This is a “preproof” accepted article for Invasive Plant Science and Management. This version may be subject to change in the production process, *and does not include access to supplementary material.* DOI: 10.1017/inp.2024.31

Short title: Phenology of Flowering Rush

Phenology and Resource Allocation Strategies of Diploid Flowering Rush (*Butomus umbellatus*) in Ohio and New York

Maxwell G. Gebhart[1,2], Ryan M. Wersal[1], Andrew R. Hannes[3], Nathan E. Harms[4], Bradley T. Sartain[5], William L. Wolanske[6], and Mia Yeager[7]

[1]Graduate Student and Associate Professor respectively, Department of Biological Sciences, Minnesota State University, Mankato, Mankato, MN, USA; [2]Research Associate, Geosystem Research Institute, Mississippi State University, Starkville, MS, USA; [3]Ecologist, Buffalo District, US Army Corps of Engineers, Buffalo, NY, USA; [4]Senior Research Biologist, US Army Engineer Research and Development Center, Lewisville, TX, USA; [5]Research Biologist, US Army Engineer Research and Development Center, Vicksburg, MS, USA; [6] Fish and Wildlife Technician 2, New York State Department of Environmental Conservation, Basom, NY, USA; [7]Mentor Marsh Habitat Restoration Manager, Cleveland Museum of Natural History, Cleveland, OH, USA

Author for correspondence: Ryan M. Wersal, ryan.wersal@mnsu.edu, ORCHiD: **0000-0002-4014-5138**

Abstract

Flowering rush (*Butomus umbellatus* L.) is an emergent perennial monocot that has invaded aquatic systems along the U.S. - Canadian border. Currently, there are two known cytotypes of flowering rush, diploid and triploid, within the invaded range. Although most studies have focused on the triploid cytotype, little information is known about diploid plants. Therefore, phenology and resource allocation were studied on the diploid cytotype of flowering rush in three study sites (Mentor Marsh, Ohio; Tonawanda Wildlife Management Area, New York; and Unity Island, New York) to understand seasonal resource allocation, environmental influences on growth, and to optimize management strategies. Samples were harvested once a month from May to November at each site from 2021 to 2023. Plant metrics were regressed to air temperature, water temperature, and water depth. Aboveground biomass peaked from July-September and comprised 50 to 70% of total biomass. Rhizome biomass peaked from September to November and comprised 40 to 50% of total biomass. Rhizome bulbil densities peaked from September to November at 3,000 to 16,000 rhizome bulbils m^{-2}. Regression analysis resulted in strong negative relationships between rhizome starch content and air temperature (r^2=0.52) and water temperature (r^2=46). Other significant, though weak, relationships were found including a positive relationship between aboveground biomass and air temperature (r^2=0.17), a negative relationship between rhizome bulbil biomass and air temperature (r^2=0.18) and a positive relationship between leaf density and air temperature (r^2=0.17). Rhizomes and rhizome bulbils combined stored up to 60% of total starch and present a unique challenge to management as these structures cannot be reached directly with herbicides. Therefore, management should target the aboveground tissue before peak production (July) to reduce internal starch storage and aim to limit regrowth over several years.

Keywords: air temperature, emergent, great lakes, starch, vegetative propagules, water depth, water temperature, wetlands

Management Implications

Flowering rush (*Butomus umbellatus* L.) is an invasive emergent monocot commonly found along the U.S. – Canadian border that can grow rapidly and cause numerous water-use issues as a monotypic population. Within the U.S., there are two cytotypes of the species, a

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

diploid and triploid, which have slightly different reproductive strategies, with diploid reproducing both sexually and asexually and triploid plants reproducing only asexually. The current study sought to describe the life history, resource allocation, and phenology of diploid flowering rush. Diploid *B. umbellatus* in this study produced 8,000 rhizome bulbils per m^{-2} on average with one site having over 15,000 rhizome bulbils per m^{-2}. Not only does diploid *B. umbellatus* produce large numbers of propagules, but up to 30% of total plant starch is found in rhizome bulbils, which will allow for longevity in the sediment. A successful management program will need to indirectly target the belowground biomass and starch storage of this plant. Since rhizomes and rhizome bulbils are at or below the sediment surface it is difficult to directly treat with an herbicide or to harvest. Management programs should target aboveground biomass before July to remove new growth. Additional in-season applications should occur as plants regrow to limit rhizome bulbil formation. Repeat applications should reduce year-to-year recruitment, though management of *B. umbellatus* will be long-term as the propagule bank will need to be exhausted to completely control this species.

Introduction

Flowering rush (*Butomus umbellatus* L.) is an invasive perennial monocot primarily found in aquatic and wetland habitats along the U.S. - Canadian border. *B. umbellatus* was first documented in the St. Lawrence River, which facilitated spread into the Great Lakes region by the mid-1950's (Bellaud 2009; Gunderson et al. 2016). *B. umbellatus* 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 Amplified fragment length polymorphism (AFLP) genotypes (Anderson et al. 1974; Gaskin et al. 2021). Both cytotypes of *B. umbellatus* are capable of vegetative reproduction through fragmentation of the rhizome and production of rhizome bulbils developed along the rhizome and leaf axils (Hroudová and Zákravský 1993; Thompson and Eckert 2004). The diploid cytotype can also undergo sexual reproduction through self-compatible flowers on an inflorescence containing 20 to 50 flowers (Thompson and Eckert 2004). Current information about *B. umbellatus* biology and management largely centers on the triploid cytotype and its invasion within lakes, whereas the diploid cytotype and impacted wetlands remain understudied.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Within aquatic systems, plants must overcome unfavorable conditions such as low light availability and limited CO_2 concentrations which can impact photosynthesis and growth (Ralph et al. 2007). *B. umbellatus* can grow both emergent and submersed leaves (often together) and can be found in water as deep as 6 m but is usually found at depths of <1.3 m (Carter et al. 2018; Madsen et al. 2016c). Triploid *B. umbellatus* is typically found growing in lake systems whereas diploid *B. umbellatus* is more commonly found in wetland systems with variable hydrology (Gunderson et al. 2016). Alongside reproductive differences, the triploid cytotype is more widespread within the U.S., with populations found from Minnesota westward in lake or reservoir systems (Gaskin et al. 2021; Liu et al. 2005). Conversely, diploid *B. umbellatus* has been documented from Minnesota eastward and is prolific especially around the Great Lakes region within wetlands (Trebitz and Taylor 2007). Differences in geography and growing environment can impact a plant's life history, resource allocation, and phenology.

Emergent wetland plants are subject to the environment which can impact life history characteristics such as growth and resource accumulation (MacNeill et al. 2017; Scofield et al. 2009). Phenological timing is studied to understand and generate a timeline for plant critical plant processes such as peak biomass and resource allocation periods that can provide managers with important information to optimize the timing of control activities (Clarke et al. 2023; Wersal et al. 2011; Wersal et al. 2013). Triploid *B. umbellatus* phenology has been documented in the Detroit Lakes system in Minnesota where it was observed that aboveground biomass peaks in late summer and rhizomatic biomass peaks in late fall (Madsen et al. 2016c; Marko et al. 2015). Due to differences in genetics between cytotypes and depths in which diploid *B. umbellatus* plants grow, characterizing phenology and resource allocation could yield important information for managing invaded sites. Whereas phenology focuses on timing of biomass changes or reproduction, resource allocation aims to understand locations within the plant where starch and other carbohydrates are typically stored (Clarke et al. 2023; Haram and Wersal 2023; Wersal et al. 2011; Wersal et al. 2013). Management targeting critical tissues and times of low resource allocation can reduce growth, prevent reproduction, impact nutrient acquisition, and limit annual recruitment (Scofield et al. 2009).

By combining environmental data with trends in plant growth and reproduction, a detailed understanding of phenological timing can be developed for a plant species. Previous studies on *B. umbellatus* phenology focused on the triploid cytotype within the Detroit Lakes,

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

MN (Madsen et al. 2016c; Marko et al. 2015), and there is a paucity of data describing life history, resource allocation, and phenological timings for diploid *B. umbellatus*. In documenting diploid *B. umbellatus* phenology, management strategies could be optimized if growth of both cytotypes are similar. Therefore, the objectives of this study were to 1) evaluate the phenology of diploid *B. umbellatus* by determining times of peak biomass and starch allocation patterns from three field populations over two growing seasons in Ohio (one population) and New York (two populations), and 2) relate *B. umbellatus* growth to air temperature, water temperature, and water depth. It is hypothesized that diploid *B. umbellatus* will have similar phenological timings with respect to peak aboveground biomass in late summer (August or September) and peak rhizome biomass in late fall (November-December). It is also hypothesized that the starch allocation within the plant will be higher in the belowground structures, similar to the pattern documented in triploid *B. umbellatus*.

Materials and Methods

Sites chosen for this study were selected based on knowledge of historical *B. umbellatus* infestations, access for sampling, and in close proximity to the Great Lakes. The *B. umbellatus* populations in these three sites were previously determined to be diploid *B. umbellatus* genotype G4 by Gaskin et al. (2021). Subsequent genetic testing by the resource managers associated with each site reconfirmed the cytotype.

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 (CMNH) that receives hydrologic input directly from Lake Erie. Mentor Marsh historically was used as a dumping site for salt mine tailings which caused widespread ecosystem degradation and native plant loss (Cleveland Museum of Natural History). The open niche space was quickly filled by phragmites haplotype M which is an aggressive invader (*Phragmites australis*; M. Yeager, personal communication, March 6, 2023; Guo et al. 2014). In 2013, CMNH began large scale habitat restoration efforts to manage phragmites in the marsh and the disturbance from the restoration work likely allowed *B. umbellatus* to expand its range within the marsh. Currently, CMNH's goal has been to reduce *B. umbellatus* populations by hand pulling the plants directly. More recently, *B. umbellatus* invaded and has begun to spread in the wetland system (Cleveland Museum of Natural History).

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Tonawanda Wildlife Management Area, NY (Tonawanda WMA; 43.10590 N, -78.48118 W) is a large emergent wetland complex managed by New York State Department of Environmental Conservation that undergoes seasonal water-level management through on-site water control structures. The wetland is naturally flooded in the spring (April) and can be manually or naturally drained before summer and, depending on water availability, reflooded in the fall. 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 waterfowl management area through seasonal flooding and draining. *B. umbellatus* was first discovered in Tonawanda WMA in 2009 and has since continued to spread throughout the marsh (Kennedy 2018; Roster 2011; U.S. Geological Survey 2023). Tonawanda WMA staff currently aim to control phragmites and water chestnut (*Trapa* spp.) through herbicide applications and hand-pulling, respectively.

Unity Island, NY (42.92999 N, -78.90453 W) is a wetland adjacent to the upper Niagara River near Buffalo, NY. The wetland was created in 2018 through beneficial use of dredged material from the Buffalo River federal navigation channel. *B. umbellatus* was first recorded within the Unity Island site in 2019. *B. umbellatus* is common in the mainstem upper Niagara River where it can grow in at least 3 m of water (Gunderson et al. 2016). It is thought that propagules recruited to the Unity Island site dispersed from the adjacent upper Niagara River, and establishment was likely aided by general site disturbance during restoration efforts. *B. umbellatus* continues to spread and be an aggressive invader at this site (U.S. Army Corps of Engineers 2021).

Biomass

Sampling was conducted once per month at all three sites between May and November over three years (2021 through 2023; n=420 samples per site). 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 (0.018 m^2) (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 and water temperature every 30 minutes throughout the sampling season each

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

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 separated into tissue types described by Hroudová and Zákravský (1993), with above ground tissue (leaves), rhizome and roots, inflorescences, rhizome bulbils, and vegetative bulbils formed at the base of the inflorescence (Hroudová and Zákravský 1993; Hroudová 1989). In the three-year span of sampling, bulbils formed at the base of the inflorescence were not recorded in any sample regardless of site. Thus, only rhizome bulbils were collected as a form of vegetative reproduction. The number of rhizome bulbils and leaves from each collected sample were also counted and recorded. Sorted tissues were put into separate paper bags and placed in a drying oven at 48 C for at least 72 hours, until dry. 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^2) to determine grams of dry weight per m^2 (g DW m^{-2}) for each tissue. Density of rhizome bulbils and ramets were calculated in a similar manner.

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. Samples were then placed in a food processor until the tissue was roughly cut. Rough-cut biomass was then ground using a Cyclone Sample Mill 3010-014 (UDY Corporation) 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. Starch percent dry weight (% DW) of each sample set was determined using the amylase/amyloglucosidase method via the commercially available STA-20 starch assay kit (Sigma Aldrich, St. Louis, Missouri). Recovery, accuracy, and precision were assessed using several repeated measures with every starch extraction assay. Wheat (*Triticum aestivum* L.*;* 89% purity) and corn (*Zea mays* L.; 93% purity) starch standards were used to assess recovery which was 90.0% ± 1.8 SE and 89.6% ± 1.4 SE for wheat and corn, respectively. The starch extraction assay accuracy was demonstrated by a 5-point (dilution) standard curve which was constructed for every assay and all absorbance points were consolidated and produced an r^2=0.98. Finally, the precision of the assays was determined by duplicating three *B. umbellatus* samples for every extraction and computing the percent difference between repeated samples, which on average was 10.0% ±1.9 SE (Wersal et al. 2011).

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Statistical Analysis

Monthly averages for tissue biomass, starch content, and environmental variables were calculated and pooled across study sites (N=13,727 datapoints). Study site was included as a random variable in the construction of the regression models to account for its influence on model variance. Due to the high variability in the data, an average and standard deviation were calculated per biomass and starch variable each month, then all data points that were ± 1 SD were removed (4,500 of 13,727 datapoints) (Aguinis et al. 2013; Osborne and Overbay 2004). Linear regression models were fit to determine the relationship between air temperature, water temperature, and water depth and diploid *B. umbellatus* biomass. 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. Kruskal-Wallis analyses were conducted between sites to determine site-specific differences in rhizome bulbil density and leaf density. If a difference was observed, a Dunn's All-Pairwise Comparison was used to separate site-specific data. All analyses were conducted at α=0.05 significance level using R statistical software 4.4.0 (R Core Team). Packages used for analyses are as follows: dplyr (Wickham et al. 2024), tidyr (Wickham et al. 2023), ggpubr (Kassambra 2023), rstatix (Kassambra 2023), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), and MuMIn (Bartoń 2024).

Results and Discussion

Overall, aboveground biomass peaked from July to September and comprised 50-70% of peak total biomass. Peak total *B. umbellatus* biomass was 1970.40, 2394.07, and 2399.28 g DW/m^2 for plants sampled at Mentor Marsh, Tonawanda, and Unity Island respectively. Aboveground biomass in Mentor Marsh peaked at 1410.18 ± 1167.22, 624.10 ± 192.98, and 390.72 ± 49.11 g DW m^{-2} July 2021, August 2022, and July 2023, respectively (Figure 1). Aboveground biomass at Tonawanda peaked at 1303.04 ± 98.77, 543.64 ± 29.26, and 574.47 ± 26.94 g DW m^{-2} in July 2021, June 2022, and July 2023, respectively (Figure 2). Aboveground biomass at Unity Island peaked at 1006.31 ± 155.65, 1306.55 ± 191.03, and 1141.65 ± 61.33 g DW m^{-2} in September 2021, September 2022, and August 2023, respectively (Figure 3). Aboveground tissues on average stored less than 2.5% starch across all sampling sites and years with the highest concentrations of starch occurring in May-June (Figures 1-3). Inflorescence

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

tissue at each site was less than 10% of the total biomass and peaked between August and October. Inflorescence starch was more variable throughout the year with low points around 0.6% and the highest concentration at 7% (Figures 1-3). Leaf density in Mentor Marsh peaked at 3011 ± 796, 3708 ± 780, and 1963 ± 371 ramets m^{-2} in July-August for all sampling years (Figure 4). Leaf density at Tonawanda peaked at 2400 ± 223, 1560 ± 114, and 1453 ± 185 ramets m^{-2} in June-July for each sampling year (Figure 4). Leaf density at Unity Island peaked at 2061 ± 254, 2629 ± 318, and 2793 ± 271 ramets m^{-2} in June-August for each sampling year (Figure 4). Overall, leaf density was not different ($p=0.93$) between Mentor Marsh and Unity Island though *B. umbellatus* had fewer ($p<0.01$) leaves m^{-2} at Tonawanda when compared to the other sampling sites.

Peak rhizome biomass occurred September-November and comprised 40-50% of total biomass. Peak rhizome biomass was 1506.57 ± 488.3, 2025.24 ± 128.82, and 936.41 ± 170.54 g DW m^{-2} for plants sampled at Mentor Marsh, Tonawanda, and Unity Island, respectively. Rhizome biomass in Mentor Marsh peaked at 593.27 ± 75.44, 540.94 ± 96.91, and 222.63 ± 33.08 g DW m^{-2} in September 2021, October 2022, November 2023, respectively (Figure 1). Rhizome biomass at Tonawanda peaked at 2025.24 ± 128.82, 372.28 ± 44.03, 371.65 ± 32.78 g DW m^{-2} in September 2021, September 2022, and October 2023, respectively (Figure 2). Rhizome biomass at Unity Island peaked at 936.41 ± 170.54, 911.60 ± 94.47, and 442.44 ± 34.25 g DW m^{-2} in October of all years (Figure 3). Rhizomes stored a large proportion of total starch (16–28%) across sites and years with peak storage between October and November. Low points in rhizome starch storage occurred from May to July.

Rhizome bulbil biomass was always less than 600 g DW m^{-2} for all sites over the course of the study (Figure 4). Rhizome bulbil densities peaked in the fall from September-November at 3,000-16,000 rhizome bulbils m^{-2} for each sampling site. Rhizome bulbil density in Mentor Marsh peaked at 15,333 ± 3153, 11,757 ± 2368, and 3,845 ± 967 bulbils m^{-2}, respectively, for 2021, 2022, and 2023 (Figure 4). Rhizome bulbil density at Tonawanda peaked at 3,026 ± 442, 3,422 ± 429, and 2,124 ± 306 bulbils m^{-2}, respectively for 2021, 2022, and 2023 (Figure 4). Rhizome bulbil density at Unity Island peaked at 12,802 ± 2023, 11,422 ± 2582, and 9,379 ± 1490 bulbils m^{-2}, respectively, for 2021, 2022, and 2023 (Figure 4). Rhizome bulbil densities at Mentor Marsh and Unity Island were not different ($p = 0.34$); however, fewer ($p<0.01$) bulbils were produced on an annual basis at Tonawanda when compared to the other sampling sites.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Even though rhizome bulbils accounted for a small proportion of total biomass, there were high amounts of stored starch that ranged from 15 to 30% throughout most of the year at each sampling site (Figures 1-3).

Rhizome starch content had strong relationships with air temperature (r^2=0.52) and water temperature (r^2=46) (Table 1). Other significant, though weak relationships were found including a positive relationship between aboveground biomass and air temperature (r^2=0.17), a negative relationship between rhizome bulbil biomass and air temperature (r^2=0.18), and a positive relationship between leaf density and air temperature (r^2=0.17). Both water and air temperature are important factors that drive photosynthesis, plant development, and phenological timing of plants (Henne et al. 2007). During this study, when water temperature approached 25 C, there was a general decrease in the production of aboveground, rhizome, and inflorescence biomass as well as a shift in the peak rhizome starch content period. Water depth has an influence on the temperature that plants will experience, in that, deeper water will disperse heat energy and thus result in cooler temperatures, while shallow water will result in higher temperatures (Erwin 2009; Jimenez et al. 2012). Temperature is a notable stressor on plants mainly due to the production of reactive oxygen species (ROS) which inhibits gas movement resulting in potential changes of phenological patterns in the plant (Hassanuzzaman et al. 2013; Yamamoto et al. 2008). The creation of ROS causes oxidative stress in plants, which can lead to damage in the leaf and shoot tissues thus reducing photosynthetic potentials (Marchland et al. 2005). Heat stress can occur from modest increases (1-2 C) in temperature. In warm dry years, heat stress may reduce aboveground biomass and change timing of starch storage and growth of rhizomes.

Deeper water (1 m) is not ideal for *B. umbellatus* growth, as a previous study reported that water depth had a negative relationship with triploid *B. umbellatus* growth (Madsen et al. 2016c). For diploid *B. umbellatus*, only plant height was related to water depth in the current study, and the relationship was positive (Table 1). Differences in growth response relating to water depth between the cytotypes may be due to waterbody characteristics they were growing in. Triploid *B. umbellatus* is often found in areas with deeper and more stable hydrology (i.e., lake, reservoirs, and slow-moving rivers). In contrast, the sites sampled in this study for diploid *B. umbellatus* were shallow wetland complexes with fluctuating hydrology. Mentor Marsh had little standing water throughout the growing season but water depth at Tonawanda WMA and Unity Island fluctuated between 10 and 60 cm (Figure 5). Differences in phenology demonstrate that *B.*

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

umbellatus is influenced by the flooding regime that is present within an invaded wetland system (Pigliucci and Kolodynska 2002). Wetlands likely offer the most optimal environment for *B. umbellatus* as water levels often remain below 1 m which allows for the species to grow well and produce high densities of rhizome bulbils from receding water levels (Hroudová 1989; Madsen et al. 2016c).

Diploid *B. umbellatus* can reproduce and spread vegetatively via rhizome fragmentation, rhizome bulbils, and inflorescence bulbils formed at the base of the inflorescence; though there were no inflorescence bulbils observed during this study. Diploid *B. umbellatus* has the potential to produce >15,000 rhizome bulbils per m^2. In contrast, triploid *B. umbellatus* produces fewer but larger reproductive structures (600 rhizome buds per m^2) (Marko et al. 2015). Diploid *B. umbellatus* also reproduces sexually through flowering and seed production. *B. umbellatus* can produce 20-50 flowers $inflorescence^{-1}$ and approximately 200 seeds $flower^{-1}$ with each seed having roughly 31% viability (Eckert et al. 2000). *B. umbellatus* can produce multiple inflorescences per individual, thus increasing the amount of seeds produced per square meter as well. Considering both reproductive strategies, diploid *B. umbellatus* could produce up to 18,000 individuals per m^2 (15,000 rhizome bulbils per m^2 with 3,000 viable seeds per inflorescence) during the reproductive season (fall) under optimal conditions, which is almost 30-fold higher than triploid *B. umbellatus*.

Overall, many of the life history traits between triploid and diploid *B. umbellatus* are similar. Rhizome biomass and peak periods are similar (Marko et al. 2015). Aboveground biomass in triploid biomass reached a peak of just over 500 g DW m^{-2} with diploid *B. umbellatus* producing between 600 to 1100 g DW m^{-2} (Marko et al. 2015). Both cytotypes had aboveground biomass peak between July and August with the rhizome and rhizome bulbils reaching peak production October-November). Management strategies for both cytotypes should be similar and focus on two goals: 1) long-term biomass reduction, and 2) reduction in vegetative propagules. *B. umbellatus* management is challenging as rhizomes and rhizome bulbils combined stored up to 60% of total starch and present a unique challenge to management as these structures cannot be reached directly with herbicides (Parsons et al. 2019; Turnage et al. 2017). However, rhizome biomass and starch content decrease as aboveground biomass increases in June and July. Management programs should target aboveground biomass before July to remove new growth and further deplete starch content in the rhizome tissue. Additional herbicide applications should

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

be administered as plants can recover, which ultimately exhausts starch reserves limits rhizome bulbil formation (Bellaud 2009; Erwin 2009). Repeat applications should be sufficient to reduce year-to-year recruitment, though management of *B. umbellatus* will be long-term as the propagule bank will need to be exhausted to completely manage this species. Sites invaded by diploid *B. umbellatus* may see slightly different trends in production with those sites further south potentially experiencing sooner biomass peaks. Monitoring populations closely would provide important information for when management should occur.

In addition to site specific management, large scale prevention programs should be developed to identify waterbodies that have a high probability of invasion. Diploid *B. umbellatus* poses some differences to its triploid counterpart due to the differences in reproductive output and environmental response (Banerjee et al. 2020; Gebhart and Wersal 2023). There is also evidence of differential diploid *B. umbellatus* growth based on site-specific characteristics. These site-specific characteristics should suggest that populations of *B. umbellatus* may need a particular approach when aiming for management and control. High reproductive output, especially through vegetative bulbils, increases the likelihood of diploid *B. umbellatus* to spread especially through aquatic systems that are associated with rivers. Based on the current distribution in the Great Lakes region, diploid *B. umbellatus* has access to the largest lakes and numerous river systems in North America that can move seeds, rhizome bulbils, and rhizome fragments. With the ability to produce almost 18,000 individuals per m^2 during peak production, diploid *B. umbellatus* is an invasive plant cytotype that poses a high potential to expand to most of North America. However, a differential response to environmental conditions may not allow diploid *B. umbellatus* to expand westward due to increased temperatures seen in the western United States (Levin 1983). Further temperature and phenology studies could be conducted to create a coherent profile for diploid *B. umbellatus* reproduction and life history strategies within the U.S.

Acknowledgments

We would like to thank the following personnel for assistance in the field or laboratory: Ian Knight, Blake DeRossette, Logan Byers, Rebecca Cantrell, Jacob Crestani, Stuart Davis, Sam Fellows, Jeremy Jones, Megan Landis, Amber Mack, Kaylie Malloy, Anna Mathis, Jessie McDonald, Charlotte Moore, Abby Penko, Aden Ricketts, James Rubish, Ryan Wilkinson, Heidi

Kennedy, Denise Appleby, Danielle Meyers, Brandan King, Braeden Schmidt, Grace C. Costello, Rebecca G. Giangreco, and Jane E. Clark. We would also like to thank many people in the Aquatic Weed Science Lab at MNSU for their help processing samples and offering support throughout this time. The manuscript was reviewed in accordance with U.S. Army Engineer Research and Development Center policy and approved for publication. Citation of trade names does not constitute endorsement or approval of the use of such commercial products. The content of this work does not necessarily reflect the position or policy of the U.S. government and no official endorsement should be inferred.

Funding

The research was supported by the Great Lakes Restoration Initiative U.S. Army Corps Buffalo District through the U.S. Army Engineer Research & Development Center under cooperative agreement W912HZ2120036.

Competing Interests

The authors declare no competing interest.

References

Aguinis H, Gottfredson RK, Joo H. 2013. Best-practice recommendations for defining, identifying, and handling outliers. *Organizational Research Methods*. 16:270-301.

Anderson LC, Zeis CD, Alam SF. 1974. Phytogeography and possible origins of *Butomus* in North America. *J.Torrey Bot. Soc.* 101:292-296.

Banerjee AK, Harms NE, Mukherjee A, Gaskin JF. 2020. Niche dynamics and potential distribution of *Butomus umbellatus* under current and future climate scenarios in North America. *Hydrobiologia*. 847:1505–1520.

Bartoń K. 2024. MuMIn: Multi-Model Inference. R package version 1.48.4

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Statistical Software*. 67: 1-48.

Bellaud, MD. 2009. Flowering rush. Pages 141–144 *in* Gettys, LA, Haller, WT, Bellaud, M, eds. Biology and control of aquatic plants: a best management practices handbook. Gainesville, FL: Aquatic Ecosystem Restoration Foundation.

Carter C, Madsen JD, Ervin GN. 2018. Effects of initial propagule size and water depth on *Butomus umbellatus* L. growth and vegetative propagation. *Aquat. Bot.* 150: 27-32.

Clarke M, Wersal RM, Turnage G. 2023. Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. *Aquat Bot.* 186:103627.

Dancey CP and Reidy JG. 2004. Statistics without maths for psychology: using SPSS for Windows. Prentice Hall, New York.

Eckert CG, Lui K, Bronson K, Corradini P, Bruneau A. 2003. Population genetic consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. *Mol Ecol*. 12:331-344.

Erwin KL. 2009. Wetlands and global climate change: the role of wetland restoration in a changing world. *Wetlands Ecol. Manage*. 17:71-84.

Gaskin JF, Andreas J, Grewell BJ, Haefliger P, Harms NE. 2021. Diversity and origins of *Butomus umbellatus* (flowering rush) invasion in North America. *Aquat. Bot*. 173:1631-1642.

Gebhart MG and Wersal RM. 2023. Ecological niche modeling of diploid flowering rush (*Butomus umbellatus L*.) in the United States. *J. Freshwater Ecol.* 39:1.

Gunderson MD, Kapuscinski KL, Crane DP, Farrell JM. 2016. Habitats colonized by non-native flowering rush *Butomus umbellatus* (Linnaeus, 1753) in the Niagara River, USA. *Aquat. Inv.* 11:369–380.

Haram AM and Wersal RM. 2023. Seasonal starch allocation of starry stonewort (*Nitellopsis obtusa*) growing in Lake Koronis, MN. *J. Freshwater Ecol*. 38.

Hassanuzzaman M, Nahar K, Mahabub Alam Md, Roychowdhury R, Fujita M. 2013. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci*. 14:9643-9684.

Henne PD, Hu FS, Cleland DT. 2007. Lake-effect snow as the dominant control of mesic-forest distribution in Michigan, USA. *J. Ecol*. 95:517-529.

Hroudová Z. 1989. Growth of *Butomus umbellatus* at a stable water level. *Folia Geobot*. 24.

Hroudouvá Z and Zákravský P. 1993. Ecology of two cytotypes of *Butomus umbellatus* II. reproduction, growth, and biomass production. *Folia Geobot*. 28:413-424.

Kassambara A. 2023. ggpubr: 'ggplot2' based publication ready plots. R package version 0.6.0.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Kassambara A. 2023. rstatix: pipe-friendly framework for basic statistical tests. R package version 0.7.2.

Kennedy H, Appleby D, Palermo M, Bonk E, Mahoney J. 2018. Habitat management plan for Oak Orchard Wildlife Management Area 2019-2028.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. lmerTest Package: tests in linear mixed effects models." *Statistical Software*. 82:, 1-26.

Levin DA. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist*. 122:1-25.

MacNeill GJ, Mehrpouyan S, Minow MA, Patterson JA, Tetlow IJ, Emes MJ. 2017. Starch as a source, starch as a sink: the bifunctional role of starch in carbon allocation. *J. Exp. Bot*. 68: 4433-4453.

Madsen J, Wersal R, Woolf T. 2007. A new core sampler for estimating biomass of submersed aquatic macrophytes. *J. Aquat. Plant Manage*. 45:31-34.

Madsen JD, Sartain B, Turnage G, Marko M. 2016a. Management of flowering rush in the Detroit Lakes, Minnesota. *Journal of Aquatic Plant Management*. 54:61-67.

Madsen JD, Turnage G, Getsinger KD. 2016b. Efficacy of combinations of diquat or triclopyr with fluridone for control of flowering rush. *J. Aquat. Plant Manage*. 54:68-71.

Madsen JD, Wersal RM, Marko MD. 2016c. Distribution and biomass allocation in relation to depth of flowering rush (*Butomus umbellatus*) in the Detroit Lakes, Minnesota. *Invasive Plant Sci. Manage*. 9:161–170.

Madsen JD, Wersal Ryan M. 2018. Proper survey methods for research of aquatic plant ecology and management. *J. Aquat. Plant Manage.* 56s:90-96.

Marko M, Madsen J, Sartain B. 2015 Ecology and phenology of flowering rush in the Detroit Lakes chain of lakes, Minnesota. *J. Aquat. Plant Manage*. 53:54-63.

Osborne JW and Overbay A. 2019. The power of outliers (and why researchers should always check for them). *Practical Assessment, Research, and Evaluation*. 9.

Parsons JK, Baldwin L, Lubliner N. 2019. An operational study of repeated diquat treatments to control submersed flowering rush. *J. Aquat. Plant Manage.* 57:28-32.

Pigliucci M and Kolodynska A. 2002. Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L.) Heynh (*Brassicaceae*). *Ann. Bot*. 90:199-207.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Ralph PJ, Durako MJ, Enríquez S, Collier CJ, Doblin MA. 2007. Impact of light limitation on seagrasses. *J. Exp. Mar. Bio. Ecol.* 350:176-193.

Roster T, McMahon S, Kahan S. 2011. Iroquois National Wildlife Refuge.

Scofield GN, Ruuska SA, Aoki N, Lewis DC, Tabe LM, Jenkins CL. 2009. Starch storage in the stems of wheat plants: localization and temporal changes. *Ann. Bot*. 103:859-868.

Thompson FL and Eckert CG. 2004. Trade-offs between sexual and clonal reproduction in an aquatic plant: Experimental manipulations vs. phenotypic correlations. *J. Evol. Biol*. 17:581-592.

Trebitz A and Taylor DL. 2007. Exotic and invasive aquatic plants in Great Lakes coastal wetlands: distribution and relation to watershed land use and plant richness and cover. *J. Great Lakes Res*. 33:705-721.

Turnage G, Wersal RM, Madsen JD. 2017. Evaluating the efficacy of granular copper and triclopyr alone and in combination for control of flowering rush (*Butomus umbellatus*). *J. Aquat. Plant Manage*. 55:120–122.

U.S. Army Corp of Engineers. 2021. Unity Island 204: beneficial reuse of dredged material for ecosystem restoration monitoring report.

U.S. Army Corp of Engineers. 2022. Great Lakes water level (1918-2022).

Wersal RM, Cheshier JC, Madsen JD, Gerard PD. 2011. Phenology, starch allocation, and environmental effects on *Myriophyllum aquaticum*. *Aquat. Bot*. 95:194-199.

Wersal RM, Madsen JD, and Cheshier JC. 2013. Seasonal biomass and starch allocation of common reed (*Phragmites australis*) (Haplotype I) in southern Alabama, USA. *Invasive Plant Sci. Manage*. 6:140-146.

Wickham H, François R, Henry L, Müller K, Vaughan D.2023. dplyr: a grammar of data manipulation. R package version 1.1.4.

Wickham H, Vaughan D, Girlich M. 2024. tidyr: tidy messy sata. R package version 1.3.1.

Yamamoto Y, Aminaka R, Yoshioka M, Khatoon M, Komayama L, Takenaka D, Yamashita A, Nijo N, Inagawa K, Morita N, Sasaki T, Yamamoto Y. 2008. Quality control of photosystem II: impact of light and heat stresses. *Photosynth Res.* 98:589-608.

Yeager M. Personal Communication March 6th 2023.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Table 1. Results of the linear regression analysis between the plant and environmental metrics of diploid *B. umbellatus* pooled across all study sites.

	Environmental Metric								
	Air Temperature			**Water Temperature**			**Water Depth**		
Plant Metric	**p-value**	**Regression Equation**	**r^2**	**p-value**	**Regression Equation**	**r^2**	**p-value**	**Regression Equation**	**r^2**
Aboveground Biomass	<0.01	y= 25.26x - 84.2	0.17	<0.01	y= 32.87x - 196	0.19	<0.01	y= 2.727x + 359.7	0.03
Rhizome Biomass	0.03	y= -5.50x + 529.6	<0.01	0.64	y= -1.76x + 473.6	<0.01	<0.01	y= -5.545x + 532.2	0.07
Inflorescence Biomass	<0.01	y= -2.101x + 80.98	0.05	0.04	y= -1.515x + 67.25	0.02	0.41	y= -0.0745x + 35.8	<0.01
Rhizome Bulbil Biomass	<0.01	y= -11.83x + 331.3	0.18	<0.01	y= -12.36x + 357.4	0.11	<0.01	y= -1.93x + 143.6	0.05
Total Biomass	0.03	y= 9.864x + 814.8	<0.01	<0.01	y= 21.09x + 671.3	0.02	0.14	y= -2.335x + 1062	<0.01
Rhizome Bulbil Density	<0.01	y= -137.9x + 5939	0.03	<0.01	y= -161.5x + 6862	0.03	<0.01	y= -38.81x + 4029	0.03
Leaf Density	<0.01	y= 93.45x – 457.2	0.17	<0.01	y= 104.3x – 656.9	0.15	0.14	y= 3.62x + 1339	<0.01
Plant Height	<0.01	y= 0.622x + 62.2	0.01	<0.01	y= 0.873x + 59.76	0.02	<0.01	y= 0.369x + 68.06	0.06

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

Aboveground Starch	0.08	y= 0.007x + 0.69	<0.01	<0.01	y= 0.015x + 0.5582	0.01	<0.01	y= 0.0034x + 0.889	0.01
Rhizome Starch	<0.01	y= -1.028x + 34.02	0.52	<0.01	y= -1.169x + 34.84	0.46	<0.01	y= -0.1064x + 15.59	0.06
Inflorescence Starch	0.02	y= -0.0757x + 4.361	0.02	<0.01	y= -0.1463x + 5.96	0.04	0.80	y= -0.00135x + 2.677	<0.01
Rhizome Bulbil Starch	<0.01	y= -0.2709x + 30.32	0.09	<0.01	y= -0.241x + 29.9	0.05	0.52	y= -0.007x + 25.33	<0.01

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

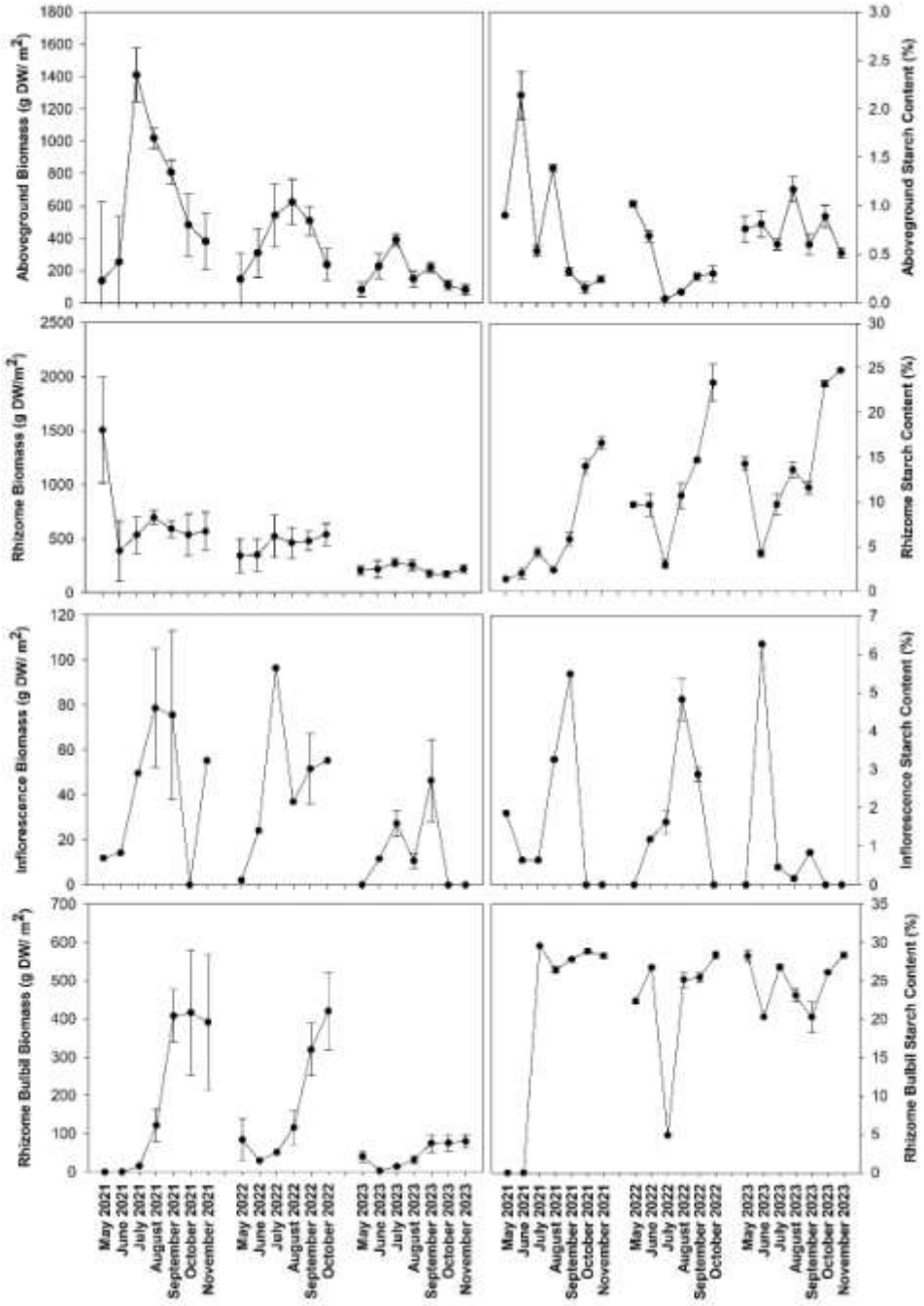

Figure 1. Mean (± 1SE) biomass and starch content in diploid *B. umbellatus* harvested from Mentor Marsh, OH. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

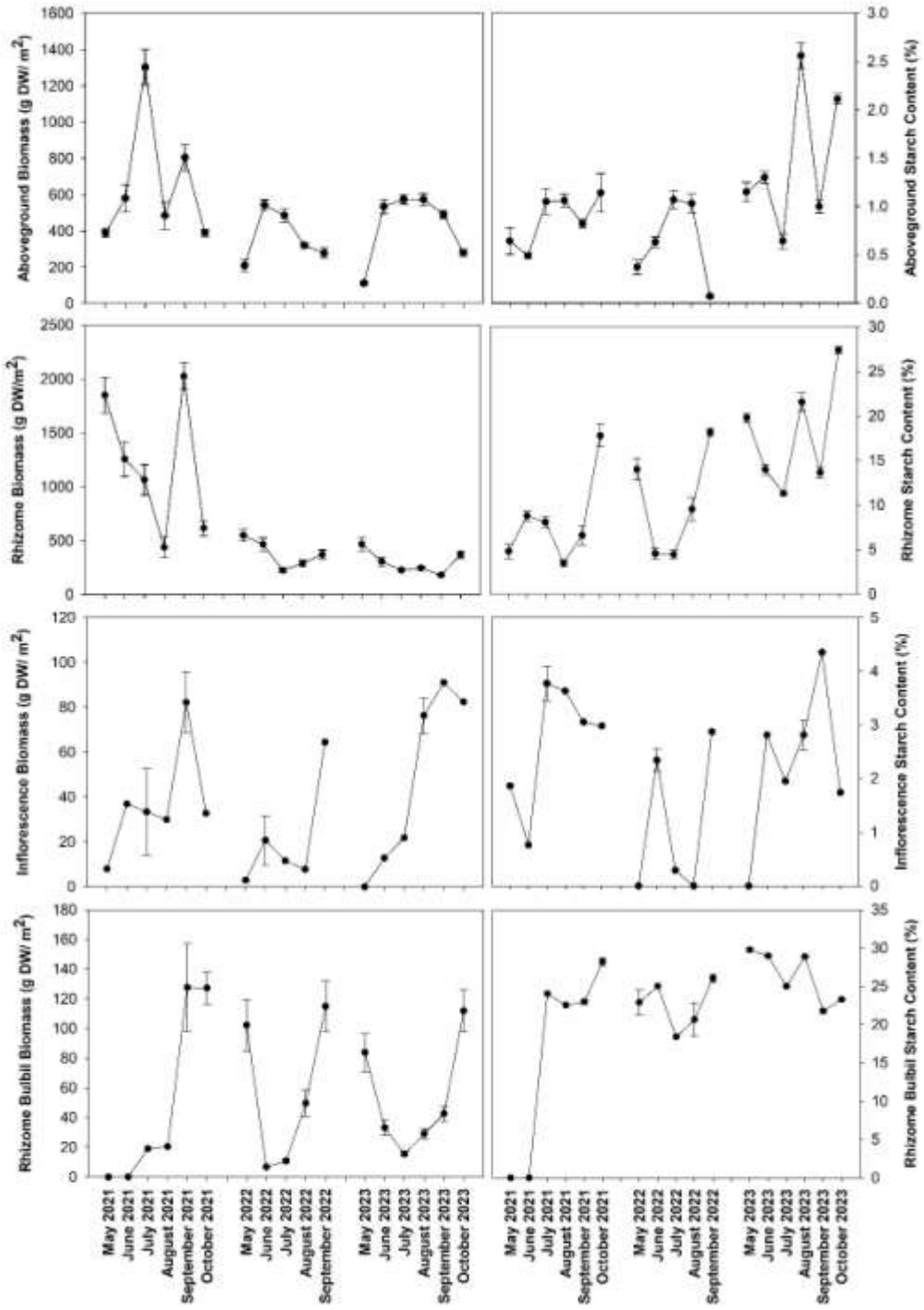

Figure 2. Mean (± 1SE) biomass and starch content in diploid *B. umbellatus* harvested from Tonawanda, NY. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

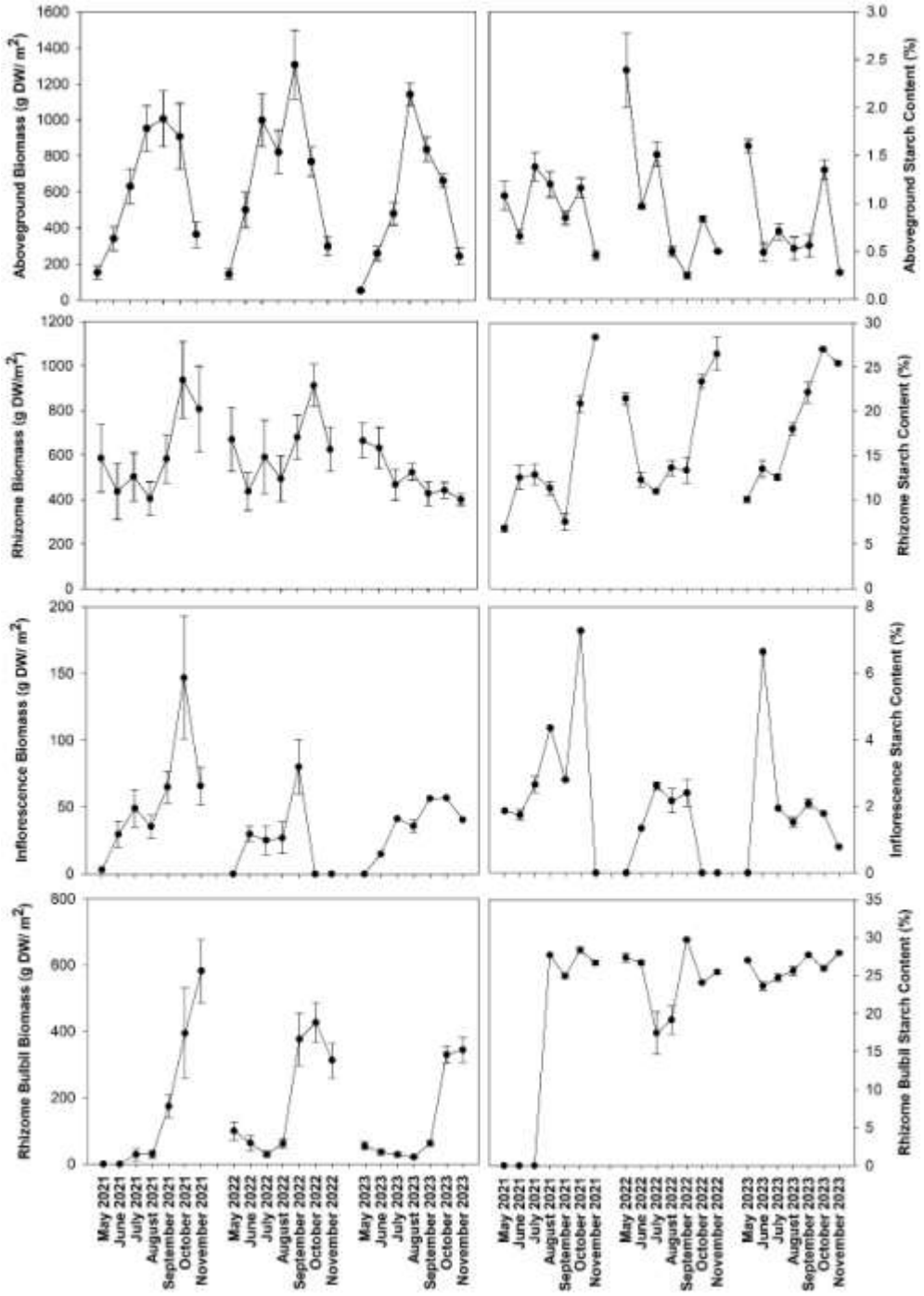

Figure 3. Mean (± 1SE) biomass and starch content in diploid *B. umbellatus* harvested from Unity Island, NY. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

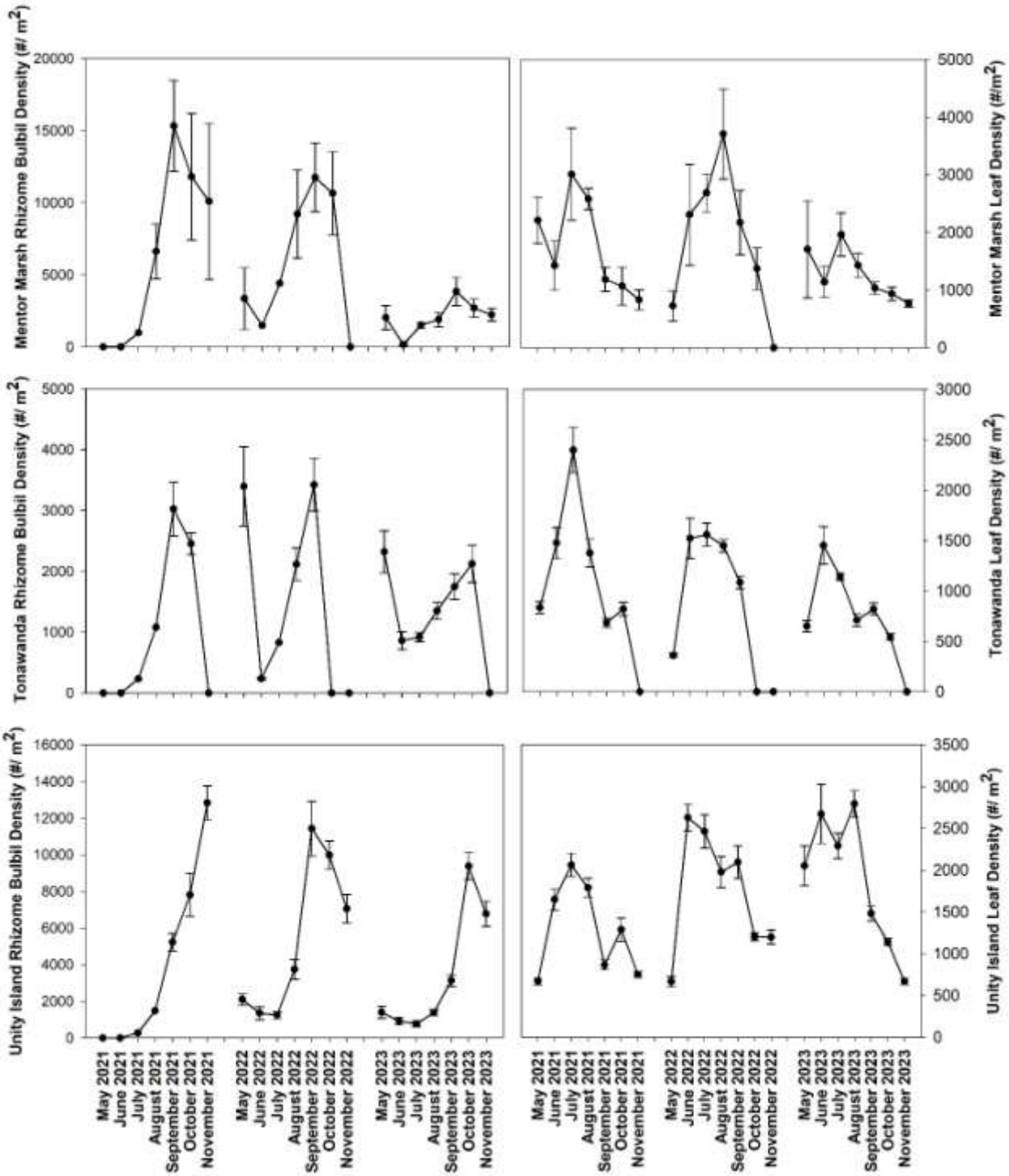

Figure 4. Mean (± 1 SE) leaf density and rhizome bulbil density in diploid *B. umbellatus* harvested from each study site. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press

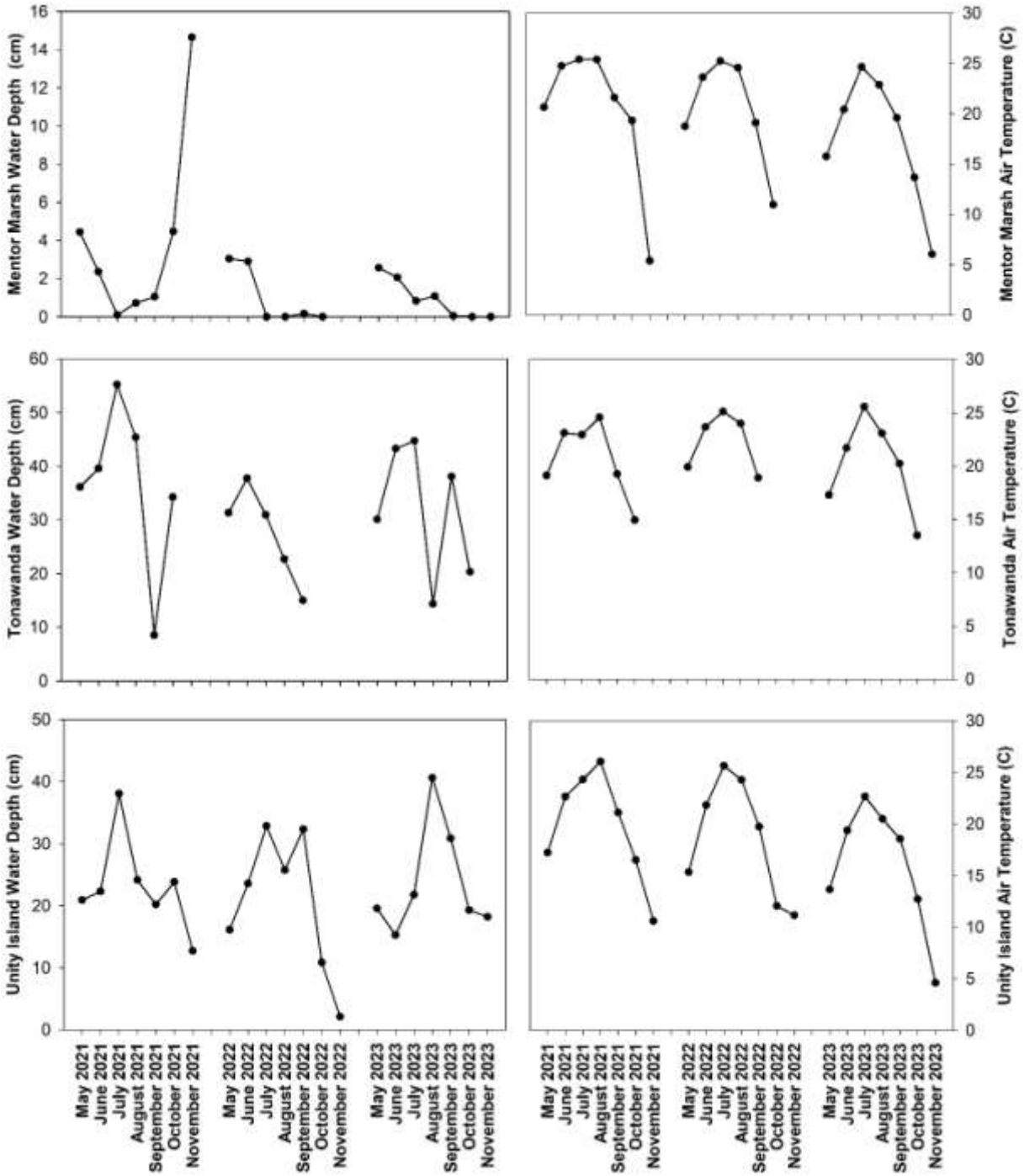

Figure 5. Water depth (cm) and mean air temperature (C) for study sites where diploid *B. umbellatus* samples were harvested. Scaling for y-axes varies based on the collected values for each measurement associated with the axis title.

https://doi.org/10.1017/inp.2024.31 Published online by Cambridge University Press