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### Life History, Resource Allocation, and Phenology of Diploid Flowering Rush (*Butomus umbellatus*) Collected from Populations in Ohio and New York

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Life History, Resource Allocation, and Phenology of Diploid Flowering Rush (*Butomus  
umbellatus*) Collected from Populations in Ohio and New York

by

Maxwell G. Gebhart

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

Minnesota State University, Mankato

Mankato, Minnesota

Date of Graduation July 2023

Life History, Resource Allocation, and Phenology of Diploid Flowering Rush (*Butomus  
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Life History, Resource Allocation, and Phenology of Diploid Flowering Rush (*Butomus umbellatus*) Collected from Populations in Ohio and New York

Maxwell G. Gebhart

Flowering rush (*Butomus umbellatus*) is an invasive wetland perennial monocot that is native to eastern Europe that invaded north America in the early 1900's. Flowering rush made its way to the Great Lakes in the mid-1950's and has now invaded lake and wetland systems along the U.S. – Canadian border causing a host of issues related to water quality and water use. Within the current invaded range two cytotypes, a diploid and a triploid, have been documented which much of the current research and developed management strategies have been largely on the triploid. Research presented here is on the diploid cytotype which is considerably understudied compared to its triploid counterpart. One study was aimed at understanding the phenology and resource allocation of diploid flowering rush from three study sites: Mentor Marsh, OH; Tonawanda, NY; and Unity Island, NY. It was found that diploid flowering rush had the aboveground tissue peak in early to mid-summer with little resource investment. Once the aboveground tissue peaked, the generated carbohydrate content was then diverted to the rhizome and rhizome bud tissues for long-term storage as starch and vegetative reproduction which peaks right before wintertime. Along with sexual reproduction occurring in the flowers, diploid flowering rush produces rhizome buds, which act as vegetative propagules, on a scale almost twenty times higher than the triploid cytotype. With high reproductive output, diploid flowering rush presents itself as a threat for further spread in the U.S.

Alongside understanding the phenological patterns of diploid flowering rush, abiotic factor influences were analyzed using ecological niche modeling. This was done to model site specific abiotic effects as well as the potential for further spread within the U.S. Diploid flowering rush displayed plastic responses to its environment with each study site being affected by different abiotic variables from levels of soil organic carbon content to precipitation seasonality. From ecological niche modeling, diploid flowering rush was determined to have high suitability along the east coast of the U.S. Records from the U.S. Geological Survey Nonindigenous Aquatic Species database were used to determine that numerous populations of flowering rush occupy areas of high suitability based on the generated ecological niche models. Research done on diploid flowering rush suggests that management should be aimed to control the aboveground tissue using herbicide treatments. This management should be done early in the growing season for effective control, otherwise, flowering rush will be minimally affected by ill-timed management. However, further research should be done to optimize management strategies further for populations of diploid flowering rush.

## CHAPTER 1 - LITERATURE REVIEW

Flowering rush (*Butomus umbellatus* L.) is an invasive perennial monocot first found within the St. Lawrence River, Quebec, Canada in 1897 (Gunderson et al. 2016). Since this discovery, this species has made its way into the Great Lakes system in the mid-1950s and has spread throughout the northern U.S. from Washington to Maine and adjacent Canadian provinces (Bellaud 2009). Flowering rush is a macrophyte that can grow submersed or as an emergent plant. It has triangular cross-section leaves and an umbel inflorescence of light pink flowers that extend above the water's surface. This species is native throughout Europe and into western Asia (Russia, Turkey, and Syria) and is believed to have several separate introductions with plants coming from several countries within these two regions (Anderson et al. 1974; Gaskin et al. 2021). Flowering rush native to Europe was found to be introduced into western North America whereas the west Asian plants were introduced into the St. Lawrence River (Anderson et al. 1974; Gaskin et al. 2021). This plant, both diploid and triploid, has been found to grow in water as deep as 4 meters; however, it is typically found between 0.3-1.3 meters deep (Madsen et al. 2016a; Carter et al. 2018). Within North America, there are two established cytotypes: the diploid variety, most common in the Great Lakes region, and the triploid variety, found from Minnesota to western Canada (Gaskin et al. 2021; Gunderson et al. 2016; Madsen et al. 2016c).

The triploid form typically reproduces vegetatively through fragmentation of rhizomes, whereas the diploid can reproduce sexually via seed and vegetatively through fragmentation of rhizomes (Thompson & Eckert 2004; Lui et al. 2005). Additionally, flowering rush seed has high germination success after winter when cold waters flood wetland areas, followed by increased spring temperatures and longer photoperiods (Hroudová and Zákřavský 2003). It was also found



that both cytotypes of flowering rush are known to grow almost to peak height a month into the growing season however the peak rhizoid bud density is seen much later (Marko et al. 2015). Despite these reproductive differences, it's been found that during periods of receding water flowering rush is stimulated to produce rhizome buds at a faster rate (Hroudová 1989). Harms et al. (2021) found that the two cytotypes had different reactions to high nutrient environments; the diploid form would create high amounts of biomass compared to the triploid form which created less biomass. This could pose a problem for sites that receive high amounts of external nutrient loading where diploid flowering rush has the potential to become an even larger nuisance due to increased nutrient access and higher growth rates (Eppinga et al. 2011). These differences in life history strategies are important for management, however most of the life history and management information available for flowering rush is specific for the triploid cytotype. These life history differences could offer further information as to the optimal time to implement management strategies to coincide with periods of low plant resources.

Flowering rush is known to cause several issues within aquatic ecosystems such as forming dense mats to exclude native species like the hardstem bulrush (*Schoenoplectus acutus* Muhl ex. Bigelow), creating large monotypic stands (Madsen et al. 2016a), and rapidly colonizing marsh lands (Bellaud 2009). Flowering rush has also been known to cause numerous economic issues with water use such as clogging water filters for irrigation canals and drainage ditches (Bellaud 2009), reducing water recreation, and providing habitation for great pond snails which host several parasites that cause swimmers' itch (Jacobs et al. 2011). Because of the concern with water use and access, as well as the threats that flowering rush poses ecologically, this species is near the top of the list for management in most states where it has invaded. Management programs have focused on removing flowering rush using mechanical harvesting

(Bellaud 2009) or with herbicides. Herbicides such as diquat (Madsen et al. 2016b; Parsons et al. 2019), triclopyr (Madsen et al. 2016b, Turnage et al. 2017), and fluridone (Madsen et al. 2016b) have all been evaluated. Turnage et al. 2019 showed clear results using sequential diquat applications on triploid flowering rush and found that above and below ground biomass was reduced after the initial treatment. Bellaud (2009) has several suggestions towards management methods that have been attempted with flowering rush such as herbicides and mechanical control. Herbicides, as mentioned earlier, are a more effective means for treating flowering rush with mechanical control not being recommended (Turnage et al. 2019b). Due to the asexual reproductive potential, hand digging can spread the rhizome buds more rapidly and clippings of leaf or flower tissue are not as effective since these tissues can grow back rapidly (Turnage et al. 2019b).

Alongside understanding the phenology of diploid flowering rush, we will be evaluating the ecological niche within each study site and across North America. Biological modelling has quickly grown in use with the goal of understanding complex pieces of biogeography. Flowering rush, like numerous invasive plants, is a continent hopping plant brought over for ornamental uses however, after escaping into the St. Lawrence River it quickly became a problem (Gunderson et al. 2016). Understanding which abiotic factors influence the current range of diploid flowering rush could lead to better management and predictions for future spread as well. A biogeographical modelling method that will be used is called Ecological Niche Modeling (ENM) which uses the geographic dispersion and abiotic variables to figure out the realized niche of a target species (Gaynor et al. 2018; Banerjee et al. 2020).

The ecological niche is an important concept within ecology often being used to understand what role an organism plays within certain environments or resources that are being

used (Grinnell 1917; Griesemer 1992). With an increase in computational power and statistical software, the ecological niche of a species can be constructed using abiotic factors from public data sources such as WorldClim (Fick and Hijmans 2017). Numerous types of ecological niches can be drawn for any species within the Plant Kingdom due to their reliance on the environment for almost all life processes (Junk and Piedade 1997). Understanding the ecological niche of a species provides an opportunity for management practices to take abiotic effects into account for a targeted plant species (Gaynor et al. 2018; Peterson 2003). Using this approach, a species can be determined to be a generalist, or a specialist based on the abiotic influences present within their ENM (Evangelista et al. 2008). From just a cursory glance at the native range of flowering rush, regardless of cytotype, it is most likely a generalist especially since most invasive plants conserve most of their niche between native and invaded range (Liu et al. 2020; Wiens et al. 2010).

This is not the first ENM study done on flowering rush. Banerjee et al. (2020) focused on using climatic variables and future climate projections to describe the current distribution of all flowering rush in North America. This study focused on the entire reported population of *B. umbellatus* that have invaded North America regardless of cytotype, aimed to describe where the species could invade further, and provided comparisons between the native and invaded ranges. This comparison showed that flowering rush mostly conserved the niche dynamics it has in the region it is found natively and within North America, flowering rush can reside in approximately 57% of the continental area (Banerjee et al. 2020).

To take this study further, the locations of the diploid cytotype samples from the phenology study were analyzed for the abiotic influences present on them within each study site. This diploid-centric study will focus solely on understanding the soil and climate niche

dimensions present for three populations (Mentor Marsh, OH; Tonawanda, NY; and Unity Island, NY.) In doing so, this study aims to forecast the current and predicted distribution of diploid flowering rush within North America.

The objective of this study is to understand the phenological variation of biomass and starch allocation patterns of diploid flowering rush over two growing seasons from three field populations in Ohio and New York. This study will then relate the growth and starch trends to the site-specific environmental factors being recorded throughout the growing season. This relation between environmental conditions and phenology will be used to create site-specific management strategies to curb the invasion of *Butomus umbellatus*. To my knowledge this will be the first phenology study conducted on field populations of diploid flowering rush. Alongside the phenology, diploid flowering rush across all three study sites will most likely be more reliant on seasonality variables that are available. Because this is an herbaceous perennial, certain seasonal cues will cause the plant to respond by producing more tissues, divert resources to other tissues, or begin senescence.

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## CHAPTER 2

# LIFE HISTORY, RESOURCE ALLOCATION STRATEGIES, AND PHENOLOGY OF DIPLOID FLOWERING RUSH (*BUTOMUS UMBELLATUS* L.) COLLECTED FROM OHIO AND NEW YORK

### 1. Introduction

Flowering rush (*Butomus umbellatus* L.) is an invasive perennial monocot species found in aquatic and wetland habitats along the U.S. and Canadian border. Flowering rush was first documented occurring in the St. Lawrence River which facilitated spread into the Great Lakes region in the mid-1950's (Gunderson et al. 2016; Bellaud 2009). Flowering rush is thought to have had multiple introductions into North America due to two separate cytotypes, a triploid and diploid, that have been identified with at least six genotypes (Anderson et al. 1974; Gaskin et al. 2021). Both cytotypes of flowering rush are capable of vegetative reproduction through fragmentation of the rhizome and buds, developed along the rhizome (Thompson & Eckert 2004; Hroudová and Zákřavský 1993). These rhizome buds are typically used as propagules for vegetative reproduction with production of buds being variable depending on environmental factors such as flooding (Hroudová 1989). The diploid cytotype of flowering rush can also undergo sexual reproduction through seed production in the self-compatible flowers found in the inflorescence (Thompson and Eckert 2004). However, between the two cytotypes of flowering rush in North America, most of the literature regarding its life history, ecology, and management has largely been focused on the triploid cytotype.

Triploid flowering rush has been noted to have several differences from the diploid cytotype namely within the reproduction methods, the geographic distribution, and some of the

ecology within invaded systems. For the most part, triploid flowering rush is only capable of asexual reproduction through the rhizome and rhizome buds however, some floral structures can be made with no viable seeds found within the U.S. (Liu et al. 2005; Thompson & Eckert 2004). Alongside reproductive differences, the triploid cytotype has been seen to be more widespread within the U.S. with populations found from Minnesota westward (Liu et al. 2005; Gaskin et al. 2021). Geographically, diploid flowering rush has been documented from Minnesota eastward and is prolific especially around the Great Lakes region (Trebitz and Taylor 2007). Triploid flowering rush ecology has been heavily studied within lakes due to the ability to grow in a submerged and an emergent form which allows it to grow in water up to 6 m deep (Marko et al. 2015).

Within aquatic systems, plants must contend with less than favorable conditions compared to their terrestrial counterparts due to light and gases being limited in water (Ralph et al. 2007). Water can attenuate light within the aquatic system which can severely limit where aquatic plants can grow effectively (Karlsson et al. 2009; Ralph et al. 2007). This zone where plants can grow is the littoral zone which needs sunlight to penetrate down to the sediment which supports several growth types of plants (Craine and Dybzinski 2013; Karlsson et al. 2009). Triploid flowering rush has been reported growing at water depths of 6 m however, as depth increases, typically around 1.3 m, the aboveground biomass, rhizome biomass, and emergent plant height decreases (Madsen et al. 2016c; Carter et al. 2018). Diploid flowering rush has often been noted to grow at similar depths as the triploid in addition to numerous wetland systems with little standing water. Increasing water depth often reduces the temperature and light availability which will limit physiological processes such as photosynthesis and gas exchange (Craine and Dybzinski 2013; Madsen et al. 2016c).

Flowering rush is reliant on the environment for many of its life processes and aquatic systems present several physiological challenges mainly related to photosynthesis and starch formation (MacNeill et al. 2017; Scofield et al. 2009). Glucose chains can be formed by the plant for long term storage of energy, which is often termed starch. Levels of starch content in a plant's tissues are often a metric for understanding a species' life history strategies and phenological trends (Clarke et al 2023; Wersal et al. 2011; Wersal et al. 2013). Starch comes in two varieties: transitory and storage, where the transitory starch is used quickly for metabolism and the storage starch is kept within seeds, tubers, and roots for long periods (Pfister and Zeeman 2016). This is the result of binding together an amylopectin and an amylose molecule within the plastids of the plant which several enzymes will begin to bind together the molecules (Pfister and Zeeman 2016; Manners 1989). The chains of molecules will then be moved into storage within the plastids as granules that can be broken down when the plant needs energy (Pfister and Zeeman 2016).

Storage starch can be assessed and displayed across a phenological timeline to better understand starch allocation patterns and life history processes like flowering or vegetative reproduction which act like energy sinks (Sun et al. 2004; MacNeill et al. 2017; Hedhly et al. 2016). Starch is often the targeted carbohydrate for phenological studies due to the availability of testing and the long-term storage potential (Sigma Aldrich, St. Louis, Missouri; Clarke et al. 2023). These storage starches usually will be broken down for physiologically intense processes like flowering, during which time the plant starch reserves are low. These low starch content periods are typically exploited for invasive plant management strategies (Clarke et al. 2023; Wersal et al. 2013; Turnage et al. 2018). For example, triploid flowering rush and several other aquatic plant species such as curlyleaf pondweed (*Potamogeton crispus* L.) and cuban bulrush

(*Oxycaryum cubense*) have been assessed for their starch allocation patterns to aid in management timing (Clarke et al. 2023; Wersal et al. 2013, Marko et al. 2015).

By combining environmental data with these trends, phenological studies can be developed for any plant species, in this case for diploid flowering rush. Phenology is particularly useful for identifying phenological weak points in a target plants life-history to focus management efforts (Turnage et al. 2018; Clarke et al. 2023). To date, most of the management strategies that have been developed for flowering rush are based on a population of the triploid cytotype within the Detroit Lakes, MN (Madsen et al. 2016b; Parsons et al 2019; Turnage et al. 2017). Many of the management strategies developed from this population of flowering rush are based on the species growing in a stable water system like a lake (Detroit Lakes, MN) and are focused solely on the triploid cytotype which is incredibly well documented. Understanding how flowering rush responds to shallow water systems such as wetlands and if there are phenological differences between the cytotypes then management strategies can be optimized.

The objective of this study is to evaluate the phenological processes of biomass and starch allocation patterns of diploid flowering rush sampled from three field populations over two growing seasons in Ohio (one population) and New York (two populations). These populations of flowering rush are placed within emergent wetland systems as well as have all been identified as diploid. Flowering rush is an herbaceous perennial species that will shed the aboveground tissue before overwintering within the rhizome and rhizome bud tissues therefore it is hypothesized that flowering rush will have peak aboveground biomass in August or September with peak rhizome biomass in November. It is also hypothesized that the starch allocation within the plant will be higher in the rhizome and rhizome bud tissues peaking in November as well.

## 2. Methodology

### 2.1 Study Site Description

Mentor Marsh, OH (41.73649° N, -81.29879° W) is a large emergent marsh complex managed by the Cleveland Museum of Natural History. Mentor Marsh receives hydrologic input directly from Lake Erie and is a biodiversity hotspot within Ohio and provides valuable habitat to numerous waterfowl and rare bird species as well as providing fishery habitat (Cleveland Museum of Natural History; M. Yeager, personal communication, March 6, 2023). Mentor Marsh historically was used as a disposal site for salt mine tailings which caused widespread ecosystem destruction and native plant loss (Cleveland Museum of Natural History). The open niche space because of native plant loss was quickly filled by phragmites haplotype M (*Phragmites australis*; M. Yeager, personal communication, March 6, 2023; Guo et al. 2014). Phragmites is an aggressive invader and has warranted aggressive management strategies however, flowering rush has become another aggressive invader within this system (Cleveland Museum of Natural History).

Tonawanda Wildlife Management Area, NY (43.10590° N, -78.48118° W) is an emergent wetland complex that has water level management through water control structures. The wetland is flooded in the spring (April) and drained before winter (November). Historically, this site was part of glacial Lake Tonawanda until the lake naturally drained through Niagara Falls (Calkin & Brett 1978). There are numerous dikes to control water flow within the system to create a large waterfowl management area through seasonal flooding and draining. Flowering rush was first documented at Tonawanda in 2009 and has continued to spread throughout the marsh (Roster 2011; Kennedy 2018; U.S. Geological Survey 2023).

Unity Island, NY (42.92999° N, -78.90453° W) is a constructed wetland in close proximity to the Buffalo River in Buffalo, New York. The site is managed by the U.S. Army Corps of Engineers to develop complex habitat for fisheries management as part of the Lake Erie system (U.S. Army Corps of Engineers 2021). Unity Island has been undergoing ecological restoration since 2018 with flowering rush recorded within the site in 2019. Flowering rush is thought to have been established via dredged materials being used in restoration efforts at Unity Island and has continued to be an aggressive wetland invader. Flowering rush poses a threat to restoration efforts by reducing native diversity, increasing sediment deposition, and decreasing open-water fish spawning habitat (U.S. Army Corps of Engineers 2021).

## *2.2 Biomass*

Sampling was conducted once per month at all three sites between May and November in 2021 and 2022. During each sampling event twenty samples were harvested from each study site using a polyvinyl chloride (PVC) coring device designed to remove the plant and soil within the area of the device specifically 0.018 m<sup>2</sup> (Madsen et al 2007; Wersal and Madsen 2018). Prior to sample collection geographic coordinates, plant height, water depth, and presence of emergent leaves and inflorescences were recorded. Data loggers (HOBO pendants Onset Computer Corporation) were deployed at two locations at each site to collect air temperature hourly throughout the sampling season each year. Plant samples were rinsed, placed into labeled plastic bags, and shipped overnight in a cooler on ice to Minnesota State University, Mankato for further processing.

In the lab, samples were washed and sorted into above ground tissue (leaves), rhizome and roots, inflorescences, rhizome buds formed on the rhizome, and vegetative bulbils formed at the base of the inflorescence (Hroudová and Zákřavský 1993; Hroudová 1989). The number of rhizome buds, vegetative bulbils, and leaf ramets from each collected sample were also recorded. Sorted tissues were put into separate paper bags and placed in a drying oven at 48° C for at least 72 hours. Once dried, samples were weighed to the nearest 0.001 g then dry weight (DW) was divided by the area of the PVC coring device (0.018 m<sup>2</sup>) to determine grams of dry weight per m<sup>2</sup> (g DW m<sup>-2</sup>) for each tissue. Counts of rhizome buds, bulbils, and ramets were calculated in a similar manner.

### *2.3 Starch Allocation*

Tissue samples from each site and month were consolidated into sets of 5; thus, tissue samples 1-5, 6-10, 11-15, and 16-20 became starch samples 1, 2, 3, 4 respectively. The samples were then placed in a food processor until the tissue was roughly cut. The rough cut biomass was then ground using a Cyclone Sample Mill and sieved through a #40 mesh screen (1 mm). After the biomass samples were ground approximately 50 to 55 mg of the sample was transferred to a plastic centrifuge tube for the starch analysis (Wersal and Madsen 2018, Wersal et al. 2011, and Wersal et al. 2013; Clarke et al. 2023). The starch analyses were done using the amylase/amyloglucosidase method via the commercially available STA-20 starch assay kit (Sigma Aldrich, St. Louis, Missouri) that generates the starch data as a percent dry weight (% DW) of each sample. Within the STA-20 kits, wheat (89%) and corn (93%) starch standards were included, and three flowering rush samples were duplicated per month for starch content reliability (Wersal et al. 2011; Wersal et al. 2013; Clarke et al. 2023). The accuracy of the

analysis as demonstrated by a standard curve (5 points) was  $\pm 2.7\%$  ( $r^2 = 0.98$ ) and the precision of the assays determined by the percent difference in duplicate samples, was  $8.9\% \pm 0.9$  SE. Starch recovery was  $90.5\% \pm 2.7$  SE and  $91.4\% \pm 2.0$  SE determined using a known mass and purity of the wheat and corn standards, respectively.

#### *2.4 Statistical Analysis*

The monthly averages for biomass, starch content, and environmental metrics were computed site by site. The Spearman's rank correlation method (0.0-1.0) was used to assess correlations between plant tissue biomasses, plant tissue starch contents, as well as plant tissues to environmental metrics (water depth and air temperature; Clarke et al. 2023). Spearman's rank correlation was chosen because it does not assume that variables have a linear relationship and there is no assumption of the frequency distribution between variables (Hauke and Kossowski 2011). Correlation strength was defined as no correlation (0–0.1), weak correlation (0.1–0.4), moderate correlation (0.4-0.6), strong correlation (0.6-0.9), or a perfect correlation (0.9-1; Dancey and Reidy 2004), using absolute values. Data were tested for normality using a Shapiro-Wilk's normality test ( $\alpha = 0.05$ ), the Kruskal-Wallis one-way test ( $\alpha = 0.05$ ), then the Dunn's pairwise comparison was run to determine if the data within each study site is normally distributed and to determine significance between the months. Months that were considered statistically significant from each other were denoted by different letters indicating different statistical groups. All statistical tests were done in the Statistix 10 statistical software.

All data collected was found to be significant ( $p < 0.0001$ ) for the Shapiro-Wilk's normality test therefore all collected data does not follow a normal distribution pattern



warranting nonparametric statistical analysis. The nonparametric Kruskal-Wallis test was used to determine significance between monthly data values measurements that were significantly different between each month for both 2021 and 2022 except for the inflorescence starch in 2022 at Unity Island. From Dunn's pairwise comparison, each month that is significant of another is denoted by different letters with months not significant of each other denoted by the same letter.

### 3. Results

#### *3.1 Seasonal Biomass Patterns*

Aboveground biomass commonly peaked between July and September making up 50 – 70% total biomass during this period. Mentor Marsh aboveground biomass peaked in July 2021 at 70% total biomass ( $F = 9.83$ ;  $p < 0.0001$ ) and August 2022 at 50% total biomass ( $F = 7.30$ ;  $p < 0.0001$ ; Figure 1). Aboveground biomass at Tonawanda peaked in July both years at 54% and 66% of the total biomass for 2021 ( $F = 11.45$ ;  $p < 0.0001$ ) and 2022 ( $F = 12.47$ ;  $p < 0.0001$ ) respectively (Figure 2). Finally, aboveground biomass at Unity Island peaked in August of 2021 at 67% of total biomass ( $F = 17.23$ ;  $p < 0.0001$ ) and September of 2022 at 53% biomass ( $F = 19.66$ ;  $p < 0.0001$ ; Figure 3). Leaf ramet density at Mentor Marsh was found to peak in August in 2021 at 2583 ramets per  $m^2$  ( $F = 10.56$ ;  $p < 0.0001$ ) and from July to August at 3708 ramets per  $m^2$  in 2022 ( $F = 18.46$ ;  $p < 0.0001$ ; Figure 4). Leaf ramet density at Tonawanda peaked in July at 2400 ramets per  $m^2$  in 2021 ( $F = 10.4$ ;  $p < 0.0001$ ) and 1560 ramets per  $m^2$  in 2022 ( $F = 19.06$ ;  $p < 0.0001$ ; Figure 4). Unity Island leaf ramet density peaked in July at 2113 ramets per  $m^2$  in 2021 ( $F = 16.29$ ;  $p < 0.0001$ ) and in June at 2629 ramets per  $m^2$  in 2022 ( $F = 11.78$ ;  $p < 0.0001$ ; Figure 4).

Rhizome biomass commonly peaked in October and November making up approximately 40 - 50% of the total biomass in that period. The rhizome biomass usually peaked in August for Mentor Marsh in 2021 at 36% ( $F = 3.24$ ;  $p = .0055$ ) and in 2022 there was no significant peak found ( $F = 1.96$ ;  $p = 0.08$ ). The rhizomatic biomass in Tonawanda peaked in September at 45% of total biomass in 2021 ( $F = 10.04$ ;  $p < 0.0001$ ) and 2022 again had no statistically significant peak ( $F = 2.28$ ;  $p = 0.067$ ). Finally, rhizomatic biomass peaked at Unity Island in October in both 2021 and 2022 at 39% ( $F = 3.66$ ;  $p = 0.0021$ ) and 42% ( $F = 3.52$ ;  $p = 0.0029$ ) of the total biomass, respectively. Rhizome bud biomass was less than 500 g DW m<sup>-2</sup> for both 2021 and 2022 across all sites. Peak rhizome bud density in Mentor Marsh was 15,333 and 11,757 rhizome buds m<sup>-2</sup> occurring between September and November for 2021 ( $F = 7.95$ ;  $p < 0.0001$ ) and 2022 ( $F = 8.4$ ;  $p < 0.0001$ ) respectively. Peak rhizome bud density in Tonawanda was 3,260 and 3,422 rhizome buds m<sup>-2</sup> in September and October for 2021 ( $F = 8.2$ ;  $p < 0.0001$ ) and 2022 ( $F = 15.65$ ;  $p < 0.0001$ ) respectively. Peak rhizome bud density in Unity Island was 12,802 rhizome buds m<sup>-2</sup> in November 2021 ( $F = 17.85$ ;  $p < 0.0001$ ) and 11,422 rhizome buds m<sup>-2</sup> in October ( $F = 23.65$ ;  $p < 0.0001$ ). Inflorescence tissue at each site was usually less than 10% of the total biomass and peaked between August and October. Biomass trends for Mentor Marsh, Tonawanda, and Unity Island are displayed in Figures 1, 2, and 3, respectively. Leaf ramet densities and rhizome bud densities are displayed in Figure 4, as well.

### *3.2 Seasonal Starch Allocation Patterns*

Aboveground tissue stored the least amount of starch in flowering rush typically below 2.5% starch with a majority of the growing season below 1%. Mentor Marsh saw peaks in aboveground starch content around June in 2021 at 2.1% ( $F = 28.85$ ;  $p < 0.0001$ ) and May in

2022 at 1.0% ( $F = 50.52$ ;  $p < 0.0001$ ; Figure 1). Tonawanda aboveground tissue peaked in starch content between July and August in 2021 ( $F = 8.59$ ;  $p < 0.0001$ ) and 2022 ( $F = 41.38$ ;  $p < 0.0001$ ) at 1.1% both years (Figure 2). Unity Island had peaks in aboveground starch content at 1.4% starch in July 2021 ( $F = 8.76$ ;  $p < 0.0001$ ) and 2.4% in May of 2022 ( $F = 102.44$ ;  $p < 0.0001$ ; Figure 3). Compared to the aboveground tissue, the rhizome typically stored more starch (16 – 28%) and peaked near the end of the sampling season in October and November. Mentor Marsh rhizome starch content peaked in November 2021 at 16.6% ( $F = 68.04$ ;  $p < 0.0001$ ) and in October 2022 at 23.3% ( $F = 24.32$ ;  $p < 0.0001$ ). Tonawanda saw rhizome starch content peak a bit earlier in the sampling period at 17.8% in October 2021 ( $F = 22.58$ ;  $p < 0.0001$ ) and 18.1% in September 2022 ( $F = 26.83$ ;  $p < 0.0001$ ). Whereas Unity Island rhizome starch content peaked in November both 2021 ( $F = 41.76$ ;  $p < 0.0001$ ) and 2022 ( $F = 31.98$ ;  $p < 0.0001$ ) at 28.4% and 26.5% respectively.

Rhizome buds stored high amounts of starch with a typical range of 15% to almost 30% throughout the entire sampling period. Rhizome buds starch content was highest near the end of the sampling period with Mentor Marsh at 28.0% starch content in October and November in 2021 ( $F = 8.20$ ;  $p < 0.0001$ ) and October in 2022 ( $F = 24.63$ ;  $p < 0.0001$ ). Tonawanda had a similar peak period in October in both years with 28.2% starch in 2021 ( $F = 75.08$ ;  $p < 0.0001$ ) and 26% starch in 2022 ( $F = 7.50$ ;  $p < 0.0001$ ) for the rhizome bud tissue. Finally, Unity Island rhizome bud starch peaked in October 2021 at 28.3% ( $F = 19.50$ ;  $p < 0.0001$ ) and 2022 saw peaks in September at 27% and 29% ( $F = 14.65$ ;  $p < 0.0001$ ), respectively.

Inflorescence starch was more variable throughout the year with low points around 0.6% and the highest concentration at 7%. Mentor Marsh inflorescence starch content peaked around September in 2021 at 5.5% ( $F = 72.82$ ;  $p < 0.0001$ ) and August in 2022 at 4.8% ( $F = 21.26$ ;  $p <$

0.0001) with Tonawanda peaking in a similar period at 3.6% in August 2021 ( $F = 7.48$ ;  $p < 0.0001$ ) and 2.9% in September 2022 ( $F = 6.02$ ;  $p = 0.009$ ). Unity Island inflorescence starch content peaked at 7.3% in October 2021 ( $F = 33.39$ ;  $p < 0.0001$ ) and did not have a statistically significant peak in 2022 ( $F = 2.07$ ;  $p = 0.1185$ ). The inflorescence was also seen to show a clear trend with starch content peaking near the late summer (August and September) around 3-5%. Starch trends for Mentor Marsh, Tonawanda, and Unity Island are displayed in Figures 1, 2, and 3, respectively.

### *3.3 Environmental Correlations*

Water depth varied widely between each study site. Mentor Marsh typically had little water present, usually between 0 to 5 cm for 2021 and 2022 with a peak in water depth at 15 cm in November 2021 (Figure 5). Tonawanda had water depth fluctuate between 20 to 40 cm with a peak at 55 cm in July 2021 (Figure 5). Finally, Unity Island usually had a water depth between 20 and 32 cm with a peak at 38 cm in July 2021 (Figure 5). In general biomass was found to have weak negative correlations with water depth ( $r^2 = -0.01$  to  $-0.35$ ) and air temperature ( $r^2 = -0.09$  to  $-0.55$ ) except for the aboveground tissue which was weakly positively correlated with both environmental variables ( $r^2 = 0.13$  to  $0.19$  and  $0.12$  to  $0.26$ ) (Figure 6). The starch contents were also found to be negatively correlated with the environmental variables except for the aboveground starch which was positively correlated with the water depth. Plant height was positively correlated with the water depth ( $r^2 = -0.03$  to  $0.52$ ) and had no correlation with air temperature ( $r^2 = -0.04$  to  $0.10$ ). Inversely, leaf ramet density was positively correlated with the air temperature ( $r^2 = 0.30$  to  $0.51$ ) and water depth had an overall weakly negative relationship

( $r^2 = -0.12$ ) like Mentor Marsh ( $r^2 = -0.36$ ) with Tonawanda showing a weakly positive relationship ( $r^2 = 0.15$ ) (Figure 6).

#### 4. Discussion

The sampled flowering rush populations offer a consistent phenological timeline. This timeline is as follows: once the air temperature begins to increase, flowering rush will expend energy to grow the aboveground tissue, leaves specifically. The aboveground tissue will continue to grow until the air temperature has peaked for the year around mid-summer which was typically July and August at these study sites. During this period of aboveground tissue growth, the rhizome will lose both biomass and starch content to make sucrose and other sugars to facilitate leaf tissue development. After the peak time of aboveground tissue, the rhizome and rhizome bud tissues will then begin to recuperate expended energy and accrue biomass and more starch to prepare for the overwintering period and any reproduction taking place. Once air temperatures begin to cool once again, the leaves of diploid flowering rush will senesce, and the plant will once again overwinter in the rhizome. The rhizome buds are vegetative propagules, however, most likely act as another carbohydrate storage tissue in the plant which can be produced on a massive scale.

Diploid flowering rush is highly capable of invading aquatic systems indicated by the high reproductive efforts shown throughout two years. Diploid flowering rush has access to vegetative reproduction through the rhizome buds, rhizome structures, and bulbils formed at the base of the inflorescence as well as sexual reproduction in the flowers. Throughout this study, bulbils formed at the inflorescence base were not found at any study site throughout 2021 and 2022.

Flowering rush can produce 20 to 50 flowers per inflorescence with approximately 200 seeds per flower being produced at 31% viability (Eckert et al. 2000; Lui et al. 2005). From this study, it was found that flowering rush can produce upwards of 15,000 rhizome buds per m<sup>2</sup>. Combining the possible sexual and asexual efforts, diploid flowering rush can make up to 18,000 individuals per m<sup>2</sup> during the peak reproductive season (fall). These high reproductive efforts show that the diploid cytotype of this plant poses a much larger threat than that of the triploid which was seen to only make about 600 rhizome buds per m<sup>2</sup> (Marko et al. 2015). Which means the diploid plant reproduces on a scale almost 30-fold of the triploid offering the diploid more dispersion potential throughout North America.

Besides the difference in reproduction, many of the life history traits between triploid and diploid flowering rush such as rhizome biomass production and peak periods are similar (Marko et al. 2015; Lui et al. 2005). Diploid flowering rush usually made about the same amount of rhizome biomass and more aboveground biomass compared to the triploid cytotype. The aboveground biomass in triploid reached a peak of just over 500 g DW m<sup>-2</sup> with the diploid producing between 600 to 1100 g DW m<sup>-2</sup>. Triploid flowering rush did not have more than about 14% starch content stored in the rhizome tissue compared to the diploid storing up to 28% by the end of the growing season. Both cytotypes had aboveground biomass peaks around similar time periods around the middle of the summer (July and August) with the rhizome and rhizome buds reaching a peak right before winter (October and November). This increase in aboveground tissue could cause an increase in the vegetative reproduction done by the diploid cytotype due to increased photosynthetic capabilities. However, one noted difference is not only the distribution of both cytotypes but the responses to the environment which show that the diploid, while

densely invaded in the Great Lakes region, cannot handle intense temperature changes (Lui et al. 2005; Levin 1983).

In 2022, the air temperature was much warmer (25° C at peak in July) and it was seen that at Mentor Marsh and Tonawanda, there appeared to be a decrease in the production of aboveground, rhizome, and inflorescence biomass as well as a shift in the peak rhizome starch content period. While inter-year comparisons were not tested for, the biomass of each tissue was reduced at Tonawanda especially. Water being present within each study site will have large influences on the temperature the plants experience as more water will disperse heat energy and less water will cause plants to deal with higher temperatures (Erwin 2009; Jimenez et al. 2012).

Heat is a notable stressor on plants mainly due to the production of reactive oxygen species (ROS), inhibiting proper gas movement, and change phenological patterns in plant species (Hassanuzzaman et al. 2013; Yamamoto et al. 2008). The creation of ROS causes oxidative stress in the plant which can lead to damage in the leaf and shoot tissues reducing photosynthetic potentials (Marchland et al. 2005). This heat stress can occur at increases of maybe 1-2 °C which could be seen between 2021 and 2022 which saw July and August as the hottest months typically (Nahar et al. 2010). In 2021, Mentor Marsh saw air temperatures around 23 °C and both Unity Island and Tonawanda saw air temperatures around 21 °C which increased by 2 and 5 °C respectively. Heat stress may have caused a reduction in aboveground biomass and changes in phenological timing within the rhizome.

This effect on the tissues from high temperatures suggests that despite the reproductive capability, flowering rush needs to be within an ideal placement of the littoral zone. Previous studies have shown that water depth can impact the growth of the aboveground and rhizome biomass (Madsen et al. 2016c). Water depth was often found to have a negative correlation with

biomass in most tissues except the aboveground tissue which had a weak positive relationship. However, the starch contents for many tissues were more mixed with many weak and no correlations present which would indicate that flowering rush can grow in deeper waters however, it would be suboptimal (Madsen et al. 2016a; Madsen et al. 2016c). Light is rapidly attenuated by water which can often limit the area where most vascular plant species can reside and as depth increases fewer plant species can get enough light for photosynthesis (Sculthorpe 1967; Grace 1989).

Triploid flowering rush has been found to be negatively impacted by increasing water depth on the biomass (Madsen et al. 2016c) however, the diploid populations show much weaker correlations between water depth and biomass. There could be a couple reasons for this such as the study sites are wetland complexes compared to the lake systems that have been more frequently studied with flowering rush. Mentor Marsh had little standing water with Tonawanda and Unity Island reaching almost 80 cm of water depth this is in comparison to the almost 3 m depth in the Detroit Lakes flowering rush population (Madsen et al. 2016c). Hydrological differences are most likely acting as the largest drivers for the phenological trends that were being seen as each population. Differences in the phenology show that flowering rush exhibits plasticity towards the flooding that is present within an invaded wetland system (Pigliucci and Kolodynska 2002). Wetlands most likely offer the most optimal environment for flowering rush as water levels below 1m deep allow for the species to grow largely as well as produce high densities of rhizome buds from receding water levels (Madsen et al. 2016c; Hroudová 1989).

These rhizome buds, while vegetatively viable, are also used by the plant to help survive overwintering and stressful periods. Most likely, the rhizome buds are used to survive overwinter as ready sources of energy which causes such a major reduction in their density at the beginning



of the next year. Then, the rhizome itself is used only to synthesize new aboveground tissue the following year. Flowering rush, especially at each study site, is probably selecting for high vegetative reproduction throughout the growing year at the expense of persisting within the environment. In 2022, tissues like the aboveground were seen to have a drop in biomass and starch content which should impact the rhizome bud production as reproductive tissue and carbohydrate storage structure (Essemine et al. 2010). However, the rhizome bud number and biomass were not heavily impacted from the heat stress that occurred throughout the year.

While not seen to directly impact native species presence, flowering rush could potentially spread other native and nonnative species by colonizing new areas and manipulating aquatic ecosystems (Madsen et al. 2016c; Gunderson et al. 2016). However, flowering rush is seen to cause more anthropogenic issues such as blocking waterways for navigation which could potentially become a larger problem once the species has become established (Jacobs et al. 2011; Bellaud 2009).

The data from this study offers management programs for flowering rush, specifically at these sites, to begin management applications during periods before peak biomass. Therefore, management programs could see management efficacy with herbicide applications between May and July with the goal of reducing rhizome bud production in the late season by removing aboveground tissue. By removing aboveground tissue, flowering rush should expend more energy growing it back which would reduce available starch contents in the overwintering periods. Reducing starch contents before winter could eliminate smaller or less established populations of flowering rush during the overwintering period or aid management the next year. Diploid flowering rush poses some differences to its triploid counterpart due to the differences in reproductive scale and environmental response. The potential for diploid flowering rush to

spread is much higher than that of the triploid due to the scale of reproduction occurring and its distribution within North America. High reproductive output, especially through vegetative reproduction, offers more chances for diploid flowering rush to spread especially through aquatic systems that are associated with rivers. Based on the current distribution in the Great Lakes region, diploid flowering rush has access to the largest lakes and numerous river systems in North America, that can potentially move seeds, rhizome buds, and rhizome fragments. With the ability to produce almost 18,000 individuals per m<sup>2</sup> during peak production, diploid flowering rush is an invasive plant cytotype that poses strong potential to expand to most of North America.

## **5. Acknowledgements**

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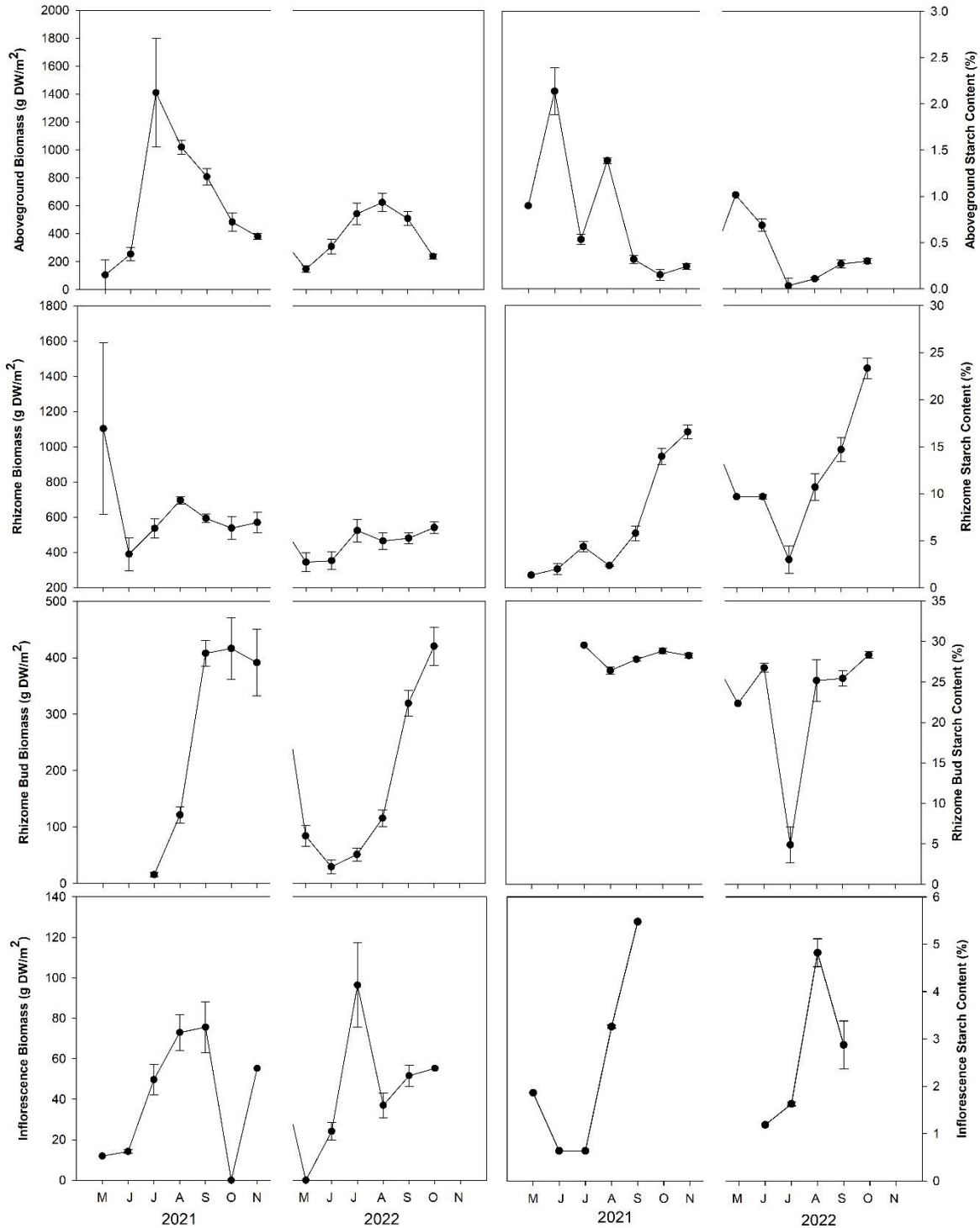


Figure 1: Mean ( $\pm 1$  SE) total biomass and starch content in diploid flowering rush harvested from Mentor Marsh, OH. Bars sharing the same letter are not different according to *Dunn's* all pair-wise comparison test at an  $\alpha \leq 0.05$  significance level. Analyses were done within tissue type and year. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

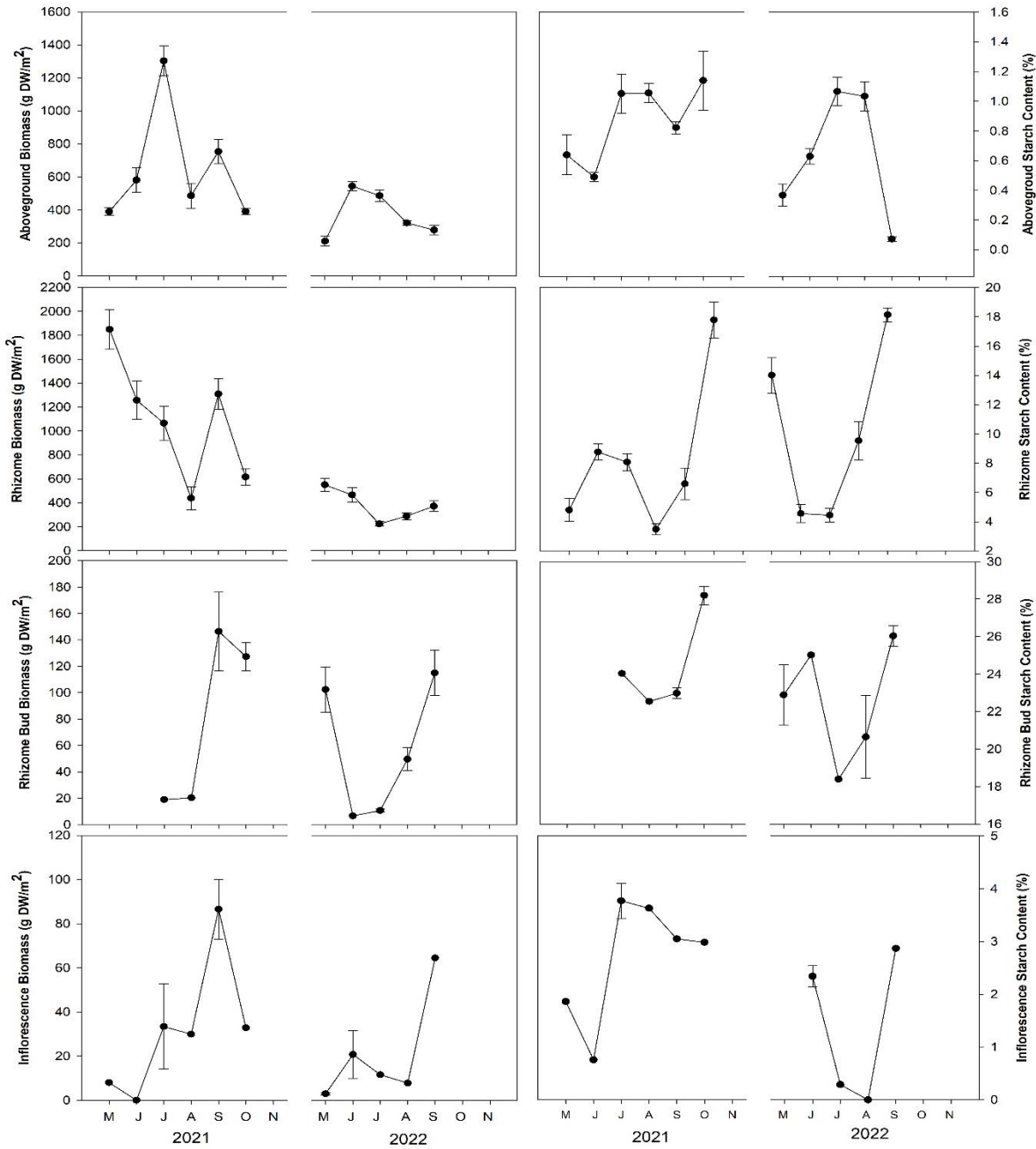


Figure 2: Mean ( $\pm 1$  SE) total biomass and starch content in diploid flowering rush harvested from Tonawanda, NY. Bars sharing the same letter are not different according to *Dunn's* all pairwise comparison test at an  $\alpha \leq 0.05$  significance level. Analyses were done within tissue type and year. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

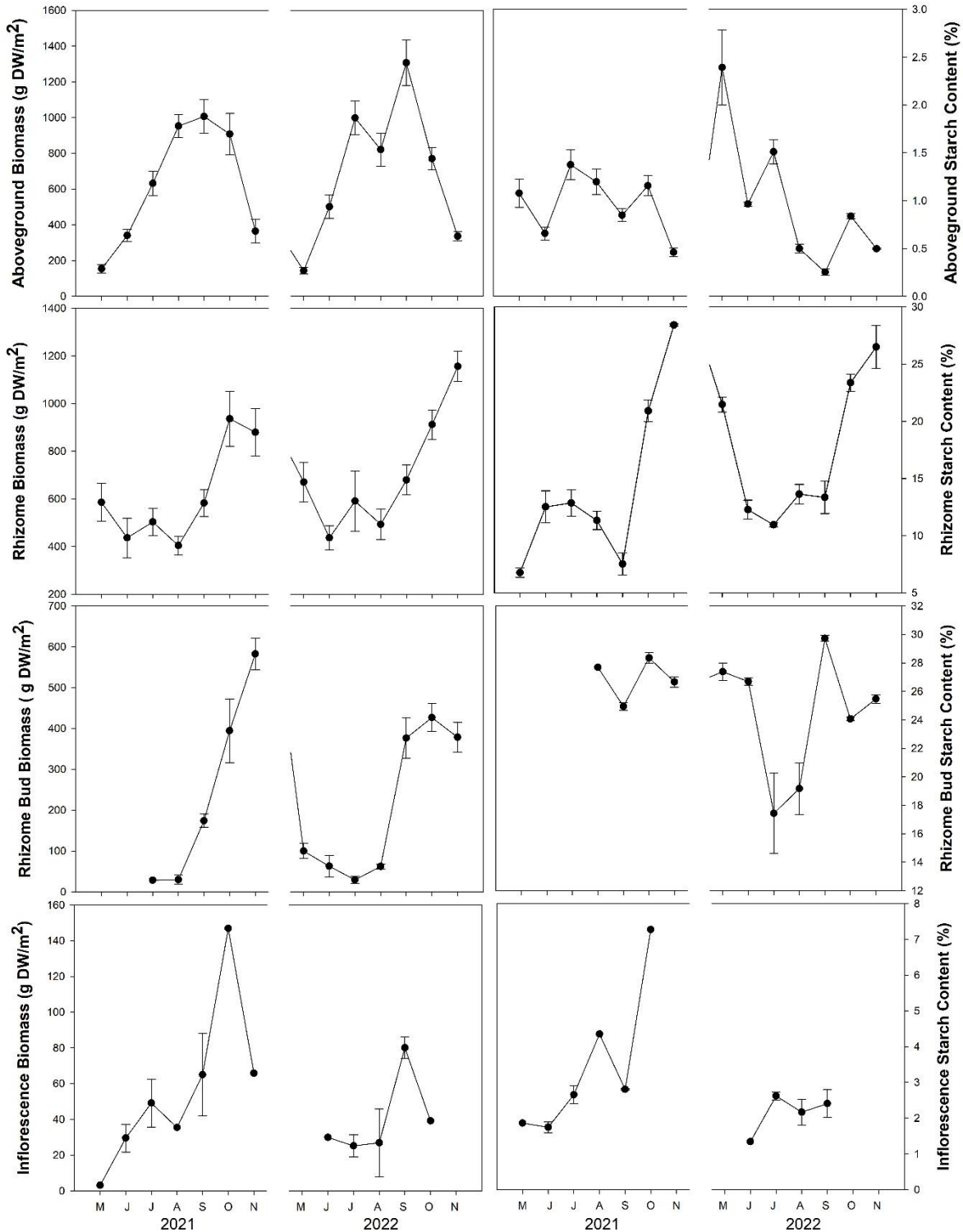


Figure 3: Mean ( $\pm 1$  SE) total biomass and starch content in diploid flowering rush harvested from Unity Island, NY. Bars sharing the same letter are not different according to *Dunn's* all pair-wise comparison test at an  $\alpha \leq 0.05$  significance level. Analyses were done within tissue type and year. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

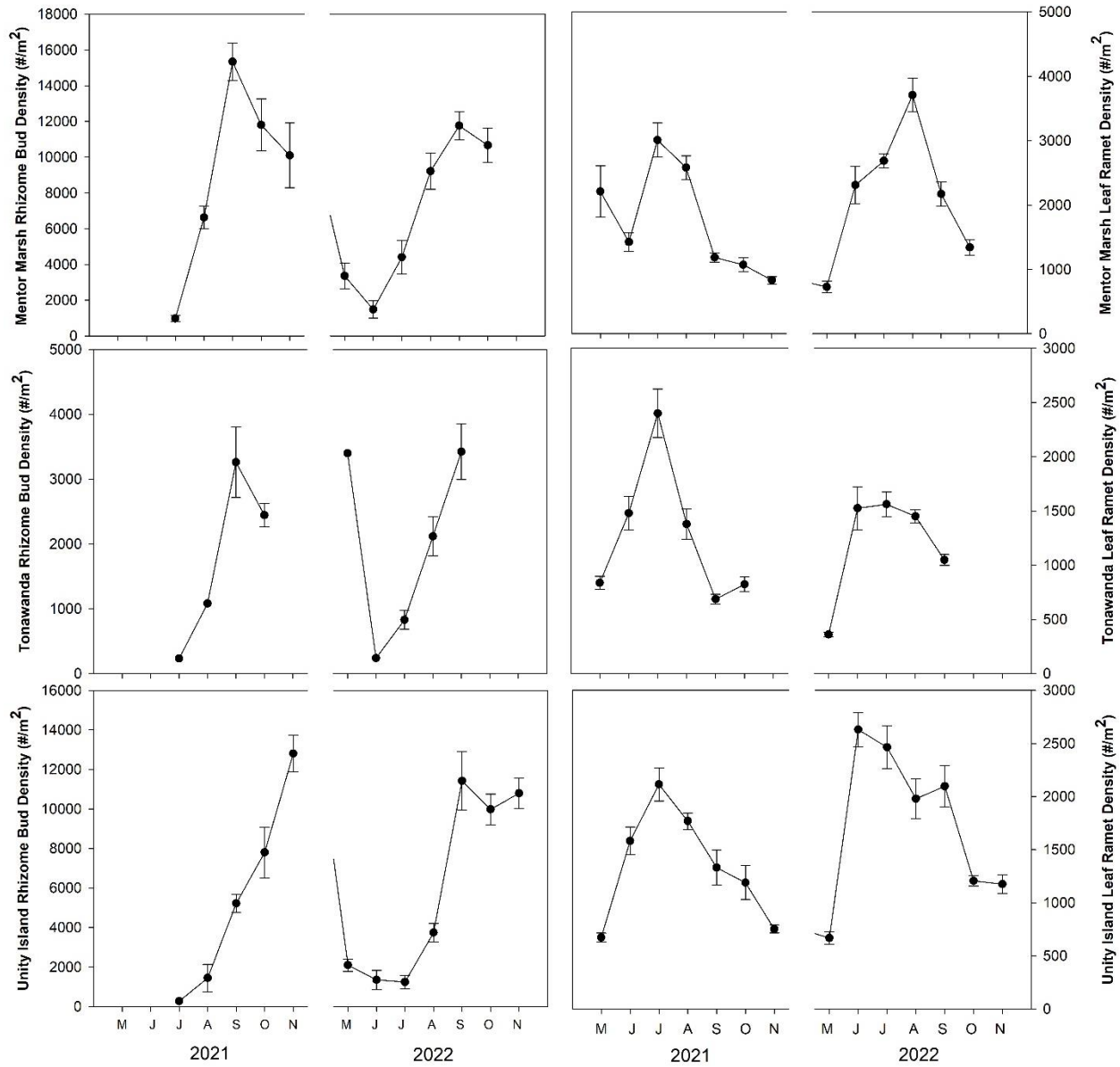


Figure 4: Mean ( $\pm 1$  SE) leaf ramet density and rhizome bud density in diploid flowering rush harvested from each study site. Bars sharing the same letter are not different according to *Dunn's* all pair-wise comparison test at an  $\alpha \leq 0.05$  significance level. Analyses were done within tissue type and year. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

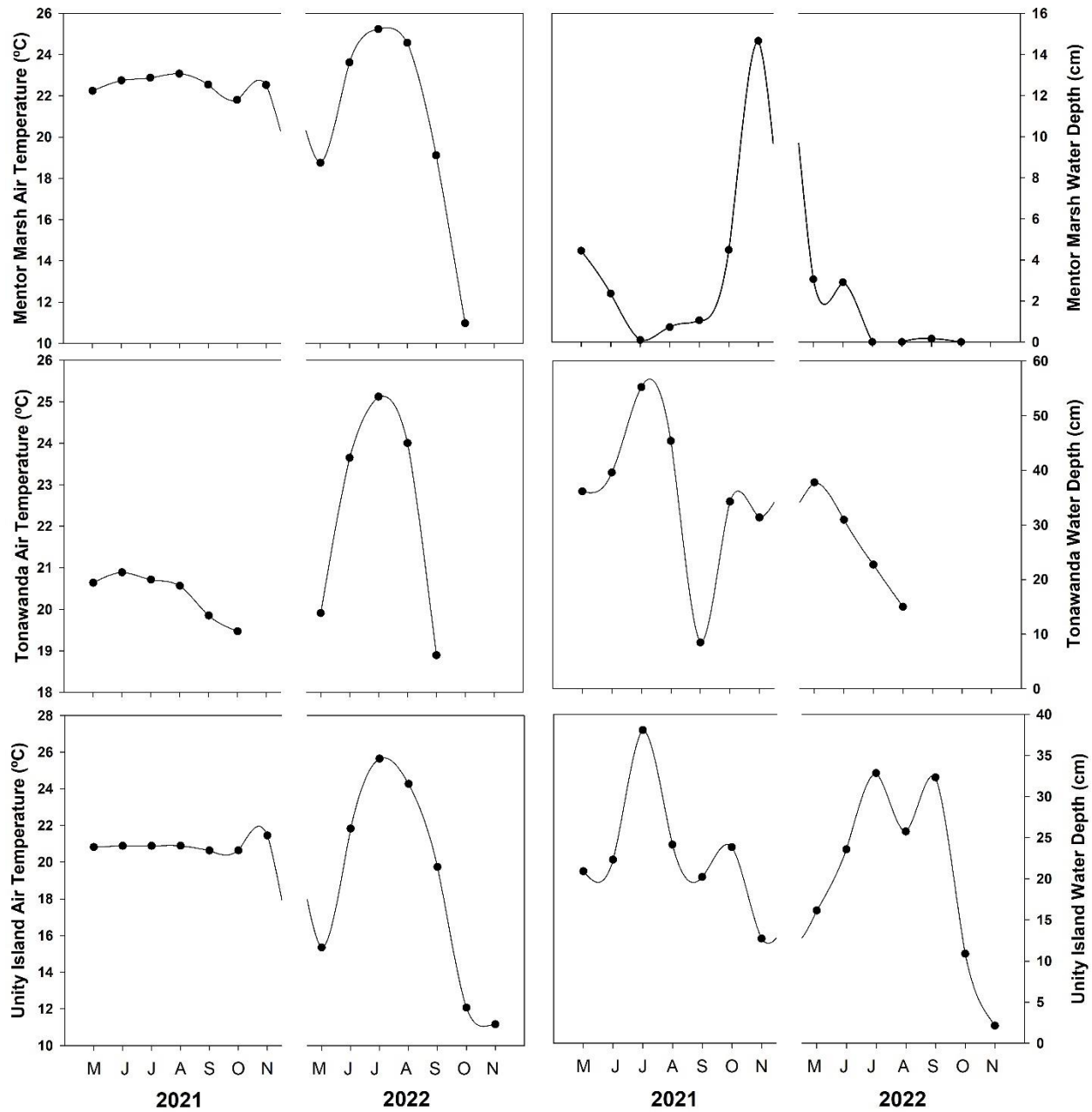


Figure 5: Mean air temperature ( $^{\circ}\text{C}$ ) and water depth for study sites where diploid flowering rush samples were harvested. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

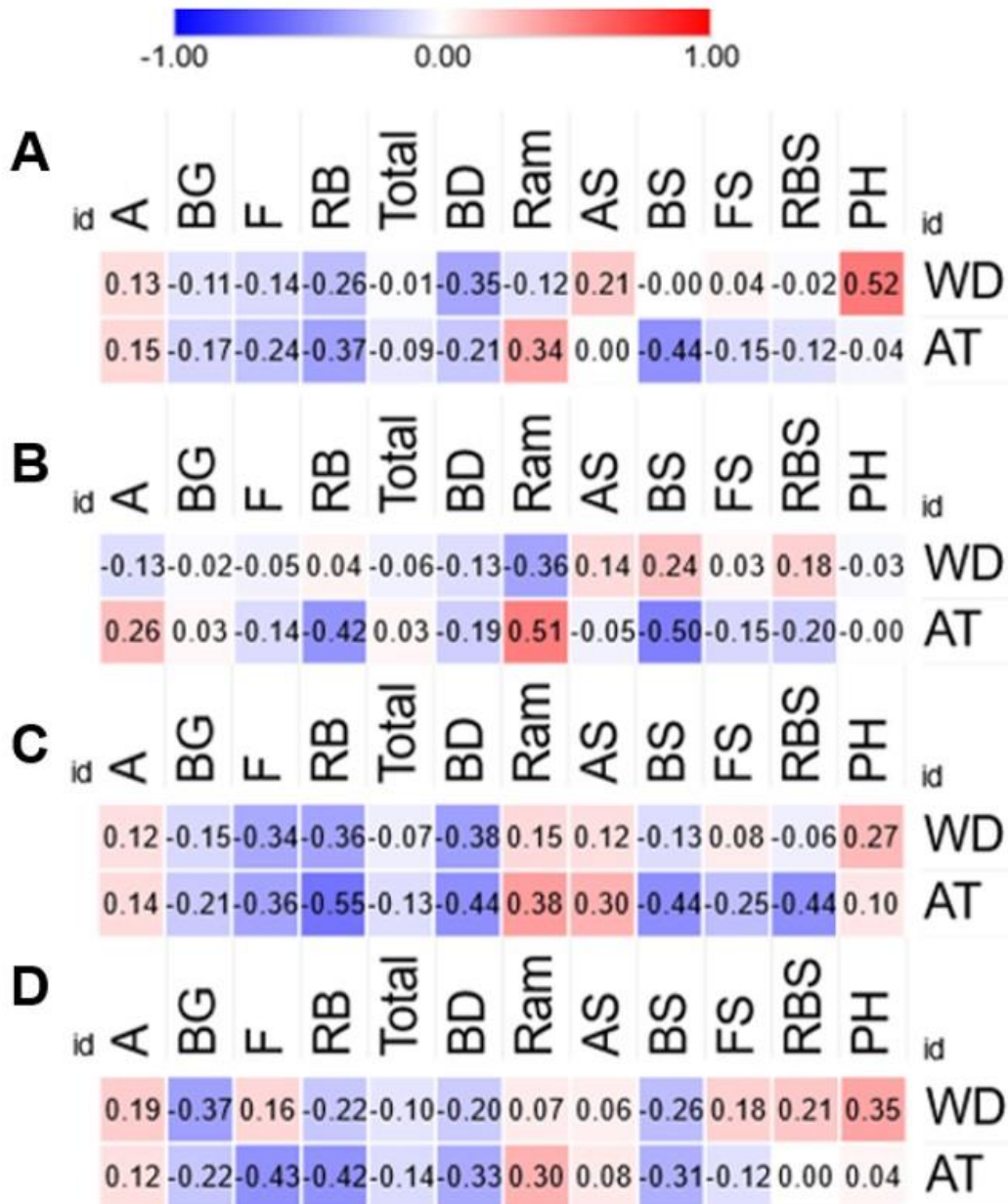


Figure 6: Multiple similarity matrices conducted via Spearman correlation tests on the collected biomass, starch, and environmental characteristics. Each matrix is for a different site with: A) All sites combined, B) Mentor Marsh, C) Tonawanda, and D) Unity Island. The abbreviations for the variables (columns) are as follows: A = aboveground biomass, BG = rhizome biomass, F = inflorescence biomass, RB = rhizome bud biomass, Total = total biomass for the sample, BD = rhizome bud density, Ram = counted leaf ramets, AS = aboveground starch content, BS = rhizome starch content, FS = inflorescence starch content, RBS = rhizome bud starch content, PH = plant height, WD = water depth, AT = air temperature. Images were made using Morpheus (<https://software.broadinstitute.org/morpheus>).



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## CHAPTER 3

ECOLOGICAL NICHE MODELING OF DIPLOID *BUTOMUS UMBELLATUS***1. Introduction**

Plant growth is highly influenced by environmental factors and these same factors will dictate plant distributions and invasion success. Environmental variables are typically soil or climate related with certain systems like wetlands, integrating water quality as another variable of influence (Bornette and Puijalon 2011). Soils are often the main source for nutrients with plant growth and distribution affected by characteristics like particle size and composition of the soil within an area (Duong et al. 2012; Schmid et al. 2022). Aquatic plant species typically grow in a wide variety of soil types with mineral and organic content, usually the drivers for soil textures (Bornette and Puijalon 2011). Several species found commonly in Minnesota see their distribution affected by soil particle size such as sago pondweed (*Stuckenia pectinate* L.) preferring high silt content comparatively to American eelgrass (*Vallisneria americana* Michx.) which was negatively affected by clay content (Schmid et al. 2022; Madsen et al. 1996). One method that has often been used to determine these pieces of environmental influence is ecological niche modeling.

Ecological niche modeling (ENM) utilizes different abiotic metrics to predict the dispersion of an interested species or populations (Evangelista et al. 2008; Gaynor et al. 2018; Phillips et al. 2006). Ecological niche modeling has been used on species such as *Galax urceolata* that are an endemic autopolyploid species (Gaynor et al. 2018) and some invasive species such *Myriophyllum heterophyllum* (Thum & Lennon 2009). The modeling method measures the suitability of the landscape based on any number of abiotic variables and



determines the highly influential factor(s) within the model (Phillips et al. 2006; Phillips and Dudik 2008). Niche modeling is a powerful tool for determining how the environment will influence a distribution of species.

Alongside soil, climate variables such as temperature and precipitation will influence plant distribution across the landscape. Plants are influenced by temperature through changes in photosynthetic rates (Hassanuzzaman et al. 2013). Additionally, temperature changes seen through seasons will influence phenological trends (Bertin 2008; DaMatta and Cochico Ramalho 2006). Usually, the more temperatures remain at an optimum the more often a plant species can effectively capture energy. Within perennial plants, like flowering rush, optimum temperatures and high photosynthetic output will lead to the generation of long-term storage carbohydrates for overwintering or vegetative propagules (Pfister and Zeeman 2016). However, out of that optimum temperature can be harmful for the plant as high heat can lead to the creation of reactive oxygen species or inactivate most of the photosynthetic enzymes (Hassanuzzaman et al. 2013; Yamamoto et al. 2008). Large waterbodies will mitigate high temperature effects, due to the specific heat of water alongside the lake effect, that plants experience (Henne et al. 2007).

Precipitation will also influence water flux and it is often a driver of water availability within most landscapes. Precipitation is another climatic factor that changes throughout the year and seasons that are often geographically dependent (Higgins et al. 1997). Within the U.S., the evaporation rates of the southwest are incredibly high compared to the high precipitation rates seen in the pacific northwest (Zhang and Villarini 2019; Hoerling et al. 2016). The Midwest and Northeast regions of the U.S. are usually temperate environments that can see anywhere between 25 to 50 inches of precipitation throughout the year (NOAA National Centers for Environmental Information). In 2021 and 2022, Midwest region saw approximately 36.7 and 35 inches,

respectively, which were lower than trends throughout 1991-2020 (NOAA National Centers for Environmental Information; Dai et al. 2015). Comparatively, the Northeast region saw 44.6 and 45.4 inches of precipitation in 2021 and 2022, respectively (NOAA National Centers for Environmental Information; Hayhoe et al. 2007). Both regions have been experiencing droughts to some degree with the Great Lakes showing reduced water levels over the past five years which has affected much of the wetlands in the area (U.S. Army Corps of Engineers 2022). The patterns of drought and precipitation within the Midwest and Northeast along with the rest of the continental U.S. create an environmental gradient which can change the distribution of plant species.

Lake and wetland systems contain hydric soils which are usually flooded, with nutrients typically in non-biologically available forms for uptake (Jackson et al. 2014). Both systems tend to have high organic content from the macrophyte communities which can reduce some vegetative reproductive capabilities in species like hydrilla (*Hydrilla verticillata*; McFarland and Barko 1987). Soils can determine how effectively wetland plants can grow and their germination opportunities with flowering rush benefitting from receding water levels that increase rhizome bud production (Hroudová 1989; Infante Mata and Moreno-Casasola 2005). Soil profiles can determine whether a plant can be present or not within an area which is typically seen in endemic species like *Ceanothus cuneatus* or Buckbrush (Lazarus et al. 2011).

*Butomus umbellatus* L. or flowering rush is an invasive perennial monocot that has spread in lake and wetland systems along the U.S. – Canadian border (Gunderson et al. 2016; Madsen et al. 2016b). Flowering rush growing in dense mats causes issues such as blocking water intake channels (Bellaud 2009; Madsen et al 2016a) and hosting parasite infected snails to pass on parasites such as swimmers' itch (*Ceraciae spp.*; Jacobs et al. 2011). Dense flowering

rush growth excludes native species like hardstem bulrush (*Schoenoplectus acutus*) and has potential to be more invasive than species like purple loosestrife (*Lythrum salicaria*; Lavoie 2003). Flowering rush is also known to have two cytotypes that have invaded North America, a diploid and triploid, which have show geographic separation with the diploid from Minnesota eastward and the triploid Minnesota westward (Gaskin et al. 2021; Anderson et al. 1974; Banerjee et al. 2020). Diploid flowering rush currently has a dearth of information regarding the life history and abiotic influences that might affect the distribution. The issues caused by flowering rush makes it a target for management strategies and further study into environmental factors that influence invasion dynamics.

Flowering rush spread has been modeled using several metrics within the recorded flowering rush invasion within North America (Banerjee et al. 2020). The study focused on comparing the native to the entire invaded range of flowering rush, describing the potential distribution within North America, and the distribution changes under future climate projections (Banerjee et al. 2020; Eyring et al. 2016). From previous studies flowering rush was found to have a potential distribution of up to 56.9% of North America, influenced primarily by the mean annual temperature and the minimum temperatures of the coldest month and coldest quarter (Banerjee et al. 2020; Liu et al. 2020). The potential distribution was also found to share similarities to the native environment in Eastern Europe as well (Banerjee et al. 2020). Previous studies have focused specifically on the entire invaded range of flowering rush across North America which is made up of two separate cytotypes (Gaskin et al. 2021). This study will focus specifically on the diploid cytotype of flowering rush that is well documented in the eastern U.S. specifically, with three populations that are identified as diploid populations (Gaskin et al. 2021). Invasive species have been largely seen to conserve their ecological niche when entering new

environments and very rarely will the species begin to overtake new niches (Wiens et al. 2010; Liu et al. 2020).

The objective for this study was to create an ecological niche model and describe the abiotic influences that act upon diploid flowering rush within each of the study sites and then all study sites as a global model. The previous study done on the realized niche of flowering rush included both cytotypes of the species across the entire invaded range of North America (Banerjee et al. 2020). Focusing specifically on the diploid cytotype will provide some explanation for specific abiotic factors and offer a site-by-site analysis of each population. It is hypothesized that suitable habitat for diploid flowering rush will be in areas that experience cooler annual temperatures and increased annual precipitation. Additionally, by analyzing each study site separately it will offer insight into whether diploid flowering rush shows plasticity in environmental influences across all assessed populations.

## **2. Methods**

### *2.1 Site Descriptions*

Mentor Marsh, OH (41.73649 N, -81.29879 W) is a large emergent marsh complex managed by the Cleveland Museum of Natural History. Mentor Marsh receives hydrologic input directly from Lake Erie. Mentor Marsh is a biodiversity hotspot within Ohio and is home to numerous waterfowl and rare bird species as well as providing fishery habitat (Cleveland Museum of Natural History; M. Yeager, personal communication, March 6, 2023). Mentor Marsh historically was used as a dumping site for salt mine tailings which caused widespread ecosystem destruction and native plant loss (Cleveland Museum of Natural History). The open

niche space because of native plant loss was quickly filled by phragmites haplotype M (*Phragmites australis*; M. Yeager, personal communication, March 6, 2023; Guo et al. 2014). Phragmites is an aggressive invader and has warranted aggressive management strategies however, flowering rush has become another aggressive invader within this system (Cleveland Museum of Natural History).

Tonawanda Wildlife Management Area, NY (43.10590 N, -78.48118 W) is an emergent wetland complex that has water level management through water control structures. The wetland is flooded in the spring (April) and drained before winter (November). Historically, this site was part of glacial Lake Tonawanda until the lake naturally drained through Niagara Falls (Calkin & Brett 1978). Today, there are numerous dikes to control water flow within the system to create a large waterfowl management area through seasonal flooding and draining. Tonawanda has been invaded by phragmites and flowering rush is known to have invaded in 2009 and has continued to grow more densely throughout the marsh (Roster 2011; Kennedy 2018; U.S. Geological Survey 2023).

Unity Island, NY (42.92999 N, -78.90453 W) is a wetland near the Buffalo River near Buffalo, New York. The site is being managed by the Army Corps of Engineers to develop complex habitat for fisheries management as part of the Lake Erie system (U.S. Army Corps of Engineers 2021). Unity Island has been undergoing ecological restoration since 2018 with flowering rush recorded within the site in 2019. Flowering rush is thought to have been established via dredged materials being used in restoration efforts at Unity Island and has continued to be an aggressive wetland invader. Flowering rush poses a threat to restoration efforts by reducing native diversity, increasing sediment deposition, and decreasing open-water fish spawning habitat (U.S. Army Corps of Engineers 2021).

## *2.2 Data Collection*

Ecological niche modeling uses abiotic variables to understand and determine specific environmental influences on the distribution of a desired species and the potential distribution as well. Twenty samples were harvested from each study site using a polyvinyl chloride (PVC) coring device designed to remove the plant within the area of the device (Madsen et al 2007, Wersal and Madsen 2018). Sampling was conducted once per month between May and November for two years and geographic coordinates were recorded for every collected sample at each study site. Modeling was done using R and MaxEnt (R Core Team 2017; Phillips et al. 2006; Phillips et al. 2008; Gaynor et al. 2018). Climatic layers were downloaded as all 19 bioclimatic variables present on WorldClim at the resolution of 30 arcseconds which is necessary for the size of the study sites being small (Fick & Hijmans 2017). Soil layers were downloaded from SoilGrids at a resolution of 250 m at a depth of 15 – 30 cm and focused typically on the physical properties of the soil as well as some nutrient profiles such as nitrogen and organic carbon content (Batjes et al. 2020). The soil depth of 15-30 cm was chosen as most of the flowering rush growing within these populations begin rooting at this depth with fewer plants found any more shallow or deeper. All abiotic variables are described in detail in Table 1.

## *2.3 Data Processing*

Data points were reduced to only one point per pixel with the altitude BioClim layer used as the resolution for pixel size (1 km). The data points were reduced to one point per pixel to avoid overloading one area of the model which could skew the results towards one location of

the study site. From the altitude layer resolution, the reduced data points were about 1 km<sup>2</sup> away from each other and a convex hull was drawn from the outermost data points. The convex hull was drawn with the outermost points at each site and was used to determine the abiotic influence within the site area. Abiotic layers were trimmed down to the extent of continental North America of -130° W to -59° W and 13° N to 59° N to determine the potential distribution of the species.

Abiotic layers, especially the climatic layers, are often measuring the same variable with a different temporal scale either by month or by quarters. Because of these similarities in measurement, a Pearson correlation was used to determine the correlational strength between all the abiotic layers being assessed. Like the one point per pixel step, it was important to determine which layers provided the most information while not over-explaining the niche model. Because the geographic coordinates for flowering rush were collected once a month the abiotic layers that described conditions monthly were kept. From those monthly abiotic variables, any layers that had a correlation higher than 0.8 were removed. If abiotic layers are highly correlated, they can overexplain parts of the generated model and by using abiotic layers with low correlations more information can be gleaned from each model. Often, layers that assess variables like precipitation as quarters or individual months of the year can explain the same information in the same time period. The abiotic variables that were kept were then made into projection and site-specific layers (Table 2). In total, there was one set of projection layers for full North America extent and there were four site specific sets of abiotic layers. Each study site was evaluated individually, and all the study sites were combined to determine site specific and general environmental effects.

## 2.4 Statistical Analysis

The MaxEnt program was utilized to determine the potential distribution of flowering rush within each study site and for the global site model as well as the individual abiotic variable contributions to the current distribution (Phillips et al. 2006; Phillips and Dudik 2008). For this study, the metrics used were: 25% test percentage of geographic data, bootstrapping, and a jackknifing analysis for variable importance. The test percentage was used to run statistical analyses such as the AUC statistic which acts as a goodness of fit test for the predictive power of each generated model. Bootstrapping reassessed the tested data across 10 replicates as well as generated averages from each of the replicates that were generated to determine what variables might explain more distribution than others. Finally, the jackknifing analysis assessed how much influence each of the abiotic variables have on the generated models by explaining how each variable will explain the species distribution (Phillip 2017).

Following the MaxEnt modeling procedure, a principal component analysis was performed to determine the specific effects attributed to each site. The rotational variables were extracted from the principal component analysis and transformed to show the relative contributions into the formation of the analysis. These rotational variables described the vector magnitude within each principal component analysis and explained the direction of each variable within the model. The resultant ecological niche models were pseudo-ground truthed by downloading known flowering rush locations across north America from the U.S. Geological Survey Nonindigenous Aquatic Species (USGS NAS) database, with no distinction between cytotypes, and overlaying point data onto the averaged niche models. The USGS NAS datapoints then had their corresponding pixel value recorded with the terra package to find how many geographic points occupy high suitability areas (Barbosa 2015). Highly suitable areas of the



raster were considered as pixel values of 1 or more for the data point (Phillip 2017). The average ASC file generated from the global sites model was loaded into ArcGIS with the USGS NAS data points. The average ASC file was then histogram equalized to improve contrast between pixels on the map and display areas with high suitability to construct Figure 3. All modelling methodology and data can be made available upon reasonable request.

### 3. Results

The generated AUC scores for each of the ecological niche models were above 0.8 with the model of every site being 0.9 and each site's model being 1.0. An AUC score is typically used to determine the reliability of a computational model with high reliability scoring closer to 1 and less reliable to -1. These values indicate that the models are adequate for predicting habitat suitability for flowering rush across North America. Though the high AUC scores are likely due to small geographic areas that were evaluated and used to generate the models. Future modelling efforts should incorporate more areas or samples collected from a larger geographic area. The current models indicate that diploid flowering rush can invade aquatic or wetland habitats throughout most of North America specifically in the U.S (Figure 1). From the terra package, it was found that of the 3742 geographic points loaded onto the model approximately 2137 data points were found to be in high suitability pixels (pixel value > 1.)

Alongside the generated heat maps, each niche model had a jackknife and principal component analysis assessing the influence of each environmental variable. From the jackknifing analysis, individual study sites saw bio 15 explain the most information singularly. Comparatively, the global site analysis as bio 4 explains the most information by itself. Within

the principal component analysis, PC1 and PC2 explain 92.7 % of the variation combined. Within the PCA, it was found that most of the variation captured in PC1 is visually explained by Soil Organic Carbon Content, Nitrogen, and Clay content with PC2 visually explained by Bio 13 and Altitude (Figure 2). These axes were loaded with the assessed abiotic variables consistently across all four analyses done. Specifically, each site's abiotic factors were: Mentor Marsh by Bio 6, Bio 12, and Altitude; Tonawanda by Nitrogen and Organic Carbon Content; and Unity Island by Bio 4, Bio 13, and Bio 14. The global site analysis was equally influenced by all variables with no specific direction towards a singular variable according to the principal component analysis. The relative loadings of each variable are displayed in Table 3 with the actual principal component analyses in Figure 2.

#### **4. Discussion**

Each model determined that each population of flowering rush is highly dependent on specific seasonal signals within each study site. Flowering rush is an herbaceous perennial plant species that needs seasonal cues for certain life processes (Greet et al. 2012, Von Holle et al. 2010; Junk and Piedade 1997). Most plants in the world need some type of signaling to occur to break seed dormancy (Hroudouva & Zákavský 2003), cue flowering (Von Holle et al. 2010), and in the case of flowering rush to start producing different tissues at different times (Marko et al. 2015; Hroudouva & Zákavský 1993). Populations of flowering rush display some plasticity towards the seasonal signals being used which is not uncommon for invasive species due to many being generalists (Emery-Butcher et al. 2020). Many invasive species are considered generalists with many being ecosystem engineers which allows them to potentially invade numerous habitats (Cuddington and Hastings 2004; Emery-Butcher et al. 2020). Being a generalist has provided flowering rush the ability to spread to numerous areas within the U.S.

with the current invasion around the U.S.-Canadian border. However, there are large areas such as the eastern U.S. that show high suitability to be invaded by flowering rush.

Based on current data, the most suitable area around the Great Lakes has already been invaded by flowering rush and the species could spread along major river systems (U.S. Geologic Survey). From the USGS NAS database, flowering rush has begun to move down the Ohio River system which offers the species high dispersion potential throughout the upper eastern U.S. By spreading through major river systems, flowering rush could display plastic responses to the numerous seasonal trends that were found within each population that was studied. Flowering rush within Unity Island was shown to be dependent on patterns of precipitation within the wettest and driest months of the year as well as the seasonal temperatures. These variables indicate that flowering rush within this site is dependent on the hydrologic flow of the Buffalo River between the rainy and dry seasons. These seasons will bring in water at different temperatures which signals flowering rush to produce a certain tissue or to set flowers (Wolfe et al. 2008; Chen and Kumar 2001). Flowering rush located in Unity Island typically grows submerged for most of the year which means that the plant will remain in its submerged form and highly reliant on the water column for temperature changes for phenological timing (Bornette and Puijalon 2010).

The Mentor Marsh population was seen to rely on temperature flux during the warmest and the coldest months of the year. Lake Erie has been under drought conditions for several years now which has caused flowering rush to grow without being submerged (U.S. Army Corps of Engineers 2022). Mentor Marsh is also an emergent wetland system which the flowering rush will usually grow in a few centimeters of water; however, due to persistent drought conditions flowering rush will grow with little to no submersion. Because the population grows exposed, air

temperatures directly affect the flowering rush rather than water temperatures from Lake Erie. This direct influence of air temperature means that the flowering rush population are more prone to temperature stressors which can change phenologic patterns and biomass production. However, air temperature can be helpful for flowering rush as the main mechanism for vegetative dispersal is the rhizome buds which could be produced more rapidly with optimal temperatures.

Compared to the other two sites, the flowering rush present at Tonawanda does not visually appear to depend on any obvious seasonality variables. The Tonawanda population is more dependent on soil aspects, specifically nitrogen and organic carbon content. Tonawanda is a waterfowl management site that is seasonally flooded in early spring then drained near the end of the year typically in October and November (Roster 2011; Kennedy 2018). Waterfowl help to facilitate the nitrogen cycle within wetlands which may be influencing nitrogen and organic carbon within the niche model (Hinckley et al. 2020; Corre et al. 2002). Often nitrogen and carbon content come from dead plant tissues and other litterfall which could come from the biodiversity that has been found within Tonawanda.

Finally, the global model considered all three study sites to determine the overall environmental influences on the distribution of flowering rush. From the principal component analysis, it was seen that the global site model had no influence in one direction or another. This is most likely due to all the geographic points being reused from each individual site for the global site model; however, temperature seasonality was found to have the most information by itself. Several studies have used this modeling to determine possible future spread under several climatic “scenarios” following different paths of carbon emissions (Banerjee et al. 2020; Eyring et al. 2016). The outcome found that flowering rush was dependent on colder climate conditions

and under almost all possible climate scenarios most of the predicted distribution would be retained. Based on this study, flowering rush is highly dependent on seasonal changes, usually changes that happen before and during winter. The current distribution of flowering rush along the U.S. - Canadian border, more northerly latitudes will see stronger seasonality patterns and colder temperatures (Oleksyn et al. 1998; Archibald et al. 2010; Banerjee et al. 2020). However, there will be physiological changes that occur from moving further south such as longer growing seasons or peak biomass periods, due to the decreased signaling in seasonal changes that occurs along a latitudinal gradient (Oleksyn et al. 1998; Lawson and Weir 2014). From these attributes diploid flowering rush could spread further south. Flowering rush can invade low suitability areas as well since there are established populations that are deemed low suitability for the species.

Diploid flowering rush displays plastic responses to the abiotic variables that were evaluated as each wetland showed a different set of impactful abiotic variables. Each site's population was found to be dependent on different environmental variables such as temperatures at the coldest months and the nitrogen content in the soil. The global analysis found that temperature seasonality was a highly explanatory variable which supports the hypothesis that flowering rush is influenced by seasonal variation in temperatures. Flowering rush is native to eastern Europe with the invaded range along a similar latitudinal line of 45° N which offers that flowering rush is most likely conserving its ecological niche (Anderson et al. 1974). Further analysis could be done to determine if the ecological niche is being largely conserved between continents; however, from a previous study that was done flowering rush is most likely already doing this (Banerjee et al. 2020). Most invasive species are thought to already conserve their ecological niche between native and invaded ranges which flowering rush is most likely already doing within North America (Wiens et al. 2010).

Based on current distribution, flowering rush occupies highly suitable environments around the Great Lakes region with numerous populations scattered throughout the rest of the U.S. This raises concern that diploid flowering rush has strong potential for further expansion in the eastern U.S. Flowering rush can reproduce sexually through flowers and asexually through vegetative propagules formed on the rhizome and inflorescence base. Reproduction, specifically sexual reproduction, offers flowering rush the chance to increase plasticity to environmental cues which could have it adapt to higher temperatures or other environmental cues (Molofsky et al. 2014). From this study, flowering rush should be monitored and maintained to prevent further invasions along river systems. Future studies could incorporate water quality information within invaded sites of flowering rush to determine that along with climate and soil if there is any water quality influence.

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Table 1: All abiotic environmental layers that were evaluated for use within the ecological niche modeling of flowering rush. The climatic variables were downloaded at 30 arcseconds and soil variables were 15 – 30 cm within the soil profile.

<b>Climate Variables</b>	<b>Soil Variables</b>
Alt – Altitude	BDOD – Bulk Density of Soil (cg/cm <sup>3</sup> )
Bio1 – Annual Mean Temperature	CEC – Cation Exchange Capacity (mmol(c)/kg)
Bio2 – Mean Diurnal Range	CFVO – Coarse Fragment Volume (cm <sup>3</sup> /dm <sup>3</sup> )
Bio3 – Isothermality	CLAY – Clay Content (g/kg)
Bio4 – Temperature Seasonality	NITROGEN – Nitrogen Content (cg/kg)
Bio5 – Max Temperature of Warmest Month	PHH2O – pH of Water in Soil (pH * 10)
Bio6 – Min Temperature of Coldest Month	SAND – Sand Content (g/kg)
Bio7 – Temperature Annual Range	SILT – Silt Content (g/kg)
Bio8 – Mean Temperature of Wettest Quarter	SOC – Soil Organic Carbon (hg/m <sup>3</sup> )
Bio9 – Mean Temperature of Driest Quarter	
Bio10 – Mean Temperature of Warmest Quarter	
Bio11 – Mean Temperature of Coldest Quarter	
Bio12 – Annual Precipitation	
Bio13 – Precipitation of Wettest Month	
Bio14 – Precipitation of Driest Month	
Bio15 – Precipitation Seasonality	
Bio16 – Precipitation of Wettest Quarter	
Bio17 – Precipitation of Driest Quarter	
Bio18 – Precipitation of Warmest Quarter	
Bio19 – Precipitation of Coldest Quarter	

Table 2: Environmental layers used for the niche model construction and principal component analysis. Soil layers were also for a depth of 15 - 30 cm (Soil Grids) and climatic layers (WorldClim) were kept for monthly values.

<b>Climate Layers</b>	<b>Soil Layers</b>
Alt – Altitude	BDOD – Bulk Density of Soil (cg/cm <sup>3</sup> )
Bio 1 – Annual Mean Temperature	CEC – Cation Exchange Capacity (mmol(c)/kg)
Bio 4 – Temperature Seasonality	CFVO – Coarse Fragment Volume (cm <sup>3</sup> /dm <sup>3</sup> )
Bio 5 – Max Temperature of Warmest Month	CLAY – Clay Content (g/kg)
Bio 6 – Min Temperature of Coldest Month	NITROGEN – Nitrogen Content (cg/kg)
Bio 12 – Annual Precipitation	PHH2O – pH of Water in Soil (pH * 10)
Bio 13 – Precipitation of Wettest Month	SAND – Sand Content (g/kg)
Bio 14 – Precipitation of Driest Month	SILT – Silt Content (g/kg)
Bio 15 – Precipitation Seasonality	SOC – Soil Organic Carbon (hg/m <sup>3</sup> )



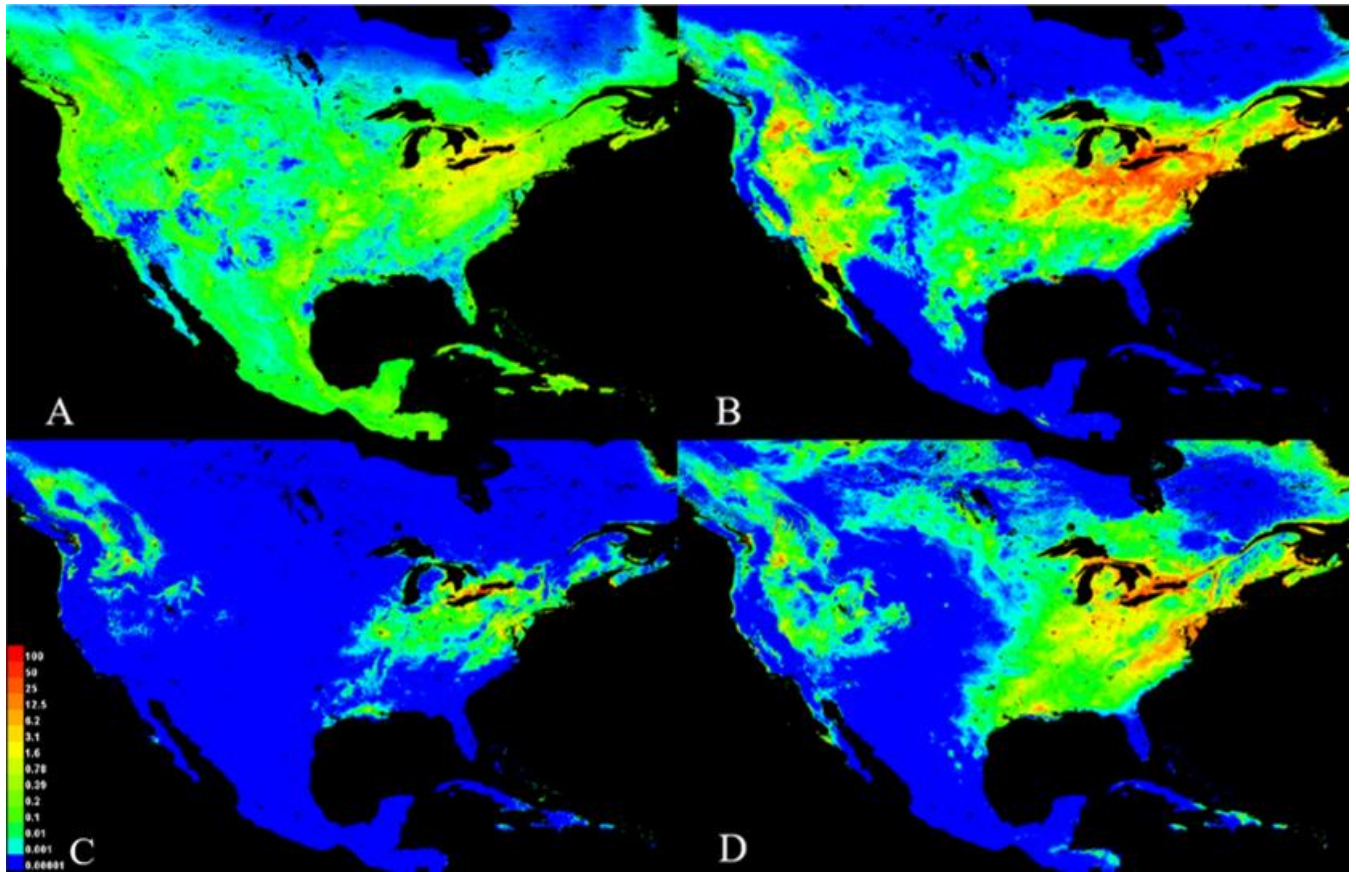


Figure 1: Generated heat maps for the ecological niche modelling of diploid flowering rush which warmer colors denote higher suitability for the population and cooler colors as more unsuitable areas. (A) Global sites heat map, (B) Mentor Marsh (Ohio) heat map, (C) Tonawanda (New York) heat map, and (D) Unity Island (New York) heat map.

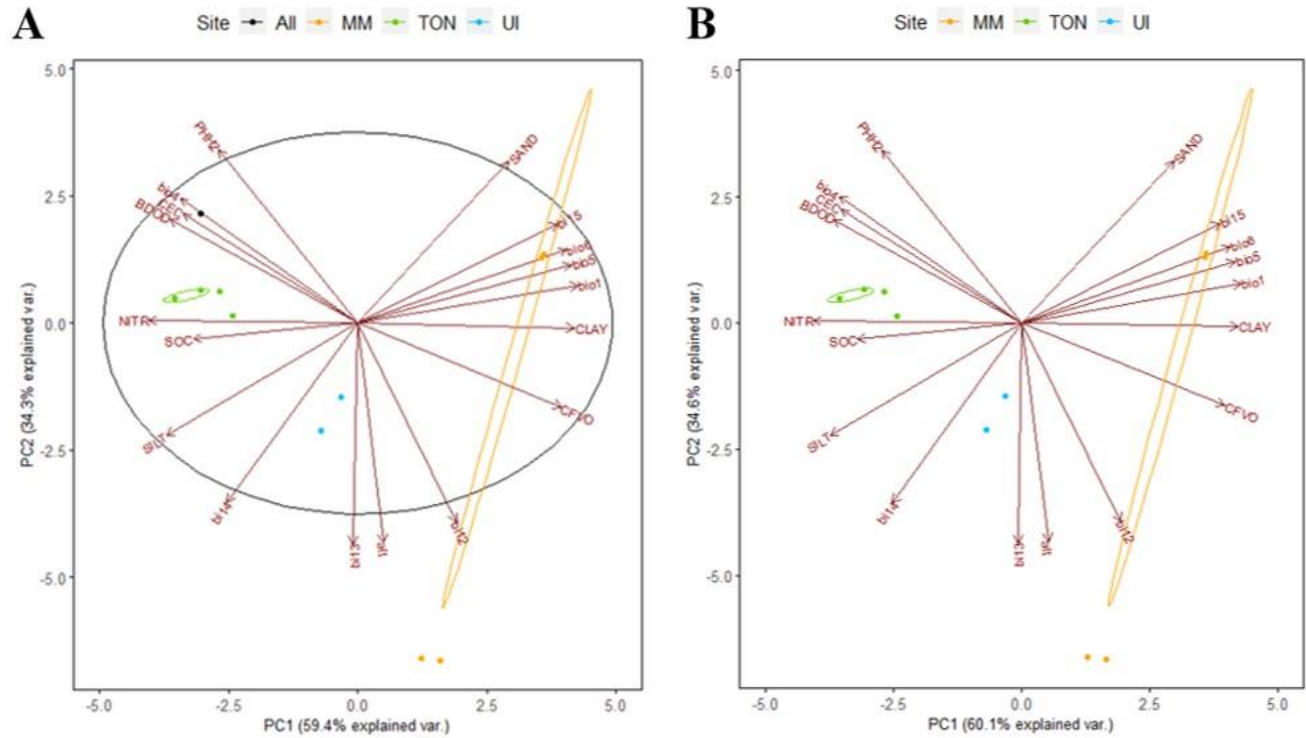


Figure 2: The principal component analysis of each site with each abiotic variable as a covariate arrow indicating the relationship to each axis. A) The principal component analysis with the global site ecological niche model included with each site. B) The principal component analysis of each individual site's model compared to each other.

Table 3: The relative loadings associated with both principal component analyses. The loadings represent the relative magnitude of the associated arrow present in the principal component analysis. Larger loading values are associated with more strength to one of the principal component axes.

	2A		2B	
	PC1	PC2	PC1	PC2
alt	0.887963	11.07166	0.91184	10.996
bio1	7.551959	1.896579	7.508688	1.995607
bio4	6.082004	6.311162	6.240002	6.374638
bio5	7.335273	2.982874	7.302437	3.10785
bio6	7.163592	3.697174	7.14135	3.846591
bio12	3.380448	10.0512	3.384594	9.969695
bio13	0.159235	11.15669	0.142538	11.08985
bio14	4.472591	9.04084	4.402393	9.038897
bio15	6.847269	5.016596	6.794854	5.016481
BDOD	6.441378	5.25689	6.415799	5.168285
CEC	5.98213	5.515876	6.188867	5.689431
CFVO	6.963472	4.226543	6.940552	4.163786
CLAY	7.436787	0.243178	7.394608	0.161318
NITROGEN	7.16192	0.163155	7.125025	0.131992
PHH20	4.757325	8.761176	4.76135	8.678206
SAND	5.184111	8.158159	5.205964	8.131859
SILT	6.563351	5.659234	6.532799	5.639385
SOC	5.629192	0.791017	5.606339	0.80013

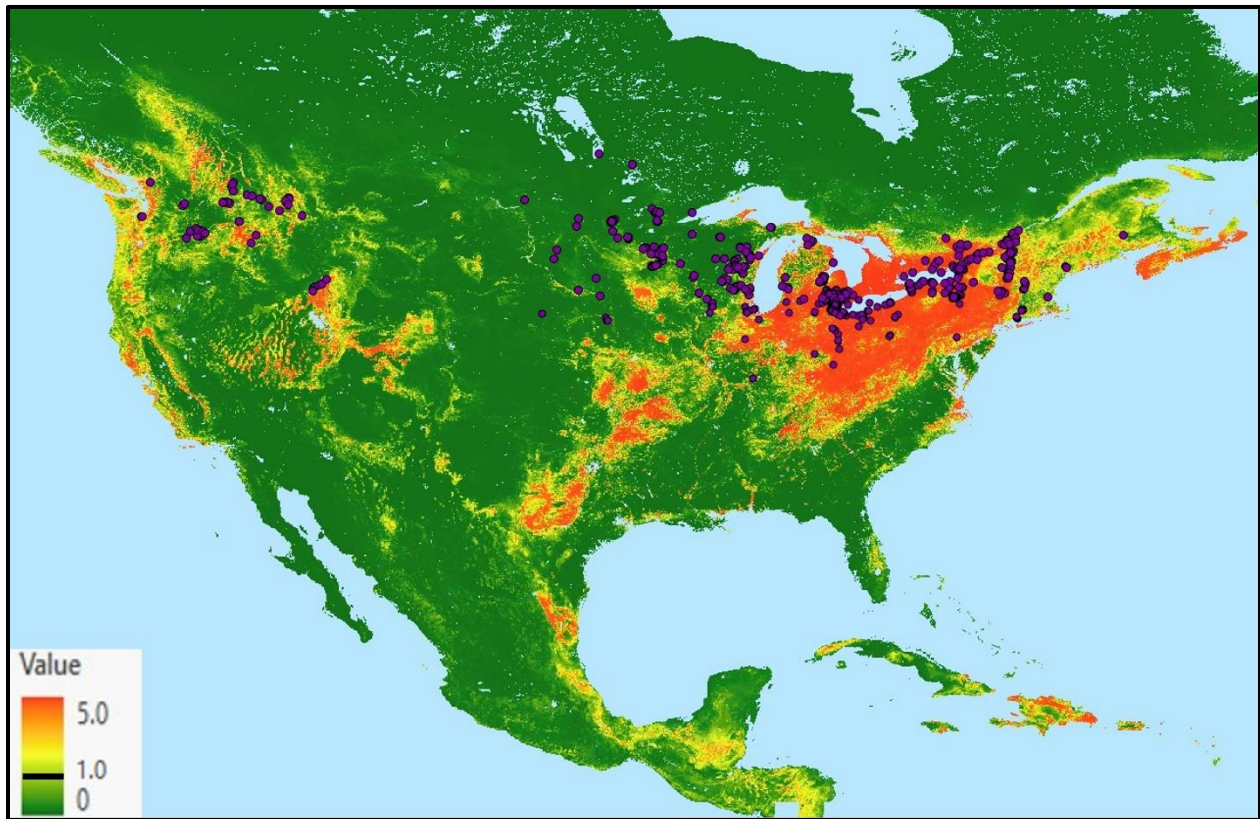


Figure 3: Global site heat map with geographic coordinates pulled from the Nonindigenous Aquatic Species database from the U.S. Geological Survey. Warmer colors indicate areas of higher suitability and better predicted conditions with greens indicating areas of lower suitability and lower predicted conditions. Values above 1.0 were considered highly suitable areas for flowering rush on this projection. This heat map was created within ArcGIS Pro.

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## CHAPTER 4 – CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

### 1. Life History

Flowering rush is an invasive monocot that displays seasonal growth patterns towards high leaf production at the beginning of the growing season with high starch content being formed by the end of the season. Leaf production was typically seen to peak, approximately 700 to 1200 g DW m<sup>2</sup>, in July and August with rhizome and rhizome bud production typically peaking in November, 1200 g DW m<sup>2</sup> and 500 g DW m<sup>2</sup> respectfully. Leaf production is most likely prioritized early season to replenish starch reserves within the rhizome after the overwintering period. The rhizome tissue as well as the rhizome buds are used to help the plant survive over winter and the rhizome will be where the leaves are grown from. The rhizomatic tissues will be the main source of energy for the winter period however, the rhizome buds are a form of vegetative reproduction for flowering rush.

Within our study of life history and resource partitioning across three study sites, diploid flowering rush is seen to make between 12,000 to 16,000 rhizome buds per m<sup>2</sup> by the end of the growing season. Both cytotypes of flowering rush are capable of making rhizome buds however, the main difference will be the scale of rhizome buds made with the triploid cytotype only making a peak of 600 rhizome buds per m<sup>2</sup> (Marko et al. 2015). These rhizome buds essentially serve the dual purpose of survival aid in stressful periods, in this case the wintertime, and acting as a form of reproduction for the plant. Unlike the triploid cytotype, diploid flowering rush can sexually reproduce and create seeds that can become new plants (Eckert et al. 2000). Flowering rush can produce 20 to 50 flowers per inflorescence with approximately 200 seeds per flower being produced at 31% viability (Eckert et al. 2000; Lui et al. 2005). Assuming maximum output

both sexually and asexually, diploid flowering rush can produce up to 19,000 individuals per m<sup>2</sup> per growing season making it a force to be reckoned with for management within invaded sites.

Despite the reproductive differences between the cytotypes, there really is not a major difference occurring within the tissue production between the diploid and triploid flowering rush. Using the study done by Marko et al. 2015, the amount of above ground and rhizomatic tissue, 1100 g DW m<sup>2</sup> and 1200 g DW m<sup>2</sup> respectfully, is being made in similar amounts and the above ground tissue peaks in the July/August period. For the rhizome bud density there are similar peaks right after November occurring in both cytotypes however, one major difference is the intensity of rhizome buds being made.

## **2. Management Goals**

Based on the information gathered during all the studies done, there are a few options that can be used to help manage diploid flowering rush. The main method for managing flowering rush will be to starve the rhizome of carbohydrates until the population crashes. This species is necessary to treat early season since by the end of the growing season flowering rush has accumulated enough resources to survive overwinter. There are two main goals when managing flowering rush: carbohydrate starvation of the rhizome and overall reduction of rhizome bud production. The rhizomatic tissues are the main energy source for the plant to survive overwintering and the leaf tissues will be used only for photosynthesis and gas exchange.

### 3. Management Method Recommendations

#### *Physical, Mechanical, and Biological Management*

Physical and mechanical management are not recommended for flowering rush as soil disturbance could spread vegetative propagules within an invaded system (Turnage et al. 2019). If physical management were to be done it should occur when water levels are low to prevent rhizome pieces and rhizome buds from spreading widely. Effective physical management methods would be benthic barriers to prevent aboveground tissue from rising into the next growing season. The benthic barriers should be placed either in April or May when the above ground mass has not started growing, is short, or while it is in its submerged form. Hand pulling and clipping are both ineffective treatments for flowering rush since they both must be done numerous times for any result. A biological control method for flowering rush has not been developed and if one were available then it is not recommended due to the general slowness of biological control.

#### *Chemical Control*

Chemical control will be incredibly effective management means for flowering rush since it can be applied widely and effectively against the population that is present in a waterbody. Diquat is the most widely used for control of flowering rush and will target the photosynthetic pathway which will destroy the above ground tissue rapidly (Poovey et al. 2012). Unfortunately, the above ground tissue does not contain much starch content compared to all other tissues and diquat will not penetrate below the soil line where the main storage tissues are for flowering rush (Turnage et al. 2020). Coming right out of winter, flowering rush focuses on building leaf tissue for the first three months of the growing season to recuperate all the used-up starch from the

winter period. During this leaf building period, the rhizome biomass and starch content sharply drops until August which is consistent across both years of the life history study.

In Figure 1, those seasonal trends are displayed with August being the lowest point in rhizome mass across all sites. This trend is seen within 2021 and 2022 with the rhizome mass increasing afterwards as the plant shifts towards preparing for winter. Because carbohydrate starvation is the main long-term strategy for managing flowering rush, applying herbicide before peak above ground biomass is key. Application in June and maybe July would cause a leaf tissue die off and force the rhizomatic tissue to continue using up stored carbohydrates for leaf production. By forcing leaf production, flowering rush will not be able to recuperate the starch lost as quickly and it could cause the plant to die over winter.



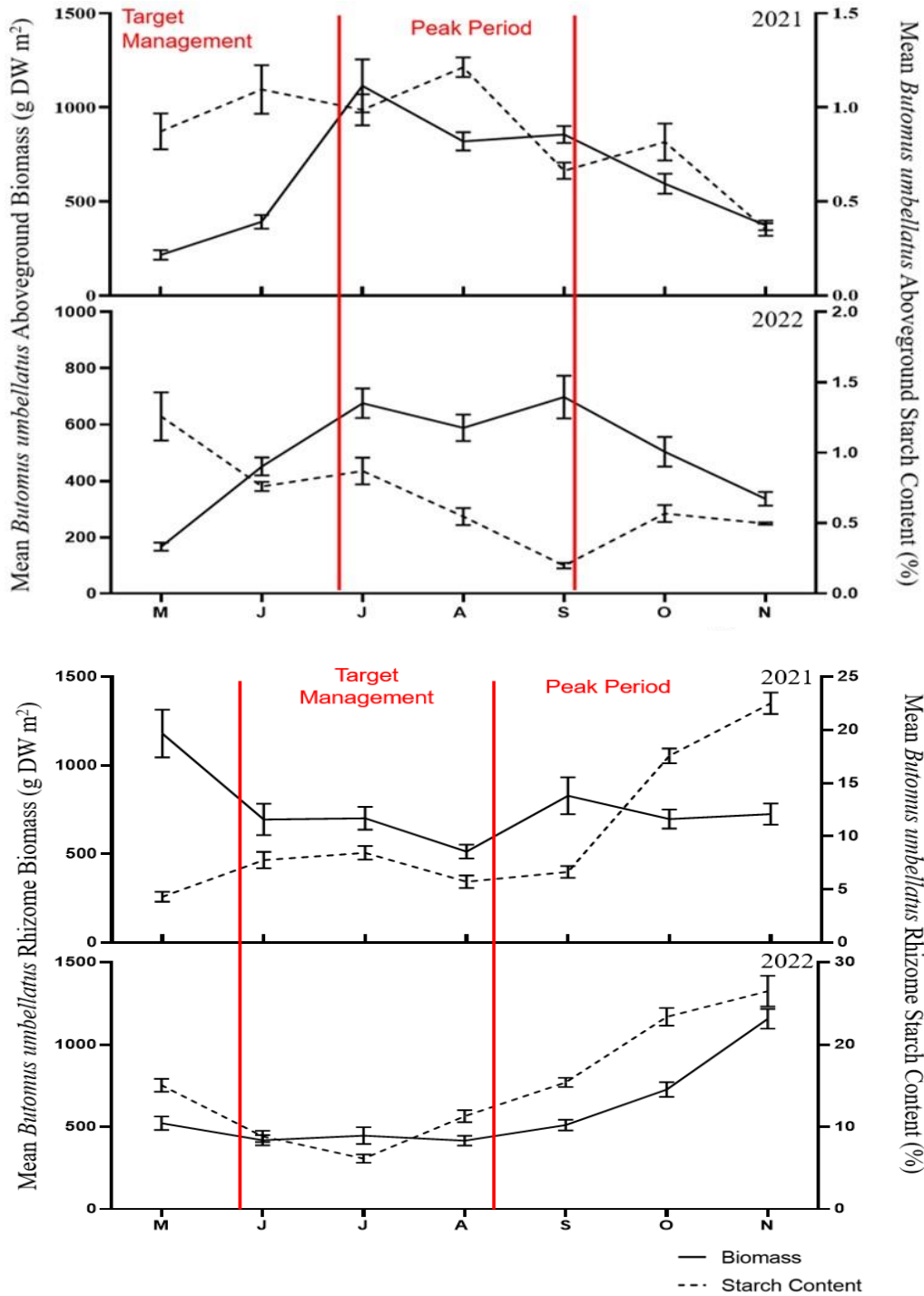


Figure 4: Biomass and starch content trends within aboveground and rhizome tissues across 2021 and 2022. Peak periods of biomass and starch are indicated by vertical red lines, as well as the recommended management period for both tissue types. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

#### 4. Site by Site Recommendations

##### *Mentor Marsh, Ohio*

Mentor Marsh is an emergent wetland that receives water from Lake Erie and is currently under heavy invasion of *Phragmites*. Mentor Marsh from the life history study was seen to be the highest producer of rhizome buds across both years. Because of an ongoing drought within Mentor Marsh, some physical control could be done to remove flowering rush from the soil however, this should be a last option to prevent rhizome fragment or rhizome bud spread. However, integrating *Phragmites* and flowering rush management could be a powerful tool at curbing the spread of both plants within the same site.

However, for managing strictly diploid flowering rush Mentor Marsh should target herbicide treatments of diquat in June or July due to the reduction in rhizome biomass and starch content. One trend that can be seen in mentor marsh is that the rhizome buds will be the main starch storage vessel for the flowering rush growing in Mentor Marsh so reducing the supply of rhizome buds and rhizomatic tissue should be a main goal. Mentor Marsh across all three study sites was seen to produce the most above ground tissue so for simple nuisance control, diquat treatments should occur in June, July, or possibly August.

##### *Tonawanda, New York*

Tonawanda is another emergent wetland system however, there are water control structures in place that allows for the wetland to be flooded in the spring and drained in the fall. Because water control structures are present flowering rush could pose the problem of blocking intake and outflow areas of these structures so management would have to be slightly different. Drawdowns are not an effective tool against flowering rush since it is an emergent plant that

preferably grows out of the water column and drawdowns can stimulate rhizome bud development (Hroudová 1989). Again, chemical control through diquat is the recommended method for controlling the flowering rush population present within Tonawanda.

Tonawanda displays similar trends to Mentor Marsh with a peak in above ground tissue occurring in August and the rhizome tissue on gradual downward trend. However, one major difference in Tonawanda is the productivity of the plants is much lower and the plants were much smaller. Because the flowering rush at Tonawanda is so much smaller using chemical control would quickly manage the population that is currently present. Based on biomass trends within this site, management should occur in June or July to again force the rhizomatic tissue to use up more carbohydrate reserves.

#### *Unity Island, New York*

Unity Island is a bit different compared to the other studied wetland sites because it sits along the Buffalo River and the main goal of the ecological restoration occurring is for complex habitat formation. The Buffalo River provides Unity Island moving water which changes management strategies for flowering rush at this site since soil disturbance could spread propagules into Lake Erie. Managing flowering rush at Unity Island will come down to using herbicide treatments during effective periods and minimizing disturbance to the rhizome of this plant.

Unity Island does not follow an entirely similar trend to Mentor Marsh or Tonawanda. This is most likely due to a difference in environments however, the above ground mass will peak around the same time period in July/August. Most likely what would need to happen is herbicide application would have to occur, again, before the peak of above ground biomass with

a possible second application in October. Flowering rush at Unity Island shows a more obvious trend in biomass with rhizome bud production reaching a peak in density and mass in September which is co-occurring with the rhizome.

## 5. Conclusions

Flowering rush is an invasive plant species that poses a big threat to systems with present populations; however, it can be easily managed through chemical control. Through our phenology study, it was found that July would be the most optimum time to manage for diploid flowering rush populations in the northern U.S. This species should be managed as soon as possible within newly invaded areas due to the massive reproductive scale occurring within one growing season. Chemical control will be the most effective method for diploid flowering rush due to the high efficacy seen in the triploid cytotype with other management methods either increasing spread or not being effective. Mechanical control is probably the worst control method for diploid flowering rush due to the volume of viable rhizome buds that get produced with chance of spreading (Turnage et al. 2019).

Mentor Marsh, OH and Tonawanda, NY are similar wetland systems which affect their flowering rush populations somewhat similarly. Because of similar environmental effects, their management methods and timing will most likely happen simultaneously to force the rhizomatic tissue to produce leaves. By forcing the rhizome of flowering rush to continually produce leaves, the population will gradually get stressed from using up starch resources and decrease overwintering survivorship. Unity Island, NY on the other hand displays slightly different trends than the other two sites which could be due to a different wetland structure and environment.

With the diploid flowering rush population at Unity Island, these plants will probably need to be stressed with chemical control more often than the other populations. Due to a consistently higher starch content, the flowering rush present will need more stressors to use up resources at a faster rate to assure a controlled population.

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