-3) and summed unit CPUE

for each dispersal method by unit. Note that a particular taxon may have more than one dispersal method, but we categorized taxon only on the highest scoring dispersal method. If a trait scored equally likely for two dispersal methods, as for chironomids, *Hesperocorixa*, *Trichocorixa*, *Branchiobdellida* (phoretic passive aerial dispersal), immature corixids, pleids, and *Clinotanypus*, then we divided the CPUE by half and added it to both dispersal methods. We followed Vieira et al. (2006) who suggested that trait affinities for families and genera without trait affinities can be derived by taking the family average from available genera. All trait affinities were established from Sarremejane et al. (2020), except in the case of *Hyalella*, *Caecidotea*, *Hydrachnidae*, and *Nehalennia* that were only available in Vieira et al. (2006) and trait affinities were established from comments.

Third, we characterized successional change and catastrophic events at SNWR by comparing taxa across years 2020 and 2021 in all units. We arbitrarily chose a minimum of 0.5 unit CPUE (which equates to at least half the number of individuals as samples taken in any given unit) because demographic and environmental stochasticity may overtake comparisons below this level of measurement. We then highlighted units where any taxa decreased across years (red), increased across years (green), or where MC was significantly higher or lower than all other units.

RESULTS

Overview

From sixty sampling sites, we counted 5842 macroinvertebrates in 97 LOTUs, representing 14 orders, 47 families, and 71 genera (Table 18). Delick et al. (2021) identified 7,763 individuals from 14 orders, 50 families, and 100 genera from 3 wetland units. Lutgen et al. (2020) identified 43 families in total, but did not identify specimens to genera. In total, Dellick et al. (2021) collected more families than did Lugten et al. (2020) or our team. However, our 2021 sampling team was the first to collect data in MC. Except for the family-level identifications in MN, there was a decline from 2020-2021 across all units at both the family and genera level.

Table 18. Raw macroinvertebrate family and genera richness, 2019-2021. 'N/A' represents years before MC was reconnected and sampling occurred. Note that while 2019-2020 shows no consistent variation in richness, 2020-2021 shows a consistent decline across all years and taxonomic levels. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

	Families			Genera	
Unit	2019	2020	2021	2020	2021
MC	n/a	n/a	35	n/a	45
MS	41	37	27	52	33
MN	32	34	37	53	40
P1A	36	39	33	58	41
Total	109	110	132	163	114

In order of greatest to least abundance, the 9 most common families are similar between all three years (Table 19). Additionally, Belostomatidae and Libellulidae, ranking in the top 9 in years 2019 and 2020, respectively, ranked 12th and 13th in abundance in 2021. Superficially, family abundance is similar between years, although major differences exist between CPUE, which for some families is highest in 2020, and for others is lowest in 2020. Overall, there is more similarity between 2019 and 2021, than between 2020 and the other two years.

Table 19. The nine most abundant families ranked by total CPUE, 2019-2021. Hyalellidae, Physidae, and Hydrachnidae only contain one genus in our sample; other families contain more than one subfamily, tribe, or genus. Note that Hydracarina in 2021 is the higher order for the family Hydrachnidae in 2020 and 2019, but represent the same LOTU.

2019		2020		2021	
Family	**CPUE**	**Family**	**CPUE**	**Family**	**CPUE**
Caenidae	28	Hyalellidae	67.8	Hyalellidae	21.2
Hyalellidae	21.9	Coenagrionidae	18	Chironomidae	13.6
Chironomidae	17	Caenidae	14.4	Caenidae	12.7
Coenagrionidae	10.8	Chironomidae	5.7	Coenagrionidae	12.0
Corixidae	6.1	Hydrachnidae	5.7	Corixidae	8.4
Pleidae	3.1	Physidae	5.4	Physidae	4.8
Physidae	2.8	Libellulidae	4.8	Hydracarina	3.0
Hydrachnidae	2.8	Corixidae	4.7	Pleidae	2.8
Belostomatidae	2.4	Pleidae	3.8	Aeshnidae	1.3

When we measured unit CPUE, we did not find that each unit had strongly different LOTUs (Table 20). We consistently found *Hyalella, Caenis*, and Chironomini in each unit, although there are significant differences in CPUE. Among the top five taxa in each unit, we only found *Neoplea* in P1A, and *Trichocorixa* and *Hydracarina* in MS, while other LOTUs we found in at least two of the units. When we separated the top nine taxa by unit CPUE, we found a higher proportion of flying taxa and a lower proportion of crawling and swimming taxa in MC, with these proportions narrowing in MS, MN, and P1A.

Table 20. The five most abundant families ranked by wetland unit CPUE. Grey indicates a taxon common between all four units. Where a LOTU did not go to genus-level identification, genus is blank and we only record the higher taxon, thus some families are shared between units, but the LOTU is not. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

MN			MC		
Higher Taxon	**Genus**	**CPUE**	**Higher Taxon**	**Genus**	**CPUE**
Hyalellidae	*Hyalella*	38.4	Caenidae	*Caenis*	14.3
Caenidae	*Caenis*	14.0	Hyalellidae	*Hyalella*	13.1
Coenagrionidae	*Coenagrion*	6.6	Coenagrionidae		8.7
Corixidae		6.1	Chironomidae	Pseudochironomini/Chironomini	6.7
Chironomidae	Pseudochironomini/Chironomini	4.6	Physidae	*Physa*	5.7
MS			**P1A**		
Higher Taxon	**Genus**	**CPUE**	**Higher Taxon**	**Genus**	**CPUE**
Hyalellidae	*Hyalella*	20.0	Hyalellidae	*Hyalella*	16.3
Caenidae	*Caenis*	8.5	Caenidae	*Caenis*	12.8
Chironomini	Pseudochironomini/Chironomini	6.9	Chironomini	Pseudochironomini/Chironomini	9.1
Corixidae	*Trichocorixa*	6.8	Physidae	*Physa*	6.3
Hydracarina		5.4	Pleidae	*Neoplea*	6.0

We produced three tables relating to macroinvertebrate community functional group and flood impact: Taxa by major dispersal method (Table 21), taxa by primary dispersal method (Table 22), and a cross year comparison of flood-affected taxa by unit CPUE (Appendix VI). We found trends in all three results. Comparing flyers to nonflyers, we found that MC had the largest spread, as well as the most overall flyers, and that wetland unit age corresponds closely to the proportion of flyers to nonflyers in each wetland unit.

Results for primary dispersal method largely follow those for flyers and nonflyers, but without the same trend by wetland unit age (Table 21 & 22). MC has the most aerial dispersers, and the fewest aquatic dispersers, although the difference between the number of aerial and aquatic dispersers has reversed, such that MC has the smallest difference between aerial and aquatic dispersers.

Table 21. Unit CPUE of nine most common taxa organized by mobility. Summed unit CPUE of the nine most common taxa, sorted by wing presence or absence of the adult stage of each of the nine most common LOTUs.

	Unit CPUE of nine most common taxa			
Wetland Unit	**MC**	**MN**	**MS**	**P1A**
Flyers	41.33	30.64	21.08	31.25
Crawlers & Swimmers	21.5	50.71	41.16	36.94

Table 22. Unit CPUE of all taxa above 0.5 CPUE 2020 & 2021, summed by primary dispersal method. We coded primary dispersal method from affinity traits (the likelihood of a dispersal method for each LOTU, scored from 0-3) and summed unit CPUE for each dispersal method by unit. Active aerial dispersers fly to colonization sites. Passive aerial dispersers are either blown to sites or are phoretic dispersers. Active aquatic dispersers swim to colonization sites. Passive aquatic dispersers either drift or are phoretic dispersers. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A); years refer to the year the unit was sampled.

	Summed unit CPUE Dispersal method of wetlands units, 2020-2021						
	P1A 2020	**P1A 2021**	**MN 2020**	**MN 2021**	**MS 2020**	**MS 2021**	**MC 2021**
Active Aerial	38.7	20.3	49.9	24.7	21.6	15.2	25.6
Passive Aerial	3	8.6	3.1	5.5	3.5	6.2	7.5
Active Aquatic	13.2	17	29.2	19.4	17	14.1	16.5
Passive Aquatic	83.7	45.8	170.4	51.8	38.1	43	30
Summed Aerial	41.7	28.9	53	30.2	25.1	21.4	33.1
Summed Aquatic	96.9	62.8	199.6	71.2	55.1	57.1	46.5
Difference	55.2	33.9	146.6	41	30	35.7	13.4

Influence of Vegetation Zone

The macroinvertebrate counts grouped by vegetation zone were not significantly different from each other (p>0.0649; Figure 25). Our normality tests showed that, except for in the *Phalaris* zone, distributions failed the assumption of normality. However, the analyses of variance tests are particularly robust to non-normal distributions, and the macroinvertebrate counts did not fail a test for equal variance. While the 25th and 75th percentiles are relatively consistent across vegetation zones, the *Typha* zone and the SAV zone showed larger 75th percentiles and very large outliers.

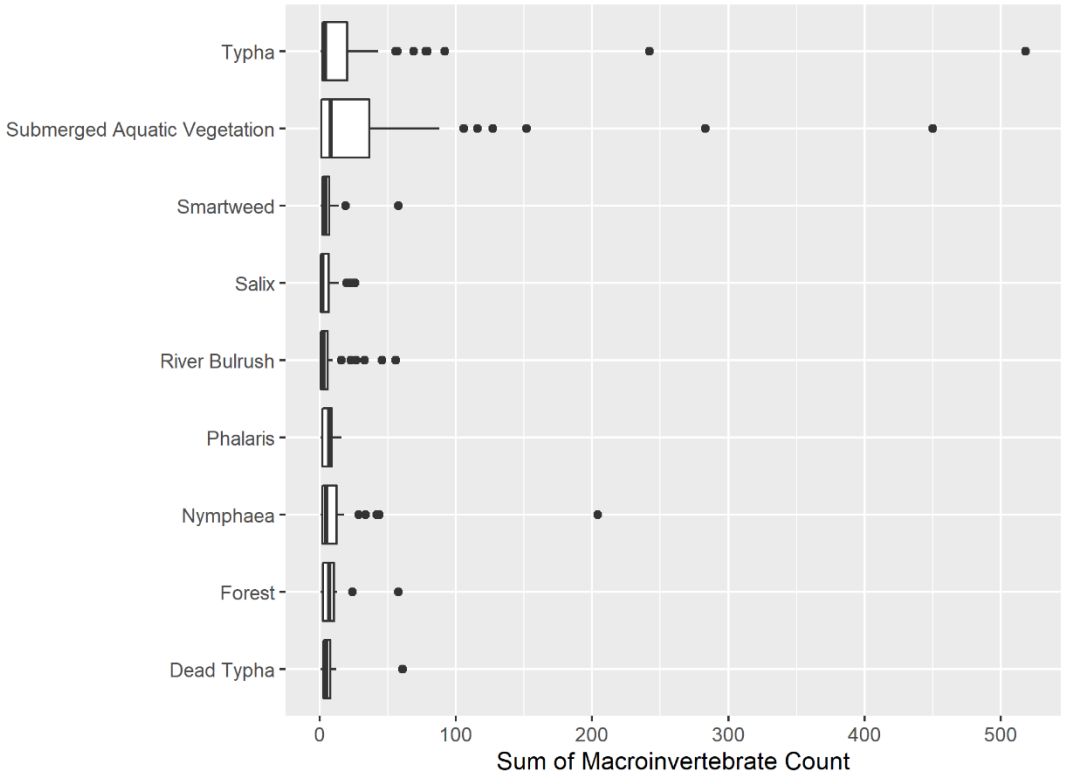


Figure 25. A histogram of summed macroinvertebrate counts divided by the vegetation zone where they were found. Points represent samples farther than X1.5 the 75th percentile. Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); channel is open flowing water with little to no vegetation; dead *Typha* is dead *Typha* sp; *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*, River Bulrush is *Schoenoplectus tabernaemontani*; Smartweed is *Persicaria amphibia*; forest is *Acer saccharum*; *Salix* is *Salix nigra* and/or *Salix exigua*; and *Phalaris* is *Phalaris arundinacea.*

Normality tests performed on LOTU CPUE showed nonnormal results across vegetation zones except *Phalaris*. Unlike for macroinvertebrate counts, however, a test of equal variance on CPUE failed. Note, however, that we used dead *Typha* zone in our ANOVA, but not in NMDS plots. The histogram of macroinvertebrate CPUE by vegetation zones shows a greater variation in quartiles than that for macroinvertebrate counts (Figure 26). Again, because analyses of variance are robust to non-normal distributions, we proceeded with our analysis. CPUE does not vary by vegetation zone for any variable except dead *Typha* (Appendix VIII). We confirmed this variation with a post-hoc test, and found significant differences between dead *Typha* and *Typha* ($p<0.0002473$), dead *Typha* and river bulrush ($p<0.0044711$), dead *Typha* and *Salix* ($p<0.0039626$), and dead *Typha* and SAV ($p<0.0005968$). Dead *Typha* and forest was nearly significant ($p<0.0562847$).

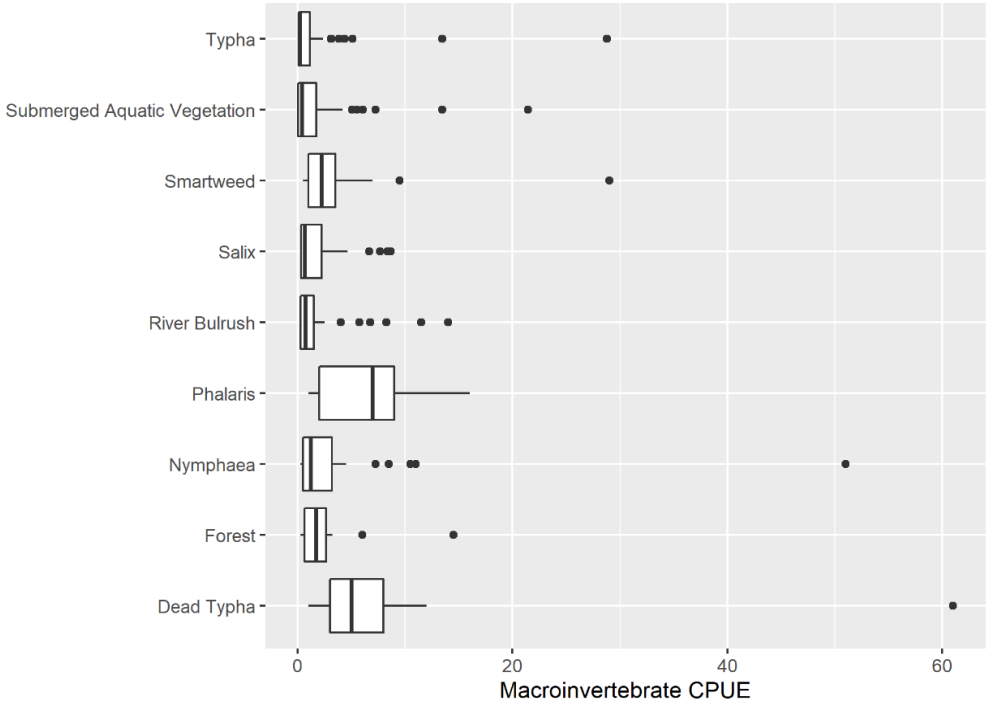


Figure 26. A histogram of macroinvertebrate CPUE divided into the vegetation zone where they were found. Points represent samples farther than X1.5 the 75th percentile. Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); channel is open flowing water with little to no vegetation; dead *Typha* is dead *Typha* sp; *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*, River Bulrush is *Schoenoplectus tabernaemontani*; Smartweed is *Persicaria amphibia*; forest is *Acer saccharum*; *Salix* is *Salix nigra* and/or *Salix exigua*; and *Phalaris* is *Phalaris arundinacea.*

An NMDS performed on macroinvertebrate communities grouped by vegetation zone showed spread of ellipses across MDS1 and MDS2 (Figure 27). The ordination showed overlap between most vegetation zones, however, MDS1 explained a high degree of variation in the forest macroinvertebrate community (Figure 27). The ellipse for forest was most dissimilar from all other ellipses. The ellipses for *Nymphaea*, *Pharalis*, smartweed, SAV, *Typha*, and river bulrush had significant overlap. MDS1 explained significant variation in the forest macroinvertebrate community, although other vegetation zones had a high degree of overlap in both dimensions. However, we did not find any significant difference based on vegetation zone (PERMANOVA, p>0.371, Table 23).

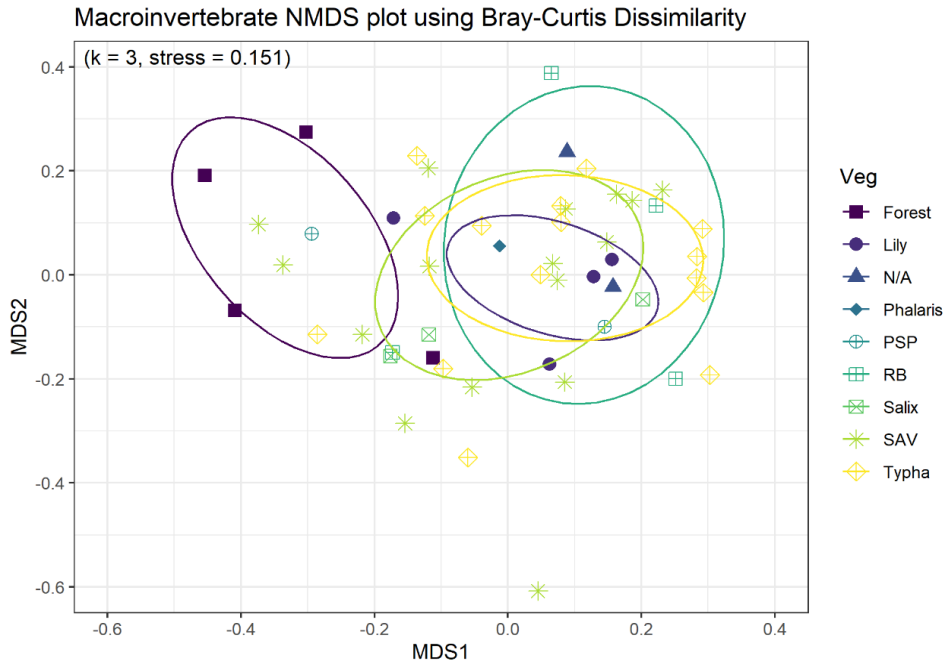


Figure 27. Macroinvertebrate NMDS plot on Bray-Curtis Dissimilarity by vegetation zone. Each colored shape represents a different vegetation zone, and ellipse colors correspond to shape colors. Note that ordination plots require a minimum of four samples, so some vegetation zones are represented without ellipses. Through computer or user error, we also produced two samples without a vegetation zone, labeled 'N/A.' Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (MEV); channel is open flowing water with little to no vegetation; dead *Typha* is dead *Typha* sp; *Typha* is *Typha spp*; *Nymphaea* is *Nymphaea odorata*, RB is *Schoenoplectus tabernaemontani*; Smartweed is *Persicaria amphibia*; forest is *Acer saccharum*; *Salix* is *Salix nigra* and/or *Salix exigua*; and *Phalaris* is *Phalaris arundinacea.*

Influence of Month

We tested normality and variance, and found an insignificant variation in CPUE between months (P> 0.697). While none of the four months were normally distributed, they did produce equal variance. A very large outlier for *Hyalella* visible in both the histogram for CPUE (38.428) may have influenced our sample. The large number of outliers may have skewed the macroinvertebrate counts, resulting in a false rejection of the null hypothesis.

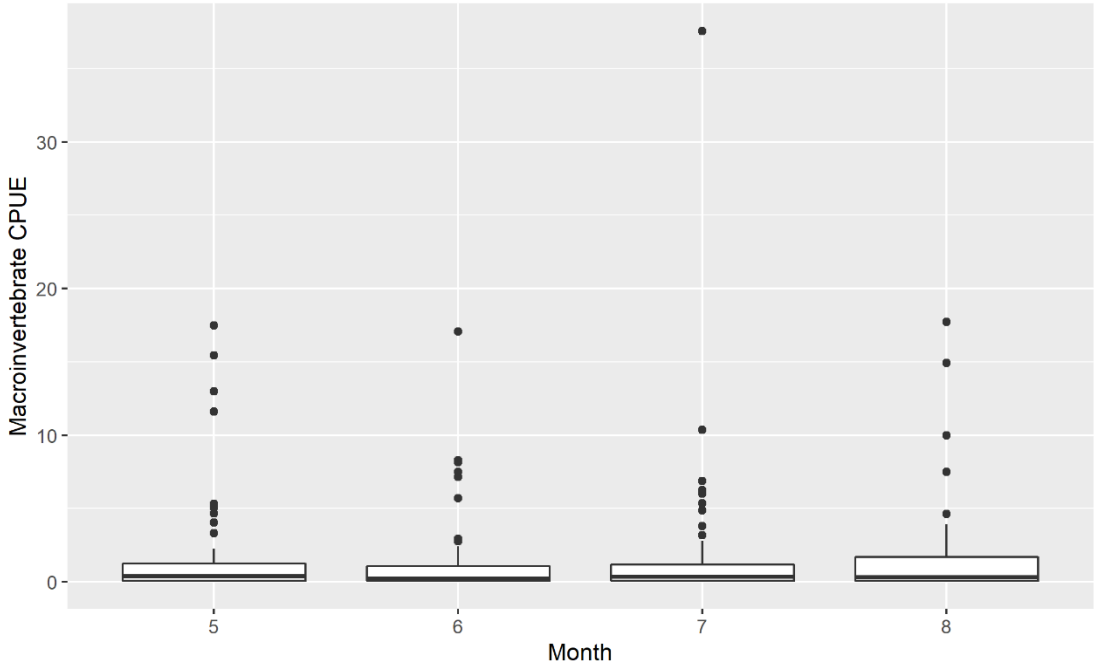


Figure 28. A histogram of macroinvertebrate CPUE by month. Months are represented by calendar month. A single large natural outlier representing *Hyalella* is visible above 35 CPUE in the histogram.

Our PERMANOVA of macroinvertebrate presence-absence by month revealed a significant difference between months ($p>0.002$, $R^2 = 0.128$, Table 23). In ordination space, ellipses of macroinvertebrate communities by month partially overlapped, with the spread of the ellipses visible across MDS1 and MDS2 (Figure 29). The community declines on MDS2 and increases on MDS1 from May to June, then increases on MDS2 and MDS1 from June to July, and finally increases on MDS2 from July to August.

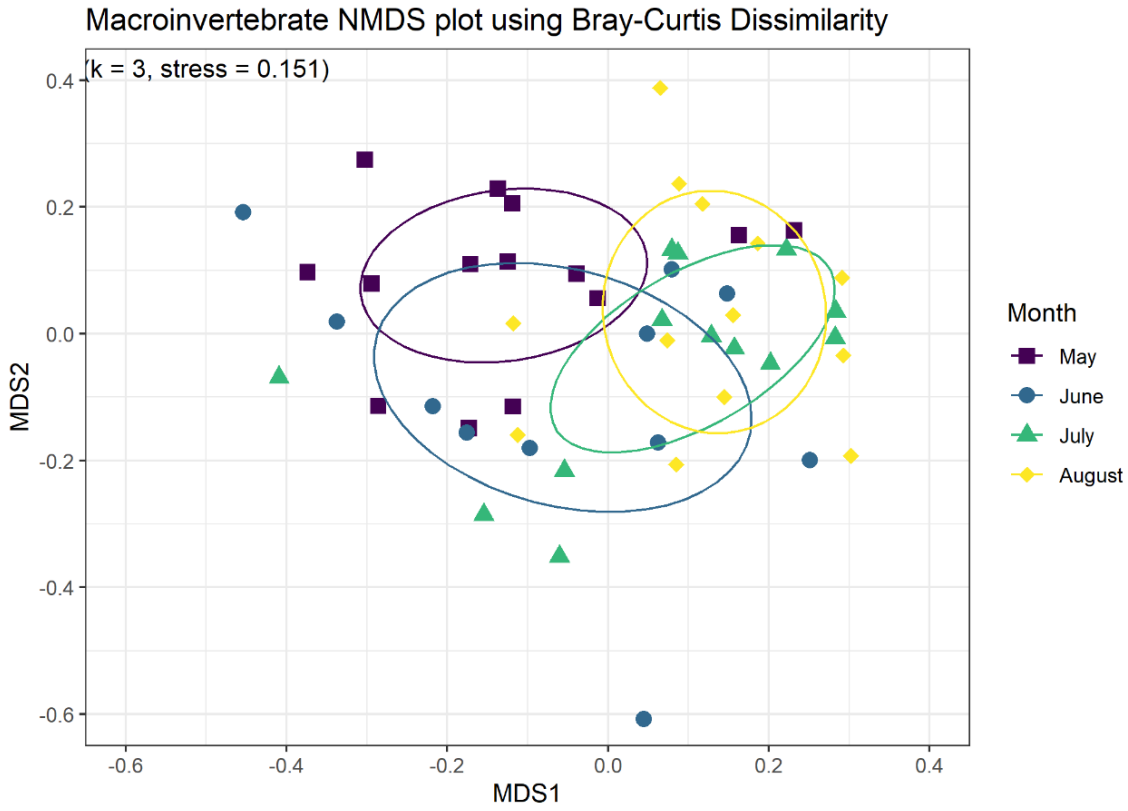


Figure 29. Macroinvertebrate NMDS plot using Bray-Curtis Dissimilarity. Months are shown as distinct shapes and colors. Ellipses are colored to correspond to each month. June shows the largest variability across MDS 1 and MDS2.

Influence of unit

We ran a PERMANOVA and found significant variation in communities between units. The PERMANOVA by unit showed a significant difference between units (p>0.011, Table 23) with an R-squared value of 0.099, meaning that variation in units explained approximately 9.9% of the variation between communities. In ordination space, there was a small difference in the ellipse centers increasing across MDS3 and MDS2 between MS, MN, P1A, and MC (Figure 30). The variation was intermediate between the ordination space for vegetation zones and that for months.

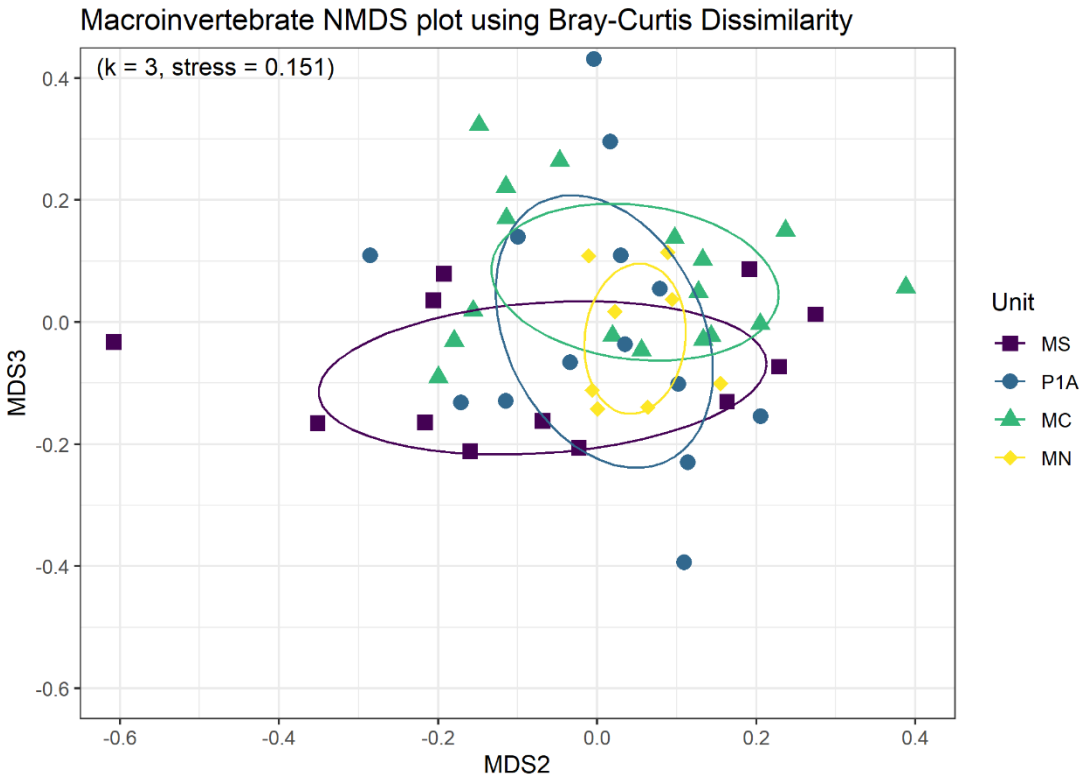


Figure 30. Macroinvertebrate NMDS plot using Bray-Curtis Dissimilarity. Wetland units are shown as distinct shapes and colors. Ellipses are colored to correspond to each month. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A).

Table 23. Permutational analysis of variance (PERMANOVA) effect size and significance (p-value), and ANOVA signficance (p-value) for macroinvertebrate communities tested against vegetation zone, month, and wetland unit. Effect size is higher for vegetation zone but still insignificant.

Permutational Analysis of Variance (PERMANOVA)

	Effect Size	P-value
Vegetation Zone	16.60%	<0.371
Month	12.20%	**<0.002**
Wetland Unit	9.90%	**<0.011**

ANOVA P-values

	CPUE	Count
Vegation	**<0.000154**	<0.0649
Month	<0.877	<0.697
Wetland unit	<0.841	<0.871

Influence of water quality

To examine relationships between water quality variables and macroinvertebrate communities, we used a linear regression model with temperature, conductivity, dissolved oxygen, turbidity, and pH as explanatory variables and macroinvertebrate count as a response variable. We found in checking model assumptions a strong correlation between dissolved oxygen and pH (73%), and a variance inflation factor of 2.78, suggesting high multicollinearity. The linear model results showed a significant correlation between macroinvertebrate count and pH ($p>0.0211$), however, the adjusted R-squared explains less than 1% of the variation (0.006). While multicollinearity and a small R-squared value (i.e., effect size) do not invalidate the results, they do suggest the effect of pH will be difficult to discern.

Restoration Success

The macroinvertebrate Index of Biotic Integrity has varied from year to year without a distinct trend. In 2021, we found that MN, P1A, and MS were moderately degraded, and that MC was moderately impacted. These values were slightly lower than in previous years. In 2020, the research teams rated all three units as mildly impacted, and in 2019 MN and MS were rated as moderately degraded, while P1A was mildly impacted, but these 2019 scores were later corrected for an error, resulting in all values being rated as mildly impacted (Table 24).

Table 24. IBI scores for each unit and year. Possible scores from most anthropogenically impacted to most pristine are 'degraded,' 'moderately degraded,' 'mildly impacted,' and 'reference' conditions. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Results are organized by sampling year. Note that these are calculated differently from IBIs for vegetation and fish.

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

DISCUSSION

Excluding microcrustacia, the most abundant nine families of macroinvertebrates represented 80% of our catch: Hyalellidae, Chironomidae, Caenidae, Coenagrionidae, Corixidae, Physidae, Hydracarina, Pleidae, and Aeshnidae. Lugten et al. (2020) and Dellick et al. (2021) (representing sampling seasons 2019 and 2020, respectively) found similar families and abundance, with some notable variation. In sampling season 2020 the nine most common families represented 86% of the total catch, and in 2020, 79.4% belonged to these families. Because most (but not all) of the nine most common families represent only one or two LOTU, we should expect similar results from past years not just among families, but also genera. Furthermore, because marshes experience large fluctuations in water levels and summer water quality, we found the majority of taxa identified are tolerant of variation in water quality and levels. We also found SNWR macroinvertebrate communities across years to be representative of Great Lakes marsh macroinvertebrate distributions, with a few exceptions.

Comparing our results to much larger datasets, Cooper et al. (2014) described ten cosmopolitan taxa that represent 61% of the community from samples across Great Lakes coastal marshes; with much smaller numbers of rare taxa representing the remainder of the sample. This is less than at SNWR, where the ten most common taxa represent 69.5% of our sample. This distribution results in what the authors called a “hollow species abundance curve,” comprising a small number (10-20) of widespread and abundant taxa and a large number of rare taxa, resulting in extremely low community evenness. Such a distribution has important implications for sampling effort, because we can represent the most common species with little effort, but a complete sample of all taxa will, in contrast, be quite difficult to obtain. Future researchers should not be surprised to field-pick most of their data from 10-20 hyperabundant LOTUs, and, conversely, find occasional, anomalous individuals that are superficially unrepresentative of the community.

However, SNWR’s ten most common taxa are not most similar to those found in Saginaw Bay marshes, but are instead a combination of taxa common to Saginaw Bay and other Great Lakes marshes (Cooper et al. 2014).These are important distinctions, because taxa can be used as indicators for several different wetland features: quality, fetch, and biogeography. For example, SNWR contains high relative abundance of *Ischnura* and coenagrionids in its ten most common taxa, which is more like Saginaw Bay communities; but also high relative abundance of *Physa* and corixids across all three years, which is more like Great Lakes marshes in general. On the other hand, the SNWR study units have never recorded *Gammarus*, *Mesovelia*, or oligochaetes in their top ten taxa, all three of which are common to both Saginaw Bay and Great Lakes wetlands (although we have recorded them low in the top twenty). Thus, SNWR may

uniquely represent an interesting combination of the most common taxa from Saginaw Bay and other Great Lakes wetlands. All the wetlands from which we are comparing results are coastal, lacustrine marshes, so we should expect differences both because SNWR is a uniquely inland floodplain wetland with coastal effects, and because it is a recently restored wetland. There are several other reasons that taxa may not exactly match Saginaw Bay taxa, but we believe the results showed that SNWR is not simply an “inland coastal estuary” (U.S. Fish & Wildlife Service 2018), but rather a restored hybrid wetland, with abiotic and biotic conditions representative of both coastal lacustrine wetlands and inland floodplain wetlands, and perhaps others.

Is fetch a defining feature of SNWR?

Fetch is the length of water over which wind has blown without obstruction, and is one of the more important physical variables influencing macroinvertebrate communities in GLCWs (Burton et al. 2002; Burton et al. 2004; Cooper et al. 2014). This hydrologic feature is likely less dominant in SNWR than in fringing marshes, drowned river mouths, open embayments, and other coastal Great Lakes marshes (Burton et al., 2004). Burton categorized three fetch communities: high-fetch marshes containing higher densities of *Sigara*, *Trichocorixa*, *Oligochaeta,* and *Bezzia*; low-fetch marshes containing higher densities of *Gammarus*, *Crangonyx*, *Caecidotea*, Chironomini, and Tanytarsini; and finally mid-fetch marshes containing higher densities of *Hyalella,* Tanypodinae*,* Libellulidae*, Caenis, Callibaetes, Physa,* and Hydracarina. We used characteristic invertebrate taxa from Burton et al. (2002), and while Burton et al. (2004) and Cooper et al. (2014) both agree with these groupings, specific taxa representative of each fetch category vary by region, and we discuss these differences below.

Overall, the low-fetch marsh taxa represented 9.48 total CPUE (number of individuals collected by each taxa divided by total number of sample sites, 60) whereas the high-fetch taxa represented 3.9 total CPUE, and the mid-fetch taxa represented 42.85 total CPUE. While there was some uncertainty with regard to identification due to differences between Burton et al. (2002) and our study (see metadata), under current biotic and abiotic conditions our data showed that SNWR macroinvertebrate communities represented a mid-fetch marsh.

When the fetch taxa are analyzed by unit CPUE, all units have more taxa representative of mid-fetch marshes than low-fetch marshes except MS, and more low fetch than high fetch taxa. The only exception is MS, where high-fetch (9.17) and low-fetch (8.17) taxa were similar, but with significantly more mid-fetch taxa (45.69). This is due in part to the large number of *Trichocorixa* present.

However, it is a very common genus in freshwater systems, so its presence is not necessarily definitive on the influence of fetch.

Although mid fetch may be a defining feature of SNWR, anthropogenic influences complicate our interpretation. Cooper et al. (2014) found that percent agriculture in the watershed and fetch have higher influence on Saginaw Bay wetlands than ambient water quality. Cooper et al. (2014) also found similar directional influences on an NMDS axis of macroinvertebrate communities for these two features. Our multiple regression analysis on four water quality parameters as explanatory variables for macroinvertebrate CPUE produced significant results for pH and dissolved oxygen, but with an effect size of only 0.6%. Such a small response supports the conclusion of Cooper et al. (2014) that ambient water quality is not an important explanatory variable for macroinvertebrate distributions in the region, and that we should look to fetch and agriculture to describe distributions, among other factors.

The four tributaries that drain into SNWR have predominantly agricultural watersheds; in fact Cooper et al. (2014) defines HUC 6 (Hydrologic Unit Code 6), the watershed that contains SNWR, as an “agricultural watershed.” So, if agriculture and fetch have similar effects on macroinvertebrate communities in SNWR, as Cooper et al. (2014) found for Saginaw Bay marshes, then SNWR should have the macroinvertebrate abundances of a high-fetch marsh. But instead, we find the extremely low levels of *Gammarus, Crangonyx,* and *Caecidotea* at SNWR, taxa that are usually more common in high fetch wetlands, and very high dominance of highly tolerant and widespread taxa such as *Hyalella*, *Physa,* and *Caenis*. SNWR is not only low in *Gammarus* relative to Saginaw Bay marshes, but as previously mentioned, also in relation to GLCW sites across Michigan. In fact, Zapelloni et al. (2021) showed that *Hyalella* density and sex ratio were not affected by phosphorus concentrations, a chemical that could be indicative of agricultural runoff. Moreover, *Physa acuta* is a globally invasive species (Van Leeuwen et al. 2013), a pattern that suggests a high tolerance to agricultural freshwater habitats. Even though SNWR does not have nutrient concentrations indicative of eutrophication, other factors upriver (low quality or absent riverine edge habitat, other less well-monitored, high sediment loads; see Water Quality, this report) could sufficiently affect macroinvertebrate metapopulations to reduce recruitment into the refuge. Indeed, regional and even global macroinvertebrate distributions may have declined so much that pristine wetlands will not provide a suitably large metapopulation to aid recruitment into the refuge of more sensitive species (Hallmann et al. 2017).

Furthermore, because agricultural runoff, watershed channelization to control farm irrigation, and sedimentation from tillage have historically negatively affected Saginaw Bay water quality; some of these effects may now be diverted into and concentrated within SNWR’s largest marsh units, meaning watershed-

level effects that were once felt at upriver sites or outlets to the Saginaw Bay are now felt in the SNWR floodplain itself. Specifically, Cooper et al. (2014) reported that 61% of Saginaw Bay wetland macroinvertebrate abundance was represented by ten cosmopolitan taxa. Although SNWR communities appear to contain a mixture of organisms common to Saginaw Bay marshes and other mid-fetch Great Lakes coastal marshes, they also show an even higher concentration of cosmopolitan taxa than most Great Lakes coastal wetlands (GLCW), with just ten taxa representing 73.5% of SNWR marsh abundance. Lugten et al. (2020) and Dellick et al. (2021) found even higher relative abundance of their top ten taxa, and although the abundances of each taxa varied, almost all families and genera remained in the top ten across all three years. This high abundance of cosmopolitan taxa could be due to the recent restoration of our sampling units, or the negative effects of agriculture in the watershed. However, another author characterized every Michigan watershed by their level of agriculture or development, and found a strong negative correlation between agriculture and macroinvertebrate functional diversity, voltinism, and lifespan (Kovalenko et al. 2014). He reported some of the highest levels of agriculture in HUC 6.

Burton et al. (2004) does suggest a different characterization of low and high-fetch taxa for Saginaw Bay. However, among the 8 taxa in that study representative of fetch distributions, they are either identified to a level beyond our expertise (e.g., *Stylaria, Ischnura verticalis*) or absent from our findings (e.g., *Hydra*, *Nectopsyche, Agraylea*). Even if recalculated using the Burton et al. (2004) taxa, we are confident the result would categorize SNWR as a low fetch wetland, because of the importance of Hydracarina and Caecidotea both in our samples and low-fetch Saginaw Bay marshes. Finally, across both studies as well as the later Cooper et al. (2014) effort in Saginaw Bay wetlands, there is a negative correlation between crustacean abundance and fetch, but SNWR has very high densities of *Hyalella*. Cooper et al. (2014) nonetheless states that his results are applicable basin-wide; this was regardless of taxonomic level or whether Saginaw Bay wetlands were considered. All three papers also note that fetch taxa are broadly categorized by vegetation zones, specifically an outer *Scirpus* zone, an inner *Scirpus* zone, and a *Typha* zone (these are also the primary vegetation zones used in IBI metrics). If fetch had any sort of dominant influence at SNWR, even if entirely distinct from its effect on GLCWs, we might observe at least some significant differentiation by vegetation zone. But our NMDS did not show any significant differences across the eight vegetation zones we sampled. Therefore, these results only further confirm the discordance between the influence of agriculture and fetch at SNWR, and the incomparability of SNWR and Saginaw Bay marshes.

The similar effects of agriculture and fetch on macroinvertebrate distributions suggest either that fetch is not a dominant influence at SNWR or that the tests for influence of fetch that are calibrated against Great Lakes marshes are not useful

at SNWR. That is, if fetch and agriculture are multicollinear, we would expect SNWR macroinvertebrate communities to reflect a high fetch wetland. A final possibility is that the presence of dikes at the refuge combined with its distance from the coast reduces fetch. But our discussion instead shows a wetland with very strong influence from agriculture and a macroinvertebrate distribution characteristic of a low or mid-fetch wetland. If diking and distance from Lake Huron also affect taxa, these effects will have to be separated out in future research. SNWR is not a Great Lakes coastal marsh, but requires a different designation.

Is Burton's IBI useful at SNWR?

Multiple abiotic explanations could account for the lack of a clear trend in IBI scores either between units or years, but here we will only offer three: methodology, flooding, and island biogeography.

First, some methodological issues exist. The marsh sites used to calibrate the IBI all around Saginaw Bay contain an inner and outer *Scirpus* zone, SNWR does not have any Scirpus vegetation zones, but only the *Typha* zone (Burton et al. 1999). SNWR instead has patches of SAV, river bulrush, *Salix*, floodplain forest, and even those that are still entirely unsampled like purple loosestrife (*Lythrum salicaria*) or undersampled like mixed emergent vegetation (MEV). GLCWs also receive more lacustrine hydrologic influence from fetch, seiche, and storm events; whereas SNWR, while receiving those lacustrine influences, primarily absorbs water from its tributaries, and most immediately the Shiawassee River. All of these factors mean that Burton et al. (1999) even if it provides a roughly accurate direction of change from year to year, does not necessarily capture refuge quality or details of macroinvertebrate abundance and change. We must stretch the usefulness of the current macroinvertebrate IBI by using only *Typha* zone communities which undoubtedly represent only a small portion of refuge richness, given the many other zones that we undersampled or tossed from the IBI analysis.

Second, so far as we are aware, none of the wetlands from which our IBI was developed have undergone quite such complete drainage, conversion to farmland, and subsequent restoration as has SNWR, although a few are otherwise heavily altered (Burton et al. 1999; Uzarski et al. 2004; Coastal Wetland Monitoring Program 2019). That is, SNWR is a complex of diked wetlands managed for waterfowl populations, ecosystem services, and wildlife conservation. Our research and current refuge managers are attempting to understand fish movements for future management goals, as well. Constructed wetlands will not behave the same way as natural wetlands, and they are not

intended to do so; the reference conditions that define a pristine GLCW will never be applicable. Wetlands near SNWR received a ‘moderately degraded’ rating, in comparison to other aquatic macroinvertebrate IBI analysis across the state (Appendix XI). It is entirely possible that a floodplain wetland with a high macroinvertebrate richness measured with some other tool will not attract desired waterfowl as well as one that artificially ranks lower or higher with the current IBI tools.

Waterfowl wetlands favor ducks and geese that prey on macroinvertebrate communities (Batzer and Boix 2016). Lamellar density and foraging habit (diving or dabbling) affect the invertebrates that waterfowl select. While the IBI assessment is modeled on lake fringing wetlands, the study units are more like floodplain wetlands (Brinson 1993; Burton et al. 1999). Dikes produce similar hydrogeomorphic effects as enclosed flooding wetlands or perhaps depressional wetlands, but when managers open water control structures or water levels are low, the SNWR marsh units can more closely mimic fringing wetlands through diel water level changes and seiche events (Batzer and Boix 2016; U.S. Fish & Wildlife Service 2018). Nonetheless, the units are never completely open to the river except through the water control structures, so less water mixing must occur than in a fringing wetland, which is completely unprotected from its lake source. Mudflats, sandflats, and shallow water on fringing wetlands attract shorebirds, but these are not as often available at SNWR as waterfowl habitat. Increasing macroinvertebrate abundance can benefit waterfowl, but tradeoffs exist between macroinvertebrate richness and abundance, and waterfowl abundance. For example, MC has the most variable habitat as measured both by our FQA and IVI (see “Results" and “Discussion,” in Vegetation section, this report) and the bathymetric report by Heitmeyer et al. (2013), and our macroinvertebrate NMDS by unit showed MC with perhaps the largest positive values for its ellipse (Figure 30), as well as the IBI that rated that unit as less impacted than the others. Because the more typical waterfowl wetlands of MN and P1A have lower IBI scores (and those animals were most often observed there during the field season), it is our thesis that management for waterfowl in MC could in fact degrade the macroinvertebrate richness of that unit. Currently MC contains large open-water habitat managed for American white pelicans, and MEV vegetation zones, as well as dense stands of *Salix* and tree cover more suitable to birds that roost and hide, rather than waterfowl that require long take off and landing water, and unobstructed views of potential predators.

While NMDS axes are not reducible to single influences, a number of abiotic and biotic explanations exist for MC’s unique plant and animal distributions. MC’s center is farthest from the distribution basin and has the most variable bathymetry. Moreover, MC’s variable bathymetry results in greater habitat variety for avian predators that segregate based on water depth, potentially resulting in top-down selection on different macroinvertebrates that segregate by water

depth, and thus resulting in the higher macroinvertebrate richness in that unit, as shown in our IBI (Brinson 1993; Heitmeyer et al. 2013). Another possible influence on macroinvertebrate abundance is benthivorous fish presence, specifically carp and bullhead, which can also exert a strong influence, but we did not find significant differences in fish presence between units, so this unlikely an explanatory factor (Batzer 1998). The most likely explanation is that MC is more protected from river influences and offers refugia to longer lived macroinvertebrates like the coenagrionids found in higher abundance there than in other units (Appendix VI). MC's variability and distance from the river also provides refuge from the negative effects of extreme river fluctuations typical of modern, anthropogenically degraded rivers, which fluctuations have a larger and more negative effect on macroinvertebrate communities than river systems with fewer dams (Moi et al. 2020). The dikes may also function like dams or obstructions to animal dispersal and water movement, adding harsh variability to the system that decreases macroinvertebrate richness and abundance more still than natural wetlands (Pollock 2017). We discuss these results on MC more in "Successional Change or Biogeography," below.

Finally, the dramatic management history within the watershed may have resulted in a significant, long-term effect on the successional trajectory of the SNWR study units (Janssen et al. 2021). The IBI was developed nearly a quarter century before our use, so climate change, invasive species introductions, changes in nutrient cycling, and novel anthropogenic pollutants could obscure the original macroinvertebrate assemblages and with them the definitions of a "healthy" wetland (Batzer & Boix 2016).

Thus, SNWR contains habitat, management decisions, and hydrogeomorphology that make it similar to waterfowl wetlands, floodplain wetlands, coastal wetlands, agricultural wetlands, and because of its human history, even constructed wetlands. This hybrid designation merits special attention and could add flexibility to management decisions. Separating out the effects of each of these to define a macroinvertebrate community is beyond the scope of this work, but strongly recommended in future efforts.

Flooding and Macroinvertebrate Communities

While we identified some of the methodological and biotic influences on IBI, it is likely that abiotic events also influenced our NMDS and IBI results. Time lags are natural to population growth and decay, Lotka-Volterra models, trophic cascades, and a number of other ecological phenomena, and large resource influxes will often result in lagged responses. We found in reviewing our data that the 2020 Edenville dam failure and subsequent flood (see introduction) induced a

widespread decline in taxonomic richness, evenness, median relative abundance of indicator taxa, and Shannon and Simpson Diversity results. This change accounts for the fact that all the units remained stable at “mildly impacted” from 2019 to 2020, and then declined (except MC—see below) to “moderately degraded” in 2021. While we have discussed the different methods that Dellick et al. (2021) used in their IBI and we later discuss the other abiotic and biotic influences on our IBI scores, our results clearly show that the 2020 flood incontrovertibly affected macroinvertebrate communities in a way consistent with the observed decline in IBI scores (Table 24 & Appendix VII). Indeed, in the case of GLCWs, large hydrologic events are a prevailing factor in macroinvertebrate community dynamics, especially large storm events when hydraulic forces restructure sediment, deposit large nutrient loads, redistribute propagules, and alter vegetation zones (Batzer & Boix 2016). Moreover, during extreme events, some taxa may increase, others decline, and still others show relatively little change. We observed many of these changes predicted by the literature across all units and major taxa, and discuss these results below. Finally, although our analysis of CPUE also showed results consistent with flooding in MC, we did not have a comparable 2020 dataset for that unit, so we will not discuss it here, but in our later section on successional change and island biogeography theory (see below). Unless otherwise specified, results below only refer to P1A, MN, and MS.

During extreme fluctuations in water levels, macroinvertebrate communities may show a delayed response of one to several years (Cooper et al. 2014). Researchers have observed both little change concurrent with extreme events (fluctuations greater than one meter), and a significant change in major taxa one to several years after those events (Burton et al. 2002; Uzarski et al. 2004; Cooper et al. 2014). After a large drop in water levels in Great Lakes marshes during 1997-2001, *Hyalella* abundance increased ~30-40% in the next several years, insect abundance decreased ~25% in a single year, and chironomid abundance decreased 10% (Cooper et al. 2014). If we assume this delayed response but with response to an extreme *increase* in water levels, the same taxa changed in the opposite direction, one year after flooding. When we compared total CPUE across years, *Hyalella* decreased by ~45 CPUE, and chironomids increased by ~8 CPUE. Contrary to the aforementioned GLCW research, however, we observed a decrease in insects like libellulids, coenagrionids, and caenids, although corixids increased. However, the differences between a lacustrine decrease in water levels and a riverine flood are likely sufficiently large to cause a different response. The late successional communities characteristic of long established GLCW would also respond differently to catastrophic events.

Our results also showed declines within units consistent with literature and assumptions about the life history of different macroinvertebrate taxa. Cooper et al. (2014) determined that crustaceans predictably increased in response to

water level decreases. We found the same strong negative relationship: *Caecidotea* and *Gammarus*, the only other major crustacean taxa at the refuge besides *Hyalella*, also declined in all wetland units from 2020 to 2021 by an order of magnitude. While *Hyalella* declined even more dramatically, we found a slight increase in MS *Hyalella* from 2020 to 2021, likely due to the slightly higher elevation of that unit. The likely lower abundance of fish (with the exception of the outlier bullhead sampled in the disconnected remnant drainage ditch, coded as the forest zone of MS) as well as low water level more suitable to *Hyalella* probably resulted in the slight increase observed in that unit, even though P1A *Hyalella* declined by nearly 70% and MN by 65%.

Another taxon that showed inconsistent variation as a result of the flood was Oligochaeta. We again found declines in all units except MS, and this may be due to the difference in elevation. Oligochaetes are bottom dwelling taxa (Vieira et al. 2006), so the higher elevation of MS may also have prevented sediment deposition by burying them after the flood. Rader et al. (2008) also report Oligochaetes as hardier organisms that could withstand the effects of flood, thus accounting for their variable response in the slightly more protected MS.

While it is possible to theorize about variable responses to the flood depending on the taxon and unit, we observed the strongest evidence of flooding in the eleven other LOTUs that declined and the nine that increased within all three units from 2020 to 2021. Along with crustaceans, and with the exception of *Physa*, all major gastropods, molluscs, and leeches declined within units from 2020 to 2021. This included *Planorbella,* Sphaeriida, Branchiobdellida, *Helobdella*, and Glossiphioniidae. The literature does not show a clear reason for Planorbella declines in response to floods (Vieira et al. 2006; Serremejane et al. 2020), and the family is reported as an aquatic passive disperser capable of living in water up to 3 m deep (i.e. the approximate depth of some flood refugia at SNWR). Nonetheless, Cooper et al. (2014) does show a negative correlation between gastropod abundance and water level, so our findings are consistent. We discuss Sphaeriida later (see “Successional Dynamics or Island Biogeography?” in this discussion), but the dramatic declines in that taxa are clearly also a result of flooding and the group’s intolerance of sedimentation. Our review of the literature did not result in a clear reason for the decline of leeches, though like gastropods that use suction to attach to substrates, they may have declined due to a simple inability to hold onto substrates and hosts, been buried by sediment, or for some other reason. Interestingly, some genera are primarily passive aerial dispersers, likely because they preferentially choose avian hosts. There is enough variation between genera and uncertainty in the life history and behavior of these animals that their declines may be due as much to environmental or demographic stochasticity as to some identifiable trait.

Finally, *Physa* (Physidae) was a superabundant LOTU both within the SNWR wetland units, nationally, and as a globally invasive organism (*Physa acuta*). Although it does not follow similar declines as other gastropods, molluscs, and leeches, its global population biology suggests a hardiness and tolerance unique among macroinvertebrates. The higher abundance of that organism could be due to a higher likelihood of selfing, a higher tolerance to poor water quality, or a seemingly unusual dispersal ability for a gastropod (Vieira et al. 2006; Sarremejane et al. 2020). In another study, flooding did not cause strong gene flow in *Physa acuta*, suggesting that floods also do not cause large movements of their populations in rivers.

Insects at the SNWR showed a more variable and complex response to flooding than did the crustaceans, gastropods, molluscs, and leeches. The already superabundant chironomids increased by 100% in MS, more than 100% in MN, and almost 200% in P1A. Although we do not have data on 2020 abundances of chironomid subfamilies and tribes, we have already mentioned that the literature supports an increase in Chironomids after floods. Nonetheless we suspect that closer examinations of the LOTUs or identifications of previous years' sample collections to our LOTUs could reveal differences at the level of subfamily and tribe.

In contrast to chironomids, coenagrionids and corixids showed variable responses. The coenagrionids were the most abundant odonates at the refuge in 2019, 2020, and 2021, and *Ishnura* and other coenagrionids are also the most abundant odonates in Saginaw Bay coastal wetlands (Cooper et al. 2014). Within SNWR, total coenagrionid CPUE (across all units) decreased by 30%, and immature coenagrionids and *Ishnura* sharply declined within all units, while *Enallagma* also declined within units except in MN where it showed a 10% increase. A substitution of *Coenagrion* apparently filled the niche, because that genus increased in all units by roughly the same CPUE as *Ishnura* decreased. The relatively smaller *Coenagrion* increase in MS may again be due to the unit's elevation. The literature on traits suggests potential mechanisms for these niche substitutions. Coenagrion have a slightly shorter life cycle, and a slightly higher affinity for passive aquatic dispersal, making them more likely to establish after catastrophic flooding. Because *Ishnura* – a dominant genus in Saginaw Bay coastal wetlands – are declining at the refuge, we can infer less recruitment from GLCW metapopulations, and a possible long term replacement of *Ishnura* with *Coenagrion* at the refuge. That replacement implies the refuge is indeed a hybrid floodplain wetland, with organisms more suitable to the shorter-term fluctuations on floodplains. Another major genus replacement also points towards the riverine regime.

Like chironomids, corixid total CPUE doubled at SNWR from 2020 to 2021, above even its 2019 levels, but some LOTUs declined while others increased.

Immature corixids and *Trichocorixa* all increased significantly to substitute for *Hesperocorixa* declines, especially in the case of MN. *Trichocorixa* have higher lifelong fecundity, more than one potential reproductive cycle per year, and a greater affinity for active aquatic and aerial dispersal; *Hesperocorixa* have lower lifelong fecundity, fewer reproductive cycles in a year, and only a weak affinity for active aerial and aquatic dispersal (Sarremejane et al. 2020). While more regular, extreme flooding and greater annual fluctuation of water levels does not preclude the continued presence of *Hesperocorixa* at the refuge, it will reduce the establishment of stable populations and continue their replacement with higher densities of *Trichocorixa*.

All these population fluctuations took place in a single year, which is much faster than the several years of population restructuring observed in Cooper et al. (2014). But the water level decline in that study was a slow three-year drop of 1m from 1998 to 2001, whereas the 2020 flood increased water levels by approximately three meters in only one to five days, and we are using three years of IBI scores, rather than a fifteen-year dataset. But researchers have also observed declines in macroinvertebrate taxa richness and density across different habitats after floods, and it is to those studies, rather than GLCW research that we should look for comparisons (Giller et al. 1991; Rader et al. 2008). SNWR also recorded a steep decline in richness and abundance from sampling season 2020 to 2021, considerably lower even than 2019 results (Table 20). While surprising, this universal drop in macroinvertebrate quality, concomitant with habitat-wide niche replacement one year after a major storm event is in fact a common result of flooding (Rader et al. 2008).

The ecological cascade caused by the flood underscores the refuge's floodplain hydrologic regime. We have already shown from comparisons of SNWR with basin-wide macroinvertebrate surveys and fetch categorizations that the refuge is only partly comparable to GLCWs. The dramatic results of flooding only further confirm SNWR is far more influenced by river events like flooding, and is not an inland coastal wetland, but a hybrid wetland.

Nonetheless, due to changes in sampling and analysis between years, as well as other confounding factors, and even regardless of other factors, at least 5-10 years of continuous monitoring would likely be necessary to observe the full effects of the catastrophic Edenville dam failure. While analysis of the anthropogenic industrial chemicals released into the refuge and the difference between anthropogenic and natural flooding is beyond the scope of this work, flooding is overall a natural process and can be beneficial in the creation of new wetlands. Moreover, community-level responses to the flood might not have long-term consequences if successional dynamics have a greater influence than this single flooding event. Before drawing any conclusions, then, we must consider that the Edenville dam failure occurred simultaneously with the reconnection of

MC to the floodplain, and only two years after restoration of MN & MS. As much as it was an environmental disaster for surrounding communities, the 2020 flood could also be seen as a natural hydrologic reconnection event of MC to the river and the natural recreation of that wetland to its precolonial condition. In order to fully understand our results, then, we will have to disentangle responses to the flood from the complex successional dynamics already present at the refuge.

Can we use successional dynamics to compare wetland units?

Research on wetland succession is extremely limited (Batzer and Boix 2016). Researchers have noted that wetland succession, particularly macroinvertebrate succession, is not a “linear, directional, deterministic process” (Batzer and Boix 2016). Instead, there are numerous possible successional paths that occur at different rates, depending on local biotic and abiotic factors, and even then, resulting differences between successional stages may not be clear. Moreover, our comparison of P1A, MS, MN, and MC represent a “space-for-time substitution,” wherein the younger units are compared to the older units to approximate how a single unit might change over time, thus ignoring the unique features of each. For those reasons, we offer only a preliminary assessment here.

Adult flying hemipterans, dipterans, and coleopterans are often early colonizers (Batzer and Boix 2016). However, unit CPUE of these orders produced a negative correlation with age: MC had 24.7, MS had 25.88, MN had 30.33, and P1A 36.69. These numbers do not rule out clear successional change initiated by those orders, because even if they are early colonizers, their initial abundance may still be smaller than in the more established populations of the older wetland units. These findings do not exclude successional change as a driving force but instead point to the dynamic, nonlinear nature of wetland succession. More importantly, the catastrophic flood restructured the entire community and SNWR may be temporarily mimicking an early successional wetland.

The most basic outline of succession involves vegetation. Our PERMANONA of macroinvertebrate counts returned significant differences between units, but we cannot easily extrapolate to real-word variation. In the NMDS ordination of counts by unit, MS has a larger ellipse than the others with a long spread across MDS2. In the ordination of counts by vegetation, the forest zone has an ellipse separate from all other zones, and we only sampled the forest zone in MS. The sampling site is likely a section of remnant irrigation channel in which large silver maples have grown, and is disconnected from the main channel parallel to the dike, such that standing water acts as a depressional wetland.

Another unique feature of MS is that the *Typha* zone did not regrow until June, months later than other units. We coded this as dead *Typha* in May, and sampled

SAV or forest zone in MS for the remainder of the season, and we did not sample living *Typha* in MS until July. MS is at a slightly higher elevation than the other units, which probably prevented regrowth of *Typha* until precipitation increased and water levels rose (Brinson 1993). The higher elevation also encouraged water to pool in the remnant channel that is now forest zone.

These factors created a unique sampling site in MS with highly skewed distributions of macroinvertebrate communities. But NMDS ordinations are robust to non normal distributions, so our ellipse for MS likely reflects the unit's high elevation, small number of vegetation zones, or some combination of other unique features, rather than failures of the model. We have already shown that the MS macroinvertebrate community did not respond to flooding like the other units. Although the metacommunity is consistent across units, within MS *Hyalella* and Oligochaetes increased unlike P1A and MN, and genera like *Anax* and *Coenagrion* increased less in MS than in P1A and MN, as well as having a much smaller overall CPUE in MS. Additionally, chironomids and hemipterans made up almost all the CPUE for those units. Their extreme abundance may also be due to the shallow, low-oxygen, warm water at SNWR throughout much of the summer. These factors suggest that short-term water level fluctuations and occasional flooding, rather than biotic succession of slower growing, competitive organisms replacing faster-growing, uncompetitive organisms, controls short term assemblage structure and population abundance at SNWR. Long term processes are likely only observable in longitudinal datasets, where, for example, P1A's faster recovery rate from flood events may become apparent.

The flooding regime and local hydrogeomorphology overtaking obvious signs of succession is visible in other taxa as well. The IBI scores are based on median relative abundance of Odonata, Gastropoda, Amphipoda, and Sphaeriida, as well as taxa richness of Crustacea and Mollusca, Shannon and Simpson indices, and overall richness and evenness. Although sphaeriids are a good indicator of GLCW wetland quality, they were almost entirely absent from our study site, and sphaeriid IBI scores were the same for all units (0). The 2020 sampling season found a CPUE of 9.1 for sphaeriids in MN (Dellick et al. 2021). They also combined their macroinvertebrate data from vegetation zones and rated it all as *Typha* zone, whereas we only used macroinvertebrate data collected in the *Typha* zone to rate our IBI. That likely produced some of the different results between IBI scores. But in 2021, we did not identify Sphaeriids in the top five or ten CPUEs for any wetland unit, and our total Sphaeriida CPUE was 0.51 (Appendix VI). Moreover, as already mentioned, 2020 reported higher total richness and abundance, suggesting that methodological differences alone cannot account for all of the change in IBI.

An alternative, ecological explanation is that sphaeriid clams prefer depths less than 2m in firm substrates, and 25 cm in soft substrates, but their abundance

declines at depth, and they are bivoltine (occasionally multivoltine) only in stable environments (Vieira et al. 2006). So a large flood depositing massive quantities of sediment would likely reduce sphaeriid abundance. In fact, that family's preference for shallow, firm sediment may exclude them from future colonization of SNWR except temporarily and in isolated areas. Thus, floodplain dynamics at SNWR may preclude permanent establishment of a sphaeriid clam population, and a successful implementation of the Burton IBI metrics. Moreover, successional stages in SNWR units are not dominated by hydraulic forcing that alters vegetation zones, as in lacustrine wetlands, but by flooding, as in floodplain wetlands.

We cannot assume a simple successional model of wetland quality, and comparisons between old units (P1A), young units (MN, MS), and new units (MC) may not reveal clear information based on temporal differences alone. At the very least, too much variation exists between these units to treat them as simply "space-for-time" substitutes of one another, and continued development of macroinvertebrate richness and abundance will likely depend on whether the macroinvertebrate communities are managed with similar care as those of waterfowl, fish, and the other wildlife on the refuge. Simply put, aquatic macroinvertebrates are the base of all wetland life.

Island Biogeography Theory in Maankiki Center (MC)

Many of the IBI scores were the same between units. MC and MS had slightly higher richness, evenness, and Shannon and Simpson Index scores than the other two units, and the same scores as each other. But MC had a higher relative abundance of odonates and Gastropods, as well as a higher taxa richness of odonates than MS, a higher number of flying taxa among its 10 most abundant taxa, and a higher number of active aerial dispersers among all taxa in all units in 2021 with unit CPUE greater than 0.5.

The higher median relative abundance and median taxa richness of odonates in MC may be due to its age, its size, or its distance from the river. However, it is striking that MC is both the youngest and the largest unit, and the only one that scored as mildly impacted. Another unique feature of MC is its greater distance from the Shiawassee River and the Spaulding Drain. While the unit itself is directly linked to a retention basin shared with MN and MS, the water from the basin releases into a long, deep, steep-banked channel that extends for ~1.5 km before opening into the central floodplain of the unit. Numerous environmental filters could act on macroinvertebrates here, especially a greater number of bird, fish, and macroinvertebrate predators. This channel might also act to increase sediment deposition before water reaches the center of the unit. Unlike the other

units, the bathymetry at MC's center is considerably varied, separating sections of MC from each other and providing more habitat patches and colonization opportunities for a variety of plants and wildlife (Brinson 1993). These features mean that water and propagules must travel twice the distance from the river or through a connection with P1A if they are to reach the varied habitat in the most protected patches of MC, and even then they are not guaranteed to find suitable habitat. However, if aquatically dispersed animals do find habitat, they will be more protected from fetch, opportunistic predation by riverine species, and other pressures. In contrast, aerial dispersers have a more direct route to colonization in MC. Such features not only make MC more variable, they also agree with Island Biogeography Theory (IBT) as it applies to macroinvertebrate communities (Pollock 2017).

Pollock applied IBT to SNWR macroinvertebrate communities in 2017, and found macroinvertebrate richness at SNWR was negatively correlated with channel distance (shortest swimming distance from the river's edge to the sample site) and dike height. However, neither MC, MN, or MS were connected in 2017, so he was not able to sample them. Several of our results now speak to the unique features of MC and implications for IBT.

We found that although the flood significantly reduced richness and abundance across all units, and subsequently wetland quality, MC still had the highest CPUE of flyers and active aerial dispersers in 2021. Note that by calculating dispersal method instead of only physical trait, we estimated *how* each LOTU accessed a wetland unit. Dispersal trait affinities estimate the likelihood of a species using active or passive and aerial or aquatic dispersal based on observations, time in each life stage, and other facts. Therefore, it is likely that a wetland will have more or fewer realized aerial dispersers in proportion to flying taxa abundance depending on environmental conditions, but it is unclear how the catastrophic flood altered dispersal method. Including both dispersal method and physical trait allowed us to examine the complexity of these functional groups.

Part of the reason for the difference between our results for physical trait and dispersal method is also the immense decline in abundance and diversity after the flood. Because water overtopped the dikes, we should expect the biogeographical signal to diminish. However, this is not sufficient evidence to ignore its influence. While 2020 saw higher unit CPUE of active aerial dispersers in MN and P1A, this only confirms the dominance of the flooding regime, with biogeographic effects swamped by the dominance of the hydrologic regime. Additionally, small ponds and microhabitats may have built up MC's flying macroinvertebrate population prior to reconnection in 2020, but we do not believe the regime shift initiated by the flood and reconnection allowed for continued maintenance and recruitment of those microhabitats, even if slower growing *Salix* and cottonwood stands remained after the flood.

Setting aside those differences, MC is particularly interesting given its age. MC had fewer total *Hyalella*, *Caecidotea*, *Neoplea,* and *Trichocorixa* than any unit in 2020 or 2021, more immature coenagrionids and chironomids than any unit in 2020-2021, and a higher number of *Leucorrhinia* (Libellulidae) than any other unit. The unit received higher FQA scores, meaning that the vegetation there is more sensitive to disturbance, and higher IVI scores, meaning it has a greater diversity of vegetation. The unit also received a 'mildly impacted' invertebrate IBI grade, and had a higher conductivity and lower pH than other units. We also found that among the nine LOTUs with the highest CPUE in each unit, MC had the most flyers and the least non-flyers, while P1A had the most similar CPUEs for flyers and non-flyers, with MN and MS having intermediate values. These tight correlations between unit age, decreasing abundance of flyers, and increasing abundance of nonflyers suggest they are each in a different successional phase corresponding to their age. Alternatively, IBT could be at play, but most likely both succession and biogeography are interacting at the refuge.

MC has both a larger abundance of flyers, and the highest richness of odonate flyers among the units, these being the strongest flyers with longer dispersal distances, and often longer life spans. For example, Aeshnid dragonflies have dispersal distances of up to 100 km, while adult sphaeriid dispersal is 11-100 m, larval dispersal is 10 m or less, and they can only exit water temporarily, for example when dispersing by attaching to larger animals (Vieira et al. 2006). Our results suggest that the colonization stage of MC succession is underway, favoring strong flyers that colonized the unit as soon as habitat became available, while weak swimmers like *Hyalella* and *Neoplea*, and crawlers like physids will take longer to fully establish a self-sustaining population. But because *Hyalella* are a multivoltine, highly tolerant taxon, they are still likely to remain at higher proportions. Nonetheless, MC *Hyalella* are lower than any other unit in 2021 or 2020, suggesting either the effects of flooding did not overwhelm the biogeography of MC, or that its early successional stage still resulted in reduced recruitment and population growth, despite any population influx from the flood. Alternatively, it may be difficult to disentangle the effects of flooding from the different age and biogeography of MC.

However, the higher richness of odonate flyers at MC is strongly suggestive of a biogeographical influence. These are longer lived species that in many LOTUs reproduce only two times or once a year, and require a richer resource pool to survive because they are the apex predators of the macroinvertebrate communities. Pollock (2017) expected only these stronger flyers to surmount higher dikes and access the interior habitats of larger wetland units, and that appears to be what is happening. Moreover, our summary of early colonizers (see above section on succesional dynamics) as predominantly dipertans, hemipterans, and coleopterans, with no clear relationship to wetland unit age

also implies that MC's communities are as much influenced by that units biogeography as its age.

Finally, the research on IBT in wetland macroinvertebrate communities is incredibly sparse, or applies to exclusively lentic habitats like ponds. So, there is no comparative literature on which to base our analyses here. But as a result of the interplay between successional dynamics and immigration rates of taxa capable of dispersing into the larger, more distant unit, two possible trajectories exist for MC. First, allogenic effects like river hydrology and sediment deposition are more characteristic of the early successional colonization phase, so they may within the next 1-3 years overtake the dispersal traits of flyers. The dominance of the flood regime may also become visible in this early colonization phase of MC and begin to homogenize its macroinvertebrate populations with those of the surrounding units, although the local characteristics of each unit will continue to play a role in their development.

A second possibility is that the longer distance from MC to the Shiawassee River, in addition to its greater size and variable bathymetry will allow macroinvertebrate communities to develop differently from those of the surrounding units, even as dispersal occurs more and more between units.

Implications for Science and Management

We have shown throughout this and other sections that SNWR is not a simple inland coastal wetland, but a hybrid wetland. Quantifying how the unique macroinvertebrate distributions fit within inland coastal wetlands, floodplain wetlands, waterfowl wetlands, and constructed wetlands will not be feasible if each of these habitat types is applied in turn. Instead, the progressive reconnection of P1A, MN, MS, and MC facilitates an incredibly detailed view of succession at SNWR, and the interactions among numerous macroecological theories such as aquatic island biogeography, and functional diversity. Framing science and management within these broader concepts will allow managers and scientists to characterize the refuge without resorting as closely to conventional wetland categories.

Recommendations for Future Research

While real time limitations exist, the literature everywhere emphasizes the segregation of macroinvertebrate communities by vegetation zones, and tests for IBI are dependent on sampling in named zones: inner *Scirpus,* outer *Scirpus,* and *Typha* zones. Many *Scirpus spp.* have been added to *Schoenoplectus spp*, of which *Schoenoplectus tabernaemontani* is present at SNWR. Greater effort should be put into sampling into any *Scirpus* or *Schoenoplectus spp*. zones to confirm the applicability of IBI to the SNWR.

The future monitoring efforts should include sampling in vegetation zones located in MC that have not yet been sampled but comprise large sections of the wetland unit, such as purple loosestrife (*Lythrum Salicaria*), angel snot (*Nostoc commune*), and a greater variety of mixed emergent and submerged aquatic vegetation zones which do not all contain the same plant communities, and therefore may harbor varying species assemblages. Field work should also include fewer samples within SAV and Typha zones, and more samples within water smartweed (*Persicaria amphibia*) and Phalaris (*Phalaris arundinacea*), for which we were not able to apply NMDS ordination ellipses due to a paucity of samples. *Typha* and *SAV* are easier to access but oversampling will impair proper and authoritative analysis. Furthermore, if vegetation zones are not determinant of macroinvertebrate communities at SNWR as they are in GLCWs, then research should depart from this model, and better incorporate water depth and hydrogeomorphology into analyses.

Finally, and with great humility, fish are dependent on macroinvertebrates and vegetation, so field researchers should give greater care to investigating these important taxa.

FISH MONITORING

INTRODUCTION

Coastal wetlands provide valuable habitat for fish species and serve as important habitats for spawning, refuge, and foraging (Uzarski et al. 2016). The floodplain wetland units of SNWR are no exception, and provide habitat for Great Lakes, riverine and resident wetland fish species. Wetland units are managed by water control structures that determine water flows between both managed wetland units and the Shiawassee River. These managed connections also impact the ability of fish to move between each respective wetland unit and the river, resulting in variable fish assemblages throughout the refuge. Similar to invertebrates, fish communities act as bioindicators and inform refuge staff of the health of each wetland unit (Cooper et al. 2018). We collected data on fish species richness, abundance, and length to inform refuge staff on the quality of each respective unit and for the SNWR floodplain complex as a whole. Refuge staff were especially interested in “species of interest”, Yellow Perch (*Perca flavescens*) and Walleye (*Sander vitreus*).

RESEARCH OBJECTIVES

- *Characterize species assemblages in each wetland unit and river and compare these to previous year's findings (2019-2020).*
- *Determine wetland health based on Indices of Biotic Integrity across units and between years.*
- *Determine what sampling effort is needed to most accurately measure fish assemblages in wetland units.*
- *Describe variations in species assemblages based on season, year, vegetation zone, and other abiotic factors.*

SAMPLING SITES

Our team monitored four wetland units and the Shiawassee River during the open water season from May to November of 2021, excluding September. Each month, within each wetland unit we set between two and four fyke nets in different vegetation zones and water depths to best sample the diverse fish communities and microhabitats. Our team also used electrofishing from May to August to sample areas that were too deep for fyke nets. Each month, within each unit we sampled two times to best sample fish communities that may be excluded from fyke net samples.

Maankiki South (MS)

We sampled for fish in three different vegetation zones in MS where sufficient water levels were available. SAV, flooded forest and *Typha*, were the predominant vegetation zones sampled. Electrofishing sites were in the perimeter channel and in the western pool.

Maankiki Center (MC)

Sampling in MC consisted of variable sampling locations and occurred in vegetation zones of *Phalaris*, *Typha*, and SAV. Some sites were accessed by boat since perimeter channel depths prevent wading. MC is the largest of the Maankiki units and had the most spatial heterogeneity which provided opportunities to sample new areas and vegetation zones. Electrofishing sites for MC were in the eastern channel along the wildlife drive and the central pool within the unit.

Maankiki North (MN)

Sampling in MN consisted of sites on the north and south ends of the unit. Sites on the south end were accessible by wading given the low water levels during the field season, while sites on the north end were only accessible by boat due to the deep perimeter channels. *Typha* and SAV were the two dominant vegetation zones sampled. Electrofishing sites for MN were along northern and southern edges.

Pool 1A (P1A)

Sampling in P1A occurred in three vegetation zones: *Typha*, *Nymphaea*, and SAV. Low water levels and dense vegetation prevented continued electrofishing surveys but fyke nets were set throughout the entire sampling season. P1A has a high amount of accumulated sediment which in combination with dense vegetation can clog fyke nets and prevent fish from being able to pass through the internal cods. Electrofishing was unable to continue because there was not enough water within the unit for the electrofishing equipment to function properly.

Shiawassee River (SHR) / Spaulding Drain (SPD)

SHR sites served as reference sites for potential fish migration sources into wetland units. Newly reconnected wetland units provide additional spawning, feeding, and refuge habitat for riverine and Lake Huron fishes so the Shiawassee River sites provide a reference as to what species could potentially move into wetland units. Fyke nets were set near the MN and P1A dikes where it was accessible by wading. Electrofishing sites flowed downstream in the SPD until SHR and continued upstream.

METHODS

Fyke Netting

Fyke Netting followed sampling protocols developed by Lugten et al. (2020) and followed by Dellick et al. (2021). Fyke nets are a passive method of fish capture that relies on fish behavior and movement during their diurnal cycles. A net has four distinct sections: a lead, two wings, and a fyke trap. The lead and wings are attached to the frame of the trap and pulled taught, and then securely staked into the sediment (Uzarski et al. 2016; Lugten et al. 2020). We placed nets in various transitional areas where fish are moving between vegetated areas and open water as fish utilize vegetated areas as nursery habitats and protection from predators while they use open water for foraging, especially as they grow larger (Munsch et al. 2016). As fish swim and encounter the lead, they follow this down into the fyke trap and into the internal cods where they are captured and cannot escape.

Sampling sites were determined by the distribution of dominant vegetation zones and water depth within target wetland units and exact placement being selected at random. Random placement sites were created by using a random number generator determining direction and step count for net placement. Direction was determined by creating ten equally sized sections while facing the desired wetland unit. One teammate would give a random number between one and ten, which would indicate direction on a plane from 0 to 180 degrees. Distance into the unit was determined by a teammate again shouting a random number (1 to 100) which determined the amount of steps into the unit for net placement. Nets varied in both frame size (large or small) and mesh type (large or small), resulting in four unique net types. Frame size was dependent on water depth. Nets were not placed where water was too deep so that fish would be able to swim over the lead and frame or where water was too shallow so that the internal cods of the net were out of the water which would not allow fish to properly enter the net.

Mesh size was selected at random and has not been shown to impact fish catch at SNWR (Lugten et al. 2020).

Sampling Protocol

We used sampling protocols from preceding field seasons (2019 and 2020) to complete our monitoring (Lugten et al. 2020; Dellick et al. 2021). Fyke nets were set up by the team at the determined random sampling site and associated water quality metrics were taken in triplicate using a YSI EXO 3 handheld multiparameter sonde. This sonde recorded temperature (ºC), pH, conductivity (µS/cm), turbidity (FNU), and dissolved oxygen (DO) (mg/L). Team members recorded sampling site information, which included: net metrics (such as frame size and mesh size), water depth, vegetation zone, and site-specific observations or comments (Lugten et al. 2020). Data were recorded in Survey123 software using an iPad and iPhone (ESRI Inc. 2021).

We set each net for two consecutive 24-hour sampling periods. After the first net set, another triplicate water quality metric was collected using the sonde. Fish not in the cod end but still in the net were manually passed through the internal funnels by team members, all fish were collected in a bucket and brought to shore. Any fish stuck in the lead or wings without passing through the frame were not counted. The fyke net was reset for the second 24-hour sampling period (Lugten et al. 2020). Fish were identified to species and the total count per species was recorded. Any fish unable to be identified was photographed and documented in order to determine species with the assistance of refuge staff and University of Michigan faculty. Lengths were recorded in centimeters for the first 30 individuals of each species; after 30, individuals of that species were counted but not measured. Other general observations of deformities, lesions, visible parasites, or mortality were recorded. All fish were released away from the fyke net after data collection to prevent immediate recapture and pseudoreplication. This process was repeated for the second net set, with the only difference being the altogether removal of the nets afterwards.

Fyke nets could be compromised by animal tampering, weather conditions, improper set up, or a combination of these factors. Our team considered compromised nets to be those with sufficiently large holes below the water surface and beyond the first cod that could allow for fish to escape, those with the cod end detached from its stake and/or open, or if wings or leads had become detached. Holes were repaired with a field kit on site, and wings reattached if there was a second set. Compromised nets were recorded and data from these sites still collected, but data from compromised nets were not included in any abundance calculations (Lugten et al. 2020). We recorded a total of 26 compromised nets out of a total of 144 nets (18%) throughout the course of our sampling in 2021 which spanned from May to November, excluding September.

Electrofishing

We used electrofishing to collect fish in the deeper water within wetland units, following protocols outlined by Lugten et al. (2020). Electrofishing was used to sample areas where fyke netting was not possible due to depth and allowed sampling of fish assemblages that utilize deeper water. We used an Smith-Root Light-Duty E-Cat, which is a platform with detachable pontoons and projectable poles at the front of the craft that is transportable over short distances which allowed for us to sample different remote locations. Anodes hung off the front of the poles and into the water, where they were electrified with a generator placed on the platform. Electrical power was controlled through a control box which was adjusted to take into account environmental conditions like water conductivity and depth. Electrofishing efforts were led by federally certified Electrofishing Crew Chief (Sasha Bozimowski) from USGS Great Lakes Science Center; we provided assistance.

Areas that were deemed suitable for electrofishing were determined by water depth where fyke nets were unusable, and where no obstacles (e.g. emergent vegetation) obstructed electrofishing. Samples were conducted as transects defined as ten minutes of shocking effort. At the beginning of each transect, water quality parameters including temperature, pH, conductivity, dissolved oxygen, and turbidity were collected using a YSI EXO3 water quality sonde. Water depth was measured at the start and the end of each transect as well as weather conditions such as temperature, cloud cover, and wind. During each transect, the electrofishing operator rowed the catamaran and controlled the electrical settings, while a student researcher operated a dead-man foot pedal controlling power supply to the system. In the event of any danger, the foot pedal operator would step off of the foot pedal, which would cut power to the system, and the electrofishing operator would also turn off the electrical box to ensure no power to the unit. As fish were shocked, they were netted using a non-conductive hand net and placed into a water filled cooler with bubbler to be measured back on shore. After the transect was complete, transect length (in seconds) and electrical settings (voltage amps, DC pulse per second, and percent power) were recorded. Additional information such as date, vegetation zone, and any observations the operator and netter may have had such as fish seen but unable to be collected were also recorded. Fish in the cooler were then brought to shore where they were identified to species, measured, and released back into the water. If more than 30 individuals of a species were caught, a random sample of 30 measurements were taken and the remaining fish counted and examined for any abnormalities.

We conducted electrofishing transects two times in each of MS, MN, MC, SPD, and once in the Shiawassee River per month. Due to low water levels, P1A was sampled once in May but was unable to be sampled in subsequent months.

DATA ANALYSES

Indices of Biotic Integrity

Indices of biotic integrity (IBI) were calculated following the CWMP IBI methods and specific vegetation calculations outlined by Cooper et al. (2018). We calculated IBI scores for each study unit based on vegetation zones and species' presence and abundance. Since IBI metrics are based upon dominant vegetation zone, but not all vegetation zones sampled within the units were represented within the metrics built in Cooper et al. (2018), we used calculated IBIs for those vegetation zones sampled within SNWR that were consistent with published IB metrics, SAV and *Typha* spp. The SAV IBI metrics were calculated from SNWR samples collected from SAV and *Nymphaea* vegetation zones, while *Typha* spp. IBI metrics were calculated from SNWR samples collected from *Typha* + *Phalaris* + *Salix* + Forest vegetation zones. Our 2021 field season IBI scores are comparable to those of the previous two field seasons (2019 and 2020) and other Great Lakes Coastal Wetlands.

Non-metric Multidimensional Scaling

Non-metric Multidimensional Scaling (NMDS) was used to assess differences in community composition across wetland units, and 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. Analyses were run using the R packages of "vegan" and "tidyverse".

Catch Per Unit Effort

Catch per unit effort (CPUE) was calculated for each species per sampling site for each month sampled. CPUE measures the relative abundance of a respective species as the ratio of total individuals collected divided by the sampling effort expended to catch those individuals. We calculated site CPUE by dividing the total number of fish collected at a site divided by the total number of nets set at the site which would be two if there were no compromised nets. We calculated species CPUE by dividing the total number of a given species by the total number of nets set.

Sampling efforts and the subsequent CPUEs were affected by compromised nets. In total there were 26 compromised nets out of 144 total nets. If one net out of a sampling period was compromised, that net and its respective fish data were excluded from the CPUE calculation.

Species Accumulation Curve

In order to evaluate whether sampling effort was sufficient to characterize fish species richness we used a species accumulation curve. Species accumulation curves show the number of unique species caught as a function of sampling effort. Curves that reach an asymptote denote that sampling efforts were sufficient to represent fish assemblages. Curves were generated in R Studio using the packages of “vegan”, “permute”, and “lattice”.

ANOVAs

To analyze differences of site fish CPUE based on wetland unit, vegetation zone, and sampling month we used ANOVAs. We ran six ANOVAS with three including an outlier and three without the outlier. The outlier was a MS site, in May, in the forest, that had a CPUE of 4569.5, which were mostly juvenile Black Bullhead (*Ameiurus melas*). That site accounted for more than half of the total fish caught during the 2021 sampling season.

Linear Regression

To analyze the effect of water quality on site CPUE, we used linear regressions to examine the effect of several variables which were temperature, dissolved oxygen (DO), conductivity, pH, and turbidity**.**

RESULTS

Overview

We gathered data from a total of 118 fyke nets over the course of the sampling season, which spanned from May to November. Nets were spread across the five different sampling areas of MS, MC, MS, P1A, and SHR.

Species Accumulation Curves

Species accumulation curves were generated for each of the respective sampling units, in order to determine whether sampling efforts were sufficient in capturing species assemblages of each respective wetland unit (Figure 31; Figure 32). Sampling efforts in MC and P1A began to reach an asymptote at 25 and 27 sites, respectively; and these appear to have been sufficient. Efforts in MS, MN, and SHR did not reach asymptotes with the number of sites for each respective unit being 22, 25, and 19; and do not appear to be sufficient.

Comparisons among years is only possible for MS, MN, and P1A because 2021 was the first year that both MC and SHR were sampled. When compared to the

previous field seasons of 2019 and 2020, our data were more similar to that of 2019 which suggested that sampling efforts were not sufficient to completely characterize fish communities throughout the SNWR wetland complex. SACs from the 2020 field season suggest that sampling efforts were sufficient to characterize fish assemblages, even though the 2019 field season found more species in each unit.

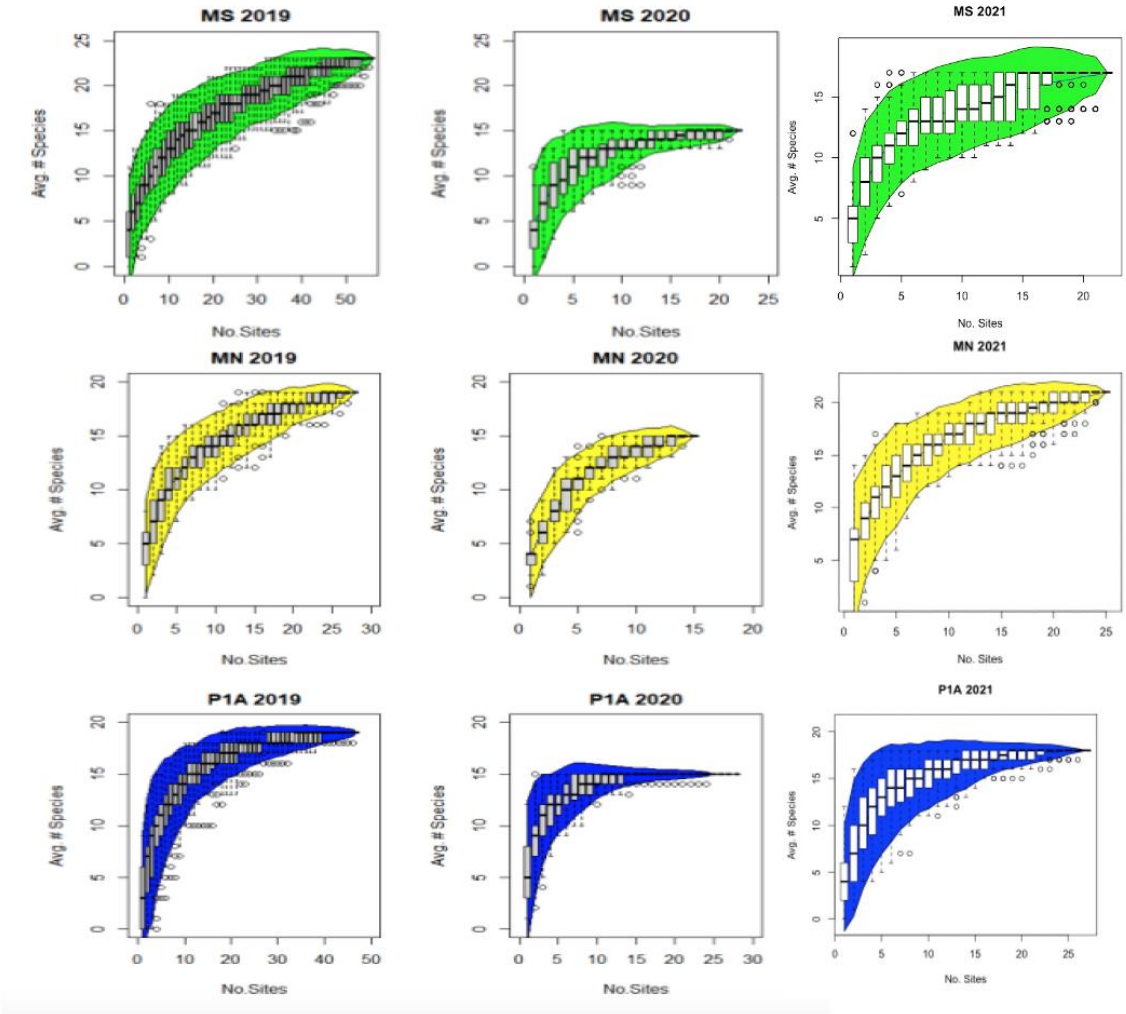


Figure 31. Across years species accumulation curve (SAC) comparison. SACs from 2019, 2020, and 2021. SACs plot number of sampled sites (No. Sites, x-axis) against average number of species (Avg # Species, y-axis). Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). SACs for 2020 may not be comparable to other years due to the difficulty of sampling after catastrophic flooding.

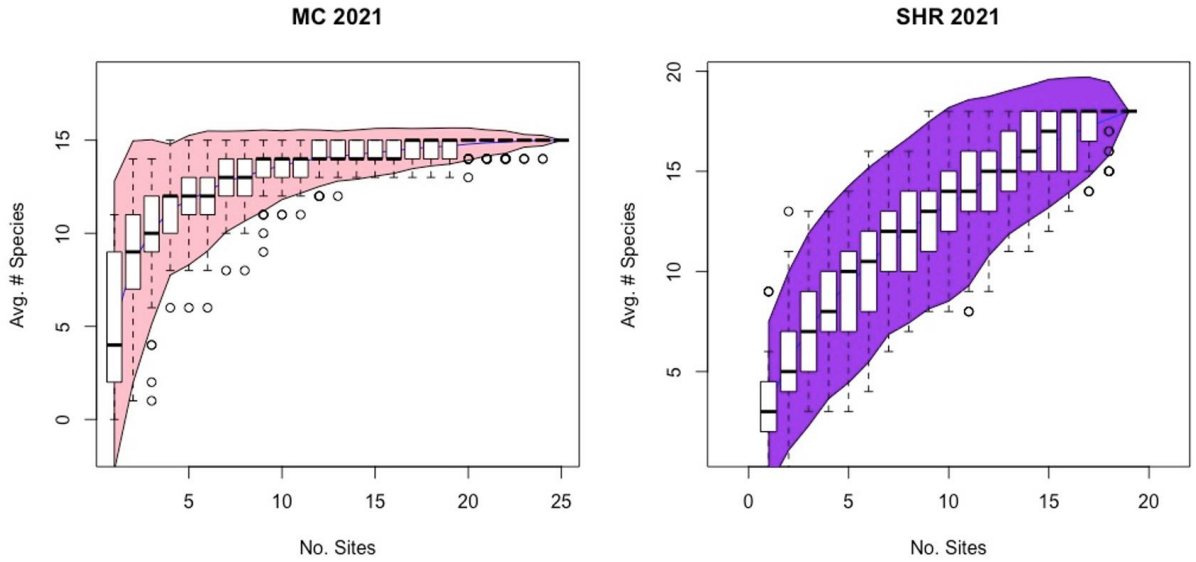


Figure 32. Species accumulation curves for MC and SHR. These sites were not sampled in previous years. Abbreviations refer to wetland units: Maankiki Center (MC), and Shiawassee (SHR). SACs plot number of sampled sites (No. Sites, x-axis) against average number of species (Avg # Species, y-axis).

Indices of Biotic Integrity

We calculated Indices of Biotic Integrity scores (IBI) for the fish communities in each wetland unit and compared scores to applicable units from 2019 and 2020 (Table 25). IBI scores range from 0 to 100, with 0 being the lowest quality and 100 being the highest quality habitats (Cooper et al. 2018). Scores are categorized by ranges from degraded (<36), moderately degraded (36-45), moderately impacted (>45-50), mildly impacted (>50-60), and reference quality (>60).

In the SAV vegetation zone, fish IBI scores for all three previously sampled units decreased in score from 2020 but stayed within the same scoring category. MC and SHR had first time scores of 31.81, placing them in the degraded category. In the *Typha* vegetation zone, IBI scores were relatively consistent with those from the previous sampling year. In MS, scores increased slightly from 40 to 45, but stayed in the same scoring category of moderately degraded. Scores for MN did not change, with 40 being the score for all three sampling years, placing it in the moderately degraded category. P1A stayed the same as the previous sampling year, with a score of 40, and a category of moderately degraded. MC had a first time score of 40, placing it in the moderately degraded category. SHR

did not have any data for the *Typha* vegetation zone. Both MC and SHR were not sampled in previous years.

Table 25. Unit IBI Scores across years in SAV and Typha. Numbers represent IBI scores and text states one of four possible qualitative scores, ranging from "degraded," to "moderately degraded," "moderately impacted," mildly impacted," and "reference conditions." MC and SHR were first sampled in 2021, so no data is available for 2019 and 2020. Abbreviations refer to wetland units: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), and Pool 1A (P1A). Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); *Typha* is *Typha spp*.

UNIT	Vegetation Zone Type: SAV			Vegetation Zone Type: *Typha*		
	2019	2020	2021	2019	2020	2021
MS	54.54 Mildly Impacted	22.73 Degraded	27.27 Degraded	55 Mildly Impacted	40 Moderately Degraded	45 Moderately Degraded
MN	N/A*	45.45 Moderately Impacted	36.36 Moderately Degraded	40 Moderately Degraded	40 Moderately Degraded	40 Moderately Degraded
P1A	22.72 Degraded	40.91 Moderately Degraded	36.36 Moderately Degraded	35 Degraded	40 Moderately Degraded	40 Moderately Degraded
MC	N/A	N/A	31.81 Degraded	N/A	N/A	40 Moderately Degraded
SHR	N/A	N/A	31.81 Degraded	N/A	N/A	N/A

NMDS

NMDS plots were made for the three grouping variables of: wetland unit, vegetation zone, and sampling month. Ellipses denote 50% variation within grouping variables explained, where sufficient data were available. PERMANOVAs were used to test for significance.

An NMDS performed for fish communities by wetland unit shows no difference in community structure with spread of ellipses across MDS1 and MDS2 (Figure 33). Ellipses for MS and MC were most similar and overlapped significantly while P1A and MN ellipses had significant overlap. There were not enough data points for SHR to create an ellipse but both points fall within the P1A ellipse, suggesting it is most similar to P1A. PERMANOVA results showed community assemblages of fish by unit were significantly different ($p < 0.05$), with an R-squared value of 0.1274.

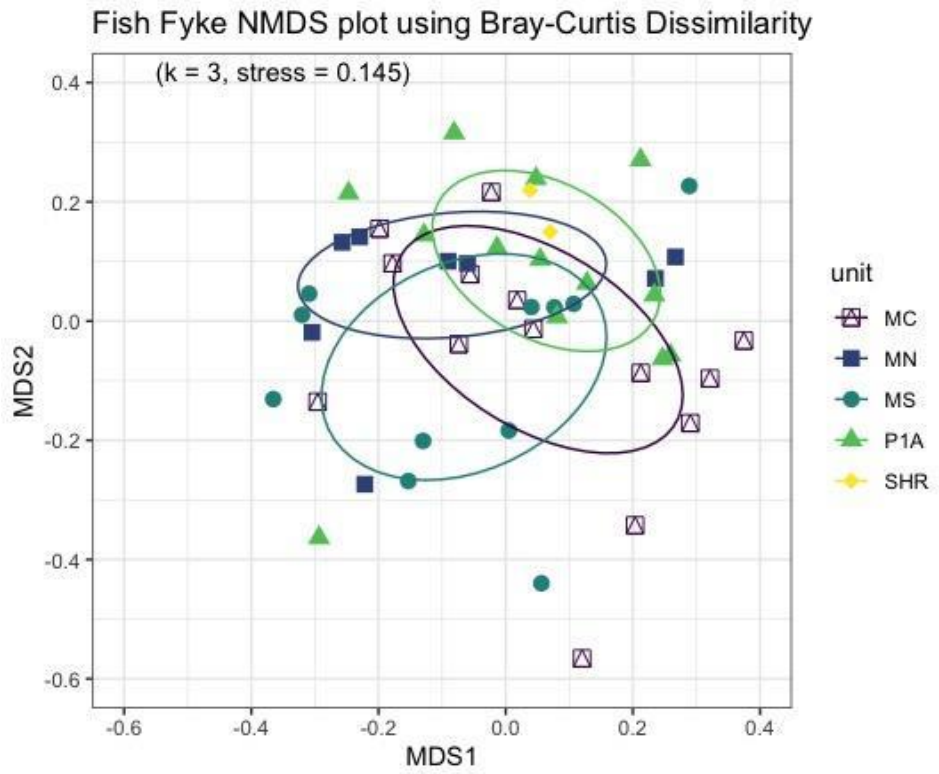


Figure 33. Fish Fyke NMDS for fish communities by wetland unit. Ellipses denote 50% variation within grouping variable. Abbreviations refer to wetland units and river locations: Maankiki North (MN), Maankiki South (MS), Maankiki Center (MC), Pool 1A (P1A), and Shiawassee River (SHR).

An NMDS performed for fish communities grouped by vegetation zone shows differences in community composition between vegetation zones (Figure 34). The ellipse for forest were most dissimilar from every other vegetation zone and had the least overlap with other ellipses. Ellipses of *Typha*, SAV, and mixed emergent have significant overlap. Ellipses were unable to be created for shore, river bulrush, forest, *Nymphaea*, and *Phalaris* due to insufficient data points, but they all broadly fall within the*Typha*, SAV, and mixed emergent vegetation zones. PERMANOVA results showed community assemblages of fish by vegetation zone were significantly different (P<0.05), with an R-squared value of 0.23365.

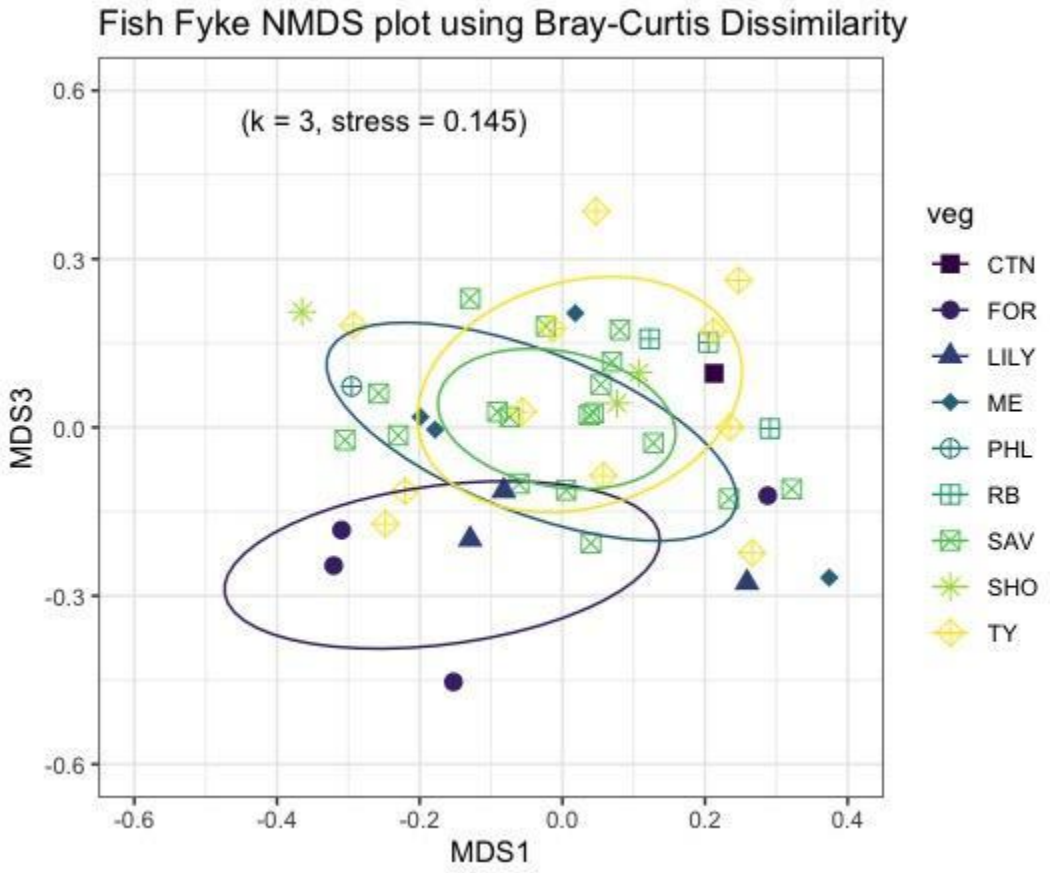


Figure 34. Fish Fyke NMDS for fish communities by vegetation zone. Ellipses denote 50% variation within grouping variable. Vegetation zones are coded for ease of use in the field and refer to dominant stands or habitat types: submerged aquatic vegetation is any mixed floating or submerged vegetation (SAV); mixed emergent is any mixed emergent vegetation, including that with SAV that is still mixed emergent dominant (ME); *Typha* is *Typha spp* (TY); Lily is *Nymphaea odorata*, RB is *Schoenoplectus tabernaemontani*; forest is *Acer saccharum*; cottonwood is *Populus deltoides* (CTN); shore is dry littoral with or without vegetation present (SHO); and *Phalaris* is *Phalaris arundinacea* (PHL).

An NMDS performed for fish communities grouped by month shows spread of ellipses across MDS1 and MDS2, with no differences in fish communities among months (Figure 35). The ellipse for the month of May was most dissimilar from the ellipses of every other month. The ellipse for November was also dissimilar but had more overlap with other ellipses than that of May. The ellipses of August, July, June, and October were similar with high overlap. PERMANOVA results showed community assemblages by month were not significantly different ($p>0.05$), with an R-squared value of 0.14163.

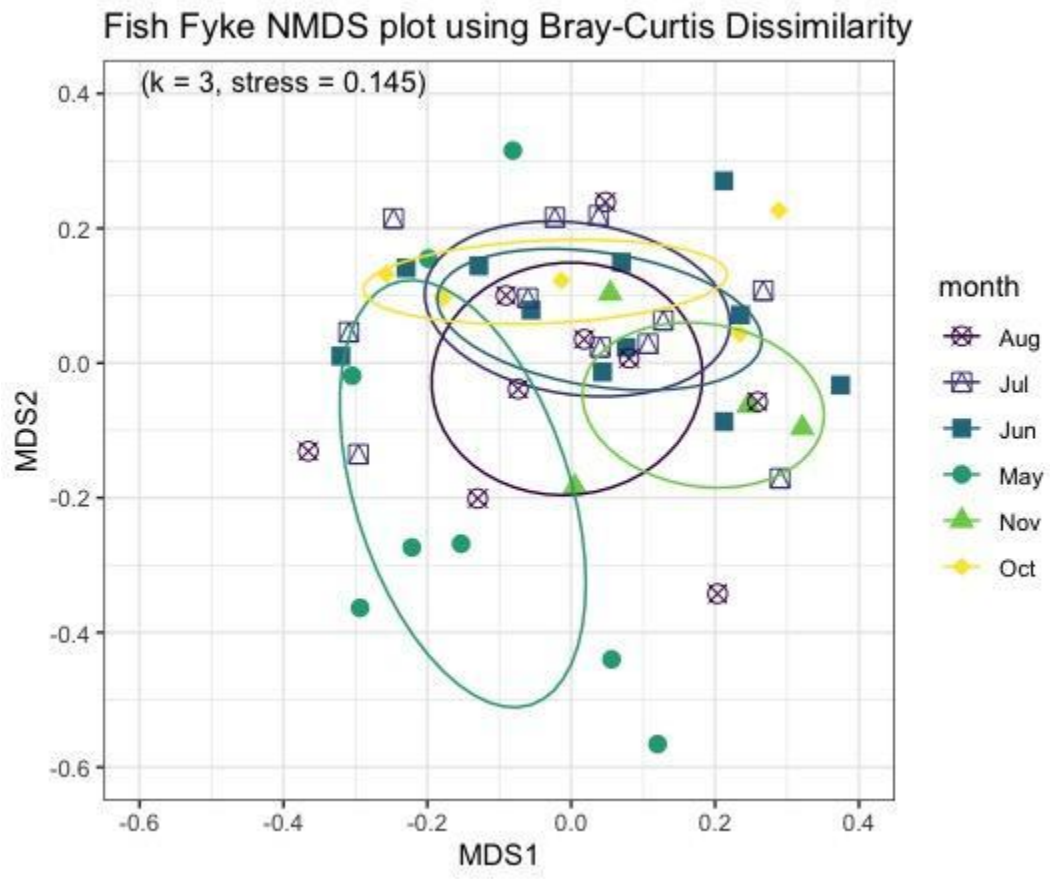


Figure 35. Fish Fyke NMDS for fish communities by month. Ellipses denote 50% variation within grouping variable. Each shape is a different color representing sample(s) taken during one month, and corresponds to an ellipse color. Abbreviations represent months.

Abundance of Common Species

We examined the most abundant fish species among wetland units and years, with Black Bullhead, Bowfin, Bluegill, Pumpkinseed, and YOY sunfish being the most abundant throughout SNWR (Figures 36; Figure 37; Figure 38). In addition to the overall most abundant species across all of the wetland units, any additional species that were the most abundant for that respective wetland unit are also shown.

CPUE for each species caught over the past sampling years in each respective wetland unit are shown in Appendix IX. Cells with zeros indicate that none of that species were caught in that respective wetland unit during the sampling year. Columns for MC and SHR for the years of 2019 and 2020 were omitted since there are no data due to no sampling. Black Bullhead CPUE in MS was higher than in most other units with every year having a CPUE of over 56. CPUE of Black Bullhead in MS for 2021 is 436.364, which is much higher than any other value, due to a site with thousands of young of year black bullhead caught. Our 2021 year caught Brook Stickleback (*Culaea inconstans*) for the first time in MS. Our 2021 year only caught two species of minnows, Emerald (*Notropis atherinoides*) and Golden Shiners (*Notemigonus crysoleucas*), which is vastly different to previous years which caught a wider diversity of minnow species.

CPUEs across sampling years for the most abundant species found in MS changed over the course of sampling seasons (Figure 36). Bluegill (*Lepomis macrochirus*), Bowfin (*Amia culva*), and Goldfish (*Carassius auratus*) stayed relatively similar across sampling years with differences ranging from 0.5 to 6. CPUE for Pumpkinseed (*Lepomis gibbosus*) was 21.510 in 2019 but decreased to 5.636 by 2021. CPUEs for Central Mudminnows (*Umbra limi*) and YOY sunfish (*Lepomis spp.*) increased greatly from 2019 to 2021 with increases being 11.935 and 62.318 respectively. CPUE for Black Bullhead showed the largest change across the years with an increase from 56.784 in 2019 to 436.364 in 2021.

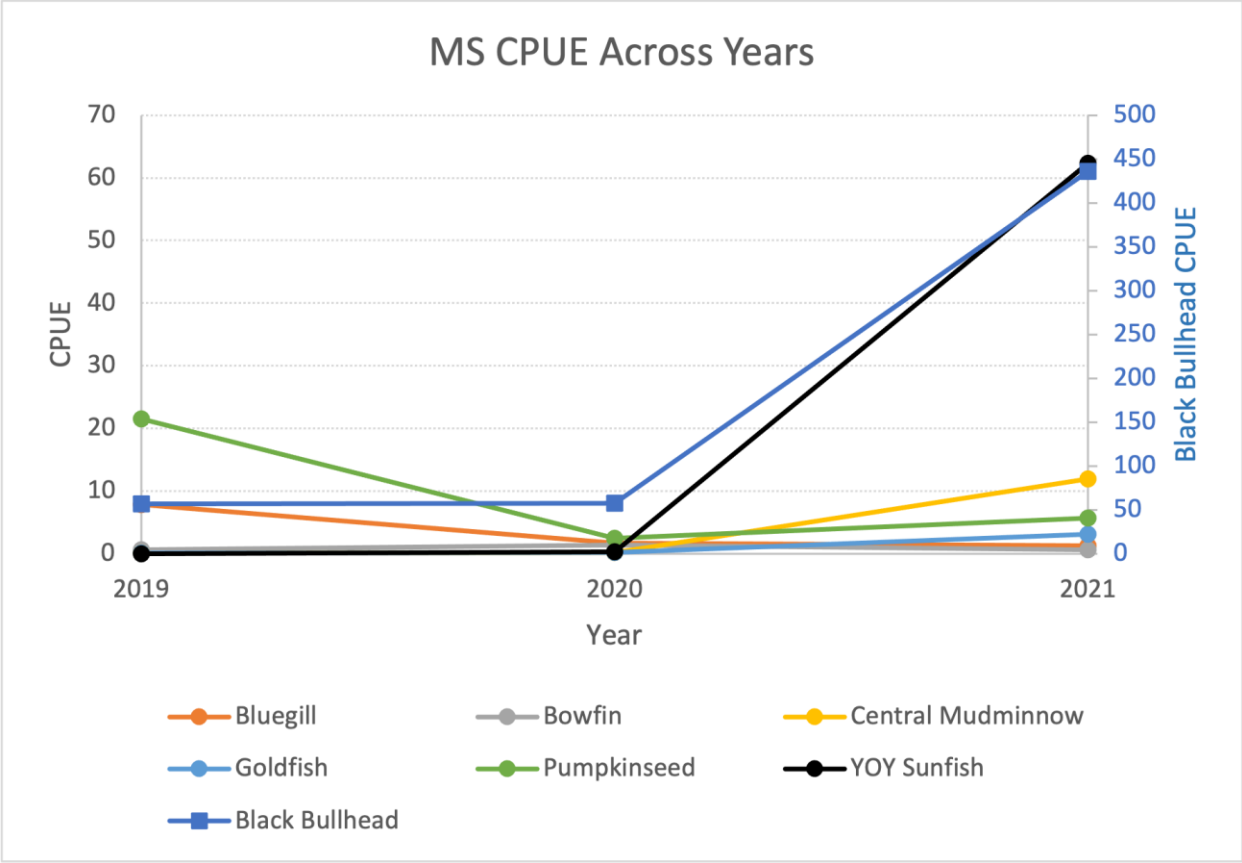


Figure 36. CPUEs across sampling years for the most abundant fish species found in MS. CPUE comparisons are shown for seven species in MS across three sampling years. Black Bullhead data points are represented as squares. Secondary axis in blue represents CPUE for Black Bullhead.

CPUEs for most abundant species in MN across years shows variability in species abundance (Figure 37). CPUEs for Bowfin, Pumpkinseed, and YOY sunfish stayed similar across sampling years with differences ranging from 2.5 to about 10. Black Bullhead CPUE decreased substantially from 2019 to 2020, going from 91.37 to 21.84, but showing almost no change in 2021 with CPUE being 21.92. CPUE for Bluegill had little change from 2019 to 2020, but increased from 2.74 in 2020 to 43.08 in 2021.

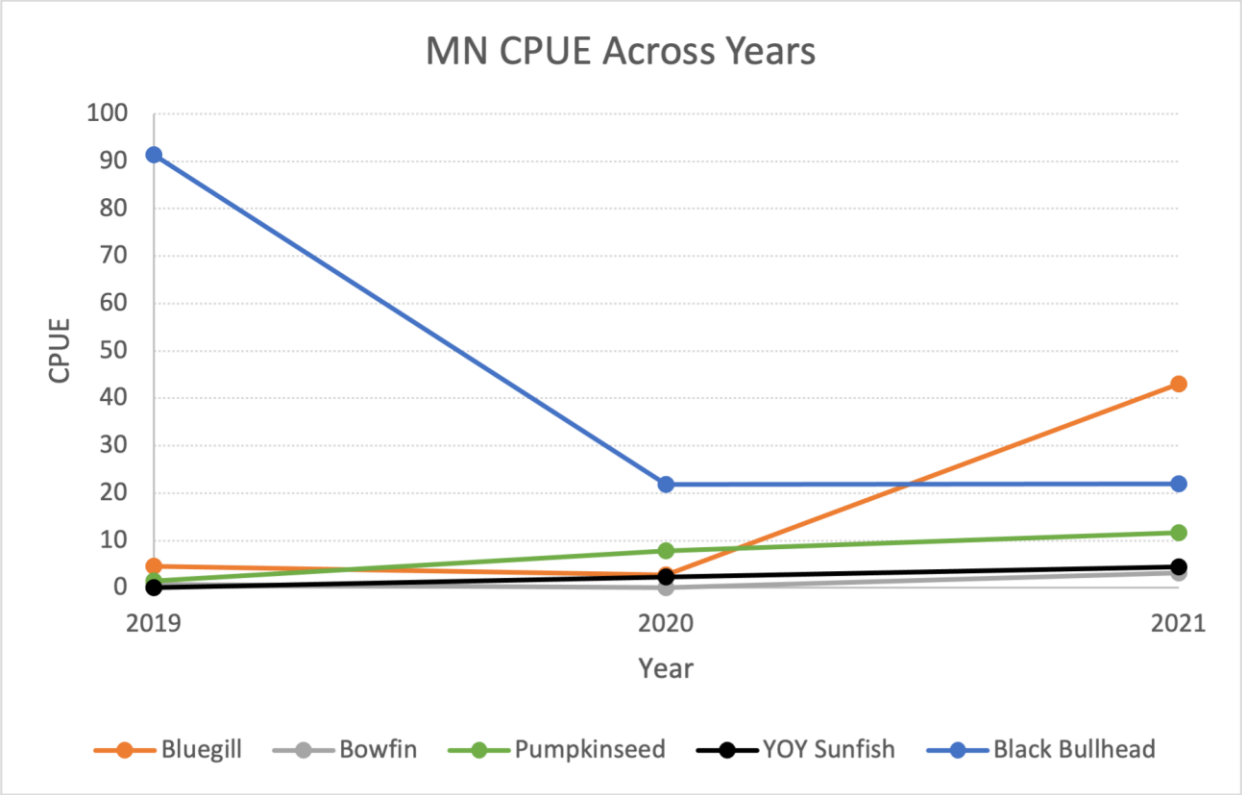


Figure 37. Total CPUE across sampling years 2019-2021 for the most abundant fish species found in MN. Lines are added to show trends, but do not refer to sampling efforts between years. Colors refer to different species (see legend).

CPUEs across sampling years for the most abundant fish species found in P1A were highly variable (Figure 38). CPUEs for Bowfin, Pumpkinseed and YOY sunfish stayed relatively similar across sampling years, with changes ranging from 1 to 3. CPUE for Emerald Shiners and Goldfish both showed little change from 2019 to 2020, but showed large increases in 2021. Black Bullhead CPUE decreased from 2019 to 2020 and then showed little change in 2021. CPUE for Bluegill increased substantially from 2.571 in 2019 to 24.88 in 2020, but then had a substantial decrease to 11.00 in 2021.

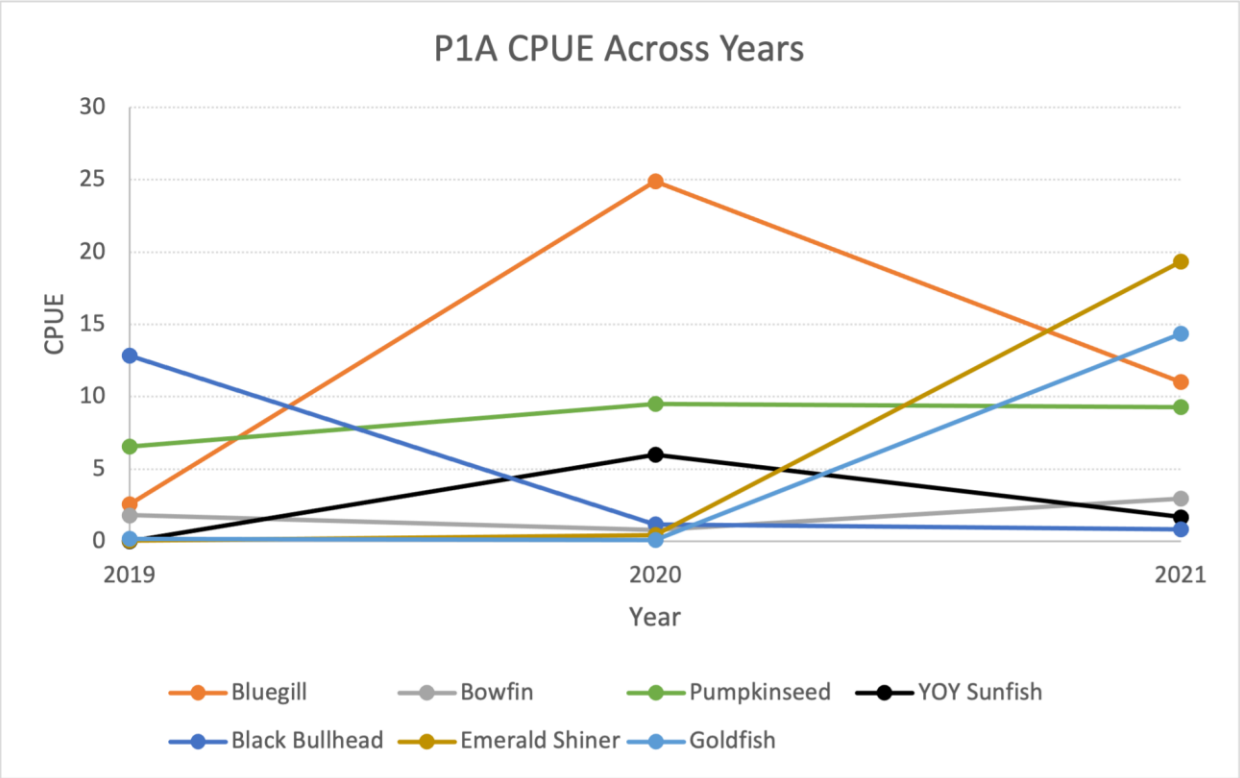


Figure 38. CPUEs across sampling years for the most abundant fish species found in P1A.

Baseline CPUEs for sampling of SHR and MC in the future were collected, as 2021 was the first year either unit was sampled (Table 26). Four new species (for the 2019-2021 SNWR monitoring) were recorded in SHR: Longnose Gar (*Lepisosteus osseus*), Round Goby (*Neogobius melanostomus*), Greater Redhorse (*Moxostoma valenciennesi*), and Quillback (*Carpiodes cyprinus*). Four important game species, Largemouth Bass (*Micropterus salmoides*), Black Crappie (*Poxomis nigromaculatus*), Northern Pike (*Esox lucius*), and Yellow Perch were recorded in SHR, and Largemouth Bass, Black Crappie and Yellow Perch were caught in MC.

Table 26. Tables of CPUEs for abundant, important, and new fish species for SHR and MC. Blue shaded boxes represent the top five most abundant species for that respective unit. Species with * represent species that were recorded for the first time. Species with ^ represent important game species.

Shiawassee River	
Species	CPUE
Bluegill	3.684
Bowfin	2.842
Pumpkinseed	2.789
Round Goby*	0.526
Channel Catfish	0.368
Longnose Gar*	0.368
Largemouth Bass^	0.211
Yellow Perch^	0.211
YOY Sunfish	0.211
Black Bullhead	0.158
Northern Pike^	0.105
Black Crappie^	0.053
Greater Redhorse*	0.053
Quillback*	0.053

Maankiki Center	
Species	CPUE
YOY Sunfish	9.320
Bluegill	9.120
Black Bullhead	6.800
Black Crappie^	5.480
Pumpkinseed	4.080
Bowfin	1.720
Largemouth Bass^	0.760
Yellow Perch^	0.320

Influence of Unit, Vegetation, and Month

Both ANOVAs for month and wetland unit were insignificant (p>0.05), meaning month or wetland unit had no impact on fish CPUE (Table 27). The ANOVA that included the MS outlier for vegetation zone was significant (p=0.031) but when the outlier was removed it became insignificant (p=0.315). To analyze specific differences between vegetation zones, a Tukey's honestly significant difference test was run, which showed forest was significantly different from all other vegetation zones.

Table 27. ANOVA results for fish CPUE based on Month, Vegetation, and Unit. Bold indicated significance (p<0.05).

Parameter	P-value (Forest Outlier included)	P-value (Forest outlier removed)
Month	0.166	0.704
Vegetation zone	**0.031**	0.315
Unit	0.248	0.304

Influence of Water Quality

Of all five variables tested on site CPUE only conductivity was significant (p=0.004) (Table 28).

Table 28. Linear model results for water chemistry on site CPUE. Bold indicated significant (p<0.05).

Water Quality Parameter	P value
Temperature (C)	0.14599
Dissolved Oxygen (mg/L)	0.64140
pH	0.39570
Conductivity (µS/cm)	**0.00381**
Turbidity (FNU)	0.64784

DISCUSSION

Abundant and New Species

Throughout fish sampling, there were species that were captured more frequently than others. Black Bullhead, Bowfin, Bluegill, Pumpkinseed, and YOY sunfish were by far the most abundant species found throughout SNWR and were found in every unit. Within these common species, Black Bullhead dominated species abundance and accounted for more than half of the total fish caught over the entirety of the field season. CPUE for Black Bullhead in MS was extremely high, due to a single net having over 4,000 individual (juvenile) Black Bullhead, and the site having 9,139 fish in total. Generally, having Black Bullhead as a dominant species within shallow wetland habitats is not surprising given their tolerance for warm, (sometimes polluted), and low DO waters (Rose 2006); however, the immense number of Black Bullhead collected was worth noting. The specific site where these Black Bullheads were caught was in a channel near the south end of MS, where waters were confined to a single small channel, essentially channeling all fish into our net.

Other studies have shown that Yellow Perch are often a dominant species within Great Lakes Coastal Wetlands (GLCW), but our results showed very few Yellow Perch caught throughout the season. Langer et al. (2018) showed that Yellow Perch were not abundant in shallow wetlands, likely due to high productivity and resulting low dissolved oxygen (Langer et al., 2018). Another explanation for the low abundance of Yellow Perch is the distance from Lake Huron. Similar studies that have analyzed fish communities within GLCW are much closer to the respective Great Lake, while SNWR is twenty miles inland from Lake Huron making it a much longer migration for any Yellow Perch coming into SNWR to spawn from Lake Huron (Langer et al. 2018). While being a species of concern for SNWR, current conditions may not be suitable for the recruitment of Yellow Perch.

There were also several new species caught for the first-time during sampling. While most new species were a result of sampling for the first time in the river and subsequently catching riverine species rather than wetland species, there were also new species caught within the wetland units. Brook Stickleback, Longnose Gar, Longear Sunfish (*Lepomis megalotis*), and Silver Lamprey (*Ichthyomyzon unicuspis*) were all caught within wetland units for the first time. The most unexpected was the Silver Lamprey due its normal habitat of larger rivers and streams and was most likely attached to a fish moving from the river into SNWR as it was in its adult parasitic stage (Fuller and Neilson 2011). Other riverine species caught that are noteworthy include Smallmouth Bass (*Micropterus dolomieu*) and Walleye, which are two important game species in the State of Michigan (Appendix X). Both were caught directly adjacent to P1A in

the spaulding drain and represent a chance for these two economically important species to enter SNWR.

Community Composition and Island Biogeography Theory

Island biogeography theory (IBT), which is described as species population dynamics as a result of size of isolated islands, or habitats, and its proximity to the mainland, or source populations, and the rates of extinction within each island; is critical in understanding fish dynamics within SNWR (Angeler and Alvarez-Cobelas 2005). Each respective wetland unit acts as an island while its connection to the Shiawassee River acts as the mainland housing the source populations. The connection timing, determined by refuge management, dictates when each wetland unit is connected to the river, and subsequently when fish can move among units and the river. Refuge units are the smallest islands in the summer when water control structures are closed, preventing the movement of fish across units and the river, and the largest during the spring and fall when water control structures are open, allowing movement across units. Extinction rates within each respective unit are also highest during the summer, when there is: no immigration due to closed control structures, challenging water quality conditions, and high numbers of piscivorous predators such as herons, kingfishers, snapping turtles etc.

The most apparent difference in community composition came from the NMDS for fish communities by vegetation zones, with the forest being dissimilar from all other zones; and the PERMANOVA showed a significant difference between community assemblages and vegetation zones. In addition, the ANOVA for site CPUE and vegetation zone also showed a significant difference, with Tukey HSD tests showing the forest vegetation zone being the only significant difference between all vegetation zones. Both results can likely be attributed to one site within MS that had 9,139 fish, with 8,859 of those fish being black bullhead. This large collection of black bullhead likely skewed the data, and when removed for site CPUE and vegetation zone, the forest vegetation zone was no longer significantly different.

Community composition across wetland units and the river was relatively homogenous with the only real difference in composition being in the MS forest vegetation zone, which can be attributed to an outlier. Communities that inhabit shallow water wetlands are more tolerant to physicochemical changes, while species that inhabit deep water wetlands are more sensitive to such changes (Langer et al. 2018). This community composition pattern is a good explanation as to why our 2021 communities were highly overlapping, given the low water levels throughout the sampling season. Water level fluctuation also facilitates the movement of fish species in and out of wetland ecosystems and also promotes

healthy biodiversity within the system (Langer et al. 2018). However, when water control structures are closed, the system is disconnected, and movement of fish is stopped. Although each wetland unit acts as a habitat island, the homogeneity of fish communities can possibly be attributed to the life history traits of fish that occupy the isolated wetlands units of SNWR. Fish that can tolerate and reproduce in the tough conditions of shallow water and isolated wetlands are more likely to persist in areas that are hydrologically disconnected for long periods of time (Baber et al. 2002).

Study Limitations

Fyke net sampling has inherent biases, previously acknowledged by both Lugten et al. (2020) and Dellick et al. (2021), and can lead to under or over representation of fish communities. Limitations stem from method bias, escaped fish, within net predation, compromised nets from Muskrats, predatory birds, and human error, and available sampling locations. Compromised nets were excluded from data analysis but they do represent a loss of data that if not compromised could have potentially changed results. Net set locations were limited by unit water depth, as our 2021 year had lower water levels throughout, preventing replication of previous years net sets. Fyke nets are also depth limited so sampling within the deeper portions of units, which may harbor different species, were limited.

Limitations for electrofishing sampling were different from fyke netting, but followed in a similar manner. The greatest limitation was finding access points to get the equipment into the wetland unit and begin sampling. Due to its weight and awkward design, the electrofishing catamaran was difficult to carry down the sides of dikes and place into the water, making it difficult to sample areas that did not have navigable slopes. Additionally, being an oar-driven vessel, maneuvering within the water, especially during windy days, was difficult at times and resulted in the loss of fish as the netter was not able to scoop them up if only a few feet away from the vessel. Certainly valuable, electrofishing can sample where fyke nets are unable, but it also has its disadvantages so its deployment must be carefully considered in order to maximize effectiveness.

Implications for Management and Science

The IBI results for fish sampling were consistent with previous years, showing very little change within wetland units in terms of habitat quality category, which ranged from degraded to moderately degraded (Lugten et al. 2020; Dellick et al. 2021). Interestingly, our results were consistent with other studies that have

covered coastal wetlands of the Great Lakes basin, showing that coastal wetlands of the Lower Peninsula of Michigan are most often moderately degraded (Cvetkovic and Chow-Fraser 2011, Appendix XI). Possible explanations are that the more southerly portions of the Great Lakes basin have higher anthropogenic impacts due to higher population density, and generally a milder climate suitable to human habitation (Cvetkovic and Chow-Fraser 2011).

Fish may also not be the best metric to measure habitat quality because they are highly mobile and many species are often generalists (Cvetkovic and Chow-Fraser 2011). Also, diked wetlands with the purpose of promoting waterfowl have adverse effects on fish communities since the connectedness of waterways has been severely reduced (Jude and Pappas 1992). This is especially present within SNWR, as units are only connected at certain locations and at certain times throughout the year, limiting species movement. Although fish can be important bioindicators of water quality, the specific purposes and goals of SNWR and limitations within the Saginaw Bay watershed do not directly align to meet the needs of wetland fish communities; meaning using fish as water quality indicators will more than likely result in some level of degraded designation.

Water quality parameters have previously been shown to influence community composition; our results for CPUE only showed one significant difference indicator, conductivity (Janetski and Ruetz 2015). Although CPUE and NMDS measured two different metrics within our study system, it is still surprising that only one water quality parameter was a significant indicator for CPUE. Unlike Dellick et al. (2021), the effect of water conductivity on abundance is not as clear, as the removal of the large black bullhead site changes the results from significant to not significant. Conductivity is shown to be higher in wetlands that are connected, compared to wetlands that are isolated, but based on our data, does not influence the abundance of species caught (Cook and Hauer 2007). This trend in conductivity has been observed not only in our summer sampling, but spring and fall samples as well. Connectivity between wetland units and the river provides more opportunities for general water movement, fish movement, abundance of species, and potential diversity; so refuge staff must identify species of priority and adjust connectivity to target their needs accordingly, regardless of conductivity. In addition, connectivity plays a role in water depth and although we did not specifically analyze the effect of water depth on fish communities and abundance, it seems that it is a major contributor to what fish are present and in what abundance(Baber et al. 2002; Langer et al. 2018). Management decisions must compromise between open connectivity and the retention of water for suitable habitat, so identifying habitat needs for species of concern, such as Yellow Perch, are critical in order for management to make the proper decisions.

Of the various vegetation zones found throughout SNWR, only forest was shown to be significantly different from other vegetation zones. Again, this may be because of the immense number of Black Bullhead caught in the confined forest section of MS, but it is worth noting (given its structure) that it is certainly different from the marsh vegetation sampled elsewhere. Habitat heterogeneity is always valuable and provides opportunities for more species diversity, but the forest significance is likely a function of how water recedes and is retained in MS during low water periods. This forested section of MS acts as a refuge for fish during low water periods and serves as another source of fish when waters rise and refloods MS.

Species accumulation curves across the wetland units and between years show a large degree of variation. Our SACs showed insufficient sampling across MS, MN, and P1A, which was consistent with sampling data from 2019 but inconsistent with the 2020 sampling. This is surprising given that there were more nets set in 2019 and 2021 compared to 2020. Typically, more samples provide better species representation composition for a given ecosystem (Moreno and Halffter 2001). A possible explanation for the differences in SACs is that there were temporal differences in sampling efforts between 2019, 2020, and 2021. The 2020 group had an abbreviated sampling season due to COVID-19, and missed the first six weeks of sampling, which were essentially the spring samples. An example of a missed species would be Brook Stickleback, which were only caught in the spring and fall of the 2021 sampling season, but were not caught in 2020, potentially because of the missed spring samples. Another possible and more likely explanation is that the highly variable nature of the wetland's changes habitat structure and the subsequent fish species that utilize SNWR. Some species, such as sunfish spp. and Bowfin, are present year round regardless of water levels (except complete drying), while other species only utilize SNWR seasonally and under specific conditions that are dependent on water levels (Jude and Pappas 1992). Habitat diversity is key in the utilization of wetlands by fish, and water levels within SNWR determine the amount of available habitat and the types of habitat available to fish to use (Jude and Pappas 1992). 2021 had lower water levels than both 2019 and 2020, potentially leading to fewer diverse habitats within SNWR for fish species to utilize. Additionally, wetlands with a longer connectivity duration have increased species richness and abundance, offering another explanation as to why the most common species are found throughout each wetland unit (Baber et al. 2002).

Our observations within and between wetland units showed variation in fish abundance and structure, likely due to connectivity and water depth. To best understand the variation within each wetland unit, continued ecosystem monitoring to increase data sets and create more robust findings. MS, MN, and P1A now have three years of sampling data which can start to show trends but more data are needed to make the best informed decisions in terms of

management. MC only has one year of data thus far, and provides a baseline for future teams to compare against, especially due to its recent reconnection to SHR and early successional stages. There is also a need for continued monitoring of SHR as knowledge about what proportion of fauna from the river utilizes SNWR can be gained. Continued monitoring is needed in both MC and SHR to fully grasp the ecosystem variation and also to implement the best conservation and management strategies for not only fish, but macroinvertebrates, vegetation, and water quality.

CONCLUSION

Ecosystem Variability

Generally, the combination of water quality, vegetation, macroinvertebrates, and fish data illustrated several different pictures across the wetland units. IBI scores of macroinvertebrates when compared to previously monitored years for MS, MN, and P1A all lowered in value from mildly impacted to mildly degraded and fish IBI scores across these units remained the same from the previous years. Conversely, IBI scores for vegetation improved from low to medium across the previously sampled units. While it seems that habitat quality has improved based on vegetation data, our results showed a decline in macroinvertebrate scores possibly because of changes in water levels, from the Midland flood, which are highly influential to wetland fauna (Langer et al. 2018). MC and SHR were unique in that MC was recently reconnected to the river and that it is by far the largest and most heterogenous unit in terms of habitat, and SHR is a warm water river site that is not suited for the application of coastal wetland biotic indices. MC was consistent with other units in terms of its fish and vegetation IBI scores but was categorized as mildly impacted for macroinvertebrates which is better than all other units. MC was also unique in that pH and conductivity were significantly lower and higher, respectively, than most other units.

Community composition varied between units, indicating differences in restoration succession. NMDS for macroinvertebrates and fish showed notable variations in community structure by unit. For fish, this variation was likely due to differences in abundances of common species as over 9,000 Black Bullhead were caught in MS. Similarly, macroinvertebrate communities in MS were most dissimilar from every other unit, with the combination of these data showing MS to be the most dissimilar unit overall. FQA analysis showed the highest amount of site-specific vegetation species in MC, showing a unique community composition compared to all other wetland units. Fish community structure in SHR was most similar to P1A even though they are two completely different ecosystem types. This was not surprising as P1A is directly connected to SHR and shows that riverine fish are accessing wetland pools. More sampling within SHR would likely change NMDS results since species accumulation curves showed inadequate sampling and thus not the representative community. Continued sampling of SHR will also shed light on what potential species could move into wetland units given the proper habitat and corridors.

Variation between units was attributed to a few key factors such as water level, water chemistry, and habitat structure. Like both Lugten et al. (2020) and Dellick et al. (2021), we attribute variation in water levels within wetland units to be the

main source of abiotic and biotic variation. On a broader scale the individual elevation, and more specifically unit micro-topography, play a large role in community differences with higher areas having shallower water and lower areas having deeper water. Water depth is extremely important in determining water chemistry, vegetation, macroinvertebrate and fish composition, and variation within each unit can alter the utilization of habitat by different species. Specific water chemistry, influenced by depth, then influences movements of fish throughout wetland units, and affects survival of both fish and macroinvertebrates. This potentially explains why IBI scores for macroinvertebrates and fish are lower than previous years, but vegetation IBI scores have increased; There is sufficient quality habitat within wetland units, but lower water depth prevents adequate utilization by wetland fauna.

We understand that there is significant annual variation within SNWR which also accounts for a large portion of the variation between units. This is especially apparent when comparing water levels between years as 2020 had a historic flooding event, while our 2021 year had low water levels; in some cases we were not able to sample in previous years' sampling locations due to low water. Uzarski et al. (2018) discussed that conflicting wetland indicator scores across each sampled taxa and water quality can be attributed to changes in temporal and spatial anthropogenic disturbance and uneven distribution of disturbance, thus creating different impacts for sampled taxa. Continued monitoring will help to understand how these annual variations influence SNWR wetland ecosystems.

Research Recommendations

To better prepare future monitoring teams, we provide recommendations on: sampling, surveying SNWR for more information, comparing SHR and SPD inputs into SNWR, and comparing results to other wetlands.

Our recommendations for sampling include: using pipettes for macroinvertebrate sampling, collecting water chemistry data for vegetation sampling, and using SACs for vegetation and macroinvertebrate sampling.

For macroinvertebrate sampling, the CWMP SOP does not include sampling recommendations on three key points. First, the SOP does not explicitly recommend pipettes, so we suggest creating clear guidelines for their use during field picking to reduce tool bias and improve CPUE accuracy. In order to accurately assess macroinvertebrate abundances between units, sampling effort must be controlled, and the preference for or against pipettes strongly effects control of sampling effort. Second, lower numbers of *Gammarus* and Oligochaete suggest a shallow water bias could be affecting macroinvertebrate distributions. Because most researchers are less than 2 m tall (~6ft), and working in waders

means they do not sample in water deeper than 1 m (3ft), we recommend including more kayak or boat based sampling. Lastly, determining the control of vegetation zones on macroinvertebrate abundance necessitates sufficient sampling more thoroughly throughout MC, where numerous vegetation zones exist that were entirely unsampled (e.g., *Lythrum salicaria*), or had a small sampling size that was unusable in NMDS ordination plots (i.e., *Phalaris*, river bulrush, and smartweed/PSP). We strongly recommend conducting more sampling events in these and other vegetation zones, especially the unique vegetation zones within MC. Although statistical methods can account for non normal distributions, the extreme bias towards edge habitat visible in our sampling map must be ameliorated in future efforts if management expects to fully understand macroinvertebrate communities at SNWR, most importantly the unique MC distributions.

We also suggest collecting water quality samples during vegetation sampling to better understand the distribution of dissolved oxygen, temperature, and sediment which can determine refugia for aquatic macroinvertebrates and fishes. Understanding of the nutrient load and water quality present can give SNWR managers a better idea of areas that are susceptible to invasive plant spread, which can also determine the presence or absence of aquatic macroinvertebrates and fishes.

Calculating SACs for vegetation and aquatic macroinvertebrates is recommended to gauge whether sampling efforts were sufficient to accurately characterize these assemblages. This would give a better understanding of potential undersampling or oversampling, depending on the results. SACs would potentially have implications for future sampling as sampling efforts may have to be adjusted to properly characterize both vegetation and aquatic macroinvertebrate assemblages.

Continued research on surrounding rivers and waterways in future SNWR monitoring would greatly contribute to a deeper understanding of how these bodies of water impact the wetland units. Previously, we have taken water chemistry and nutrient samples from the SHR and SPD, this information has given our monitoring team a better understanding of the water, sediments, and nutrients moving into the refuge. We suggest directly comparing the difference in water quality sampling between the SNWR units and the river sites (SHR and SPD) to best understand the difference in sediment and nutrient deposition and transportation between these areas. We also suggest conducting more research on the water exchange dynamic after connecting wetland units with rivers. Some potential questions include: “How do the sediments from rivers get transported in different wetland units and how will the process change the bathymetry of each wetland unit?”; and “How will DO and water temperature change in different wetland units after connection with the river?”. Additionally, our team was the first

to sample the SHR and SPD for fish communities present, this research highlights species found on the edge of large and fast-moving bodies of water. Continuing this research would better help teams and managers understand the proportion of river fishes that utilize SNWR, helping managers toward the goal of supporting Yellow Perch populations. We also suggest that future teams consider sampling river sites for aquatic macroinvertebrates. Like fish, invertebrates are mobile and can be carried along the river by its flow; the communities of aquatic invertebrates may be vastly different than those in the sampled SNWR units.This information can better portray which species prefer lentic waters of wetland units or those that prefer lotic waters in SHR. Feasible river locations may be similar to those sampled by Coastal Wetland Management Program (CWMP) during their sampling event. Data collected from fish sampling, and potentially aquatic macroinvertebrate sampling, can also explain the migration of species into SNWR units.

We recommend conducting a thorough bathymetry survey across all wetland units and that the succeeding monitoring team read and apply findings from the hydrogeomorphic analysis (GWS 2013) .This can help address questions of where sediment will accrue and what to expect for the new hydrologic flow across MC. During our sampling season, we noticed that there was a significant amount of silt in P1A, which may have accumulated from direct access to SPD over 70 years of connection. This accumulation alters multiple water chemistry variables and influences the biota living in this unit. If the approximately 2 ft of silt from P1A and dissolved solids from SPD will be dispersed throughout MC over time, it would be worthwhile to study the depths within of this unit to determine silt accumulation. A wide variety of water depths supports a larger variety of species, whereas a narrow range of water depths would no longer support as many species. Sediment redistribution creates different microhabitats (Angélibert et al. 2004), with gradual changes in water depth and chemistry impacting wetland succession (Batzer and Boix 2016). These sediments can determine macrophyte communities, which is one of the most important factors in succession (Van de Meutter et al. 2008 ; Kim et al. 2014).

We recommend comparing annual results for SNWR summer monitoring to additional older and established wetlands to help clarify hypothetical outcomes for SNWR in the future. Whether the biotic communities currently viewed in the control unit, P1A, will be similar to the other three remaining wetland units, after succession stabilization, is yet to be determined. Monitoring the health of P1A as well as comparing and contrasting the succession of surrounding units can help determine if species richness will greatly increase or decrease over time. Whether within Michigan or farther abroad, referencing well-studied floodplain wetlands will help set expectations for late successional wetlands and estimate what biotic communities will look like in the future. Additionally, we recommend that future teams and managers reconsider the metrics used for sampling

SNWR, most of the IBI metrics for fish and invertebrates were created for coastal wetlands. Results from IBI metrics used in our study may not be as relevant to the refuge and may portray a much more degraded status than intended.

Management and Science

Overland flow has a larger influence on barrier protected marshes, stream flow has a larger influence on riverine marshes, and lake water levels have a higher influence on lacustrine marshes. But all of those flows may be slowed or accelerated considerably by closing or opening water control structures in managed marshes. So, perhaps the most influential factor on the SNWR marsh units is their managed water budget and regime. Because of the strong influence of hydraulic forces from extreme events creating overland flow such as from floods and storm events; and diel, seasonal and irregular water movement from seiche events and fluctuating river levels; managerial decisions will inevitably be reactive, even though further research will allow increasingly fine tuned responses to those events.

Describing SNWR as one specific wetland type is difficult because of the features unique to this location such as its topography, hydrology, and historic disturbances. The uniform, flat topography of the study units and their proximity to Lake Huron result in relatively strong lacustrine influences at SNWR. But channelization and development between SNWR and the Saginaw Bay also likely alters seiche events in complex ways (Schrouder et al. 2009). Although seiche events affect wetland unit dynamics, their effect is probably dampened by distance from Lake Huron (Trebitz 2006). Global change, and local anthropogenic change along the major tributary rivers in the SNWR watershed, create a flashy condition with strong annual riverine influences, and complex water movement within and around the refuge. These anthropogenic effects; along with invasive species, runoff, land alterations, and the refuge's already complex hydrology; mean that it is difficult to model SNWR solely as an inland coastal marsh. Despite being located where a hybrid floodplain and Great Lakes marsh is expected, the study units were so historically altered that the Shiawassee flats were for a time functionally extinct. It is our thesis that historic and current alterations to SNWR in combination with the already hybrid categorization of watershed characteristics are so large that researchers will not properly understand SNWR dynamics if the only reference is Great Lakes coastal wetlands.

The future of SNWR is quite variable, but with each successive year of monitoring, we try to depict what this revitalized wetland and floodplain will look like. We recommend that management use as a model not just inland estuarine

wetlands, but also floodplain wetland restoration, barrier protected wetlands, constructed wetlands, and novel anthropogenic factors like the effect of dike height on dispersal. SNWR's marsh units are both too innovative in restoration ecology, too important to Michigan water management, and too complex in hydrogeomorphology and history to reference only a single model for future development. Global change has accelerated so quickly since 1953 when the federal government first purchased the land that models for future management and research should include realistic projections about management's ability to recreate functionally extinct habitat and restore water quality, vegetation zones, as well as macroinvertebrate and fish assemblages as they once might have been. Nonetheless, we hope this report will provide guidance on those topics for the benefit of the ecosystem, wildlife, and the people who hope to guide SNWR forward to an abundant, rich condition.

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APPENDICES

Appendix I

Introductions for each monitored location

Pool 1A (P1A)

Since its reconnection to the Shiawassee River in 1958, P1A has served as a reference unit for floodplain restoration. P1A is open to SPD spring through fall and then closed in the winter to retain adequate water levels for overwintering species in the unit. Several vegetation zones occur throughout P1A, including *Salix* (willow), *Nymphaea* (water lilies), *Typha* (cattails), and submerged aquatic vegetation (SAV). The unit is relatively flat with similar topography and several inches of sediment accumulation, but there is some variation due to modifications made by Canada Geese and Muskrats during nest construction.

Maankiki South (MS)

MS hydrologic reconnection construction began in 2016 and was first flooded in the spring of 2018. This unit is primarily closed, with reconnection to the river typically occurring in the spring to promote flooding and later closed to retain water levels throughout the summer. Connection to the Shiawassee River has varied however, with the control structure being opened in the March of 2019 to raise water levels, in May of 2020 to receive anticipated flood waters from heavy precipitation and dam breach upstream on the Tittabawassee, in October of 2020, and in July of 2021 after heavy precipitation. Water levels are often lower compared to other units since MS is at a higher elevation. Water levels were also lower in 2021 due to less snow melt and spring precipitation; resulting with only the perimeter channels and a small area on the north side of the dike being inundated. The center area of MS was saturated but not inundated during monitoring. Vegetation throughout MS is characterized by zones of flooded forest, *Typha*, *Phalaris*, and SAV.

Maankiki North (MN)

Similar to MS, MN hydrologic reconnection construction began in 2016 but was flooded for the first time in the spring of 2017. It was also opened in May of 2019 for several days to raise water levels, May of 2020 to accept flood waters, and in July of 2021 after heavy and prolonged precipitation. MN is the lowest in terms of elevation and as a result has the highest water levels throughout the season, which resulted in limited fyke sets for the 2019 teams, but lower water levels allowed for net sets in 2020 and 2021. Similarly to MS, the perimeter of MN has deep channels that is the main cause for variable topography, with the interior of the unit being very uniform. Vegetation zones within MN include *Typha*, *Phalaris*, and SAV, but also has a row of dead trees on the north end which serves as a roosting site for various bird species, most noticeably double-crested cormorants (*Phalacrocorax auritus*).

Maankiki Center (MC)

Like both MS and MN, MC was reconnected to the Shiawassee River in 2016 but was not fully flooded until 2020. Initial plans were to flood MC in the spring of 2020, but because of flooding and COVID-19 delays construction in adjacent units was delayed, requiring water control structures to remain closed, but it received flood waters in May of 2020 from the Tittabawassee dam breaks. MC is a new study unit and all data collected in 2021 will be the first collected since reconnection to the Shiawassee River. MC is unique in that it is directly connected to P1A, in addition to the distribution basin. MC varies topography with deep channels around the perimeter and a set of interior berms that prevent erosion from wave fetch on the outer dikes and wildlife drive. Vegetation zones within MC vary and include Mixed Emergent, *Typha*, *Salix*, *Phalaris*, and SAV.

Shiawassee River (SHR) & Spaulding Drain (SPD)

The Shiawassee River and Spaulding Drain serves as a reference point for potential species movements into the SNWR wetland units. The rivers also supply the wetland with water through the various water control structures around SNWR. The Shiawassee also serves as a migratory pathway into the wetland for species coming from Saginaw Bay and Lake Huron. These sampling sites were added in 2021 and were not sampled in 2019 or 2020. Nets set in the river were within 100 feet of the shore given the topography of the river and increasing depth towards the middle. No macroinvertebrate or vegetation sampling were conducted due to time and manpower constraints, in addition to sampling in the rivers being primarily for reference of potential fish sources.

Appendix II

Tukey HSD test for water quality parameters and months.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.Temp) ~ Month, data = H2Oanalysis)
##
## $Month
##                diff         lwr          upr     p adj
## May-Apr  0.43298816  0.03402329  0.831953026 0.0236539
## Jun-Apr  0.37044615 -0.02648786  0.767380152 0.0852024
## Jul-Apr  0.48647624  0.08870688  0.884245606 0.0060848
## Aug-Apr  0.46020216  0.06193242  0.858471899 0.0121024
## Oct-Apr  0.23054127 -0.17229542  0.633377965 0.6172557
## Nov-Apr -0.90935165 -1.31218834 -0.506514956 0.0000000
## Jun-May -0.06254201 -0.13435397  0.009269947 0.1341578
## Jul-May  0.05348808 -0.02280622  0.129782380 0.3664979
## Aug-May  0.02721400 -0.05164750  0.106075507 0.9480814
## Oct-May -0.20244689 -0.30183378 -0.103059993 0.0000001
## Nov-May -1.34233981 -1.44172670 -1.242952915 0.0000000
## Jul-Jun  0.11603010  0.05118829  0.180871903 0.0000044
## Aug-Jun  0.08975602  0.02191224  0.157599786 0.0020408
## Oct-Jun -0.13990487 -0.23079709 -0.049012658 0.0001451
## Nov-Jun -1.27979780 -1.37069001 -1.188905580 0.0000000
## Aug-Jul -0.02627408 -0.09884570  0.046297534 0.9350461
## Oct-Jul -0.25593497 -0.35040855 -0.161461392 0.0000000
## Nov-Jul -1.39582789 -1.49030147 -1.301354313 0.0000000
## Oct-Aug -0.22966089 -0.32621955 -0.133102232 0.0000000
## Nov-Aug -1.36955381 -1.46611247 -1.272995154 0.0000000
## Nov-Oct -1.13989292 -1.25383234 -1.025953498 0.0000000
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.DO ~ Month, data = H2Oanalysis)
##
## $Month
##                diff         lwr         upr     p adj
## May-Apr -1.3886232 -13.5356103 10.75836388 0.9998766
## Jun-Apr -1.4913258 -13.5764807 10.59382919 0.9998071
## Jul-Apr -3.3284844 -15.4390729  8.78210411 0.9832195
## Aug-Apr -3.5148788 -15.6407018  8.61094421 0.9779559
## Oct-Apr -4.6954028 -16.9602725  7.56946694 0.9163173
## Nov-Apr  0.8044444 -11.4604253 13.06931416 0.9999954
## Jun-May -0.1027026  -2.2891080  2.08370289 0.9999994
## Jul-May -1.9398612  -4.2627370  0.38301465 0.1706569
## Aug-May -2.1262556  -4.5272933  0.27478212 0.1210660
## Oct-May -3.3067796  -6.3327386 -0.28082059 0.0220650
## Nov-May  2.1930676  -0.8328914  5.21902663 0.3254196
## Jul-Jun -1.8371586  -3.8113490  0.13703174 0.0869590
## Aug-Jun -2.0235530  -4.0891419  0.04203588 0.0591219
## Oct-Jun -3.2040770  -5.9714048 -0.43674919 0.0118281
## Nov-Jun  2.2957702  -0.4715576  5.06309803 0.1768647
## Aug-Jul -0.1863944  -2.3959285  2.02313967 0.9999794
## Oct-Jul -1.3669184  -4.2432853  1.50944851 0.7957339
## Nov-Jul  4.1329288   1.2565619  7.00929573 0.0005311
## Oct-Aug -1.1805240  -4.1203737  1.75932577 0.8967762
## Nov-Aug  4.3193232   1.3794735  7.25917299 0.0003554
## Nov-Oct  5.4998472   2.0308182  8.96887624 0.0000788
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.Cond ~ Month, data = H2Oanalysis)
##
## $Month
##                 diff        lwr        upr     p adj
## May-Apr   -0.8744203 -312.27562  310.52678 1.0000000
## Jun-Apr  -79.2053031 -389.02137  230.61077 0.9885019
## Jul-Apr -204.2755209 -514.74361  106.19256 0.4468442
## Aug-Apr -226.2278788 -537.08652   84.63076 0.3203926
## Oct-Apr -244.9069445 -559.33020   69.51631 0.2415064
## Nov-Apr -352.7736111 -667.19686  -38.35036 0.0167648
## Jun-May  -78.3308827 -134.38176  -22.28000 0.0008558
## Jul-May -203.4011005 -262.95054 -143.85166 0.0000000
## Aug-May -225.3534585 -286.90667 -163.80025 0.0000000
## Oct-May -244.0325242 -321.60627 -166.45878 0.0000000
## Nov-May -351.8991908 -429.47294 -274.32545 0.0000000
## Jul-Jun -125.0702178 -175.68073  -74.45970 0.0000000
## Aug-Jun -147.0225758 -199.97619  -94.06896 0.0000000
## Oct-Jun -165.7016414 -236.64509  -94.75819 0.0000000
## Nov-Jun -273.5683081 -344.51176 -202.62485 0.0000000
## Aug-Jul  -21.9523580  -78.59616   34.69145 0.9116351
## Oct-Jul  -40.6314236 -114.37021   33.10737 0.6591559
## Nov-Jul -148.4980903 -222.23688  -74.75930 0.0000001
## Oct-Aug  -18.6790657  -94.04531   56.68717 0.9902442
## Nov-Aug -126.5457323 -201.91197  -51.17949 0.0000218
## Nov-Oct -107.8666667 -196.79899  -18.93434 0.0067621
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.pH) ~ Month, data = H2Oanalysis)
##
## $Month
##                  diff          lwr          upr     p adj
## May-Apr -0.012237718 -0.225795887  0.201320451 0.9999979
## Jun-Apr  0.020429452 -0.192041636  0.232900541 0.9999553
## Jul-Apr -0.007480456 -0.220398695  0.205437783 0.9999999
## Aug-Apr -0.014759058 -0.227945138  0.198427022 0.9999936
## Oct-Apr -0.063877602 -0.279508286  0.151753081 0.9753925
## Nov-Apr -0.022596652 -0.238227336  0.193034031 0.9999259
## Jun-May  0.032667170 -0.005772382  0.071106723 0.1548592
## Jul-May  0.004757262 -0.036081598  0.045596122 0.9998622
## Aug-May -0.002521340 -0.044734378  0.039691697 0.9999973
## Oct-May -0.051639885 -0.104839765  0.001559996 0.0635673
## Nov-May -0.010358935 -0.063558815  0.042840946 0.9973832
## Jul-Jun -0.027909908 -0.062618472  0.006798656 0.2076392
## Aug-Jun -0.035188511 -0.071503968  0.001126946 0.0644448
## Oct-Jun -0.084307055 -0.132959899 -0.035654211 0.0000101
## Nov-Jun -0.043026105 -0.091678948  0.005626739 0.1221069
## Aug-Jul -0.007278602 -0.046124783  0.031567578 0.9978893
## Oct-Jul -0.056397147 -0.106967024 -0.005827269 0.0178763
## Nov-Jul -0.015116196 -0.065686074  0.035453681 0.9742508
## Oct-Aug -0.049118544 -0.100804524  0.002567436 0.0746892
## Nov-Aug -0.007837594 -0.059523574  0.043848386 0.9993648
## Nov-Oct  0.041280950 -0.019708617  0.102270517 0.4108685
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.Turb) ~ Month, data = H2Oanalysis)
##
## $Month
##                diff          lwr         upr     p adj
## May-Apr -1.08228750 -3.926702915  1.76212792 0.9185568
## Jun-Apr -1.28770912 -4.117828276  1.54241004 0.8270920
## Jul-Apr -1.79767392 -4.633566014  1.03821818 0.4939201
## Aug-Apr -1.62430988 -4.463769384  1.21514963 0.6176941
## Oct-Apr -1.46631504 -4.338334604  1.40570453 0.7351999
## Nov-Apr -1.11200000 -3.986515893  1.76251589 0.9123208
## Jun-May -0.20542162 -0.718413267  0.30757002 0.8979926
## Jul-May -0.71538642 -1.259325737 -0.17144710 0.0022302
## Aug-May -0.54202238 -1.104264578  0.02021982 0.0670313
## Oct-May -0.38402754 -1.092605264  0.32455018 0.6766046
## Nov-May -0.02971251 -0.748341478  0.68891647 0.9999997
## Jul-Jun -0.50996480 -0.973371030 -0.04655856 0.0205468
## Aug-Jun -0.33660076 -0.821360125  0.14815861 0.3785250
## Oct-Jun -0.17860592 -0.827418483  0.47020665 0.9830745
## Nov-Jun  0.17570912 -0.484065811  0.83548405 0.9857474
## Aug-Jul  0.17336404 -0.344034453  0.69076253 0.9548625
## Oct-Jul  0.33135888 -0.342189412  1.00490717 0.7678601
## Nov-Jul  0.68567392  0.001559515  1.36978832 0.0490570
## Oct-Aug  0.15799484 -0.530418997  0.84640868 0.9935393
## Nov-Aug  0.51230988 -0.186445320  1.21106507 0.3113777
## Nov-Oct  0.35431504 -0.466796969  1.17542704 0.8601977
```

Appendix III

Tukey HSD test for water quality parameters and units.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.Temp ~ Unit, data = H2Oanalysis)
##
## $Unit
##               diff       lwr       upr     p adj
## MN-MC   -0.6651245 -3.245617  1.915368 0.9768498
## MS-MC   -1.6085248 -4.254142  1.037092 0.5038048
## P1A-MC  -0.1207508 -2.701243  2.459742 0.9999939
## SHR-MC   0.1429193 -2.911016  3.196854 0.9999939
## SPD-MC   2.5069891 -5.200966 10.214944 0.9377193
## MS-MN   -0.9434003 -3.616837  1.730037 0.9136548
## P1A-MN   0.5443737 -2.064634  3.153381 0.9910806
## SHR-MN   0.8080438 -2.270023  3.886111 0.9749077
## SPD-MN   3.1721136 -4.545434 10.889661 0.8466250
## P1A-MS   1.4877740 -1.185663  4.161211 0.6016427
## SHR-MS   1.7514441 -1.381421  4.884309 0.5967985
## SPD-MS   4.1155139 -3.624053 11.855080 0.6482088
## SHR-P1A  0.2636701 -2.814397  3.341737 0.9998772
## SPD-P1A  2.6277399 -5.089808 10.345288 0.9250625
## SPD-SHR  2.3640698 -5.524410 10.252550 0.9556445
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.DO) ~ Unit, data = H2Oanalysis)
##
## $Unit
##                diff           lwr        upr     p adj
## MN-MC    0.34893262  0.0005737905  0.6972915 0.0493525
## MS-MC   -0.14118130 -0.4983317003  0.2159691 0.8669177
## P1A-MC   0.20365774 -0.1447010866  0.5520166 0.5481310
## SHR-MC   0.58949599  0.1772238619  1.0017681 0.0007488
## SPD-MC   0.39901286 -0.6415380693  1.4395638 0.8811477
## MS-MN   -0.49011392 -0.8510199490 -0.1292079 0.0016773
## P1A-MN  -0.14527488 -0.4974830931  0.2069333 0.8446862
## SHR-MN   0.24056337 -0.1749664857  0.6560932 0.5589666
## SPD-MN   0.05008024 -0.9917657135  1.0919262 0.9999930
## P1A-MS   0.34483905 -0.0160669778  0.7057451 0.0704217
## SHR-MS   0.73067730  0.3077498863  1.1536047 0.0000179
## SPD-MS   0.54019416 -0.5046242518  1.5850126 0.6753876
## SHR-P1A  0.38583825 -0.0296916085  0.8013681 0.0858307
## SPD-P1A  0.19535512 -0.8464908364  1.2372011 0.9945695
## SPD-SHR -0.19048313 -1.2554044556  0.8744382 0.9956481
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.Cond) ~ Unit, data = H2Oanalysis)
##
## $Unit
##                 diff         lwr         upr     p adj
## MN-MC   -0.2455372888 -0.36602563 -0.12504895 0.0000002
## MS-MC   -0.1419556593 -0.26548477 -0.01842655 0.0138819
## P1A-MC  -0.0001206499 -0.12060899  0.12036769 1.0000000
## SHR-MC   0.0886787406 -0.05391556  0.23127304 0.4777464
## SPD-MC   0.1690795956 -0.19082014  0.52897933 0.7580572
## MS-MN    0.1035816295 -0.02124646  0.22840972 0.1665578
## P1A-MN   0.2454166390  0.12359690  0.36723638 0.0000003
## SHR-MN   0.3342160294  0.19049497  0.47793709 0.0000000
## SPD-MN   0.4146168844  0.05426923  0.77496454 0.0136987
## P1A-MS   0.1418350095  0.01700692  0.26666310 0.0156344
## SHR-MS   0.2306343999  0.08435471  0.37691409 0.0001282
## SPD-MS   0.3110352549 -0.05034050  0.67241101 0.1368155
## SHR-P1A  0.0887993905 -0.05492167  0.23252045 0.4852819
## SPD-P1A  0.1692002454 -0.19114741  0.52954790 0.7584703
## SPD-SHR  0.0804008550 -0.28792797  0.44872968 0.9890281
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.pH ~ Unit, data = H2Oanalysis)
##
## $Unit
##                diff         lwr        upr     p adj
## MN-MC    0.78981335  0.51621071  1.0634160 0.0000000
## MS-MC    0.30172872  0.02122115  0.5822363 0.0267691
## P1A-MC   0.40501537  0.13141273  0.6786180 0.0004155
## SHR-MC   0.59813030  0.27432986  0.9219307 0.0000034
## SPD-MC   0.28716184 -0.53009168  1.1044154 0.9150944
## MS-MN   -0.48808463 -0.77154189 -0.2046274 0.0000193
## P1A-MN  -0.38479798 -0.66142394 -0.1081720 0.0011634
## SHR-MN  -0.19168305 -0.51804212  0.1346760 0.5429588
## SPD-MN  -0.50265152 -1.32092215  0.3156191 0.4919774
## P1A-MS   0.10328665 -0.18017062  0.3867439 0.9021555
## SHR-MS   0.29640158 -0.03576757  0.6285707 0.1107324
## SPD-MS  -0.01456689 -0.83517211  0.8060383 1.0000000
## SHR-P1A  0.19311493 -0.13324414  0.5194740 0.5346149
## SPD-P1A -0.11785354 -0.93612417  0.7004171 0.9984492
## SPD-SHR -0.31096847 -1.14736260  0.5254257 0.8942777
```

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.Turb) ~ Unit, data = H2Oanalysis)
##
## $Unit
##                diff        lwr        upr     p adj
## MN-MC    0.31480969 -0.1622221 0.79184147 0.4084387
## MS-MC    0.36169291 -0.1295897 0.85297551 0.2839477
## P1A-MC   0.06240065 -0.4165030 0.54130427 0.9990435
## SHR-MC  -0.21469963 -0.7792523 0.34985305 0.8847731
## SPD-MC  -0.04257967 -1.4674779 1.38231851 0.9999993
## MS-MN    0.04688322 -0.4495193 0.54328574 0.9998014
## P1A-MN  -0.25240904 -0.7365635 0.23174543 0.6674306
## SHR-MN  -0.52950932 -1.0985230 0.03950439 0.0845779
## SPD-MN  -0.35738936 -1.7840609 1.06928219 0.9795877
## P1A-MS  -0.29929226 -0.7974938 0.19890932 0.5174607
## SHR-MS  -0.57639254 -1.1574053 0.00462025 0.0532280
## SPD-MS  -0.40427258 -1.8357721 1.02722697 0.9655347
## SHR-P1A -0.27710028 -0.8476841 0.29348359 0.7311986
## SPD-P1A -0.10498032 -1.5322788 1.32231820 0.9999422
## SPD-SHR  0.17211996 -1.2861503 1.63039020 0.9994084
```

Appendix IV

Tukey HSD test for water quality parameters and vegetation zones

1.Tukey HSD test for average temperature and vegetation zones.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.Temp ~ Vegetation, data = H2Oanalysis)
##
## $Vegetation
##                                  diff        lwr        upr     p adj
## Nymphaea-SAV                3.1000648  -1.3189489  7.5190785 0.4698220
## Typha-SAV                   0.6609784  -1.9296984  3.2516551 0.9995144
## Dead Typha-SAV              5.2220439 -11.5147788 21.9588666 0.9969176
## Shore-SAV                   3.9145439  -1.1172202  8.9463080 0.3049913
## Smartweed-SAV               8.3203773  -3.5597745 20.2005290 0.4725495
## RB-SAV                      4.0924798  -0.7589956  8.9439552 0.1940219
## Mixed Emergent-SAV          1.2961828  -3.7355813  6.3279469 0.9994680
## Cottonwood-SAV              1.0694884  -8.6675678 10.8065446 0.9999999
## Salix-SAV                   5.0900439  -4.6470123 14.8271001 0.8558168
## Forest-SAV                 -2.7973727  -6.6430999  1.0483544 0.4107152
## Phalaris-SAV                6.4117106  -2.0527086 14.8761298 0.3459454
## Typha-Nymphaea             -2.4390864  -7.1220054  2.2438326 0.8587972
## Dead Typha-Nymphaea         2.1219792 -15.0634611 19.3074195 0.9999997
## Shore-Nymphaea              0.8144792  -5.5523705  7.1813288 0.9999996
## Smartweed-Nymphaea          5.2203125  -7.2839320 17.7245570 0.9670426
## RB-Nymphaea                 0.9924151  -5.2329314  7.2177615 0.9999956
## Mixed Emergent-Nymphaea    -1.8038819  -8.1707316  4.5629677 0.9987068
## Cottonwood-Nymphaea        -2.0305764 -12.5200181  8.4588654 0.9999679
## Salix-Nymphaea              1.9899792  -8.4994626 12.4794209 0.9999738
## Forest-Nymphaea            -5.8974375 -11.3753698 -0.4195052 0.0226607
## Phalaris-Nymphaea           3.3116458  -6.0084677 12.6317594 0.9906424
## Dead Typha-Typha            4.5610656 -12.2473633 21.3694945 0.9991325
## Shore-Typha                 3.2535656  -2.0114794  8.5186105 0.6671966
## Smartweed-Typha             7.6593989  -4.3214214 19.6402192 0.6174626
## RB-Typha                    3.4315015  -1.6615194  8.5245224 0.5354194
## Mixed Emergent-Typha        0.6352045  -4.6298405  5.9002494 0.9999998
## Cottonwood-Typha            0.4085100  -9.4511204 10.2681404 1.0000000
## Salix-Typha                 4.4290656  -5.4305648 14.2886960 0.9445457
## Forest-Typha               -3.4583511  -7.6046345  0.6879323 0.2080456
## Phalaris-Typha              5.7507322  -2.8544081 14.3558726 0.5484827
## Shore-Dead Typha           -1.3075000 -18.6606071 16.0456071 1.0000000
## Smartweed-Dead Typha        3.0983333 -17.3210124 23.5176791 0.9999974
## RB-Dead Typha              -1.1295641 -18.4312545 16.1721263 1.0000000
## Mixed Emergent-Dead Typha  -3.9258611 -21.2789682 13.4272460 0.9998481
## Cottonwood-Dead Typha      -4.1525556 -23.4040994 15.0989883 0.9999051
## Salix-Dead Typha           -0.1320000 -19.3835438 19.1195438 1.0000000
## Forest-Dead Typha          -8.0194167 -25.0664485  9.0276151 0.9244656
## Phalaris-Dead Typha         1.1896667 -17.4505605 19.8298938 1.0000000
## Smartweed-Shore             4.4058333  -8.3278660 17.1395327 0.9924972
## RB-Shore                    0.1779359  -6.4963361  6.8522079 1.0000000
## Mixed Emergent-Shore       -2.6183611  -9.4248097  4.1880875 0.9822841
## Cottonwood-Shore           -2.8450556 -13.6069957  7.9168846 0.9993206
## Salix-Shore                 1.1755000  -9.5864402 11.9374402 0.9999999
## Forest-Shore               -6.7119167 -12.6951190 -0.7287143 0.0137067
## Phalaris-Shore              2.4971667  -7.1286052 12.1229386 0.9994313
## RB-Smartweed               -4.2278974 -16.8914381  8.4356432 0.9944519
## Mixed Emergent-Smartweed   -7.0241944 -19.7578938  5.7095049 0.8062992
## Cottonwood-Smartweed       -7.2508889 -22.4705706  7.9687928 0.9180110

## Salix-Smartweed            -3.2303333 -18.4500151 11.9893484 0.9999191
## Forest-Smartweed          -11.1177500 -23.4310788  1.1955788 0.1212081
## Phalaris-Smartweed         -1.9086667 -16.3473245 12.5299912 0.9999994
## Mixed Emergent-RB          -2.7962970  -9.4705690  3.8779749 0.9661746
## Cottonwood-RB              -3.0229915 -13.7018266  7.6558437 0.9987168
## Salix-RB                    0.9975641  -9.6812710 11.6763992 1.0000000
## Forest-RB                  -6.8898526 -12.7222512 -1.0574539 0.0068100
## Phalaris-RB                 2.3192308  -7.2135363 11.8519979 0.9996919
## Cottonwood-Mixed Emergent  -0.2266944 -10.9886346 10.5352457 1.0000000
## Salix-Mixed Emergent        3.7938611  -6.9680791 14.5558013 0.9912338
## Forest-Mixed Emergent      -4.0935556 -10.0767579  1.8896468 0.5106601
## Phalaris-Mixed Emergent     5.1155278  -4.5102441 14.7412997 0.8417467
## Salix-Cottonwood            4.0205556  -9.5923416 17.6334527 0.9981117
## Forest-Cottonwood          -3.8668611 -14.1279684  6.3942462 0.9849387
## Phalaris-Cottonwood         5.3422222  -7.3914771 18.0759215 0.9658380
## Forest-Salix               -7.8874167 -18.1485240  2.3736907 0.3232403
## Phalaris-Salix              1.3216667 -11.4120327 14.0553660 1.0000000
## Phalaris-Forest             9.2090833   0.1467187 18.2714480 0.0425750
```

2.Tukey HSD test for average dissolved oxygen and vegetation zones.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.DO ~ Vegetation, data = H2Oanalysis)
##
## $Vegetation
##                                    diff          lwr        upr     p adj
## Nymphaea-SAV                 -0.5480640  -4.01197245  2.9158445 0.9999960
## Typha-SAV                    -1.1616773  -3.19241716  0.8690625 0.7667701
## Dead Typha-SAV                7.8965194  -5.22288374 21.0159225 0.7034219
## Shore-SAV                     2.8401305  -1.10409135  6.7843523 0.4271779
## Smartweed-SAV                 1.1381860  -8.17424459 10.4506167 0.9999997
## RB-SAV                       -4.8047627  -8.60766260 -1.0018627 0.0024271
## Mixed Emergent-SAV           -3.0309806  -6.97520247  0.9132412 0.3236595
## Cottonwood-SAV                0.3509638  -7.28157002  7.9834977 1.0000000
## Salix-SAV                    -3.5234806 -11.15601447  4.1090532 0.9333052
## Forest-SAV                   -4.9433291  -7.95785858 -1.9287996 0.0000092
## Phalaris-SAV                  2.5773527  -4.05760601  9.2123114 0.9808873
## Typha-Nymphaea               -0.6136134  -4.28438792  3.0571611 0.9999928
## Dead Typha-Nymphaea           8.4445833  -5.02647526 21.9156419 0.6466128
## Shore-Nymphaea                3.3881944  -1.60255374  8.3789426 0.5232016
## Smartweed-Nymphaea            1.6862500  -8.11538485 11.4878849 0.9999904
## RB-Nymphaea                  -4.2566987  -9.13652754  0.6231301 0.1555687
## Mixed Emergent-Nymphaea      -2.4829167  -7.47366485  2.5078315 0.8924054
## Cottonwood-Nymphaea           0.8990278  -7.32327448  9.1213300 0.9999999
## Salix-Nymphaea               -2.9754167 -11.19771892  5.2468856 0.9891313
## Forest-Nymphaea              -4.3952652  -8.68922246 -0.1013078 0.0395104
## Phalaris-Nymphaea             3.1254167  -4.18029060 10.4311239 0.9606456
## Dead Typha-Typha              9.0581967  -4.11733597 22.2337294 0.5027806
## Shore-Typha                   4.0018078  -0.12527462  8.1288903 0.0669253
## Smartweed-Typha               2.2998634  -7.09147773 11.6912045 0.9996718
## RB-Typha                     -3.6430853  -7.63532423  0.3491536 0.1117064
## Mixed Emergent-Typha         -1.8693033  -5.99638573  2.2577792 0.9413293
## Cottonwood-Typha              1.5126412  -6.21597427  9.2412566 0.9999641
## Salix-Typha                  -2.3618033 -10.09041872  5.3668122 0.9974362
## Forest-Typha                 -3.7816518  -7.03177661 -0.5315269 0.0084280
## Phalaris-Typha                3.7390301  -3.00623502 10.4842951 0.8012814
## Shore-Dead Typha             -5.0563889 -18.65887556  8.5460978 0.9864956
## Smartweed-Dead Typha         -6.7583333 -22.76433602  9.2476694 0.9642005
## RB-Dead Typha               -12.7012821 -26.26346497  0.8609009 0.0906163
## Mixed Emergent-Dead Typha   -10.9275000 -24.52998668  2.6749867 0.2582565
## Cottonwood-Dead Typha        -7.5455556 -22.63615961  7.5450485 0.8890532
## Salix-Dead Typha            -11.4200000 -26.51060406  3.6706040 0.3474500
## Forest-Dead Typha           -12.8398485 -26.20241357  0.5227166 0.0726253
## Phalaris-Dead Typha          -5.3191667 -19.93058121  9.2922479 0.9885894
## Smartweed-Shore              -1.7019444 -11.68344081  8.2795519 0.9999912
## RB-Shore                     -7.6448932 -12.87661881 -2.4131675 0.0001522
## Mixed Emergent-Shore         -5.8711111 -11.20644534 -0.5357769 0.0174391
## Cottonwood-Shore             -2.4891667 -10.92507079  5.9467375 0.9981278
## Salix-Shore                  -6.3636111 -14.79951523  2.0722930 0.3524658
## Forest-Shore                 -7.7834596 -12.47348020 -3.0934390 0.0000066
## Phalaris-Shore               -0.2627778  -7.80807980  7.2825242 1.0000000
## RB-Smartweed                 -5.9429487 -15.86945019  3.9835528 0.7104850
## Mixed Emergent-Smartweed     -4.1691667 -14.15066303  5.8123297 0.9669229
## Cottonwood-Smartweed         -0.7872222 -12.71739224 11.1429478 1.0000000
## Salix-Smartweed              -4.6616667 -16.59183669  7.2685034 0.9799939
## Forest-Smartweed             -6.0815152 -15.73349793  3.5704676 0.6391294
## Phalaris-Smartweed            1.4391667  -9.87878637 12.7571197 0.9999996
## Mixed Emergent-RB             1.7737821  -3.45794359  7.0055077 0.9936761
## Cottonwood-RB                 5.1557265  -3.21503453 13.5264875 0.6718530
## Salix-RB                      1.2812821  -7.08947898  9.6520431 0.9999971
## Forest-RB                    -0.1385664  -4.71037736  4.4332445 1.0000000
## Phalaris-RB                   7.3821154  -0.09028346 14.8545142 0.0562400
## Cottonwood-Mixed Emergent     3.3819444  -5.05395968 11.8178486 0.9756483
## Salix-Mixed Emergent         -0.4925000  -8.92840412  7.9434041 1.0000000
## Forest-Mixed Emergent        -1.9123485  -6.60236909  2.7776721 0.9723316
## Phalaris-Mixed Emergent       5.6083333  -1.93696869 13.1536354 0.3758907
## Salix-Cottonwood             -3.8744444 -14.54511290  6.7962240 0.9888289
## Forest-Cottonwood            -5.2942929 -13.33761192  2.7490261 0.5725195
## Phalaris-Cottonwood           2.2263889  -7.75510748 12.2078853 0.9998678
## Forest-Salix                 -1.4198485  -9.46316747  6.6234705 0.9999875
## Phalaris-Salix                6.1008333  -3.88066303 16.0823297 0.6825529
## Phalaris-Forest               7.5206818   0.41701483 14.6243488 0.0274480
```

3.Tukey HSD test for average conductivity and vegetation zones.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = avg.Cond ~ Vegetation, data = H2Oanalysis)
##
## $Vegetation
##                                  diff         lwr       upr     p adj
## Nymphaea-SAV                154.131621   36.883284 271.37996 0.0012312
## Typha-SAV                    44.572673  -24.164958 113.31031 0.5958070
## Dead Typha-SAV               94.152455 -349.920535 538.22544 0.9999200
## Shore-SAV                   -27.653101 -161.159356 105.85315 0.9999366
## Smartweed-SAV               201.269121 -113.943307 516.48155 0.6193171
## RB-SAV                      158.831942   30.109231 287.55465 0.0035475
## Mixed Emergent-SAV          100.611621  -32.894634 234.11788 0.3540078
## Cottonwood-SAV               33.819121 -224.531209 292.16945 0.9999994
## Salix-SAV                   175.485788  -82.864542 433.83612 0.5223451
## Forest-SAV                  163.784273   61.746772 265.82177 0.0000162
## Phalaris-SAV                132.069121  -92.514721 356.65296 0.7335127
## Typha-Nymphaea             -109.558948 -233.809404  14.69151 0.1442504
## Dead Typha-Nymphaea         -59.979167 -515.955190 395.99686 0.9999994
## Shore-Nymphaea             -181.784722 -350.714394 -12.85505 0.0227798
## Smartweed-Nymphaea          47.137500 -284.633790 378.90879 0.9999987
## RB-Nymphaea                  4.700321 -160.474890 169.87553 1.0000000
## Mixed Emergent-Nymphaea    -53.520000 -222.449672 115.40967 0.9964740
## Cottonwood-Nymphaea        -120.312500 -398.625645 158.00065 0.9575968
## Salix-Nymphaea              21.354167 -256.958979 299.66731 1.0000000
## Forest-Nymphaea              9.652652 -135.691648 154.99695 1.0000000
## Phalaris-Nymphaea          -22.062500 -269.350219 225.22522 1.0000000
## Dead Typha-Typha            49.579781 -396.393114 495.55268 0.9999999
## Shore-Typha                -72.225774 -211.921599  67.47005 0.8646820
## Smartweed-Typha            156.696448 -161.186987 474.57988 0.8983333
## RB-Typha                   114.259269  -20.872296 249.39083 0.1911651
## Mixed Emergent-Typha        56.038948  -83.656877 195.73477 0.9755325
## Cottonwood-Typha           -10.753552 -272.356106 250.84900 1.0000000
## Salix-Typha                130.913115 -130.689439 392.51567 0.8884992
## Forest-Typha               119.211600    9.199532 229.22367 0.0209892
## Phalaris-Typha              87.496448 -140.821106 315.81400 0.9828062
## Shore-Dead Typha          -121.805556 -582.230231 338.61912 0.9993160
## Smartweed-Dead Typha       107.116667 -434.663581 648.89691 0.9999603
## RB-Dead Typha               64.679487 -394.380964 523.73994 0.9999988
## Mixed Emergent-Dead Typha    6.459167 -453.965509 466.88384 1.0000000
## Cottonwood-Dead Typha      -60.333333 -571.128649 450.46198 0.9999998
## Salix-Dead Typha            81.333333 -429.461982 592.12865 0.9999957
## Forest-Dead Typha           69.631818 -382.671856 521.93549 0.9999970
## Phalaris-Dead Typha         37.916667 -456.658771 532.49210 1.0000000
## Smartweed-Shore            228.922222 -108.937122 566.78157 0.5263631
## RB-Shore                   186.485043    9.398629 363.57146 0.0291310
## Mixed Emergent-Shore       128.264722  -52.328694 308.85814 0.4496348
## Cottonwood-Shore            61.472222 -224.071040 347.01548 0.9999069
## Salix-Shore                203.138889  -82.404373 488.68215 0.4469395
## Forest-Shore               191.437374   32.686899 350.18785 0.0050396
## Phalaris-Shore             159.722222  -95.675436 415.11988 0.6500559
## RB-Smartweed               -42.437179 -378.435025 293.56067 0.9999996
## Mixed Emergent-Smartweed  -100.657500 -438.516844 237.20184 0.9979577
## Cottonwood-Smartweed      -167.450000 -571.269154 236.36915 0.9686453
## Salix-Smartweed            -25.783333 -429.602487 378.03582 1.0000000
## Forest-Smartweed           -37.484848 -364.190630 289.22093 0.9999999
## Phalaris-Smartweed         -69.200000 -452.296487 313.89649 0.9999841
## Mixed Emergent-RB          -58.220321 -235.306734 118.86609 0.9951371
## Cottonwood-RB             -125.012821 -408.351082 158.32544 0.9510285
## Salix-RB                    16.653846 -266.684416 299.99211 1.0000000
## Forest-RB                    4.952331 -149.796916 159.70158 1.0000000
## Phalaris-RB                -26.762821 -279.692810 226.16717 0.9999999
## Cottonwood-Mixed Emergent  -66.792500 -352.335762 218.75076 0.9997894
## Salix-Mixed Emergent        74.874167 -210.669096 360.41743 0.9993708
## Forest-Mixed Emergent       63.172652  -95.577823 221.92313 0.9769845
## Phalaris-Mixed Emergent     31.457500 -223.940158 286.85516 0.9999997
## Salix-Cottonwood           141.666667 -219.520165 502.85350 0.9794011
## Forest-Cottonwood          129.965152 -142.289667 402.21997 0.9169533
## Phalaris-Cottonwood         98.250000 -239.609344 436.10934 0.9983593
## Forest-Salix               -11.701515 -283.956333 260.55330 1.0000000
## Phalaris-Salix             -43.416667 -381.276011 294.44268 0.9999995
## Phalaris-Forest            -31.715152 -272.164096 208.73379 0.9999994
```

4.Tukey HSD test for log(average pH) and vegetation zones.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.pH) ~ Vegetation, data = H2Oanalysis)
##
## $Vegetation
##                                  diff          lwr          upr     p adj
## Nymphaea-SAV                -0.006882924 -0.066127166  0.052361318 0.9999998
## Typha-SAV                   -0.033516636 -0.068248974  0.001215702 0.0698998
## Dead Typha-SAV               0.058327886 -0.166057110  0.282712882 0.9994204
## Shore-SAV                    0.025990965 -0.041468217  0.093450147 0.9820683
## Smartweed-SAV                0.023796606 -0.135476624  0.183069836 0.9999978
## RB-SAV                      -0.114191437 -0.179233548 -0.049149325 0.0000013
## Mixed Emergent-SAV          -0.078577764 -0.146036946 -0.011118582 0.0083007
## Cottonwood-SAV               0.056790360 -0.073751103  0.187331823 0.9556486
## Salix-SAV                   -0.064437017 -0.194978480  0.066104446 0.8974685
## Forest-SAV                  -0.090690297 -0.142248678 -0.039131916 0.0000012
## Phalaris-SAV                -0.035933598 -0.149413241  0.077546045 0.9964901
## Typha-Nymphaea              -0.026633712 -0.089416045  0.036148620 0.9629611
## Dead Typha-Nymphaea          0.065210810 -0.165188652  0.295610273 0.9987189
## Shore-Nymphaea               0.032873888 -0.052484340  0.118232117 0.9821249
## Smartweed-Nymphaea           0.030679530 -0.136960703  0.198319763 0.9999819
## RB-Nymphaea                 -0.107308513 -0.190769655 -0.023847371 0.0018117
## Mixed Emergent-Nymphaea     -0.071694840 -0.157053069  0.013663389 0.1993099
## Cottonwood-Nymphaea          0.063673284 -0.076955161  0.204301729 0.9414693
## Salix-Nymphaea              -0.057554093 -0.198182538  0.083074351 0.9715538
## Forest-Nymphaea             -0.083807373 -0.157248183 -0.010366563 0.0109673
## Phalaris-Nymphaea           -0.029050674 -0.154002327  0.095900978 0.9998016
## Dead Typha-Typha             0.091844522 -0.133500474  0.317189519 0.9724206
## Shore-Typha                  0.059507601 -0.011079100  0.130094301 0.1947613
## Smartweed-Typha              0.057313242 -0.103309617  0.217936101 0.9903130
## RB-Typha                    -0.080674800 -0.148955232 -0.012394369 0.0067923
## Mixed Emergent-Typha        -0.045061128 -0.115647828  0.025525573 0.6196452
## Cottonwood-Typha             0.090306996 -0.041877778  0.222491771 0.5130018
## Salix-Typha                 -0.030920381 -0.163105155  0.101264393 0.9997894
## Forest-Typha                -0.057173661 -0.112761498 -0.001585823 0.0375756
## Phalaris-Typha              -0.002416962 -0.117783207  0.112949283 1.0000000
## Shore-Dead Typha            -0.032336922 -0.264984237  0.200310394 0.9999990
## Smartweed-Dead Typha        -0.034531280 -0.308286634  0.239224074 0.9999996
## RB-Dead Typha               -0.172519323 -0.404477311  0.059438666 0.3748932
## Mixed Emergent-Dead Typha   -0.136905650 -0.369552965  0.095741665 0.7326441
## Cottonwood-Dead Typha       -0.001537526 -0.259636549  0.256561497 1.0000000
## Salix-Dead Typha            -0.122764903 -0.380863926  0.135334120 0.9188555
## Forest-Dead Typha           -0.149018183 -0.377562049  0.079525683 0.5872742
## Phalaris-Dead Typha         -0.094261484 -0.344164789  0.155641820 0.9848288
## Smartweed-Shore             -0.002194358 -0.172910815  0.168522099 1.0000000
## RB-Shore                    -0.140182401 -0.229662138 -0.050702664 0.0000300
## Mixed Emergent-Shore        -0.104568728 -0.195820513 -0.013316944 0.0103726
## Cottonwood-Shore             0.030799396 -0.113482344  0.175081136 0.9999144
## Salix-Shore                 -0.090427982 -0.234709722  0.053853758 0.6468981
## Forest-Shore                -0.116681261 -0.196896058 -0.036466465 0.0001680
## Phalaris-Shore              -0.061924563 -0.190974074  0.067124949 0.9141452
## RB-Smartweed                -0.137988043 -0.307763906  0.031787821 0.2421109
## Mixed Emergent-Smartweed    -0.102374370 -0.273090827  0.068342087 0.7083294
## Cottonwood-Smartweed         0.032993754 -0.171051440  0.237038948 0.9999949
## Salix-Smartweed             -0.088233623 -0.292278817  0.115811570 0.9575059
## Forest-Smartweed            -0.114486903 -0.279567593  0.050593787 0.4885786
## Phalaris-Smartweed          -0.059730204 -0.253304472  0.133844063 0.9972059
## Mixed Emergent-RB            0.035613673 -0.053866064  0.125093410 0.9769530
## Cottonwood-RB                0.170981797  0.027814218  0.314149376 0.0058151
## Salix-RB                     0.049754419 -0.093413159  0.192921998 0.9922157
## Forest-RB                    0.023501140 -0.054691882  0.101694162 0.9977902
## Phalaris-RB                  0.078257838 -0.049544789  0.206060465 0.6800175
## Cottonwood-Mixed Emergent    0.135368124 -0.008913616  0.279649864 0.0892538
## Salix-Mixed Emergent         0.014140747 -0.130140993  0.158422487 1.0000000
## Forest-Mixed Emergent       -0.012112533 -0.092327330  0.068102264 0.9999975
## Phalaris-Mixed Emergent      0.042644166 -0.086405346  0.171693677 0.9949193
## Salix-Cottonwood            -0.121227377 -0.303730947  0.061276192 0.5581379
## Forest-Cottonwood           -0.147480657 -0.285047899 -0.009913416 0.0237948
## Phalaris-Cottonwood         -0.092723958 -0.263440416  0.077992499 0.8216476
## Forest-Salix                -0.026253280 -0.163820521  0.111313962 0.9999722
## Phalaris-Salix               0.028503419 -0.142213038  0.199219876 0.9999929
## Phalaris-Forest              0.054756699 -0.066739399  0.176252797 0.9432871
```

5.Tukey HSD test for log(average turbidity) and vegetation zones.

```
##   Tukey multiple comparisons of means
##     95% family-wise confidence level
##
## Fit: aov(formula = log(avg.Turb) ~ Vegetation, data = H2Oanalysis)
##
## $Vegetation
##                                  diff        lwr          upr     p adj
## Nymphaea-SAV                0.20287733 -0.6213019  1.027056587 0.9996548
## Typha-SAV                  -0.27585586 -0.7617626  0.210050913 0.7753542
## Dead Typha-SAV             -0.06269806 -3.1842413  3.058845126 1.0000000
## Shore-SAV                   1.05330321  0.1148414  1.991765025 0.0136207
## Smartweed-SAV              -0.10885976 -2.3245973  2.106877817 1.0000000
## RB-SAV                     -0.38986793 -1.2947045  0.514968690 0.9585513
## Mixed Emergent-SAV          0.08582597 -0.8526358  1.024287780 1.0000000
## Cottonwood-SAV              1.28074405 -0.5352901  3.096778194 0.4610977
## Salix-SAV                  -1.47174079 -3.2877749  0.344293355 0.2460300
## Forest-SAV                 -0.08166923 -0.8133702  0.650031783 0.9999999
## Phalaris-SAV               -0.28789683 -1.8665746  1.290780949 0.9999825
## Typha-Nymphaea             -0.47873318 -1.3536438  0.396177475 0.8143455
## Dead Typha-Nymphaea        -0.26557539 -3.4707891  2.939638355 1.0000000
## Shore-Nymphaea              0.85042588 -0.3370394  2.037891188 0.4359643
## Smartweed-Nymphaea         -0.31173708 -2.6438726  2.020398476 0.9999993
## RB-Nymphaea                -0.59274526 -1.7538191  0.568328634 0.8742898
## Mixed Emergent-Nymphaea    -0.11705136 -1.3045167  1.070413943 1.0000000
## Cottonwood-Nymphaea         1.07786672 -0.8784930  3.034226425 0.8074496
## Salix-Nymphaea             -1.67461812 -3.6309778  0.281741586 0.1766063
## Forest-Nymphaea            -0.28454655 -1.3164136  0.747320511 0.9989914
## Phalaris-Nymphaea          -0.49077416 -2.2290454  1.247497053 0.9987463
## Dead Typha-Typha            0.21315779 -2.9221618  3.348477408 1.0000000
## Shore-Typha                 1.32915907  0.3458444  2.312473758 0.0007406
## Smartweed-Typha             0.16699610 -2.0681080  2.402100169 1.0000000
## RB-Typha                   -0.11401207 -1.0652883  0.837264182 0.9999998
## Mixed Emergent-Typha        0.36168182 -0.6216329  1.344996513 0.9875846
## Cottonwood-Typha            1.55659990 -0.2830134  3.396213240 0.1902521
## Salix-Typha                -1.19588494 -3.0354983  0.643728401 0.5918387
## Forest-Typha                0.19418663 -0.5942191  0.982592345 0.9996528
## Phalaris-Typha             -0.01204098 -1.6177871  1.593705187 1.0000000
## Shore-Dead Typha            1.11600128 -2.1204836  4.352486141 0.9927021
## Smartweed-Dead Typha       -0.04616169 -3.8545231  3.762199727 1.0000000
## RB-Dead Typha              -0.32716986 -3.5540651  2.899725395 1.0000000
## Mixed Emergent-Dead Typha   0.14852403 -3.0879608  3.385008895 1.0000000
## Cottonwood-Dead Typha       1.34344211 -2.2471155  4.933999691 0.9857603
## Salix-Dead Typha           -1.40904273 -4.9996003  2.181514852 0.9793017
## Forest-Dead Typha          -0.01897116 -3.2016603  3.163717963 1.0000000
## Phalaris-Dead Typha        -0.22519877 -3.7017412  3.251343657 1.0000000
## Smartweed-Shore            -1.16216297 -3.5370936  1.212767638 0.9027907
## RB-Shore                   -1.44317114 -2.6879730 -0.198369269 0.0088609
## Mixed Emergent-Shore       -0.96747725 -2.2369311  0.301976561 0.3363760
## Cottonwood-Shore            0.22744084 -1.7797419  2.234623542 0.9999999
## Salix-Shore                -2.52504400 -4.5322267 -0.517861297 0.0026070
## Forest-Shore               -1.13497244 -2.2602230 -0.009721907 0.0459108
## Phalaris-Shore             -1.34120005 -3.1364788  0.454078744 0.3677258
## RB-Smartweed               -0.28100817 -2.6428537  2.080837316 0.9999998
## Mixed Emergent-Smartweed    0.19468572 -2.1802449  2.569616328 1.0000000
## Cottonwood-Smartweed        1.38960380 -1.4489812  4.228188808 0.9025490
## Salix-Smartweed            -1.36288104 -4.2014660  1.475703969 0.9137800
## Forest-Smartweed            0.02719053 -2.2738899  2.328270965 1.0000000
## Phalaris-Smartweed         -0.17903708 -2.8719553  2.513881106 1.0000000
## Mixed Emergent-RB           0.47569390 -0.7691080  1.720495766 0.9831687
## Cottonwood-RB               1.67061198 -0.3210710  3.662294969 0.2009483
## Salix-RB                   -1.08187286 -3.0735559  0.909810131 0.8214821
## Forest-RB                   0.30819870 -0.7891652  1.405562584 0.9988055
## Phalaris-RB                 0.10197109 -1.6759616  1.879903789 1.0000000
## Cottonwood-Mixed Emergent   1.19491808 -0.8122646  3.202100787 0.7177723
## Salix-Mixed Emergent       -1.55756676 -3.5647495  0.449615948 0.3087472
## Forest-Mixed Emergent      -0.16749519 -1.2927457  0.957755338 0.9999979
## Phalaris-Mixed Emergent    -0.37372280 -2.1690016  1.421555989 0.9999333
## Salix-Cottonwood           -2.75248484 -5.2913925 -0.213577227 0.0208792
## Forest-Cottonwood          -1.36241327 -3.2816470  0.556820481 0.4503924
## Phalaris-Cottonwood        -1.56864088 -3.9435715  0.806289725 0.5669869
## Forest-Salix                1.39007156 -0.5291622  3.309305320 0.4176007
## Phalaris-Salix              1.18384396 -1.1910866  3.558774563 0.8910590
## Phalaris-Forest            -0.20622761 -1.9026066  1.490151397 0.9999997
```

Appendix V

All plant species across sampled vegetation zones and units.

Wetland Unit	MC	MC	MC	MC	MC	MN	MN	MN	MS	MS	MS	MS	P1A	P1A	P1A	P1A
Vegetation Zone	Mixed Emergent	Phalaris	Salix	SAV	Typha	Phalaris	SAV	Typha	Forest	Phalaris	SAV	Typha	Nymphaea	Salix	SAV	Typha
Abutilon theophrasti																x
Acer saccharinum									x							
Algae	x			x			x					x	x			
aquatica												x		x		x
Amaranthus										x						
Asclepias incarnata														x		
Atriplex prostrape														x		
Barbaric orthoceris												x				
Bidens									x					x		
Bidens spp.																
Boehmeria cylindrica		x							x					x		x
Brasenia schreberi							x	x								
Butomus umbellatus		x														
Carex									x							
Carex spp.														x		
Carya ovata																
demersum	x	x		x			x	x			x	x	x		x	x
Cirsium										x						
Cirsium arvence	x					x			x	x						
Cirsium spp.														x		
Convolvulaceae														x		
Convolvulaceae spp.														x		
crepidioides														x		
Cuscuta										x						
Cyperus						x										
Cyperus strigosus										x		x				x
Dead typha	x				x							x			x	x
Digitaria spp.										x						
Echinochloa crus-galli						x						x				
Eleocharis palustris						x						x				
Elodea canadensis				x								x	x		x	
Elodea nuttallii				x			x	x								
Erechtites hieraciifolius									x							
Fontinalis spp.										x						
Galium triflorum														x		
Impatiens capensis									x							
Juncus tenuis						x										
Lactuca spp									x							
Lemna minor	x	x	x	x	x		x	x	x		x	x	x		x	x
Lemna trisulca	x	x	x	x	x		x	x	x			x	x		x	x
Lolium perenne	x															
Ludwigia palustris										x						
Lycopus americanus	x					x										
Lycopus spp.										x						
Lycopus uniflorus										x						
Lysimachia nummularia									x							
Lythrum salicaria	x	x								x		x		x		x
Mentha aquatica										x						x

Wetland Unit	MC	MC	MC	MC	MC	MN	MN	MN	MS	MS	MS	MS	P1A	P1A	P1A	P1A
Vegetation Zone	Mixed Emergent	Phalaris	Salix	SAV	Typha	Phalaris	SAV	Typha	Forest	Phalaris	SAV	Typha	Nymphaea	Salix	SAV	Typha
Mimulus ringens				x						x						
Moss									x					x		x
Myriophyllum spicatum							x	x					x			
Najas minor	x			x			x	x			x		x		x	
Nostoc commune		x														
Nymphaea odorata				x									x		x	x
Oxalis spp.														x		
Panicum									x							
Panicum spp.									x							
Panicum spp.									x							
Panicum spp.										x		x				
Penthorum sedoides																x
Persicaria amphibia	x	x				x							x		x	x
Persicaria lapathifolia						x				x	x	x		x		x
Persicaria maculosa						x										
Persicaria pensylvanica										x		x		x		x
Persicaria punctata										x				x		x
Phalaris arundinacea	x	x	x	x		x				x				x		x
Phragmites australis										x						
Pilea pumila									x							
Poaceae						x										
Populus deltoides						x	x									
Potamogeton natans	x						x	x			x					
Potamogeton nodosus	x	x		x							x	x	x		x	
Potentilla norvegica										x						
Ranunculus recureatus														x		
Riccia fluitans		x	x	x	x			x		x		x				
Ricciocarpus natans																
Rorippa spp.														x		
Sagittaria latifolia												x				
Salix interior	x	x		x										x		
Schoenoplectus tabernaemontani															x	
Scutellaria galericulata						x										
Setaria pumila									x							
Setaria viridis										x		x				
Solanum dulcamara														x		
Sonchus spp.										x						
Spirodela polyrhiza	x	x	x	x	x			x	x	x	x	x	x		x	x
Stellaria media														x		
Stuckenia pectinata	x	x	x	x							x	x	x		x	
Symphyotrichum puniceum									x							
Tanacetum vulgare									x							
Toxicodendron radicans														x		
Triadenum virginicum	x															
Typha angustifolia	x				x			x		x		x				x
Typha x glauca																x
Ulmus americana									x							
Utricularia vulgaris		x	x	x	x		x	x				x			x	
Vallisneria americana											x				x	
Verbena hastata		x								x		x				
Vitis riparia														x		
Wolffia spp.	x						x	x			x	x	x		x	
Xanthium orientale														x		

Appendix VI

Major taxa associated with high and low fetch wetlands by unit. Taxa associated with wetlands that have stronger fetch, mid fetch, or low fetch. Total CPUE for the three functional groups, as well as relative unit CPUE is presented. **Bezzia* or *Palpomia* is our SNWR 2021 LOTU, as the two genera are indistinguishable with our tools, but the high fetch taxa is solely *Bezzia.* This does not change the conclusion.

	High Fetch LOTU CPUE			
	MC	**MN**	**MS**	**P1A**
Sigara	0.22	0	0.08	0.19
Trichocorixa	1.06	2.21	6.75	1.13
Oligochaeta	0.56	0.14	1.42	0.63
Bezzia or Palpomia	0.83	0.43	0.92	0.19
Total	2.67	2.79	9.17	2.13
Relative Unit CPUE	0.03	0.03	0.1	0.02
	Low Fetch LOTU CPUE			
	MC	**MN**	**MS**	**P1A**
Gammarus	0.39	0.5	0	0.63
Crangonyx	0	0	0	0
Caecidotea	0.06	0.14	0.08	2.69
Chironomini	6.67	4.64	6.92	9.13
Tanytarsini	1.83	0.71	1.17	1.69
Total	8.94	6	8.17	14.13
Relative Unit CPUE	0.11	0.06	0.09	0.13
	Mid Fetch LOTU CPUE			
	MC	**MN**	**MS**	**P1A**
Hyalella	13.11	38.43	20	16.25
Tanypodinae	1.22	1.43	0.92	0.56
Libellulidae	1.61	0.43	0.42	0.5
Caenis	14.28	13.11	13.11	13.11
Callibaetes	0.28	0.5	0.5	0.38

Physa	5.67	1.64	5.33	6.31
Hydracarina	0.56	3.64	5.42	3.44
Total	36.72	59.18	45.69	40.55
Relative Unit CPUE	0.45	0.55	0.52	0.36

Appendix VII

Macroinvertebrate unit CPUE from 2020 to 2021 ranked by Pool 1A unit CPUE. We arbitrarily included all taxa recorded above 0.5 unit CPUE in any one pool and year. We highlighted taxa records that declined within each wetland unit from 2020-2021 (red) or increased within each wetland unit from 2020-2021 (green). We also highlighted MC records where the taxa was lower than all other wetland units and years (gold), lower than 2021 wetland units (yellow), higher than all other wetland units and years (dark blue), and higher than 2021 wetland units (light blue). Note that other trends not highlighted here are analyzed in the discussion section.

	P1A 2020	P1A 2021	MN 2020	MN 2021	MS 2020	MS2021	MC2021
Hyalella	44.71	16.25	135.21	38.43	15.38	20	13.11
Enallagma	12	0.19	2.71	3	3.06	0.08	0.78
Caenis	11.86	12.81	15.14	14	15.75	8.5	14.28
Ischnura	9.64	5.06	7.79	2.43	2.63	0.5	3.78
Physa	9.14	6.31	2.07	1.64	5.19	5.33	5.67
Neoplea	8.29	6	1	1	3.06	2.25	0.72
Caecidotea	7.57	2.69	0.21	0.14	1.19	0.08	0.06
Chironomidae*	5.93	17	4.29	9.57	6	12.08	14.72
Immature Coenagrionidae	4.07	1.38	8.36	2.43	3.19	2.83	8.72
Belostoma	2.79	1.94	1.14	0.57	1.19	1.17	1.17
Oligochaeta	2.71	0.63	1.29	0.14	0.31	1.42	0.56
Pachydiplax	2.57	0	1.14	0.07	0.69	0	0
Planorbella	2.07	0.69	0.57	0.14	1.44	0.33	0.56
Hydrachnidae	1.79	3.44	11.57	3.64	3.75	5.42	0.56
Helobdella	1.64	0	0.86	0	2.31	0	0
Hesperocorixa	1.57	1.56	23.14	2.5	1.25	0.75	0.61
Sympetrum	1.57	0	0.07	0.14	0.88	0.08	0.17
Gammarus	1.43	0.63	3.14	0.5	0.06	0	0.39
Nannothemis	1.21	0	0	0.07	0.25	0.17	0
Branchiobdellida	0.86	0	0	0	0	0	0

Erythemis	0.71	0.19	0.86	0.07	1.44	0.08	0.11
Nehalennia	0.64	0	0	0.36	0.19	0	0
Sphaeriidae	0.57	0.31	9	0.21	0.13	0	0
Notonecta	0.5	0.19	0.21	0.36	1.63	0.33	0.78
Anax	0.5	1.75	0.5	1.86	0.63	0.75	0.67
Coenagrion	0.43	3	0.21	6.57	0	0.75	3.72
Buenoa	0.43	0.19	0	0.21	0	0.17	0.5
Libellulidae	0.36	0.13	1.86	0	0.88	0	0.11
Immature Corixidae	0.29	4.94	5.07	6.14	0.88	3.67	2.72
Glossiphoniidae	0.29	0.13	2.93	0	1.63	1.25	0.11
Lestes	0.14	0.25	4.71	0	3.13	0	0.28
Merragata	0.07	0	4.5	0	0.06	0	0
Berosus	0.07	0.63	0.21	0.43	0	1.25	1.25
Leptocerus	0.07	0.75	0	0.14	0.31	0.83	0
Heliosoma	0.07	0	0	0.71	0.19	0	0.11
Drunella	0	0	1.21	0	0	0	0
Trichocorixa	0	1.13	0	2.21	0.31	6.75	1.06
Bezzia or Palpomia	0	0.19	0	0.43	0.44	0.92	0.83
Leucorrhinia	0	0.13	0.14	0.07	0.06	0.08	1.11
Callibaetis	0	0.38	0.14	0.5	0.13	0.5	0.28
Anopheles	0	0.06	0.64	0.71	0.5	0.17	0.17
Immature Pleidae	0	0.69	0	0	0	0	0
Clinotanypus	0	0	0.71	0	0	0	0

Appendix VIII

Tukey Honestly Significant Difference Test of multiple comparisons of means for macroinvertebrate CPUE by Vegetation Zone.

```
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = CPUE ~ Vegetation.Type, data = VegCPUE)

$Vegetation.Type
                                                 diff        lwr         upr     p adj
Forest-Dead Typha                          -6.53809524 -13.163780  0.08758971 0.0562847
Nymphaea-Dead Typha                        -4.77678571 -10.612890  1.05931854 0.2091370
Phalaris-Dead Typha                        -2.60989011  -9.477215  4.25743515 0.9585208
River Bulrush-Dead Typha                   -6.98319328 -12.645046 -1.32134051 0.0044711
Salix-Dead Typha                           -7.04201681 -12.703870 -1.38016404 0.0039626
Smartweed-Dead Typha                       -5.42559524 -11.421622  0.57043189 0.1116882
Submerged Aquatic Vegetation-Dead Typha    -7.36789555 -12.643696 -2.09209507 0.0005968
Typha-Dead Typha                           -7.67243867 -12.918706 -2.42617110 0.0002473
Nymphaea-Forest                             1.76130952  -3.943630  7.46624861 0.9886233
Phalaris-Forest                             3.92820513  -2.828005 10.68441512 0.6704893
River Bulrush-Forest                       -0.44509804  -5.971652  5.08145550 0.9999995
Salix-Forest                               -0.50392157  -6.030475  5.02263197 0.9999987
Smartweed-Forest                            1.11250000  -4.755937  6.98093749 0.9996344
Submerged Aquatic Vegetation-Forest        -0.82980031  -5.960131  4.30052989 0.9998890
Typha-Forest                               -1.13434343  -6.234298  3.96561154 0.9988209
Phalaris-Nymphaea                           2.16689560  -3.816982  8.15077271 0.9688967
River Bulrush-Nymphaea                     -2.20640756  -6.756485  2.34367000 0.8474028
Salix-Nymphaea                             -2.26523109  -6.815309  2.28484647 0.8273708
Smartweed-Nymphaea                         -0.64880952  -5.608544  4.31092539 0.9999781
Submerged Aquatic Vegetation-Nymphaea      -2.59110983  -6.650753  1.46853298 0.5479581
Typha-Nymphaea                             -2.89565296  -6.916841  1.12553499 0.3757955
River Bulrush-Phalaris                     -4.37330317 -10.187359  1.44075301 0.3152843
Salix-Phalaris                             -4.43212670 -10.246183  1.38192948 0.2973649
Smartweed-Phalaris                         -2.81570513  -8.955657  3.32424713 0.8839508
Submerged Aquatic Vegetation-Phalaris      -4.75800544 -10.196824  0.68081274 0.1405197
Typha-Phalaris                             -5.06254856 -10.472724  0.34762666 0.0871250
Salix-River Bulrush                        -0.05882353  -4.383135  4.26548795 1.0000000
Smartweed-River Bulrush                     1.55759804  -3.195867  6.31106355 0.9832873
Submerged Aquatic Vegetation-River Bulrush -0.38470227  -4.189589  3.42018455 0.9999971
Typha-River Bulrush                        -0.68924540  -4.453075  3.07458464 0.9997174
Smartweed-Salix                             1.61642157  -3.137044  6.36988708 0.9788783
Submerged Aquatic Vegetation-Salix         -0.32587874  -4.130766  3.47900808 0.9999992
Typha-Salix                                -0.63042187  -4.394252  3.13340817 0.9998554
Submerged Aquatic Vegetation-Smartweed     -1.94230031  -6.228666  2.34406508 0.8907876
Typha-Smartweed                            -2.24684343  -6.496806  2.00311904 0.7744903
Typha-Submerged Aquatic Vegetation         -0.30454313  -3.457940  2.84885352 0.9999979
```

Appendix IX

Fish CPUE for all species recorded by fyke netting across all sampling years. CPUEs for each unit are grouped by colors, MS = green, MN = yellow, P1A = blue, MC = red, SHR = purple. Represents across year CPUEs and also total species list for fish sampling by fyke net.

Species	MS19	MS20	MS21	MN19	MN20	MN21	P1A19	P1A20	P1A21	MC21	SHR21
Banded Killifish	0.667	0.000	0.682	0.000	0.000	1.320	0.400	0.000	0.778	0.000	0.000
Black Bullhead	56.784	57.220	436.364	91.370	21.840	21.920	12.857	1.170	0.815	6.800	0.158
Black Crappie	0.922	0.960	2.455	1.481	0.420	1.760	0.543	0.830	1.333	5.480	0.053
Bluegill	7.882	1.700	1.273	4.593	2.740	43.080	2.571	24.880	11.000	9.120	3.684
Bluntnose Minnow	0.118	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Bowfin	0.608	1.390	0.636	0.704	0.110	3.120	1.800	0.790	2.963	1.720	2.842
Brook Silverside	0.000	0.000	0.000	0.000	1.210	0.000	0.000	0.000	0.000	0.000	0.000
Brook Stickleback	0.000	0.000	0.273	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Brown Bullhead	0.039	0.000	0.045	0.111	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Central Mudminnow	0.020	0.130	11.955	0.000	0.000	0.440	0.000	0.040	0.037	0.000	0.000
Channel Catfish	0.000	0.000	0.000	0.000	0.000	0.040	0.229	0.000	0.333	0.000	0.368
Common Carp	0.098	0.130	1.318	0.296	0.210	0.320	0.257	0.000	0.296	0.480	0.105
Emerald Shiner	0.176	0.000	0.591	0.000	0.000	0.080	0.029	0.420	19.333	0.720	0.053
Fathead Minnow	0.412	0.000	0.000	0.000	0.000	0.000	0.143	0.000	0.000	0.000	0.000
Gizzard Shad	0.000	0.000	0.000	0.296	1.470	0.440	0.971	1.330	0.667	1.040	0.000
Golden Shiner	0.373	0.090	0.818	0.259	0.320	0.360	0.057	2.290	1.222	0.040	0.000
Goldfish	0.098	0.130	3.136	0.111	0.050	1.440	0.171	0.080	14.370	0.760	0.053
Greater Redhorse	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053

Green Sunfish	6.353	1.000	0.909	0.704	0.580	1.400	0.857	1.000	1.704	1.960	0.263
Johnny Darter	0.020	0.000	0.000	0.037	0.110	0.000	0.000	0.000	0.111	0.000	0.000
Largemouth Bass	0.255	0.740	0.136	0.407	0.630	0.440	0.371	7.170	2.481	0.760	0.211
Longear Sunfish	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.160	0.000
Longnose Gar	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.368
Northern Pike	0.157	0.000	0.045	0.407	0.000	0.280	0.343	0.330	0.000	0.000	0.105
Pumpkinseed	21.510	2.390	5.636	1.519	7.840	11.640	6.543	9.500	9.296	4.080	2.789
Quillback	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.053
Round Goby	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.526
Silver Shiner	0.039	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Spottail Shiner	0.059	0.000	0.000	0.037	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tadpole Madtom	0.020	0.000	0.000	0.074	0.000	0.080	0.000	0.000	0.111	0.000	0.053
Warmouth	0.059	0.170	0.000	0.148	0.480	0.000	1.429	3.380	0.000	0.000	0.000
White Crappie	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Yellow Bullhead	0.000	0.000	0.136	0.037	0.000	1.360	0.086	0.000	0.000	0.320	0.000
Yellow Perch	0.000	0.040	0.045	0.333	0.370	0.240	0.571	0.250	0.889	0.320	0.211
YOY Crappie	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000
YOY Sunfish	0.000	0.260	62.318	0.000	2.370	4.440	0.000	6.000	1.667	9.320	0.211

Appendix X

Species caught via electrofishing in each wetland unit, Shiawassee River, and spaulding drain. Represents total species list by electrofishing. X in table represents species was caught, blanks represent species were not caught.

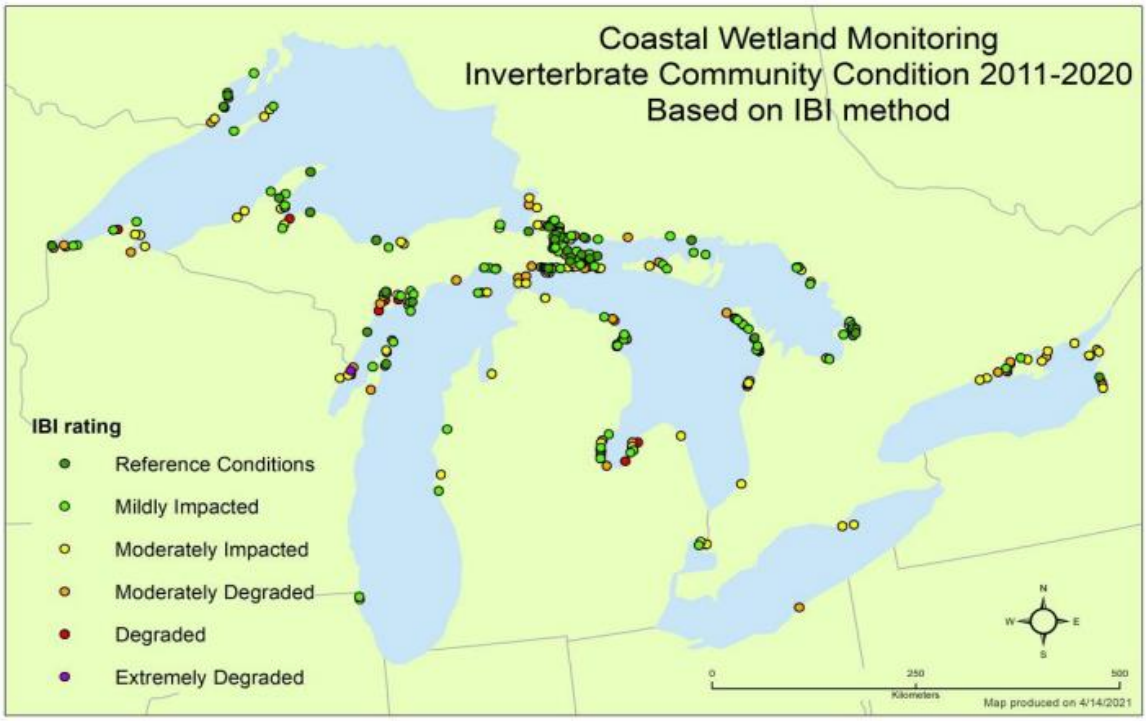
Species	MS	MN	P1A	MC	SHR	SP
Bigmouth Buffalo	X					
Black Bullhead	X	X		X		
Black Crappie	X	X	X	X		X
Bluegill	X	X	X	X	X	X
Bowfin	X	X	X	X	X	X
Channel Catfish					X	X
Common Carp	X	X	X	X	X	X
Emerald Shiner	X					X
Freshwater Drum						X
Gizzard Shad	X			X	X	X
Golden Redhorse					X	X
Golden Shiner	X	X				
Goldfish	X	X		X	X	
Green Sunfish	X		X	X		
Johnny Darter					X	
Largemouth Bass	X	X	X	X	X	X
Longnose Gar				X		
Longear Sunfish				X		
Pumpkinseed	X	X		X	X	X
Quillback					X	
Silver Lamprey			X			
Smallmouth Bass		X				X

Walleye						**X**
Yellow Perch	**X**	**X**		**X**	**X**	**X**
YOY Sunfish	**X**					

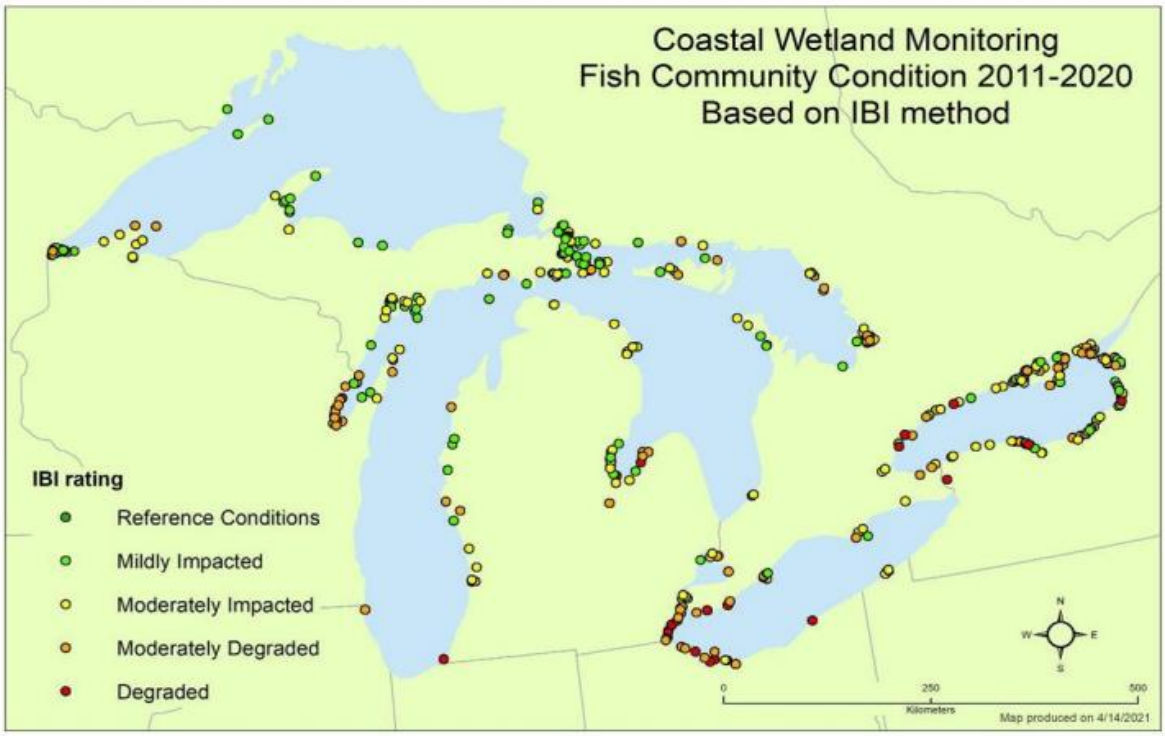
Appendix XI

These images were taken from Uzarski et al. (2022) and describe all Great Lakes coastal wetland communities with aquatic macroinvertebrate and fish IBI scores. Information was compiled from sampling efforts spanning 2011-2020. With ‘Reference Conditions' being the highest quality of wetland, located mainly in the Upper Peninsula of Michigan, sites near industrialized areas show more degraded conditions. Saginaw Bay coastal wetlands ranged from moderately degraded to degraded (third and second lowest ranking, respectively).

1.IBI scores for Invertebrate Communities in Great Lakes Coastal wetlands from 2011-2020



2.IBI scores for Fish Communities in Great Lakes Coastal Wetlands from 2011-2020



UNIVERSITY OF MICHIGAN, SCHOOL FOR ENVIRONMENT AND SUSTAINABILITY

Continuing Machine Learning Model Development for Automated Fish Counting using Adaptive Resolution Imaging Sonar at Shiawassee National Wildlife Refuge

By

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

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Abstract

Conventional fish sampling methods, such as fyke netting and electrofishing, pose safety concerns in rivers with high flows, deep water, or highly turbid waters. Imaging sonars, such as the Adaptive Resolution Imaging Sonar (ARIS), provide an alternative sampling method that can overcomes these limitations. In 2020 and 2021, an ARIS (Adaptive Resolution Imaging Sonar; Sound Metrics Corp.) unit was placed within the Shiawassee National Wildlife Refuge in Eastern Michigan to record fish activity between the Shiawassee River and the floodplain wetland units within the refuge. In 2020, a machine learning model was developed to analyze ARIS footage for fish activity (i.e. bi-directional movement). By the end of 2020, the model was able to count fish with a precision of 64.94%. In 2021, our objectives were (1) to process and store all current ARIS data; (2) to retrain the machine learning model to improve accuracy and to identify fish schools in addition to individual fish; and (3) to add a function to the post-processing code that calculates individual fish length.

The machine learning model is trained on single images and takes .mp4 files as inputs, thus all ARIS footage (produced from the sonar unit as .aris format) must be converted to .mp4 file format. The conversion process took place on Open Science Grid. All .aris files and converted .mp4 files were stored on the Great Lakes Slurm High-Performance Computing Cluster at the University of Michigan. In 2021, scientists at Advanced Research Computing (ARC) within UM trained two machine learning models on the Great Lakes cluster. The first model was trained on 3909 examples of fish and 207 examples of schools. The second model was trained on the same training set as the first model but with an additional 331 examples of fish and 901 examples of schools for a total of 4240 examples of fish and 1108 examples of schools. The models were evaluated by true positive rate, false negative rate, false positive rate, and overall precision. Individual fish lengths were calculated using a formula provided by Sound Metrics Corp.

I converted 6,856 .aris files spanning 2020 and 2021 to .mp4 format. The length function was implemented in the code but was not evaluated. The two 2021 models improved on the true positive rate and false negative rate from 2020, but the false positive rate and overall precision worsened. The poor false positive rate and precision likely stems from the model identifying small fish within schools as individual fish, thus one fish is counted as part of a school and as an individual. To rectify this, I recommend using fish length as a determiner of whether a fish should be counted as an individual.

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Introduction

The Shiawassee National Wildlife Refuge (SNWR) contains a large, hybrid river floodplain and coastal wetland that is undergoing a historic reconnection of long-diked floodplain areas with the Shiawassee River in central-eastern Lower Peninsula of Michigan. After being used for agricultural purposes for many years, the refuge was established in 1953 as a sanctuary for migratory birds. Today, the refuge is designated as a United States Important Bird Area, providing critical stopover habitat for Canada geese, ducks, and other migratory waterfowl and water birds (USFWS, no date.). In addition to bird watching, the refuge provides opportunities for other recreational activities, such as hunting and hiking. Refuge wetlands also provide flood mitigation, drought prevention, and water quality protection for the surrounding areas such as the city of Saginaw (Mitsch and Gosselink, 2000). The U.S. Fish and Wildlife Service (USFWS), as managers of the refuge, is invested in preserving and expanding these ecosystem services through continued restoration efforts.

The USFWS has partnered with the U.S. Geological Survey, Great Lakes Science Center and the University of Michigan, School for Environment and Sustainability (SEAS) to study the effects of hydrologic reconnection to the Shiawassee River on the ecology of the floodplain wetlands, including the dynamic relationships between Lake Huron, the Saginaw River and its tributaries, and the refuge wetlands. Restoration efforts on the refuge began in 2011 when Ducks Unlimited was awarded $1.5 million from Sustain Our Great Lakes to restore wetlands. The refuge's current Habitat Management Plan includes four phases, with phase I being the initial wetland restoration. Phases II, III, and IV include further restoration, enhancement, and hydrologic connectivity and are funded by the Great Lakes Restoration Initiative (Dunton 2018). Since 2019, annual student teams from SEAS have conducted post-restoration monitoring on the refuge by collecting data on fish, invertebrates, vegetation, and water quality. At the time of the 2021 student team's field study, restoration phases I and II had been completed.

Many riverine and Great Lakes fish species are known to utilize lower river floodplain and coastal wetlands for a variety of reasons, but research on movements of fish between the Great Lakes, the rivers, and these wetlands - especially those recently reconnected - has been limited. However, we have a general idea of what we think is driving fish movements in and out of wetlands during specific seasons. When water levels are high in spring, fish can move upriver and into the wetlands for spawning. However, conditions become harsh and stressful for many fish species when water levels drop in summer and movement between the river and the wetland pools is thought to be limited. In fall, water levels rise, and fish may again move upriver and into the wetlands, but it is unknown how they utilize the wetlands during this season (Eggleston et al. 2020). Knowing how many fish, what size fish, and when fish move in and out of wetlands would enhance understanding of how fish are utilizing the wetlands, and thus associated evaluation of ecological restoration (Kowalski 2010). Monitoring of fish activity can be done using hands-on methods such as fyke netting or electro-fishing. These methods allow for fish identification and fish counting, but these have practical limitations among other biases related to assessing fish community. Hands-on sampling methods are limited by access and safety concerns in rivers with high flows, deep water, highly turbid waters, or in low light. Thus,

fish sampling has been limited during the critical spring and fall seasons when water levels are high and fast due to run-off and increased rainfall. It is during these times also that fish activity is equally high. Furthermore, it is difficult to track fish activity over time using aforementioned methodologies because they represent fish activity/community composition at discrete time intervals (e.g. one net set for 24 hours).

Hydro acoustic imaging sonars provide an alternative method of collecting data on fish movements that overcomes the limitations of other sampling methods in fast-moving, deep, or turbid waters. Imaging sonar can be placed underwater at various depths and conditions, and record fish activity continuously for months. Because imaging sonars use sound waves instead of light to form images, sonar cameras can capture fish activity in highly turbid or low to no light conditions. Sonar cameras, such as the Dual-Frequency Identification Sonar (DIDSON), have been used to study fish abundance and activity between the Great Lakes and coastal wetlands via visual inspection of the sonar footage and proprietary software's, such as Echoview (Kowalski 2010; Eggleston et al. 2020). The Adaptive Resolution Imaging Sonar (ARIS) has been used to detect migrating sea lamprey with a machine learning model (Zang 2019).

In 2020 and 2021, an ARIS unit was placed in SNWR at a water control structures that allows water and fish to move between the parent river system and the restored floodplain wetland units. We used the ARIS Explorer 3000 with a frequency of 3 MHz. In 2020, the ARIS camera was placed at a water control structure connecting one of the wetland pools (Pool 1A or "P1A") and the Spaulding, which connects nearby to the Shiawassee River (see Study Area, p. 4). The camera collected data from March 18 to November 19, 2020. In 2021, the ARIS unit was also placed in the same spot as 2020 from April 9 to December 6, 2021. The ARIS unit was placed at a water control structure in the Maankiki Distribution Basin from March 8 to April 8 in an attempt to image spawning adult Northern Pike accessing three newly opened emergent marsh units (see Study Area, p. 4).

The enormous amount of data collected by the sonar cameras is impractical to analyze by visual inspection alone, so in 2020 an initial machine learning model for the analysis of ARIS data was developed and tested (Dellick et al. 2021). The machine learning model was developed by the University of Michigan, Division of Information Technology Services, Advanced Research Computing (ARC) group to count fish and determine the direction that individual fish were swimming (i.e. into or out of the wetland units.) The 2021 UM SEAS student team labeled approximately 3,500 images to train and test the model. Of the images labeled, 80% were used to train the model while 20% were used to test the model's performance. The model had a true positive rate of 57.13%, a false negative rate of 42.87%, a false positive rate of 34.06%, and an overall precision rate of 64.94%.

This initial version of the machine learning model was below that required by SNWR managers, and they desired additional functionality. The model was unable to detect fish when there were large schools of fish and had trouble detecting fish that overlapped one another. The model was also unable to calculate fish length or provide any metrics to indicate what types of fish the camera was capturing.

My objectives were: (1) to process and store all current ARIS data within a complex computing infrastructure; (2) to retrain the machine learning model to improve accuracy and to identify fish schools in addition to individual fish; and (3) to add a function to the post-processing code that calculates fish length.

How the ARIS works

Imagining sonars or acoustic cameras such as the ARIS use sound waves to produce images. Each transducer element emits an acoustic signal into the water. When the sound wave reaches an object, the wave bounces off the object and returns to the transducer element (Figure 1). The element then records the strength of that signal as well as the time between the emission and reception of the signal. The longer the time between emission and reception, the further away the object is from the camera. Hard, high-density objects, such as rocks, produce stronger signals than soft, low density objects and stronger signals will appear brighter in the resulting image. Objects in sonar images cast "shadows," meaning that they block sound waves from traveling any further in that direction so any objects behind the initial object struck by the sound wave will not appear in the image.

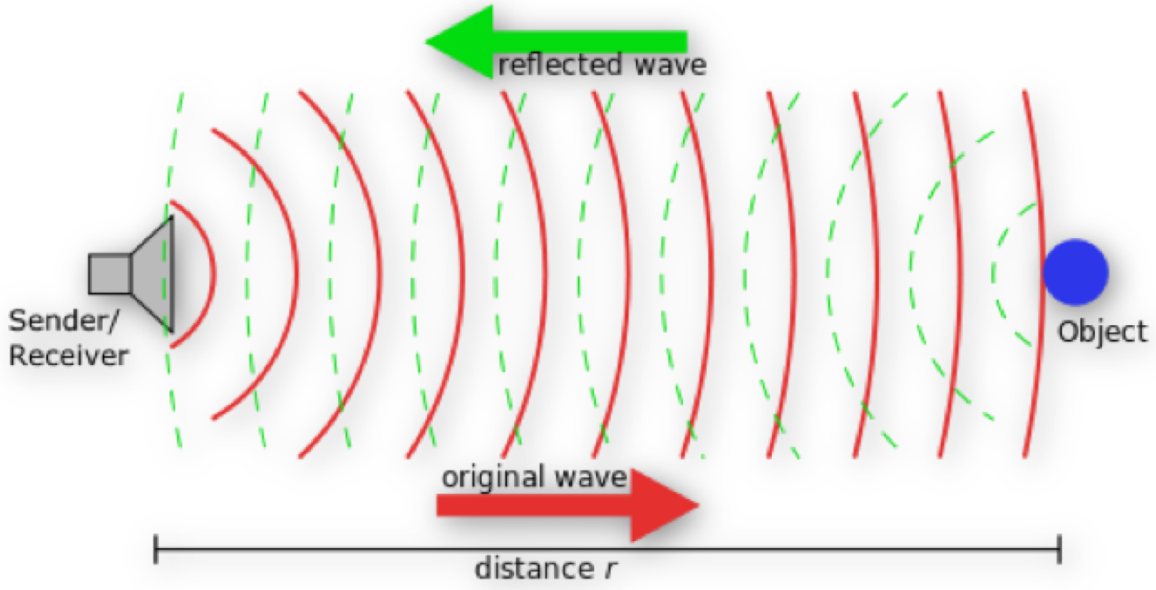

Figure 1. A transducer element (sender/receiver) emits a sound wave. The sound wave hits an object and returns to the transducer. The sound wave traveled a distance of *r*, meaning that the object is also a distance of *r* from the camera (Sound Metrics Corp. 2020).

The ARIS consists of 128 transducer elements forming a linear array. Each element emits an acoustic beam and receives the returned signal. The transducer elements do not transmit or receive at the same time. Rather, they transmit and receive in a specific order within groups. All 128 beams are then compiled together to form one image (Figure 2). The elements are oriented such that the beams are wider further from the camera. This produces a conical-shaped image,

with the cross-range or width being shorter near the camera and longer further from the camera. The areas in the bottom of an ARIS image are areas that are physically closer to the camera, while areas near the top of the image are physically farther from the camera. The range of the acoustic beam is determined by the frequency of the signal. A higher frequency produces a shorter range (e.g. 5 meters), while a lower frequency produces a longer range (e.g. 15 meters). Our ARIS model uses a higher frequency (3 MHz) with a range of 4 meters.

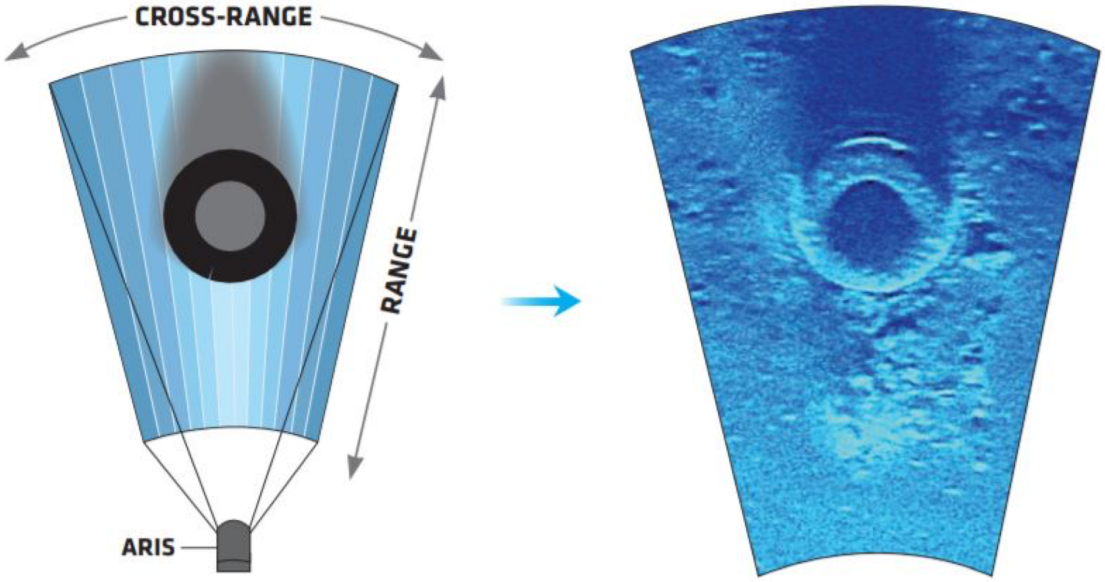

Figure 2. A diagram showing how an image of a tire is formed and the resulting image. The ARIS beams are indicated by the “slices" of the conical-shaped image on the left. The tire within the range of the camera appears in the resulting image but forms a shadow behind it (Sound Metrics Corp. 2020)

Study Area

The Shiawassee National Wildlife Refuge (SNWR) contains a large, hybrid river floodplain and coastal wetland with many management units (Figure 3). It is located within the Saginaw River watershed and is adjacent to the city of Saginaw, Michigan. The Cass, Shiawassee, Tittabawassee, and Flint rivers converge in the refuge to form the Saginaw River, which flows into Saginaw Bay and, ultimately, Lake Huron. When the area that is now the SNWR was agricultural land, many dikes were constructed to hold back the water from these rivers. As part of the restoration effort ongoing at SNWR, water control structures have been constructed to allow hydrologic reconnection between the historical floodplain areas and the river. These structures allow refuge managers to control the amount of water that flows into specific units at specific times of year. Opening the water control structures allows water and fish to move between the river and wetland the units. The ARIS camera was placed at open water control structures to capture this fish movement. For most of 2020 and 2021, the ARIS was placed at the water control structure connecting Pool 1A and the Shiawassee River (Figure 4). The ARIS was placed at the water control structure entering the Maankiki Distribution Basin connecting

the three Maankiki units (North, Center, and South) to the Shiawassee River for one month during 2021 (Figure 4). To access the Maankiki Distribution Basin, fish must swim along a channel constructed perpendicular to the Shiawassee River.

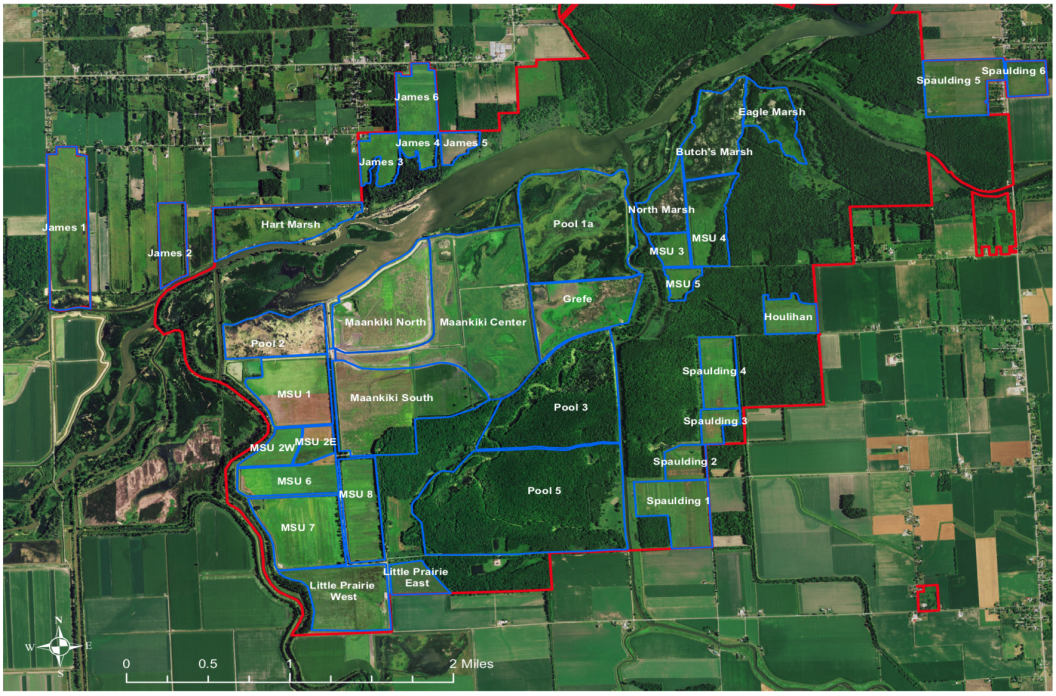

Figure 3. Named management units and locations within SNWR. The red boundary encloses the area owned by USFWS. The blue boundaries enclose individual management units (Dunton 2018).

Figure 4. ARIS locations indicated by the star at the entrances to Pool 1A (left) and Maankiki Distribution Basin (right). The channel flowing from the Shiawassee River into the Maankiki Distribution Basin can be seen along the left edge of the Maankiki North unit (Google Maps 2022)

Methods

Converting File Formats: .aris to .mp4

The machine learning model is trained on single images and takes .mp4 files as inputs, thus all ARIS footage (produced as .aris format) must be converted to .mp4 file format. This conversion process requires moving all ARIS files and the resulting .mp4 files across three separate computing or storage resources: the Great Lakes Slurm High-Performance Computing Cluster at the University of Michigan, Open Science Grid (OSG), and Amazon Simple Storage Service (S3). A high-performance computing cluster is a collection of servers or computers networked to complete tasks resulting in much higher processing speeds than those of a standard desktop computer. The Great Lakes cluster uses Slurm, a free and open-source application that manages the allocation of resources across the servers within the computing cluster. The Great Lakes cluster was used to store ARIS footage and execute simple jobs. OSG comprises thousands of computers distributed across the world (Pordes et al. 2007; Sfiligoi et al. 2009). These massive computing resources are accessible to OSG users. Because computing time on the Great Lakes cluster is a paid service, we applied for and received a grant from Extreme Science and Engineering Discovery Environment (XSEDE) for 200,000 CPU-hours on OSG to convert .aris format files to .mp4 format. S3 is a cloud storage service and S3 “buckets” are containers used to store files, similar to a folder on a standard computer. S3 buckets were used to store files as a “middle man” service between the Great Lakes cluster and OSG because we were not able to directly transfer files between them. We used an S3 compatible file service provided by the Open Storage Research Infrastructure (OSiRIS) to move files between the Great Lakes cluster and S3 and between OSG and S3. OSiRIS is a pilot project funded by the National Science Foundation to evaluate a software-defined storage infrastructure for their primary Michigan research universities.

Footage from the ARIS was downloaded in the field and stored on several 5 TB hard drives. Each hard drive contained approximately one month of footage and each file contained approximately one hour’s worth of footage. The hard drives were then plugged into a desktop computer connected to the University of Michigan network. The ARIS footage was then uploaded via Windows Secure Copy (WinSCP) to the Great Lakes Cluster. WinSCP is a free software that facilitates the transfer of files from a local computer to a remote server, such as the Great Lakes cluster.

ARC scientists created a tool to convert .aris format to .mp4 format and we housed this tool on OSG. Transfer the .aris files to OSG, we first added them to an S3 bucket – one for every day of ARIS footage. For example, we placed the 24 .aris files for each hour recorded on September 12, 2021 in the “ARIS 9/12/2021” S3 bucket. As each .aris file was converted to .mp4, the .mp4 files were outputted to a new S3 bucket corresponding to the day and year. The 24 .mp4 files for each hour on September 12, 2021 were placed in the MP4 9/12/2021 bucket. At the end of the conversion process, every .aris file in the ARIS S3 buckets had a corresponding .mp4 file in the MP4 S3 buckets. Next, the .mp4 files were transferred from S3 to the Great Lakes cluster.

Once the .mp4 files were on the Great Lakes cluster, they could then be converted to single frames for labeling or processed by the machine learning model.

Documentation for this process as well as the scripts used can be found here: https://github.com/umich-seas-snwr-fish-count/osg-workflow

Improving the Machine Learning Model

To increase the accuracy and precision of the machine learning model, other members of the 2022 UM SEAS student team and I labeled approximately 2,000 images containing single fish and approximately 2,000 images containing fish schools (Figure 5). Within each image, there may be multiple examples of fish or fish schools. We used the Python graphical image annotation tool labelImg to label the fish and fish schools within the images. Once the images were labeled, ARC scientists trained two machine learning models on the Great Lakes cluster. The first model was trained on 3909 examples of fish and 207 examples of schools. The second model was trained on the same training set as the first model but with an additional 331 examples of fish and 901 examples of schools for a total of 4240 examples of fish and 1108 examples of schools.

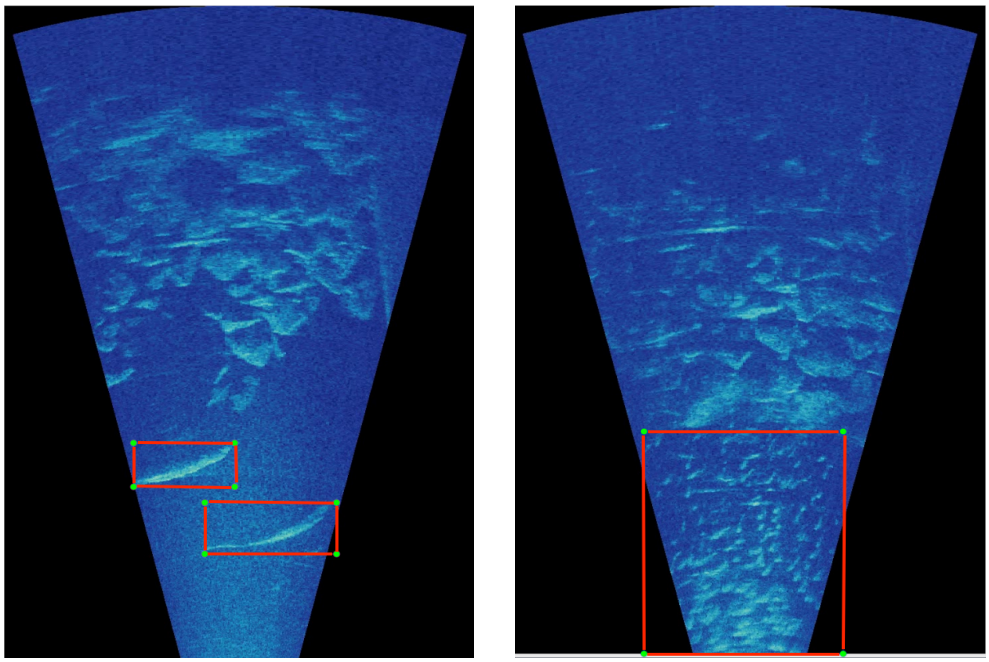

Figure 5. An example of training images containing labeled single fish (left image) and a labeled school of fish (right image). The red box was drawn by the labeler to indicate the area of the image containing a fish or fish school.

Both the 2020 and 2021 machine learning models utilized a convolutional neural network called ResNet50 from the Keras software library. A convolutional neural network is an algorithm using mathematical operations from functional analysis, such as convolution, to identify features or objects from an image in a way that mimics the human brain. The models were trained using

supervised learning. This means that fish were labeled in thousands of images and then 80% of the labeled dataset was used to show the computer what a fish looks like and the other 20% of the dataset was used to test accuracy and precision of the model's ability to identify fish in new images. In the 2020 model, only single fish were labeled (Dellick et al. 2021), while in 2021 we labeled single fish and fish schools.

The 2020 machine learning model took an .mp4 file as input and subsamples, using every other frame of the .mp4 video, to increase processing speed while maintaining information. The .mp4 videos have a frame rate of 15 frames per second. The fish do not move dramatically within one second, thus subsampling for 7.5 frames per second retains the fish's movement across the video. The machine learning model utilizes methods from PyImageSearch to keep track of the fish identified in the image. PyImageSearch is a website that publishes free algorithms and methods to complete computer vision and deep learning tasks. If an object is identified in the frame and the model is greater than 40% confident that the object is a fish, then a bounding box is drawn around the object (Figure 6). The object's centroid, the center point of the bounding box, and the coordinates of the corners of the bounding box are calculated and recorded. The object is then assigned an ID number. For example, the first object identified and tracked is Object 1. In the next image, if 1) an object, Object 2, is identified, 2) the model is over 40% confident that the object is a fish, and 3) the centroid of this object's bounding box is close to the original Object 1's centroid, then Object 2 is not assigned a new ID. Instead, Object 1's centroid is updated with Object 2's centroid because the model has determined that Object 1 and Object 2 are the same. In other words, the fish identified in the first frame is determined to be the same fish in the next frame, but in another location. This is how the model tracks a fish as it moves across frames. A fish is counted (i.e., the total fish count increases by one) when the fish has been identified for more than two frames. Thus, in this example, the fish count would increase by one. In the next frame, if there is no identified object whose centroid is close to Object 1's centroid, then Object 1 is marked as having disappeared. An object is deregistered if it has disappeared for more than 40 frames. To deregister means that the object's ID number is removed from the list of current ID numbers. The model also counts how many fish are swimming towards the left and right of the camera. When a fish is first identified, it's position in the image frame is calculated. If the fish is to the right of the midline in the image, then the total right fish count is increased by one. The total left fish count is incremented similarly. The model outputs a .csv file containing the total fish, total right fish, and total left fish counted as well as a video with bounding boxes drawn around the fish identified by the model.

Length Calculation

We calculated fish length using a formula for sonar beam width provided by Sound Metrics Corp. (Formula 1). This formula takes the fish's depth (distance) from the camera and beam angle as inputs. For our ARIS model, the beam spacing or beam angle was 0.25 degrees. We calculated depth from the camera by first locating the depth markers one, two, three, and four meters already provided on the .aris files (Figure 6). The depth markers correspond to arcs on the image, not straight lines, and the arcs intersect the bounds or the straight edges of the conical .aris video. The arcs also all have the same sagitta, the height of an arc. Thus, for any

arc intersecting the bounds of the image with the same sagitta as the depth arcs, we could calculate that arc's depth from the camera. As mentioned above, when a fish is identified, its centroid is calculated and a bounding box is drawn around it (Figure 6). To determine the fish's depth from the camera, we calculated the arc going through the centroid with the same sagitta as the depth arcs and then determined where that arc intersects the bounding lines of the image. The bounding lines are used as a kind of ruler because they have one, two, three and four meters marked.

$$beam\ width\ (cm)\ =\ depth\ (cm)\ \ x\ \ sin(beam\ angle\ °) \qquad \text{(Formula 1)}$$

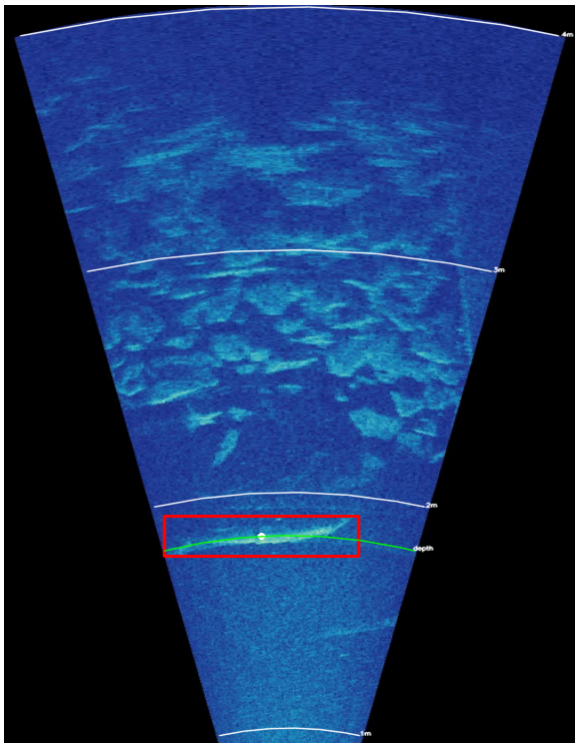

Figure 6. An .aris image containing one fish. The fish is enclosed within the red bounding box and the fish's centroid is the white circle in the middle of the red bounding box. The green arc represents the arc of consistent depth passing through the centroid of the fish. The white arcs are depth markers for one, two, three, and four meters distance from the sonar camera.

Once we calculated depth, we computed the width of each sonar beam in centimeters. Based on our ARIS unit model, we know that there are 128 beams. Thus, the length of the whole arc intersecting the fish's centroid is the beam width multiplied by 128. However, we needed the length of the arc within the fish's bounding box. To find this, we first determined how long the arc within the bounding box is in pixels, which was calculated by a simple geometric formula (Formula 2). We computed the whole arc passing through the centroid the same way. Then we divided the whole arc length in pixels by 128 to get the beam width in pixels. Then we divided the length of the arc in the bounding box by the width in pixels. This gave us the number of beams covering the arc within the bounding box. Then, we multiplied the number of beams by beam width in centimeters to get the length of the arc within the bounding box in centimeters.

This length, as represented by the portion of the green arc within the red bounding box in Figure 2, was our proxy for fish length.

$$arc\ length\ = radius\ x\ central\ angle \qquad \text{(Formula 2)}$$

The length calculation function was within the post-processing portion of the codebase, meaning that length was calculated after the .mp4 video had been analyzed by the model. When the model was running, the centroids and the coordinates of the corners of the bounding boxes for every fish were recorded as the fish moved across the camera. Thus, for every frame a fish appeared in, there was a record of the fish's centroid and bounding box coordinates. To calculate the best estimate of the fish's length, we used the bounding box with the largest total area. Since many fish could be identified when they were only partially in frame, the largest bounding box likely ensured that the full fish was in frame. Once the largest bounding box was determined, then the length was calculated and recorded. When the model was run, the lengths for every fish identified by the model were reported in a .csv output file.

Evaluation of Model Performance

The machine learning models are evaluated by: True Positive Rate, False Negative Rate, False Positive Rate, Precision, and mean Average Precision (mAP) values. Both machine learning model versions built in 2021 were tested on 1830 examples of known (visually identified) fish and 847 examples of known fish schools. All these metrics except mAP were calculated for each identification class (fish or fish schools). True Positive Rate is the rate at which the model recognizes an actual fish or a fish school. It is calculated as the ratio of false positives to the sum of the true positives and false negatives (Formula 3). False Negative Rate is the rate at which the model fails to recognize a fish or school of fish. It is calculated as the ratio of false negatives to the sum of the true positives and false negatives (Formula 4). False Positive Rate is the rate at which the model identifies a fish or school of fish incorrectly. It is calculated as the ratio of false positives to the sum of false positives and true positives (Formula 5). Precision is the ratio of true positives to the sum of the true positives and false positives. It is calculated as the ratio of true positives to the sum of true positives and false positives (Formula 6). Mean Average Precision is the average of the precisions for each identification class.

$$True\ Positive\ Rate\ (TPR)\ = \frac{TP}{P} = \frac{TP}{TP + FN} \qquad \text{(Formula 3)}$$

$$False\ Negative\ Rate\ (FNR)\ = \frac{FN}{P} = \frac{FN}{TP + FN} \qquad \text{(Formula 4)}$$

$$False\ Positive\ Rate\ (FPR)\ = \frac{FP}{FP + TP} \qquad \text{(Formula 5)}$$

$$Precision\ = \frac{TP}{TP + FP} \qquad \text{(Formula 6)}$$

The 2020 model did not have a mean Average Precision because it only had one identification class – fish. The 2021 models had a mean Average Precision, which was the average of the precision for fish and the precision for fish schools. A good model maximizes True Positive Rate, Precision, and mAP, while minimizing False Negative Rate and False Positive Rate

Results

I converted 3137 .aris format files – spanning from March, 18 2020 to November, 19 2020 and 3719 .aris files spanning from March 8, 2021 to December 2, 2021 – to .mp4 format. All .aris files and their corresponding .mp4 files were stored on the Great Lakes cluster.

I implemented the length calculation function within the post-processing code, but it was not evaluated.

ARC trained two machine learning models in 2021. The first model performed modestly at identifying individual fish with a precision rate of 57% and was very good at identifying schools of fish with a precision rate of 90%. The second model is considered more robust because it was trained on more data and with a more even distribution between examples of individual fish and fish schools. However, performance in identifying individual fish decreased to a precision rate of 45%, while performance in identifying fish schools remained strong with a precision rate of 91% (Table 1). Compared to the 2020 model for individual fish, both 2021 models increased the True Positive Rate and decreased the False Negative Rate. However, the False Positive Rate and overall Precision decreased substantially (Table 1).

	2020 Model	2021 Model 1		2021 Model 2	
	Fish	Fish	School	Fish	School
True Positive Rate	57%	67%	98%	73%	99%
False Negative Rate	43%	33%	2%	27%	1%
False Positive Rate	34%	51%	11%	90%	10%
Precision	66%	57%	90%	45%	91%
mAP	N/A	74%		77%	

Table 1. Mean Average Precision for the 2021 models, and True Positive Rate, False Negative Rate, False Positive Rate, and Precision metrics for the 2020 model and for both 2021 models and both classes (fish and fish schools).

Discussion

Machine Learning Model Performance

The model performance for individual fish is below what has been achieved in similar studies, while the performance for fish schools is on par. Similar studies have achieved False Positive Rates of 5% and False Negative Rates of 7% (Bothmann et al. 2016). Our model's low

Precision for individual fish could be due to training image quality, a noisy background, or a confusing overlap in the individual fish and fish school classes.

The effect of poor training image quality can negatively affect the model's ability to identify fish and lead to poor test results. The labeling team for individual fish consisted of four people labeling images independently, none with prior experience in the process. Although we each had a brief training on labeling images, we did not conduct any systematic quality control methods to determine if we were labeling consistently and accurately across labelers.

One factor that could contribute to training inconsistency is the difficulty of visually identifying individual fish against the rocky background of the sonar images. The sonar range captures rocks on the benthos of the sampling area, resulting in an image background for the camera containing many rocks that are of a similar shape and size as the larger fish (Figure 5). When looking at a single frame, it can be difficult to tell if a certain object is a fish or a rock. I would toggle between multiple images, and I would then be able to see a fish moving amongst the rocks. Even when I did determine that there was a fish amongst the rocks it was still a challenge to determine where exactly the fish began and ended. Sometimes the fish's tail or head would merge with a rock making it impossible to tell the exact boundary of the fish. Subsequently, if the labelers failed to label many fish in the rocks, then the model's ability to identify fish amongst the rocks decreases because it lacks examples of fish amongst the rocks. By visually inspecting some labeled output videos, I found that the model does sometimes label rocks as fish and misses fish in the rocks, more than fish in the clear areas. This likely contributes to the high False Positive Rate and high False Negative Rate of individual fish. In addition, the model may correctly identify a fish, but if the testing image is mislabeled then that identification will be recorded as a false positive.

The machine learning model has also been evidenced to identifying small fish within schools as individual fish. In response to the high False Positive Rate for individual fish, I examined the labeled output videos for three .mp4 videos I knew contained fish schools. I found that the model tends to label more individual fish within schools when the school contains fewer fish (Figure 7). Most likely this is due to my labeling methodology. When there were two or three small fish in a frame, I chose to label them as individual fish rather than a school. Edge cases where there are ten or fifteen individual fish that are visually distinct are simultaneously being labeled as a school and labeled for individual fish. For larger schools, the fish mostly blur together, but the model can still identify an individual fish if it is distinct enough. The model is trained to identify small fish as individual fish regardless of if they are in school or not. The model is correctly identifying a small fish as an individual, but the test images did not have small fish within schools labeled as individuals, thus resulting in a false positive.

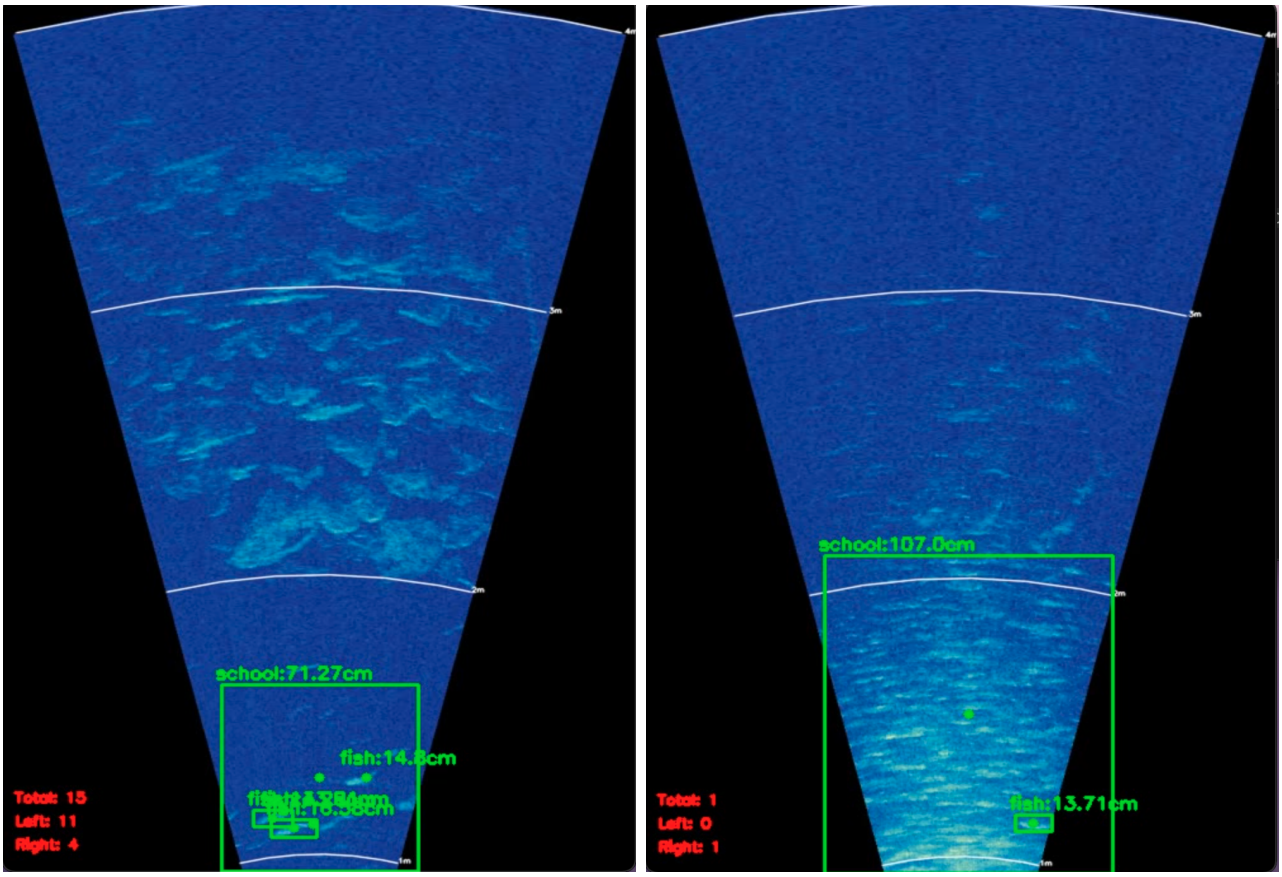

Figure 7. Frames from model output videos where the model labeled individual fish within a small school (left) and a large school (right).

Future of File Management

The time and labor-intensive process of converting two years of .aris footage to .mp4 format has shown the importance of frequent and scheduled file conversions within USGS or the UM SEAS student team. Going forward, someone within the USGS or the UM SEAS team should have the regular responsibility of converting the previous month's .aris footage to .mp4 format. ARC staff and I wrote the scripts necessary to semi-automate the conversion process, so the person responsible for converting the files would not need to have much coding knowledge or experience. ARC staff is also working with the USGS to replicate our workflow within their computing infrastructure so that the conversion and storage process can be done entirely within the USGS without the need for UM resources.

Recommendations

Installing a Flat Background to Minimize Noise

Installing a flat background over the rocks would likely yield many benefits for model performance. With no rocks obscuring the fish, it will be easier and faster to label accurately.

With better training images and less background noise from the rocks, the model will also likely become more accurate. A flat background covering the rocks will also discourage the fish from milling and hiding in the rocks thereby focusing the ARIS work on studying fish actively swimming in and out of the wetland pools. Since having too many fish in one frame may decrease the model's accuracy, keeping the fish moving past the camera will prevent the frame from becoming too crowded.

Since we are using a sonar camera, the coloration of the background has no effect. My recommendation is to find a flat material that would not degrade in the water and is heavy enough to stay in place, such as a concrete slab.

Quality Control and Training Two Models

If retraining the model is required, I recommend performing some form of quality control on the training image process. The mislabeled images are likely from failing to label fish rather than mislabeling a fish (i.e. labeling a rock as a fish). There could be a review process in which two labelers review each other's images with a focus on finding any fish in the rocky areas that are unlabeled. In addition, several labelers could label the same set of images and a script could be written with the help of ARC to compare how each person labeled the images.

It may also be beneficial to explore training two separate models – one for individual fish and a separate one for fish schools. It may increase the accuracy of identifying individual fish if the model is optimized to only identify individual fish. Then both models could be run on the same .mp4 file to get counts of individual fish and fish schools. One potential drawback of this approach is overcounting small fish. Creating separate models would not eliminate the problem of counting individual fish within schools. However, it still may be interesting to see if having two separate models improve overall performance.

Preventing the Overlap of Individual Fish and Fish Schools

There are two avenues to explore in preventing the overlap of individual fish and fish schools. First, the model could be retrained with stricter parameters guided by ARC and the USGS on what should be labeled as an individual fish versus a fish school. However, I think there would still be edge cases where the model will identify the individual fish within a school. The second option is to use the length of the fish identified within a school to determine if it should be counted as an individual fish. Simply discarding all fish identified within schools would lead to an undercount of individual fish because larger fish do swim within the schools of small fish and can be identified by the model as distinct from the school. If the fish is larger than a specified length, then it gets counted as an individual. If not, then it is assumed to be a small fish within a school and not counted as an individual.

Improve Directionality Function

The directionality function should be changed to determine what direction the fish is swimming. Currently, the model simply records the fish's position relative to the midline (right or left) when it is first identified by the model and uses that as a proxy for directionality. If a fish is to the left of the midline when it is first identified, but leaves the frame to the right of the midline, then the fish was swimming to the right, but its direction is recorded as left. Because the model gives each fish a unique ID and tracks it through successive frames, we could instead determine the fish's position relative to the midline in the last frame that it appears. This would not be difficult to change with the assistance of ARC.

Ground Truthing Length, Species, and Camera Properties

The length function needs to be tested for accuracy via ground truthing. In order to test length, an object of known length (e.g. a piece of wood, etc.) could be placed certain distances from the camera and then the .aris footage from that recording would need to be converted to .mp4 format. A script could then be written with the assistance of ARC to use our length function to measure the object and compare the true measurement to the code-calculated length. Additionally, fish lengths estimated from the ARIS imagery could be compared with actual fish lengths collected via sampling (e.g. netting) near the camera. These would not be "the same" fish as in the images but would represent the populations found in the vicinity.

We would like to be able to identify fish species from ARIS images. The use of gill-netting or cast-netting could also be used to determine what species of fish are active near the camera and during what weeks or seasons. We briefly tried to visually identify fish species using the sonar footage, but quickly realized that we were unable to do so. We noticed that we were seeing the underside of the fish rather than the top view that we were expecting. For example, we could often see paired ventral fins plus the anal fin, or the long anal fin of a Channel Catfish *Ictalurus punctatus*. This limited our ability to identify fish species because many of the identifying features (i.e., distinctive dorsal fins) cannot be seen from the bottom view. It appears that the ARIS produces a "wire-mesh" image that characterizes a 3-D view of the fish, often emphasizing the bottom view. It could be useful to place objects (or perhaps actual fish) with an identifiable top and bottom in front of the camera at different distances, times, and conditions to research what could be causing the camera to image the bottom view of an object rather than the top. It may also be beneficial to contact Sound Metrics, the producer of the ARIS, to discuss how the ARIS image formation process may be causing the bottoms of the fish to be seen.

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