J. Aquat. Plant Manage. 61: 22–29

Phenology of two-horned water chestnut (*Trapa bispinosa* Roxb. var. *iinumai Nakano*) in northern Virginia ponds

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ABSTRACT

Species of water chestnut, specifically Eurasian water chestnut (*Trapa natans*), have plagued the northeastern United States, including the tidal Potomac, for over 100 years. In 2014 a new species of invasive water chestnut identified as two-horned water chestnut (*Trapa bispinosa* Roxb. var. *iinumai Nakano*) was discovered in the Potomac River, and in subsequent years it has spread to nearby waterbodies. The purpose of this study is to describe the phenology of *T. bispinosa* to assist managers in developing effective approaches for management. Structured observational studies were conducted at two ponds in northern Virginia in 2019 and 2020. *Trapa bispinosa* initiated growth in late April, increasing rapidly to a maximum of 100% cover in June. Rosette diameters increased gradually from late April to a maximum in August and September. This increase in rosette size was strongly correlated with degree days and calendar days and is consistent among ponds and between years. Flower counts were zero from April through June, then increased rapidly to maximum in late August. Fruit counts were zero from April through June; fruit started to appear in July, and counts increased to a maximum in early September. Since the species is annual and dependent on sexual reproduction, control efforts for *T. bispinosa* should be initiated before fruits are produced. Based upon our data, in the mid-Atlantic region, May would be an ideal time to begin because rosettes should be observable, but flowers and fruit should not appear until late June. These studies indicate aquatic managers may have a 4-to-6-wk window in the late spring to prevent seed production and should focus resources on management during that period.

Key words: degree days, EDRR, invasive plants, *Trapa natans*, water chestnut.

INTRODUCTION

Water chestnut, or water caltrop as it is sometimes called, *Trapa* spp. are native to Eurasia and have been used as a food crop in that part of the world for centuries. Four-horned water chestnut (*Trapa natans*) found its way to North America in the early part of the 20th century and has spread throughout the northeastern part of the continent (Naylor 2003; Hummel and Kiviat 2004). Recently a two-horned species has turned up in the Potomac River and other sites in eastern North America. DNA taxonomic studies recently revealed that this new two-horned water chestnut is a form of *Trapa bispinosa* Roxb. var *iinumai* Nakano. The speculation is that this plant was introduced at some undetermined time and place and remained cryptic for a number of years until a large enough outbreak occurred to merit notice (Chorak et al. 2019; Dodd et al. 2019). *Trapa bispinosa* Roxb. var *iinumai* Nakano (herein after referred to simply as *T. bispinosa*) bears fruits with two sharp, straight horns.

T. bispinosa has pink flowers and forms two-horned fruits with two small pseudo-spines, and the fruit has no prominent crown (Figures 1 and 2). The vast majority of *T. bispinosa* rosettes have leaves with pink to dark red undersides. The long anchoring stems of *T. bispinosa* can reach up to 4 m deep. Like other *Trapa* species, in addition to the floating leaf rosette, *T. bispinosa* has feathery underwater leaves at each node of the submersed stem. Adventitious roots grow down into the substrate from the lower nodes (Muenscher 1944).

Water chestnut (*Trapa* spp.) includes a set of species that are annual floating leaved macrophytes native to tropical, subtropical, and temperate zones of the Eastern Hemisphere with a native range that includes southern Europe, Africa, and Asia (Hsieh 1993). It is found in the shallows of slow-moving rivers, ponds, and lakes and is widely cultivated in Asia (Hummel and Kiviat 2004). Most of the ecological data available for this genus has been derived from studies of *T. natans* that has spread throughout the northeastern United States (Naylor 2003). These studies indicate that *T. natans* (and in all likelihood *T. bispinosa*) has a major effect on other aquatic plant species, on water quality, and on other aquatic organisms. *Trapa natans* can undergo explosive growth, with its biomass in Lake Champlain growing 10-fold in one year (Bogucki et al. 1980). Water chestnut creates a canopy that interrupts the passage of light through the water (Groth et al. 1996) thereby inhibiting photosynthesis by submersed aquatic vegetation trying to grow below it. During their growing season of late May until September, more than 65% of the total plant biomass may concentrate in the top 15 cm of the water column (Galanti et al. 1990). Light levels were reduced by over 95% below a *T. natans* bed in the tidal Hudson River as compared with a nearby *Vallisneria americana* bed (Caraco and Cole 2002). Floating

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DOI: 10.57257/JAPM-D-22-00009

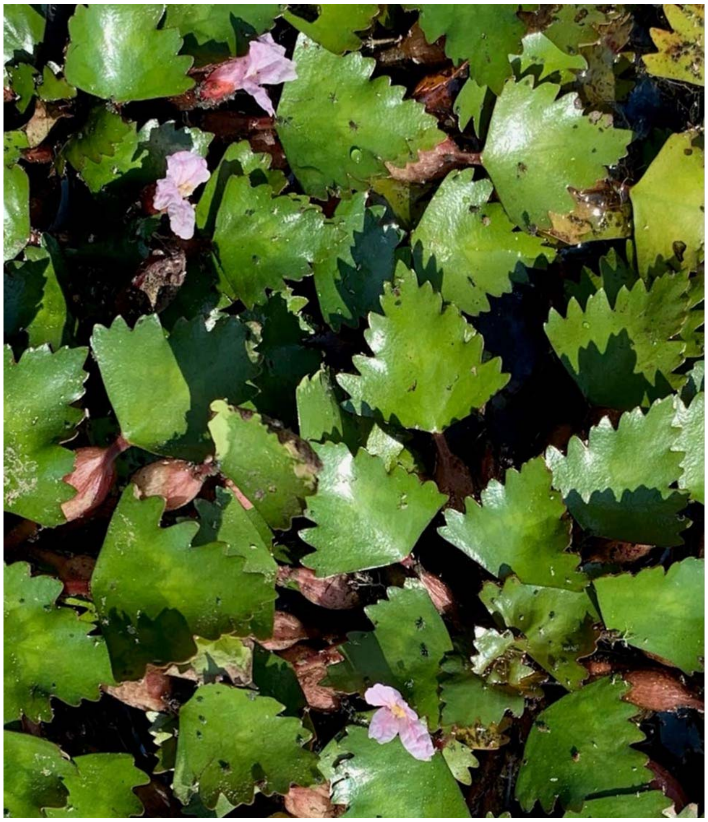

Figure 1. *T. bispinosa* with pink flowers.

Figure 2. *T. bispinosa* with different stages of two-spined fruit.

rosettes of *T. natans* release oxygen directly into the atmosphere while depleting oxygen from the surrounding water, resulting in hypoxia and possible anoxia. In the tidal Hudson River *T. natans* reduced dissolved oxygen concentrations to below 2.5 mg/L 50% of the time, and anoxia occurred on a regular basis (Caraco and Cole 2002; Strayer 2010). Water chestnut colonies displace native species (Strayer et al. 2003; Hummel and Kiviat 2004) and alter aquatic ecosystem biodiversity and function. In a Massachusetts lake that previously had a variety of species of aquatic plants, *T. natans* became a monoculture 5 yr after it was introduced (Groth 1988). Macrophyte beds with *T. natans* had lower biomass of epiphytic algae and invertebrates than adjacent areas of submersed macrophytes (Cattaneo et al. 1998). By outcompeting native submerged aquatic vegetation, water chestnut may degrade waterfowl habitats (Martin and Uhler 1939). However, Hummel and Kiviat (2004) and Yozzo and Odum (1993) cite several studies that suggest that *T. natans* beds may harbor substantial numbers of chironomids and enhance total fish production in the tidal Hudson River. Despite those benefits it has impeded recreational boating and swimming in lakes and tidal freshwater (Naylor 2003; Hummel and Kiviat 2004). Control and removal have cost over $8 million in just the Potomac River and Lake Champlain (Naylor 2003).

The dispersal of water chestnut is facilitated by both natural (Pemberton 2002; Hummel and Kiviat 2004; Swearingen and Fulton 2022) and human-mediated vectors (Dementeva and Petushkova 2010). Its barbed seeds can attach to fish nets, boats, birds, and other animals (Hummel and Kiviat 2004; Dementeva and Petushkova 2010). A probable mode of dispersal is resident Canada geese (*Branta canadensis*); *Trapa* spp. seeds cling to goose plumage and may be transported locally by geese. Managers in Virginia and Maryland are concerned that if *T. bispinosa* in the Potomac River watershed is allowed to establish in ponds, it could soon spread back to tidal waters. This would create a management challenge that would undo past decades of successful eradication and undermine more recent estuarine water quality improvements. Thus, continuous monitoring and early detection and rapid response are needed to control its invasion.

The study of phenology is important because the phenological differences between native plants and invasives may contribute to the success of such invasives. The period of flowering and fruiting helps to determine how the communities assemble and how new species invade (Wolkovich and Cleland 2011). The time of seed production is crucial to long-term control, especially for annual species. Potential control methods for *T. bispinosa* include hand removal of plants and herbicide applications. Both have effectiveness if caught early in invasion of individual waterbodies (Rybicki and Swearingen 2019; Poudel 2021). According to Rector et al. (2015), the application of herbicide may significantly hinder the surviving plant's ability to produce viable seeds. The management process of *T. bispinosa* is difficult because it is an annual plant and seeds may remain viable in sediments for up to 10 yr. Thus, the success of the management approaches depends on the removal of plants before seed production for as much as a decade (Hummel and Kiviat 2004). The phenology of *T. bispinosa* in the United States has not been studied in detail, and assumptions are made with reference to its closest relative, Eurasian water chestnut (*T. natans*). Knowledge of the vegetative and reproductive phenology of *T. bispinosa* is crucial for the successful implementation of management options. Hence, the objective of this study is to describe the growth and development of *T. bispinosa* in two ponds in northern Virginia.

Table 1. Description of study sites. Maximum pond coverage is the greatest extent covered by *T. Bispinosa* as a percent of total pond area. VGA = Virginia Golf Academy pond; HP = H-mart pond.

Pond name	VGA	HP
Latitude (°)	38.82867N	38.83673N
Longitude (°)	77.40161W	77.42897W
Pond area (ha)	0.38	0.12
Frequency of observation	Weekly	Biweekly
Maximum pond coverage (%)	85	100
Initial year of colonization	2016	2015

MATERIALS AND METHODS

Seasonal growth and development of phenological traits of *T. bispinosa* were determined for two ponds in the western part of Fairfax County, VA (Table 1; Figure 3). These ponds are located in the Bull Run subwatershed of the Potomac River about 2.5 km apart and were constructed as stormwater management ponds. Virginia Golf Academy (VGA) had an area of 0.38 ha with inputs consisting mostly of golf course runoff, and H-mart Pond (HP) had a smaller area of 0.12 ha and received inputs mainly from a shopping center parking lot. The whole pond maximum percent cover of *T. bispinosa* was approximated by direct visual observation. The surface area of both ponds was dominated by *T. bispinosa* at its maximum extent in late summer.

Sites VGA and HP were sampled in 2019 from early June until late October and in 2020 from late April until early November. VGA was sampled weekly, whereas HP was sampled biweekly in both years. Before sampling began in 2020, VGA and HP were visited frequently from February to April to note the onset of *T. bispinosa* so that early development of the population could be followed. During the growing season, we evaluated several vegetation parameters including overall percent coverage of the pond, percentage cover of the sampled quadrats in the pond, rosette diameter (RD), flowers per rosette (FL), and fruits per rosette (FR).

At each pond, three locations at least 10 m apart were chosen along a consistent stretch of the vegetated shoreline. Three replicate quadrats were sampled at each location. Quadrat coverage of *T. bispinosa* was assessed by using 1 m^2 quadrat divided into 100 (10 by 10) cells (Figure 4). Cells with any *T. bispinosa* leaves present were noted and tallied to get a number from 0 to 100 (the total number of cells) for each quadrat. Water temperature was measured in each pond on each date using a YSI ProDDS minisonde.

To study the phenology of *T. bispinosa* in more detail, three representative plants were chosen randomly from each of the three quadrats at each of the three sampling locations in each pond. Thus, nine plants were sampled from each pond whenever possible. Any debris on the rosette was rinsed, and the plants were placed in plastic bags and processed within 24–48 hr of collection. The bags were chilled in a refrigerator at 4 C until phenological variables could be measured within 48 hours of collection.

Rosettes were approximately circular, and rosette diameter (RD) was measured for the largest rosette on each of the nine plant samples in the longest dimension with a ruler. Mean and standard error of RD was calculated for each site visit. The number of flowers on each plant (FL) was recorded. Flowers included complete flowers, flower buds, and recently submerged flowers without petals. Broken fruit stubs, locations where fruit had recently been attached, were added to fruit count. Mean and standard error of RD, FL, and FR were calculated.

The concept of degree days is used extensively in ecological literature, and 10 C is a frequently used index temperature for these calculations (Alexander et al. 2020; Vercaemer et al. 2021; Blatt et al. 2022). Air degree days (ADD) above an index temperature of 10 C were calculated by subtracting the daily mean air temperature from the index temperature for each day and accumulating those over the course of the year. Daily mean air temperatures were measured at the nearby Dulles International Airport National Weather Service station (https://www.ncdc.noaa.gov/cdo-web/search). Calculations were started on 1 April and ended on 30 November. Days with mean daily temperature below 10 C were not included in the calculation. Water degree days (WDD) involved a similar calculation using estimated daily water temperature in lieu of daily mean air temperatures. Estimated daily water temperature for each day was calculated by interpolating between the observed water temperature at the sampling sites on sampling dates. Calendar days (CD) were also computed beginning 1 April in both years, and this parameter was used as an additional measure of seasonality. Statistical analyses were done using Systat 13.0, and graphs were constructed using Sigmaplot 14.5 and Systat 13.0.

RESULTS AND DISCUSSION

For *T. bispinosa* quadrat coverage, a consistent seasonal pattern was observed with a rapid increase from the first appearance of rosettes in late April to a maxima of 85 to 100% by mid-June in 2020 (Table 2). For the remainder of the year quadrat coverage remained at or near its maximum value. In contrast to the rapid increase of quadrat coverage, rosette diameter (RD) increased in a roughly linear fashion into or through August in both ponds and both years (Figures 5a–d and 6a). At VGA, RD was highest in mid-September in both years, whereas at HP maximum RD was observed in August. In October RD declined somewhat, and by November the rosettes had started to decompose and sink to the bottom.

Flowers were absent during the early growth period of the rosettes and were first observed in mid- to late June in both years and both ponds (Table 2; Figures 5 and 6b). Once flowers were present, FL increased rapidly within 2 to 4 wk and reaching a maximum of approximately 2 to 3 flowers $plant^{-1}$ in August with substantial variability. Subsequent values declined to approximately 1 to 1.5 flowers $plant^{-1}$.

Fruits first appeared in late June to mid-July, about 2 wk after flowers appeared (Table 2). Like flowers, the density of fruits increased rapidly, quickly attaining values of 2 to 3 fruits $plant^{-1}$ within about 2 wk of first incidence (Figures 5 and 6c). Fruits continued to increase at VGA in 2020 reaching a value of 4 to 5 fruits $plant^{-1}$ in September. At HP

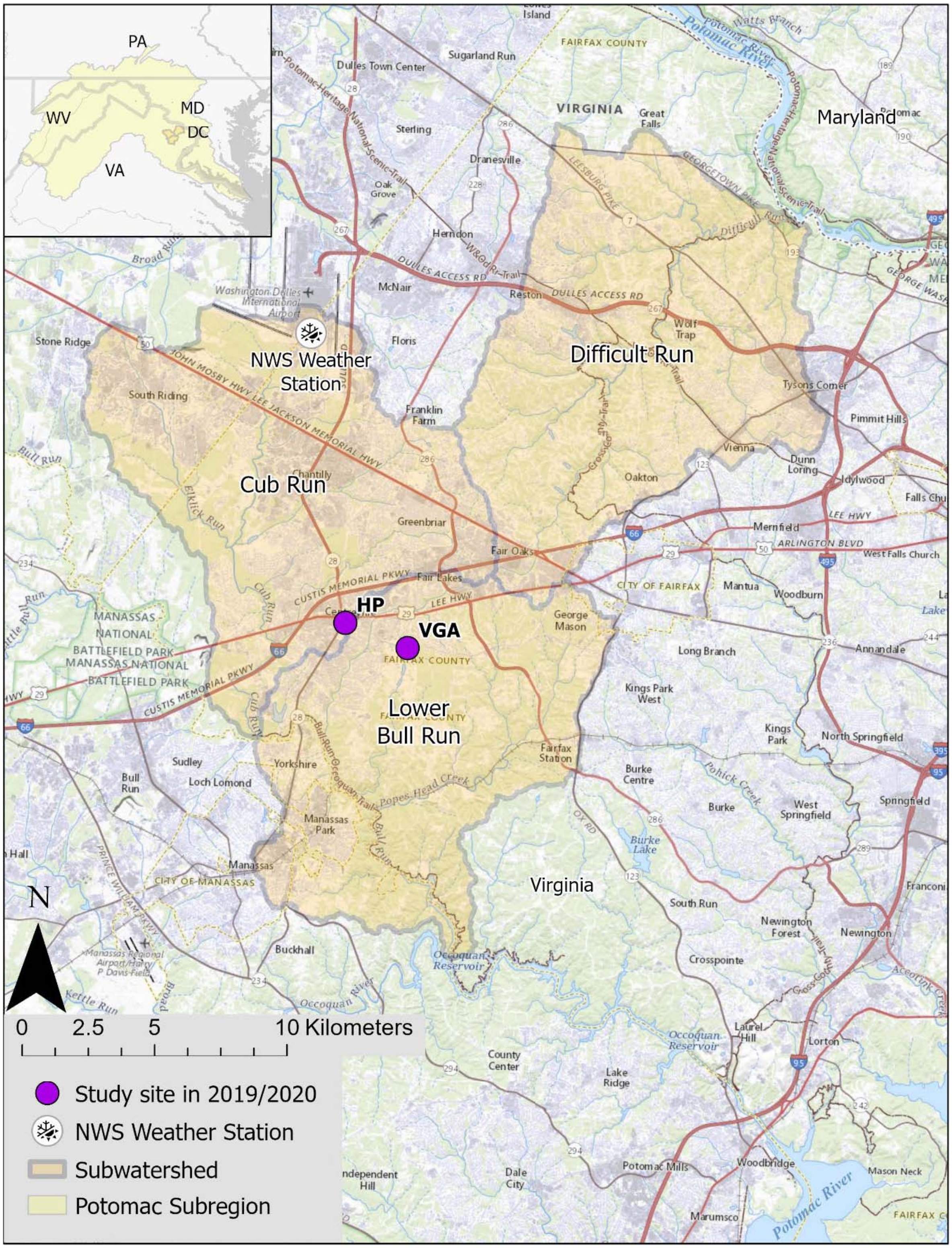

Figure 3. *T. bispinosa* study ponds and weather station in Fairfax County, VA.

further increases were also seen to approximately 5 fruits plant^{-1} in 2019 and 3.5 fruits plant^{-1} in 2020. FR then decreased sharply but were still present in September and October in both ponds over both years.

Correlation analysis was conducted on phenological variables, air and water temperature, CD, ADD, and WDD for the 2020 data from both ponds (Table 3). A highly significant correlation was observed between RD and FL

Figure 4. Quadrat used for sampling *T. bispinosa* coverage (QC). Whole quadrat is 1 m by 1 m and is divided into 100 squares each 0.1 m by 0.1 m.

and between RD and FR. All three measures of *T. bispinosa* phenology were highly correlated with CD, ADD, and WDD with CD having the strongest coefficient. The correlations with the climatic variables were highest for RD because FL and FR remained at zero for over a month in the spring as degree days were accumulating. ADD and WDD were very highly intercorrelated because these were shallow ponds, and there was a very strong correlation between air temperature and water temperature. Interestingly, neither air temperature nor water temperature correlated with either of the degree day measures, with calendar days, or with plant phenology measures. This may be explained by the fact that temperature is an instantaneous measure while the other measures reflect cumulative conditions, and plant development is responding to accumulated warmth and light energy.

Phenological data on *Trapa bispinosa* are very limited, and so comparisons with the more extensively researched *Trapa natans* are useful. In this study *T. bispinosa* rosettes first appeared in late April when water temperature was 13.6 C, WDD was 56, and ADD was 46. Several researchers have reported that *T. natans* fruits start to germinate when temperatures reach 12 C (Hummel and Kiviat 2004). In contrast one field study germination occurred at 16 C in late April (Galanti et al. 1990). *T. bispinosa* rosette diameter continously increased from late April into August in our study with little variation between ponds or years. During this period, rosette diameter increase was strongly correlated with measures of seasonality including calendar days and degree days for both water and air. Since both temperature and day length were increasing from April to 21 June, it is hard to tell which one is the driver of the phenological parameters. However, because rosette size continued to increase with increasing degree days into August, temperature is likely more important. In a 5-yr study of *T. natans*, Tsuchiya et al. (1987) found that rosette density of *T. natans* reached its seasonal peak in June while biomass was greatest in July in Lake Kasumigaura, Japan. The closest analog to rosette density in our study was coverage, which peaked in June. Rosettes continued to be present into October and early November. The flower and fruit production continued throughout the study which ended in Mid-November. Plants survived until December when a hard frost caused senescence.

Table 2. Date of observation of first rosettes in ponds, date when quadrat used for sampling *T. bispinosa* coverage reaches 100%, and dates of first flowers and first fruits in quadrats. VGA = Virginia Golf Academy pond; HP = H-mart pond.

Pond name	VGA	HP
Rosettes first observed in 2020	23 April	30 April
Quadrat coverage reaches 100% in 2020	12 June	19 June
First flower observed in 2019	11 June	21 June
First fruit observed in 2019	21 June	12 July
First flower observed in 2020	26 June	3 July
First fruit observed in 2020	10 July	16 July

Although there was appreciable variability in the seasonal growth pattern of rosette size, when data from both years and both ponds were pooled (Figure 6a), there was a very clear pattern of nearly linear increase in rosette size from April through June. This was followed by a tapering increase in rosette size in July and August, reaching a plateau or even slightly decreasing in October. There was little difference in this overall pattern between years and ponds.

Flower and fruit phenology followed a different pattern. *T. bispinosa* started flowering in mid-June to July. At that time water temperature was 22 to 26 C and air temperature was 21 to 25 C with ADD running from 590 to 1,200 and WDD from 725 to 840. FL increased rapidly to a maximum of about two flowers per rosette in August and then declined through October. There was substantial variability in FL during the summer that was not attributable to pond or year. FL was highly correlated with RD, which may indicate more resources for flower production when rosettes are larger. In an experimental study comparing the two species, flower production of *T. natans* was observed by 12 June, whereas *T. bispinosa* lagged behind with flowers noted by 17 July (Dodd and Schad 2021), which was later than in our study. Those authors noted that *T. natans* reached the surface faster than *T. bispinosa*, and they attributed the earlier appearance of flowers in *T. natans* to that rapid growth.

In our study *T. bispinosa* fruits began to appear soon after flowers, typically 1 to 2 wk later. Air temperatures at this time were 24 to 27 C, while water temperatures were 25 to 28 C, ADD was 810 to 1,200, and WDD was 960 to 1,070. FR increased quickly after the first appearance and peaked at 4 to 5 fruits per rosette in late August followed by a marked decline into early November. Like FL, FR was highly correlated with CD, ADD, WDD, and RD, again suggesting

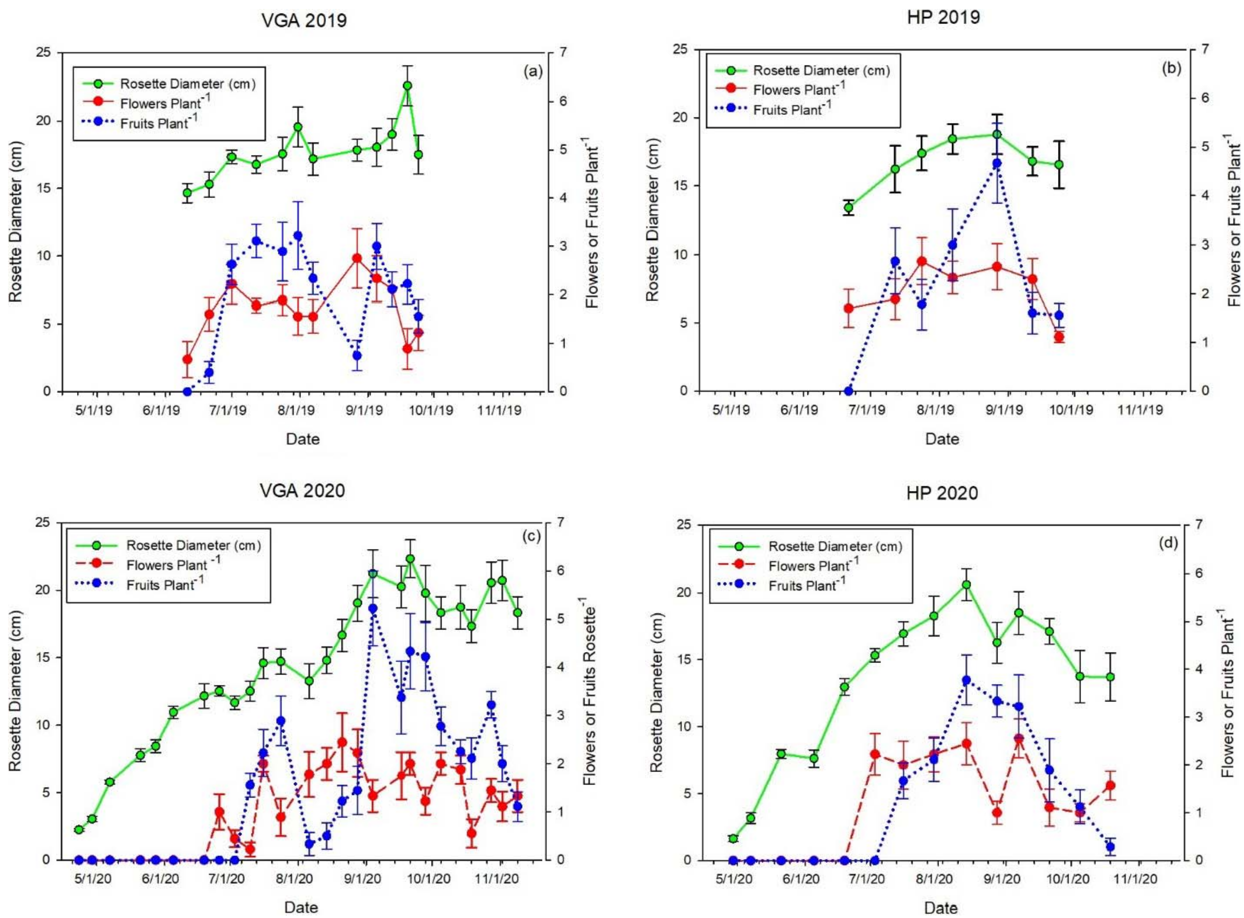

Figure 5. Seasonal progression of rosette diameter (RD), flowers plant^{-1} (FL), and fruits plant^{-1} (FR) at each pond (VGA = Virginia Golf Academy pond; HP = H-mart pond) in each year. (a) VGA 2019, (b) HP 2019, (c) VGA 2020, (d) HP 2020. Mean with standard error.

that larger rosettes may produce more fruit. Interestingly, CD, ADD, and WDD were very highly intercorrelated and strongly correlated with plant phenology parameters. Although it might be expected that plant phenology parameters should be most highly correlated with WDD due to the plants residing in the water, the simpler measures of CD and ADD were essentially equal to WDD in their predictive power.

A 12-wk (May to August), outdoor mesocosm study comparing the two species indicated that *T. natans* and *T. bispinosa* differed in phenology and light requirements (Dodd and Schad 2021). Flower production of *T. natans* was observed by 12 June, whereas *T. bispinosa* lagged behind the congener, with flowers noted by 17 July. Water temperature in the mesocosms ranged from 27.4 to 35.8 C with a mean of 31.5 ± 1.3 C. Those authors noted that *T. natans* reached the surface faster than *T. bispinosa* to which they attributed the earlier appearance of flowers in *T. natans*.

Dodd and Schad (2021) also found that *T. natans* had mature fruit observed by 17 July (35 days from the start of the experiment), but no fruit was recorded for *T. bispinosa* until 14 August (28 days later). Mean attached fruits rosette^{-1} at the end of their 84-day study, in August, was between 1 and 2 fruits rosette^{-1}, whereas in the current field study, over a whole growing season, the fruit density was in the 2 to 3 fruits plant^{-1} range with values as high as five fruits plant^{-1}.

Overall, *T. bispinosa* in our studies exhibited a similar phenology to *T. natans* in previously published studies but was slightly delayed in its development and was capable of maintaining fruit production a little later into the fall. This phenological synthesis will aid in the management and control of *T. bispinosa* as there is limited field information available. To effectively control *T. bispinosa* it is necessary to time harvesting or herbicide application before fruits are produced. Since flower production is almost immediately followed by fruit production, it will be necessary to implement control measures before or soon after observing the flowers. Based on our studies, this would mean initial treatment operations should be conducted during the month of May. Rosettes should already be observable by that time, but flower production should not begin until June. Based upon our data, May would be an ideal time to begin treatment and/or removal in the mid-Atlantic region because rosettes should be observable, but flowers and fruit should not appear until late June. Poudel (2021) reported on findings from herbicide treated ponds as well as the untreated ponds reported here. However, the treatments were not for immediate management purposes and not

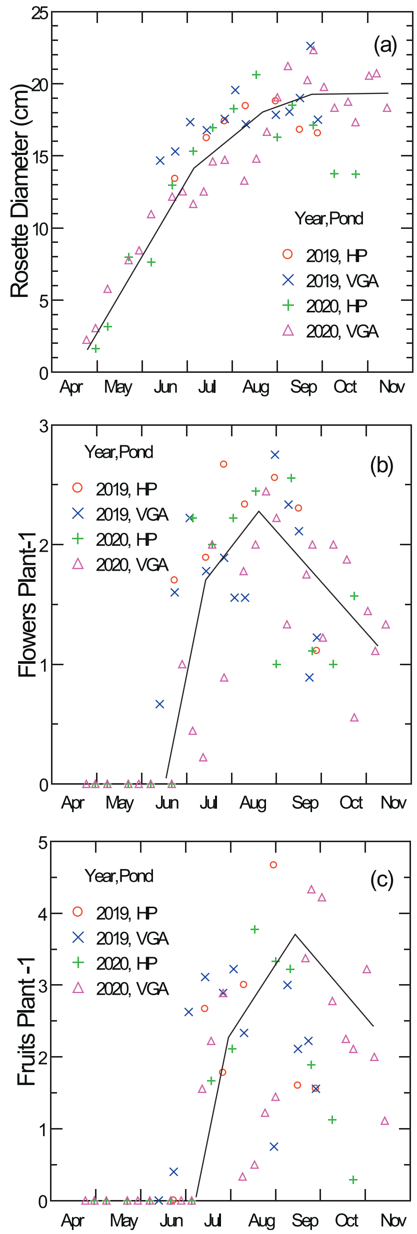

Figure 6. Seasonal progression of rosette diameter (RD), flowers plant^{-1} (FL), and fruits plant^{-1} (FR) with individual means from each pond and year. (a) RD, (b) FL, (c) FR. Trend line was added manually.

done in a structured way. Experimental research should be conducted to validate the effectiveness of the treatment regime proposed here and examine the plants' regrowth potential both later in the year and in subsequent years and the need for retreatment. Research is direly needed to understand the retreatment methods and intervals to prevent the seed production and control this species successfully.

ACKNOWLEDGEMENTS

Funding and support and for this research was provided through George Mason University (GMU) and Potomac Environmental Research and Education Center (PEREC). The authors would like to thank David Tolentino for helping with the filed work, Ian Pfingsten (USGS) for the map, and the late Dr. Thomas Lovejoy and the late Dr. Lee Talbot for their support as committee members.

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Table 3. Correlation analysis for variables for both ponds on sampling dates through the end of September 2020 ($N = 32$).

Pearson correlation matrix	Rosette diameter (RD)	Flowers plant^{-1} (FL)	Fruits plant^{-1} (FR)	Calendar days (CD)	Air temperature (C)	Water temperature (C)	Air degree days (ADD)	Water degree days (WDD)
Rosette diameter (RD)	1							
Flowers plant^{-1} (FL)	0.797	1						
Fruits plant^{-1} (FR)	0.788	0.574	1					
Calendar days (CD)	0.937	0.762	0.799	1				
Air temperature (C)	0.532	0.47	0.224	0.415	1			
Water temperature (C)	0.389	0.351	0.031	0.244	0.951	1		
Air degree days (ADD)	0.901	0.782	0.808	0.977	0.355	0.179	1	
Water degree days (WDD)	0.92	0.779	0.816	0.995	0.393	0.217	0.983	1

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