Water chestnut biomass estimates using density as a proxy: Facilitating multiyear comparisons with a streamlined approach

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ABSTRACT

Water chestnut (*Trapa natans)* is an invasive macrophyte negatively impacting native aquatic communities in the United States. In New York state, water chestnut occurrence is monitored through iMapInvasives, a public database that includes several data fields for all records, such as distribution type (or categorical density). Biomass is not regularly recorded in iMapInvasives but is important as a secondary measurement to gauge primary production, nutrient uptake, and invasive impact. Lack of biomass data in iMapInvasives may be addressed with alternative methods of acquiring biomass information from records. The primary goal of this project was to develop methods that allow comparable biomass estimates to be made using a measured area and an observed distribution type in the iMapInvasives database. Nine locations were sampled for water chestnut in June and July 2021. Areas of sparse, dense, and monoculture growth were recorded along with trace points. Collected plants were cleaned, measured, and dried to obtain final dry biomass density values for each distribution type. Density values were highest in monoculture and lowest in sparse but also varied based on location and date. ANOVA testing indicated that plant density, rosette growth, and rosette width varied among distribution types. Our water chestnut measurements were used to create formulas that can estimate biomass using presence- and distribution-type data in iMapInvasives. These formulas may be useful for stakeholders and managers seeking to understand the invasive impact of water chestnut and assess its change in abundance over time.

Key words: iMapInvasives online database, invasive species, macrophyte management, monoculture, *Trapa natans*.

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INTRODUCTION

Water chestnut (*Trapa natans*) is an invasive macrophyte that is negatively impacting the integrity of native aquatic communities in the United States. Native to Africa and Eurasia, water chestnut aggressively grows in dense monocultures that displace other macrophytes, alter habitat structure for aquatic animals, and reduce recreational opportunity in U.S. waterways (Hummel and Kiviat 2004; Nieder et al. 2004). Water chestnut must be persistently managed by removing or treating full plants or the fruiting rosettes from a waterbody. Management must be consistent for up to 12 yr to deplete the seed bank (Naylor 2003). If management is incomplete or intermittent, then the water chestnut will release new nutlets and resupply the seed bank, thereby allowing them to persist in a waterbody (Naylor 2003). The difficulties of proper water chestnut management are further exacerbated by the movement of water, boats, and animals between waterbodies or within a large interconnected system (Hummel and Kiviat 2004; Marsden and Hauser 2009). Aggressive growth, persistence in the seed bank, and complex invasion pathways of water chestnut have all contributed to its rampant growth across the northeast United States, despite many years of management efforts.

Due to the sheer volume of water chestnut that must be managed, it is important for invasive species managers to have access to spatial data to track presence and prioritize management of certain areas. The iMapInvasives[1] database is one such asset available to managers in participating jurisdictions (Arizona, Maine, New York, Oregon, Pennsylvania, Saskatchewan, and the Maritime Provinces of Canada). Spatial data for invasive species are collected by managers, scientists, and the public, then gathered and curated in iMapInvasives (https://www.imapinvasives.org/). Data from iMapInvasives extend beyond presence records, also including information on treatments, area searched, and distribution type (or categorical density) of invasive species. Together, these data can be a useful tool for managers of water chestnut.

One important metric for water chestnut management is biomass, because biomass is related to production, nutrient uptake, and invasive impact (Bolpagni et al. 2006; Santos et al. 2011; Zhu et al. 2011). During water chestnut treatment efforts, the amount of water chestnut removed is often reported in iMapInvasives. Methods of water chestnut collection and measurement vary widely across reports, including the number of plants harvested, number of

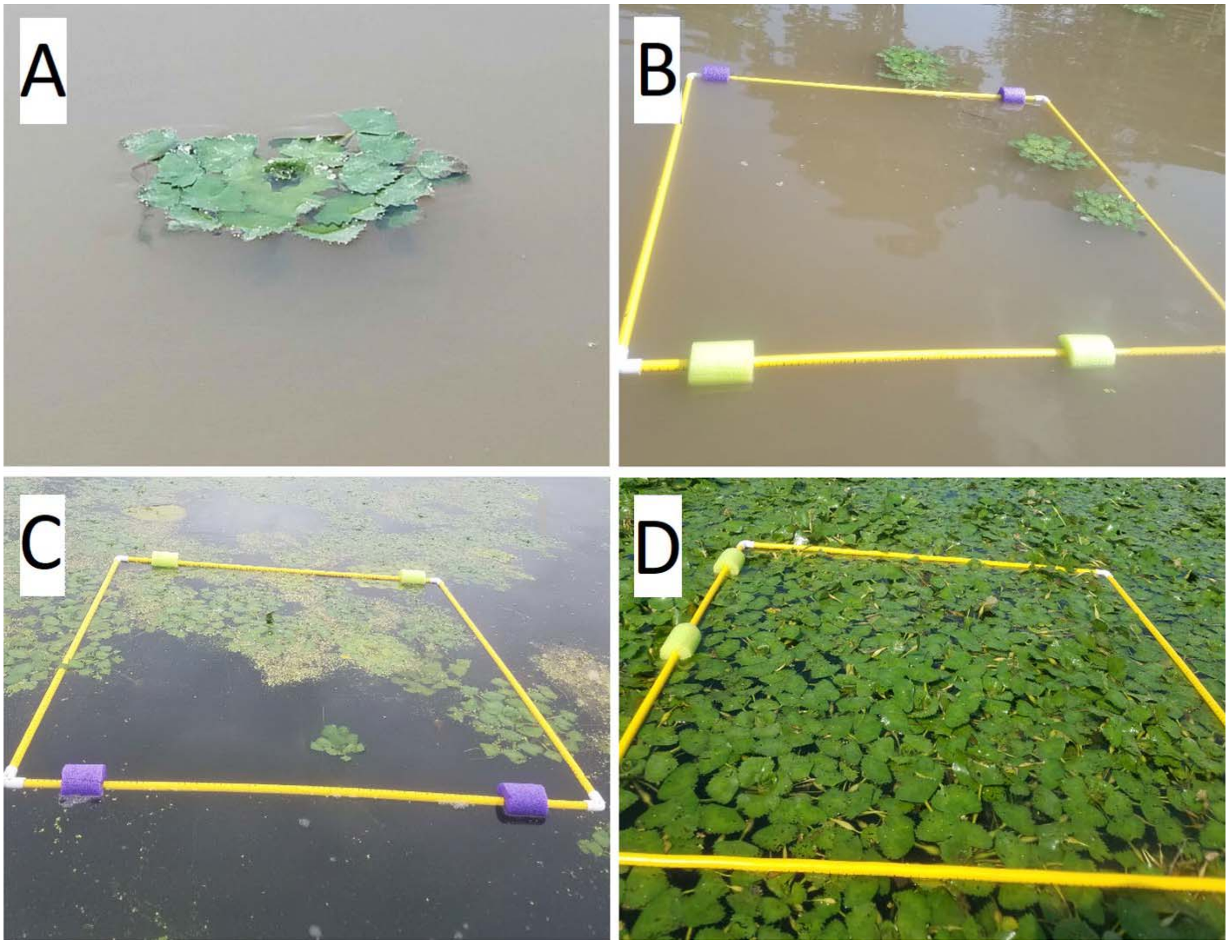

Figure 1. Representative examples of the four water chestnut distribution types used to categorize summer 2021 field samples. A = trace (single plant/clump), B = sparse (scattered plants/clumps), C = dense (dense plants/clumps), D = monoculture.

garbage bags filled, or volume of harvested water chestnut. In many cases, no measurements are taken that enable an estimation of biomass. In addition, chemical control methods of water chestnut management do not result in any harvest or biomass measurement. Finally, there is notable variation in water chestnut density, both between waterbodies and within a waterbody (Hummel and Kiviat 2004). In iMapInvasives this density variation is categorized into four distribution types: monoculture, dense (dense plants/clumps), sparse (scattered plants/clumps), and trace (single plant/clump) (Figure 1). Some reports in iMapInvasives utilize a fifth distribution type (linearly scattered), but this is usually applied to terrestrial or riparian species that grow along a road or waterway and is not applicable to water chestnut. Distribution type is a qualitative assessment of macrophyte abundance, so there is not an absolute cutoff between some distribution types. Although this does introduce some variation in data collection, adopting a qualitative assessment that can be conducted visually is more accessible and efficient for managers than a quantitative assessment. Data collectors select the distribution type based on how their observation aligns with the written descriptions in iMapInvasives. To maintain consistency between data collectors, webinar training and training documents are provided, including pictures of infestations for each distribution type (Figure 1).

The described variation in water chestnut management and iMapInvasives reports creates further difficulty in the estimation of water chestnut biomass for management purposes. Alternative estimation methods are required to address management needs, and methods that can be used in conjunction with iMapInvasives are ideal. Our primary research goal was to develop standard methods that facilitate water chestnut biomass estimation using measured area and an observed distribution type in the iMapInvasives database. Our secondary research goal was to test application of our biomass estimation methods to iMapInvasives records, first by utilizing 2021 statewide survey and treatment records, and second in a two-year case study for Delegan Pond, NY.

MATERIALS AND METHODS

Biomass collection

Field collection of water chestnut occurred June 21 to July 28, 2021. Water chestnut was sampled from nine sites within six waterbodies (Figure 2), with a total of 21 samples

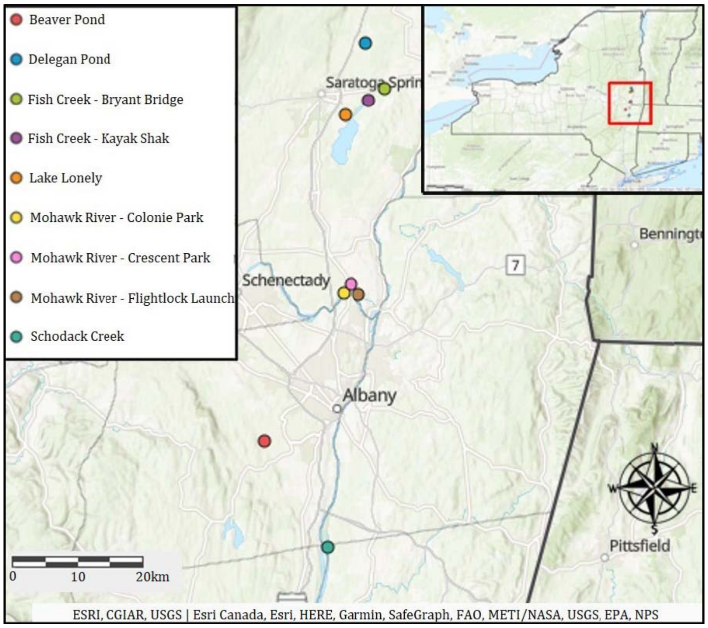

Figure 2. Map of nine study sites surveyed for water chestnut, summer 2021.

collected across all sites (six trace, five sparse, five dense, and five monoculture; Table 1). Selection criteria for sites included boat accessibility, reported 2020 presence of water chestnut in iMapInvasives (categorized by listed distribution types), prior management activity, and distance from the New York State Forest Health Diagnostic Laboratory in Delmar, NY. Two of the waterbodies (Fish Creek and the Mohawk River) were broken up into different sites (Fish Creek into Kayak Shak and Bryant Bridge; Mohawk River into Flightlock Launch, Crescent Park, and Colonie Park) due to their relatively large size, multiple points of access, and the presence of individual water chestnut populations throughout the habitat matrix.

Prior to arrival at each site, the survey area was plotted with a polygon in ArcMap,[2] and random sampling points were generated using the "Create Random Points" data management tool at a density of 100 points ha^{-1} and a minimum 1-m distance between points. Upon arrival at each site, the previously defined area was surveyed for trace, sparse, dense, and monoculture distributions of water chestnut (Figure 1) using a two-person canoe. If any sparse, dense, or monoculture distributions of water chestnut were observed, they were mapped out by distribution type using Locus GIS.[3] Any random sampling points that fell within each distribution of water chestnut were recorded, and a subsample of those points was selected using a random number generator.

At each selected point, a floating square meter quadrat was dropped, and all water chestnut inside was harvested via hand pulling from the boat, pulling up as much of each plant as possible. Plants were harvested only if the center of the rosette was inside the quadrat. To better capture the heterogeneity of each distribution type, sparse samples were composites utilizing five points (or five m^2 total per sample), and dense samples were composites of three points (or three m^2 total per sample). Monoculture samples consisted of one point (or one m^2 total per sample) due to processing time and space limitations in the drying oven. Trace distribution requires a single plant/clump to be present, and so trace samples could not be measured on an area (m^2) basis, but rather on a presence-by-presence basis. If water chestnut trace distribution was present, it was georeferenced as a presence point via the iMapInvasives mobile app[4] and hand pulled. All water chestnut samples were collected in polyester mesh bags, placed in a cooler to minimize water loss, and transported to the NYSDEC Forest Health Diagnostic Laboratory for further processing.

Sample processing

Upon arriving at the laboratory, water chestnut samples were rinsed with a hose to remove sediment and entangled nontarget organisms. After rinsing, the number of individual plants per sample were counted along with number of rosettes. In trace, sparse, and dense samples, all rosette widths were measured to the nearest cm. Due to the high density of rosettes in monoculture samples, a subsample of 30 rosettes was measured.

Following initial processing, samples were placed in preweighed, dry, labeled mesh bags (mass of empty bag = M_b), then weighed to the nearest gram to obtain wet biomass (M_w) of the water chestnut (M_w = mass on scale − M_b). Samples (still inside mesh bags) were oven-dried at 110 C until a constant mass was achieved (no decrease in mass over a minimum 30-min drying period). The final measured mass was then used to obtain dry biomass (M_d) of the water chestnut (M_d = mass on scale − M_b).

Table 1. Schedule summary of water chestnut samples collected June to July 2021.

Site	Date	Samples collected (no. × distribution type[s])
Beaver Pond	21 June to 23 June 2021	1 × sparse, 1 × dense
Delegan Pond	29 June 2021	1 × trace, 1 × sparse
Fish Creek–Kayak Shak	1 July 2021	1 × trace, 1 × sparse, 1 × monoculture
Fish Creek–Bryant Bridge	7 July 2021	2 × monoculture
Lake Lonely	13 July 2021	1 × dense, 1 × monoculture
Mohawk River–Flightlock Launch	15 July 2021	1 × sparse, 1 × dense
Mohawk River–Crescent Park	19 July 2021	1 × monoculture
Schodack Creek	22 July 2021	4 × trace
Mohawk River–Colonie Park	28 July 2021	1 × sparse, 2 × dense

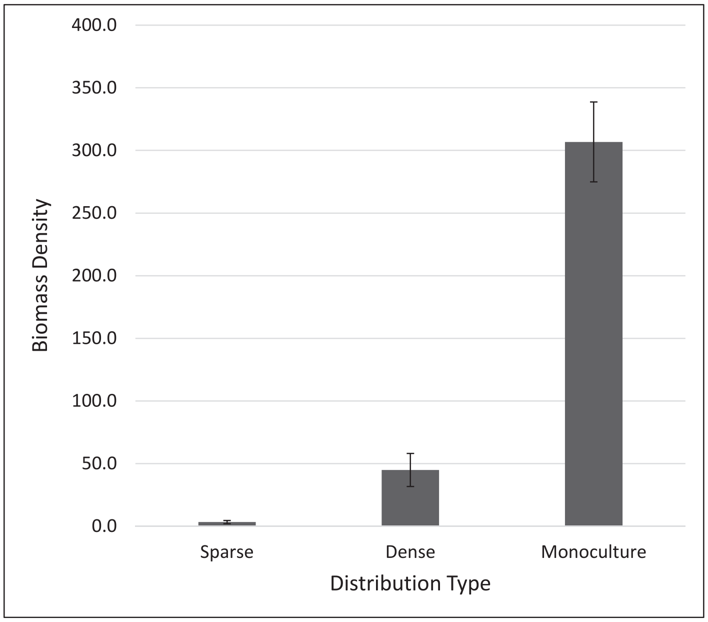

Figure 3. Mean biomass density ± standard error for three distribution types of water chestnut as estimated from summer 2021 samples. Biomass density = g dry plant mass m^{-2}.

Data analyses

After drying all samples and taking final measurements, mean plant density (mean no. plants m^{-2}), rosette growth (no. rosettes $plant^{-1}$), rosette width (cm), individual biomass (g dry biomass $plant^{-1}$), dry biomass percentage (M_d/M_w), and biomass density (M_d per number of points in sample for trace; and M_d per area of sample for sparse, dense, and monoculture) were calculated in Microsoft Excel. Measure of variability was standard error. A record of searched areas and presence records/polygons for all four distribution types was then submitted to iMapInvasives.

The mean biomass density values for each distribution type calculated in this study (Figure 3) were used to create a set of water chestnut biomass estimation formulas (Figure 4). Reported area (in m^2) or number of trace points may be utilized along with distribution type in these formulas to estimate dry biomass of water chestnut in any given iMapInvasives record.

One-way ANOVA tests ($\alpha = 0.05$) were conducted to compare mean plant density and biomass density between sparse, dense, and monoculture samples. One-way ANOVA tests ($\alpha = 0.05$) were also conducted to compare mean rosette growth, rosette width, individual biomass, and dry biomass percentage between trace, sparse, dense, and monoculture samples.

Estimating water chestnut biomass

In 2021 aquatic invasive species program managers across the state were asked to document their water chestnut survey and treatment efforts using a standard data collection protocol to facilitate the estimation of standing and harvested biomass. The data collection protocol

$$B_T = 7.3*n$$

$$B_S = 3.3*A_S$$

$$B_D = 44.9*A_D$$

$$B_M = 306.8*A_M$$

Figure 4. Formulas to estimate water chestnut biomass present using information from iMapInvasives. B_T = estimated biomass in trace distributions; B_S = estimated biomass in sparse distributions; B_D = estimated biomass in dense distributions; B_M = estimated biomass in monoculture distributions; n = number of trace points observed; A_S = area of sparse observed; A_D = area of dense observed; A_M = area of monoculture observed.

included the following steps using iMapInvasives: (1) create a presence record delineating the extent of an infestation, (2) indicate the distribution type in the presence record, and (3) create a treatment polygon delineating where water chestnut rosettes were pulled. A total of 43 sites were included in this statewide assessment.

Biomass was estimated for each site in ArcGIS Pro[5] using the 2021 area of presence and treatment records in iMapInvasives along with the formulas developed from summer sampling (Figure 4). For point records indicated as trace, the estimated standing biomass was set to the average weight of one dried water chestnut individual, since trace indicates a single plant. This value was also used for the estimate of harvested biomass if the point fell within a treatment polygon. If the point did not intersect with a treatment polygon, then the harvested biomass was zero. For sparse, dense, and monoculture patches, the estimated standing biomass was calculated by multiplying the mean biomass density for the distribution type by the area of the presence polygon (Figure 4). The estimated biomass harvested was calculated by multiplying that same mean biomass density by the area of the presence polygon intersecting with a treatment record (calculated via a spatial union). In the rare instances where sparse, dense, or monoculture presence records were recorded as points, a 1-m buffer was used to derive a measure of area. For two sites where data collectors counted the number of scattered individuals across entire waterbodies, we multiplied the average trace biomass by the number of rosettes reported for an estimate (since trace is equivalent to a single plant).

The results of these calculations were aggregated at the site level. Sites were delineated in an ArcGIS Online interface[6] by aquatic invasive species program managers across New York state, and typically consisted of an entire waterbody (e.g., a pond or lake) or a part of a waterbody (e.g., a segment of a river or one bay of a large lake).

Delegan Pond in Wilton, NY, was one site with sufficient data for water chestnut biomass estimation of both 2020 and 2021 records, allowing us to assess change over time. Records from 2020 were collected early summer during treatment efforts, similarly to 2021 records. Water chestnut

TABLE 2. SUMMARY OF WATER CHESTNUT DATA COLLECTED JUNE TO JULY 2021. MEASURE OF VARIABILITY IS MEAN ± STANDARD ERROR. *TRACE SAMPLES WERE NOT MEASURED WITH M^2 AND CANNOT BE COMPARED TO SPARSE, DENSE, AND MONOCULTURE DATA IN SOME CASES (N/A).

Distribution type	Overall	Monoculture	Dense	Sparse	Trace
Plant density (no. plants m^{-2})	N/A	76.6 ± 9.1	9.9 ± 0.8	1.7 ± 0.6	N/A
Rosette growth (no. rosettes $plant^{-1}$)	1.1 ± 0.050	1.1 ± 0.0	1.4 ± 0.2	1.1 ± 0.0	1.0 ± 0.0
Rosette width (cm)	20.5 ± 0.4	23.6 ± 0.6	17.6 ± 0.7	15.7 ± 1.0	18.2 ± 1.7
Individual biomass (g dry biomass $plant^{-1}$)	3.5 ± 0.42	4.2 ± 0.5	4.5 ± 1.2	2.1 ± 0.6	3.3 ± 0.8
Dry biomass percentage (% dry biomass/wet biomass)	9.5 ± 0.79	8.1 ± 0.7	10.6 ± 0.4	8.8 ± 1.5	10.4 ± 2.7

biomass was calculated for each year in ArcGIS Pro using methods identical to those applied to the 2021 statewide summary.

RESULTS AND DISCUSSION

Overall mean dry biomass of water chestnut samples was 9.5 ± 3.6% of wet biomass (Table 2). Monoculture samples had a mean plant density of 76.6 ± 9.1 plants m^{-2}, notably higher than dense (9.9 ± 0.8 plants m^{-2}) and sparse (1.7 ± 0.6 plants m^{-2}) samples (Table 2). Dense samples had a slightly elevated rosette growth (1.4 ± 0.2 rosettes $plant^{-1}$) compared to other distribution types, although rosette width was highest in monoculture samples (23.6 ± 0.6 cm). (Table 2). Individual biomass was highest in dense (4.5 ± 1.2 g) and monoculture (4.2 ± 0.5 g) samples (Table 2).

Mean water chestnut biomass density was 3.3 ± 1.3 g dry plant mass m^{-2} (g_{DW} m^{-2}) in sparse samples, 44.9 ± 13.2 g_{DW} m^{-2} in dense samples, and 306.8 ± 31.9 g_{DW} m^{-2} in monoculture samples (Figure 3 and Table 3). Mean trace water chestnut biomass density was 7.3 ± 2.8 g dry mass per trace point. These biomass density values are integrated in our set of biomass estimation formulas as constants that can be multiplied by number of trace points, or area of sparse, dense, or monoculture to estimate water chestnut biomass of any iMapInvasives record that identifies distribution type (Figure 4).

One-way ANOVA tests indicated that mean plant density and biomass density were significantly different between sparse, dense, and monoculture samples (Table 4). Rosette growth and mean rosette width were significantly different between trace, sparse, dense, and monoculture samples (Table 4). No significant difference between distribution types was indicated for mean individual biomass or dry biomass percentage (Table 4).

Of the 43 sites across New York surveyed in the 2021 water chestnut management efforts, 36 had treatment records, allowing for the estimation of biomass harvested. At two sites, the number of individual rosettes pulled was recorded rather than a polygon. A total of 1,352 acres of water chestnut growth was recorded between these 43 sites, containing an estimated 53,301 kg of standing biomass. From this area, a total of 884 acres was treated, with an estimated water chestnut harvest of 27,107 kg.

In 2020 two main patches were mapped at the center of Delegan Pond (one classified as sparse, the other as monoculture), along with several sparse patches and trace individuals along the northern and western shores (Figure 5). In 2021 one main patch was mapped at the center of Delegan Pond (classified as sparse), along with several sparse patches and trace individuals along the western and northern shore (Figure 5). While it is likely that the 2020 treatment effort is the primary cause of observed differences between 2020 and 2021 records, both the area and distribution types changed over time, making it difficult to rely on either metric alone to assess treatment effectiveness. In this case, biomass estimates are incredibly useful since they integrate both area and distribution type information. Utilizing the area and distribution type of records with the estimation formulas (Figure 4) provides a standardized biomass estimate that can be compared across years. In the case of Delegan Pond, the estimated amount of biomass went from 124.5 to 8.6 kg, a 93% decrease.

When using the results presented here and their application to determining water chestnut biomass, it is important to consider some factors that could have impacted the results. From initial hand-pull harvesting to final drying of water chestnut samples, small fragments of water chestnut were lost during each transfer. In addition, small, attached fragments of non–water chestnut plants were occasionally thoroughly entangled and may not have been completely removed from samples, primarily in cases where *Lemna* spp. occurred together with water chestnut. These minor losses or gains to the sample are not likely to have appreciably impacted the results. Another limitation of the data is sample size, as the small size of the oven (0.079 m^3) limited the amount of water chestnut that could be processed at any given time. Finally, we observed that the water chestnut plants were notably larger in mid- to late July

TABLE 3. RANGE SUMMARY OF WATER CHESTNUT DATA COLLECTED JUNE TO JULY 2021. *INDIVIDUAL BIOMASS WAS NOT ACTUALLY MEASURED FOR INDIVIDUAL PLANTS, RATHER REPRESENTING THE RANGE OF MEAN SAMPLE VALUES.

Measured variable	Units	Minimum observed value	Maximum observed value
Plant density	No. plants m^{-2}	0.2	96.0
Rosette growth	No. rosettes $plant^{-1}$	1.0	1.9
Rosette width	cm	3.0	44.0
Individual biomass*	g dry biomass $plant^{-1}$	0.9	8.7
Dry biomass ratio	(g dry biomass/g wet biomass) × 100%	2.9	21.4
Biomass density	g dry plant mass m^{-2}	0.2	392.0

Table 4. Summary of one-way ANOVA tests conducted to compare measurements of water chestnut samples across different distribution types. Distribution types: T = Trace; S = Sparse ; D = Dense ; M = Monoculture.

Mean subject of ANOVA test	Units	Distribution types compared	F	P value	Critical F
Plant density	No. plants m^{-2}	S, D, M	60.62	5.34E-07	3.89
Rosette growth	No. rosettes $plant^{-1}$	T, S, D, M	5.69	0.0069	3.20
Rosette width	cm	T, S, D, M	19.91	8.38E-12	2.63
Individual biomass	g dry biomass $plant^{-1}$	T, S, D, M	1.65	0.21	3.20
Dry biomass percentage	(g dry biomass/g wet biomass) × 100%	T, S, D, M	0.55	0.66	3.20
Biomass density	g dry plant mass m^{-2}	S, D, M	68.09	2.82E-07	3.89

than in June. This growth contributed to the variability observed in water chestnut samples, particularly in biomass and rosette width measurements. A major goal of this study was to create a tool that enables water chestnut managers to estimate the amount of water chestnut removed during a control event. Water chestnut management typically occurs during the period when plants breach the surface and seeds mature. Precisely when within this timeframe varies between site and year as weather conditions and schedules allow. The biomass estimates developed here for each density can be considered an average across the early plant growth and seed maturation period.

The biomass density values estimated in this study were different from those reported by other studies. Hummel and Kiviat (2004) reported between 104 and 1,575 g dry mass m^{-2} in the Hudson Valley of New York state, and Pierobon et al. (2010) reported between 47.1 and 504.8 g dry mass m^{-2} in northern Italy. Our study ranged between 0.2 and 392.0 g dry mass m^{-2} (averages between 33 and 306 g dry mass m^{-2}) (Table 3). On the high end, the values found within this study fall within the range of those previously reported, whereas on the low end, this study reports lower biomass than in previous studies. This is likely because of our focus on comparing biomass of different densities. Most other studies have focused on determining biomass in monoculture and dense populations because they impose the greatest impact on native habitats. The variability observed in the findings must also be considered, as well as the difference from some biomass densities estimated in other studies (Hummel and Kiviat 2004; Pierobon et al. 2010). For example, the four trace points collected at Schodack Creek totaled 9 g dry biomass, lower than the 29 g dry biomass estimated using the biomass estimation formula derived from our overall findings (Figure 4). The expansion

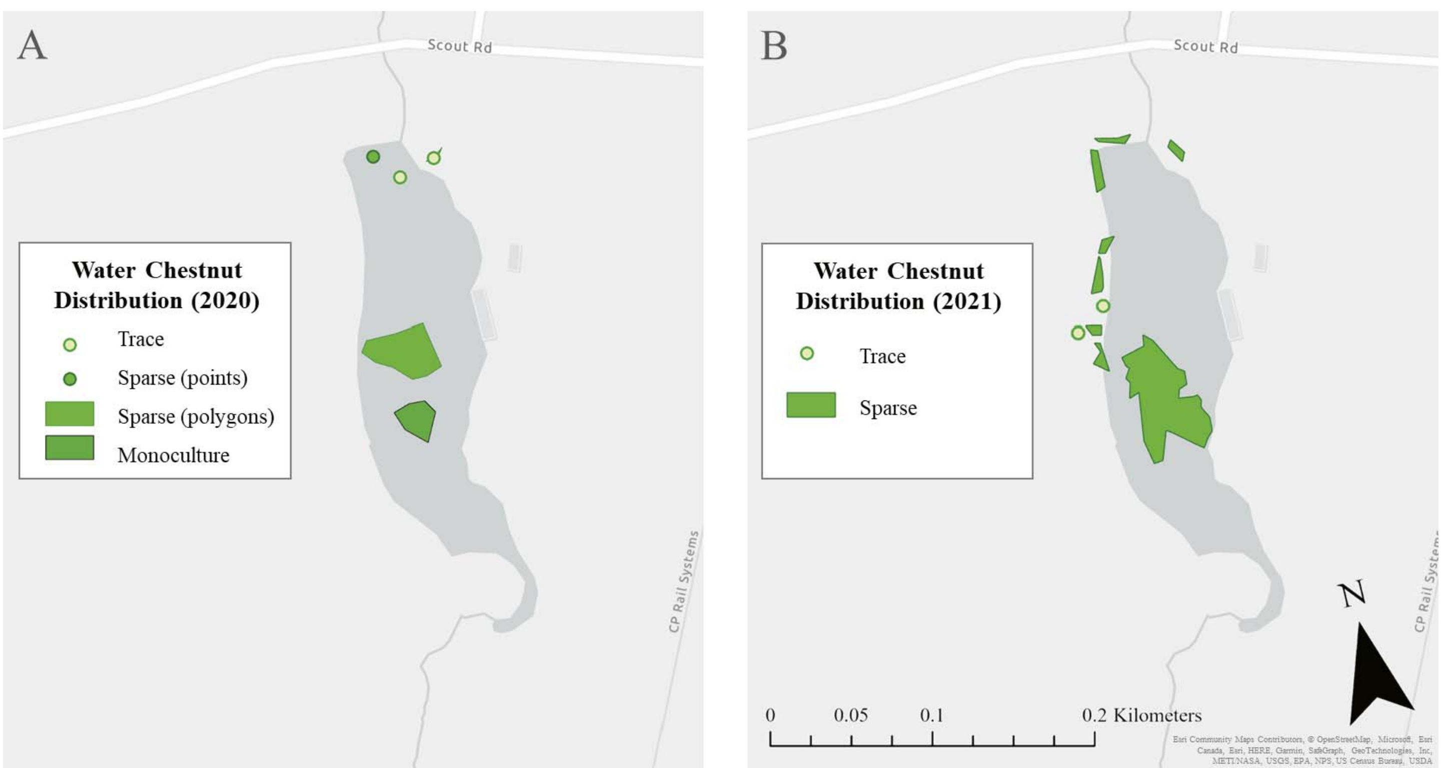

Figure 5. Map of water chestnut survey data at Delegan Pond in 2020 (A) and 2021 (B) reported to iMapInvasives; circles indicate point records. In 2020 there were 395.0 m^2 of monoculture, 989.15 m^2 of sparse patches (including the buffered point), and two reports of single plants (data collected 20 August 2020). Therefore, the biomass estimate calculation for 2020 is (306.8 g m^{-2} × 395.0 m^2) + (3.3 g m^{-2} × 395.0 m^2) + (7.3 g/plant × 2 plants) = 124,455.6 g, or 124.5 kg. In 2021 there were 2,596.1 m^2 of sparse patches and three reports of single plants (data collected 20 June 2021). Therefore, the biomass estimate calculation for 2021 is (306.8 g m^{-2} × 395.0 m^2) + (3.3 g m^{-2} × 2,596.1 m^2) + (7.3 g/plant × 3 plants) = 8,588.9 g, or 8.6kg, a 93% decrease from 2020.

of our understanding of the contribution of water chestnut biomass at lower densities may allow for a fuller understanding of how water chestnut may impact the plant community and nutrient cycling.

Density-dependent survivability is another factor that influences growth of water chestnut. Hummel and Kiviat (2004) determined that reduced intraspecific competition led to larger plants with more numerous rosettes in low-density growth. In contrast, our study resulted in dense plots having both the highest individual biomass and rosette growth. The difference in observed results may be because of more optimal habitat in observed dense and monoculture plots.

These biomass estimates are highly valuable to the assessment and planning of invasive species management efforts, allowing for consistent quantitative comparisons across multiple years without requiring labor-intensive biomass measurements in the field for each site. By conducting the field and lab work outlined in this study to derive biomass density estimates, we can apply the results to any water chestnut survey and treatments in the future, removing the need for aquatic invasive species managers to measure biomass for each site. Aquatic invasive species managers simply need to delineate an infestation and select the appropriate distribution type, rather than spending time counting rosettes, weighing wet biomass, or drying harvested biomass to obtain dry biomass. These biomass estimates could potentially be used to assess posttreatment effectiveness, identify infestations where more management resources are needed, and influence regional strategies.

One potential use of water chestnut is for bio-gas production. Biogas is an alternative energy source that relies on the microbial digestion of organic matter to create a usable fuel (Sudhakar et al. 2013). A bio-gas digester can use multiple fuel sources, and harvested water chestnut may be a seasonal source for energy production (Sudhakar et al. 2013). Estimates of water chestnut biomass present using records in iMapInvasives could help biogas harvesters better prioritize efforts.

Accurate monitoring of water chestnut biomass is important in considering its potential use as an atmospheric carbon sink. Pierobon et al. (2010) observed that water chestnut acted as a net carbon sink until mid-August, when lakes supporting large crops of water chestnut became net carbon sources. If managers harvested water chestnut when biomass reaches its peak, this would achieve the added benefit of removing carbon from the atmosphere before it is reintroduced back to aquatic or terrestrial ecosystems through decomposition.

Water chestnut management will continue to be a part of lake and river management in areas where it becomes a nuisance species. Reducing the size and scale of infestations is possible with annual effort and tracking. Utilizing a standardized technique to calculate the biomass of plants removed or of a standing bed provides managers the capability to track changes over time and compare between management strategies between and within sites. Additionally, this tool may be useful for those looking to harvest water chestnut for other reasons.

SOURCES OF MATERIALS

[1]iMapInvasives, http://www.imapinvasives.org.

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