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Evaluating the suppression of *Hydrilla verticillata* by manual removal and planting native aquatic plants

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Abstract

Hydrilla is an invasive aquatic macrophyte found throughout the San Marcos River and every continent except Antarctica colonizing rivers and lakes. Multiple hydrilla management options are available to control hydrilla. Due to the high number of endemic and listed species in the San Marcos River, management options are limited. We examine the ability of the three native species (water stargrass, Illinois pondweed, and Texas wild rice) to suppress or outcompete hydrilla in the greenhouse and in the San Marcos River interaction studies. In *ex situ* studies, there were decreasing root and shoot biomass and relative growth rates for hydrilla as the ratio of water stargrass and Illinois pondweed increased. Hydrilla allocated a significantly greater biomass to its shoots compared to its roots in both the winter and summer *ex situ* studies. The large amount of biomass allocated to hydrilla allows its shoots to reach the upper water column blocking sunlight and reducing photosynthesis of native plants. Water stargrass and Illinois pondweed had greater relative growth rates compared to hydrilla when planted in higher numbers under *ex situ* conditions. The results of the *ex situ* studies found that water stargrass and Illinois pondweed planted collectively or alone cannot suppress or outcompete hydrilla if planted in smaller ratios than hydrilla. In the *in situ* study in the San Marcos River, no strong correlations were found between river discharge and the coverage of each plant, but water stargrass was found to have a moderate positive correlation (0.47) as discharge increased the last 4 months of the study. Hydrilla was present in 100% of the plots (n = 24) at the end of the study regardless of percent removal, while Texas wild rice and water stargrass were present in 50% (n = 12) and 42 % (n = 10) of the plots, respectively. We found no evidence that water stargrass or Texas wild rice gains a competitive advantage over hydrilla in plots where hydrilla was removed at percentages between 0-75% in 0.25 m^2 plots. In plots where 100% hydrilla was removed, Texas wild rice exhibited equal or greater coverage to hydrilla. An unexpected result from this

study indicated that hydrilla coverage was among the highest in plots where 100% of the hydrilla was removed at the start of the study indicating its ability to quickly invade a disturbed site. Moreover, contrasting results were observed for the percentage cover and final dry weight biomass of each macrophyte in this study. The mean percent coverage was greater for hydrilla than Texas wild rice and water stargrass except in plots where Texas wild rice was planted and 100% of the hydrilla removed. While the overall survival percentage in plots was 50% for Texas wild rice, the mean root and shoot mean dry weights was significantly greater than hydrilla which occurred in all plots. While water stargrass coverage was low in all plots and only documented in 42% of the plots at the end of the study, there was no difference in the root and shoot dry weights of water stargrass and hydrilla. The *in situ* study results indicate morphological differences with hydrilla allocating greater biomass into its shoots while Texas rice allocates ca. equal amounts of biomass into its shoots and roots. The results of the *ex situ* study indicate that greater number of native aquatic plants must be planted to suppress hydrilla. In the *in situ* study, Texas wild rice was found to be competitive with hydrilla in plots where 100% of the hydrilla was removed, but hydrilla quickly reinvaded. Additional studies are needed *in situ* to determine the area of hydrilla to be removed and the number of native aquatic plants required to suppress hydrilla.

Introduction

Submerged aquatic plants are an integral component of freshwater ecosystems. They perform essential functions that enhance their ecosystem by reducing eutrophication through nutrient retention (Kao et al., 2003), improving water clarity (Mukhopadhyay and Dewanji, 2004), and providing food, habitat, and shelter for aquatic species (Carlsson and Lacoursiere, 2005; Jeppesen and Sondergaard, 1998). Aquatic plants have also demonstrated the ability to absorb toxic metals and elements from the water aiding in the water purification process (Alvarado et al, 2008). One of the greatest threats aquatic plants face is the introduction of exotic plant species, which have the ability to form monocultures and outcompete native plants (Havel et al., 2015).

Exotic aquatic plant species originate from different geographical locations than where they are currently present, and although many plants are introduced into new geographical regions, only a small percentage become invasive outside their native range (Larson, 2008). Invasive aquatic plants possess traits that allow them to occupy a wide range of habitats and to out-compete native species by rapid growth rates and usurping resources (Blumenthal and Hufbauer, 2007). Exotic plants introduced into freshwater systems with diverse and abundant native vegetation are likely to result in drastic changes in ecosystem functioning (Hussner et al., 2016), have a negative impact on native plant abundance and associated animal diversity, and can interfere with human activities such as recreation and flood control (Dayan and Netherland.,2005). The most invasive aquatic plants can cause irreversible damage to an aquatic ecosystem by increasing sedimentation rates, interfering with light penetration, decreasing river water velocity, and ultimately changing the banks of river channels from lotic to lentic systems (Santos et al., 2011). Aquatic environments have shown to be particularly vulnerable to invasive exotic weed species (Netherland et al., 2005). The clear and shallow water, favorable water velocity, and constant water chemistry and cool temperatures make the San Marcos River more susceptible to invasion compared to other freshwater ecosystems.

Lemke (1989) found that 8 of the 31 plant taxa collected in the upper San Marcos River were non-native; and in 2001, it was reported that 16 of the aquatic plant species inhabiting the San Marcos River and the headwaters of Spring Lake were non-native (Bowles and Bowles, 2001). In response to the growing number of non-native species invading the San Marcos River, in 2013 the City of San Marcos, as part of the Edwards Aquifer Habitat Conservation Plan (EARIP, 2012), hired contractors to manually remove invasive aquatic plants and initiated replanting native aquatic plants to expand the range of the federally endangered Texas wild rice (*Zizania texana* Hitchc.; Poaceae) and habitat for the endangered fountain darter (*Etheostoma fonticola*). One of the invasive plants designated for removal was hydrilla.

Hydrilla [*Hydrilla verticillata* (L.F.) Royal] (Figure 1) is an aquatic plant native to the warm areas of Asia and Africa (Balciunas et al., 2002). Hydrilla is a submersed ascending macrophyte with slender stems up to 9 m in length that is multiple branching with small whorled leaves 2-4 mm (Langeland et al., 2008). It was first discovered in the United States in 1960 in Florida (Blackburn et al., 1969) and has expanded its range throughout much of the United States. Hydrilla was first documented in the San Marcos River in 1975 (Flook, 1975), and was so abundant that it was being harvested and marketed for use in aquariums. Lemke (1989) recorded it as the most abundant aquatic plant in the San Marcos River. In a 2001 study to assess the volume of floating aquatic vegetation in the San Marcos River, hydrilla made up 34-64% of the aquatic vegetation collected over different seasons (Owens et al., 2001). During 2010, hydrilla made up 26.63% of the San Marcos River aquatic macrophytes (Hardy et al., 2010).

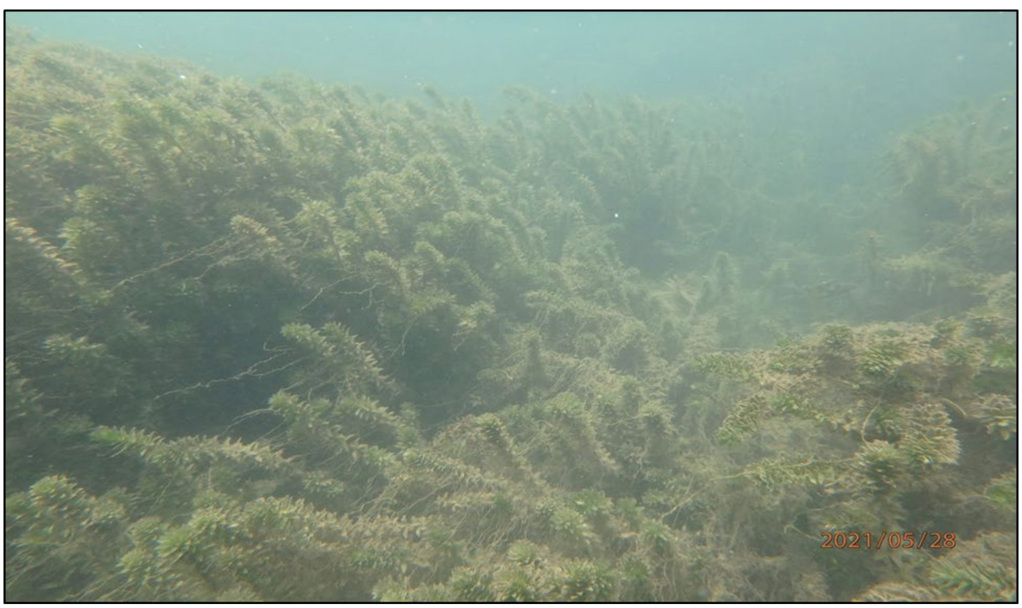


Figure 1. Monoculture of hydrilla in the San Marcos River.

Hydrilla is considered one of the most invasive species of aquatic weeds in the United States (Madsen, 1997) and one of the most successful invasive aquatic species in the world (Langeland, 1996). This is largely attributed to hydrilla's rapid growth rates, multiple modes of reproduction, and ability to adapt to disturbed conditions, which allows it to pullulate. Glomski and Netherland (2012) found that a 10 cm fragment can increase to > 8000 cm of shoot tissue in five weeks. Due to its rapid growth rates, hydrilla has the ability to outcompete other submerged plants for sunlight (Langeland, 1996) by forming monocultures at the water surface by blocking sunlight from other submersed species (Haller and Sutton, 1975). Hydrilla's modes of reproduction include fragmentation, tubers (Figure 2), and turions (Langeland, 1996). Owens et al. (2001) found that 70-83% of hydrilla fragments become established in one month in controlled conditions. Fragments of hydrilla present a threat to native species like Texas wild rice. Floating fragments of hydrilla form large mats covering stands of Texas wild rice and other aquatic plants blocking sunlight, reducing flow velocity, and reducing nutrient uptake by native species (Power, 1996). Hydrilla tubers are subterranean and have been documented to lie dormant for over four years (Van and Steward, 1990).

Figure 2. Hydrilla tuber collected during the winter *ex situ* study.

In addition to rapid growth and multiple reproduction modes, hydrilla also exhibits physiological characteristics and adaptations that allow this plant to grow in a wide range of conditions. Hydrilla is typically a C_3 species but can utilize a modified C_4 photosynthetic pathway in extreme environmental conditions (Salvucci and Bowes, 1981). Exposure to increased irradiance and higher temperature can prompt hydrilla to switch from the C_3 to the C_4 pathway (Salvucci and Bowes, 1981; Bowes and Salvucci, 1984, 1989). Hydrilla is also found in conditions ranging from oligotrophic to eutrophic and in environments with very low light levels (Cook and Lüönd, 1982). Hydrilla's resilience, rapid growth rate, and multiple modes of reproduction make it a difficult species to manage in aquatic ecosystems.

There is a wide range of available hydrilla management options. Biocontrol agents for hydrilla have displayed promising results (Del Fosse et al., 1976). However, studies have indicated that most biological control agents either do not reduce the biomass of hydrilla enough to limit its presence in aquatic sites (Purcell et al., 2019), or result in unwanted non-target damage (Sailer, 1978). Grass carp (*Ctenopharyngodon idella)* have historically been used for aquatic vegetation management and are successful in reducing hydrilla populations (Courtenay and Stauffer, 1984). However, grass carp can result in detrimental impacts by reducing native aquatic plants and increasing turbidity (Dibble and Kovalenko, 2009).

Herbicides are an effective method for invasive aquatic plant control but often result in non-target damage. Herbicide use within river systems is not recommended due to rapid dilution and dispersion downstream (Getsinger et al., 1996). Due to the flowing water, multiple herbicide treatments would be required to control hydrilla from target areas, and the quick dispersal of the herbicide through flowing water presents a threat to nearby native aquatic plants. The San Marcos River provides habitat to eight endangered species, including Texas wild rice. Because of the vulnerability of Texas wild rice to herbicide treatment and runoff, Texas Parks and Wildlife recommends avoiding the application of herbicide in the upper 4 km of the San Marcos River and all tributaries that enter the upper 4 km of the San Marcos River (TPWD, 2009).

The ineffective application of herbicide in flowing water results in hydrilla resprouting from the roots and tubers in the sediment. Manual removal and planting of native plants are the only viable management options to control in the San Marcos River. The use of hand cutters, rakes, or bare hands to remove vegetation is the most common form of mechanical control in the world and the lakeshores of the United States (Madsen, 2000). The use of hand-weeding is labor intensive and requires the use of scuba divers at depths >1m; however, it allows the successful eradication of small populations of invasive species with minimal disturbance to the aquatic plants in the near vicinity (De Winton et al., 2013).

Non-native aquatic plant removal efforts continue in the San Marcos River under the EARIP (2012); however, because of the reproductive capabilities of hydrilla, additional management options are essential to control hydrilla. Planting native aquatic plants in sites with no initial competition offers a potential management alternative. A competition study conducted by Doyle et al. (2007) measured the competition between hydrilla and the native *Vallisneria americana*. The study found that hydrilla grown with *V. americana* developed lower total biomass, total basal stems, and smaller tubers relative to control plants. The study indicated that the presence of *V. americana* resulted in a 30–40% reduction of total hydrilla biomass. One method the authors suggested for hydrilla suppression is the planting of fast-growing native plants capable of shading out hydrilla.

Texas wild rice (Figure 3) is a federally endangered aquatic plant endemic to the upper 3-4 km of the San Marcos River, Hays County, Texas. The upper reach of the San Marcos River is a consistent temperature and quality which allows for diverse and prolific spring flow-dependent native plant communities and endemic species. Texas wild rice is a perennial, C_3 grass (Poole, 2002 and Waller and Lewis, 1979) that grows in areas with a coarse sandy substrate with low organic matter, moderate current velocity, and in shallow water that is ≤ 1 m in depth (Poole and Bowles, 1999). It is characterized by its long ribbon-like leaves and produces emergent inflorescence (Power and Doyle, 2004). Texas wild rice is anemophilous reproducing by seed (Poole, 2002) and asexually via tillers (Tolley-Jordan and Power, 2007). Sexual reproduction can be limited in Texas wild rice as pollen rarely travels > 1.5 m from a flowering plant, and it was rare for the next clump of Texas wild rice to be within that distance (Oxley et al., 2008). However, with planting and the increase of Texas wild rice since 2013, the species populations have now increased by over 50% and seed production is now common (Poole, TPWD, personal communication).

Figure 3. Texas wild rice plants in the San Marcos River, Hays County, Texas with submerged elongated ribbon-like blades and emergent stems with inflorescence.

When Texas wild rice was described in 1932, it was abundant in the San Marcos River, Spring Lake, and adjacent ditches (Silveus, 1933). The abundance of Texas wild rice rapidly declined in the subsequent decades due to floating debris that damaged the grass's inflorescence, regular mowing by the city, commercial plant collection, and periodic influxes of raw sewage (Emery, 1967). By 1967, there was only one plant in Spring Lake, no plants in the upper 0.8 km of the San Marcos River, and only scattered plants in the next 2.4 km (Emery, 1967). Due its rapid decline and restricted population, Texas wild rice was added to the U.S. Federal Endangered Species List in 1978 (USFWS, 1978). The replanting efforts under the EARIP (EARIP, 2012) have increased the population of Texas wild rice from 5,497 m^2 in 2013 to 9,804 m^2 in 2018 based on Texas Parks and Wildlife Department and U.S. Fish and Wildlife's (USFWS) annual survey in 2018. A further increase in Texas wild rice population was estimated to be > 15,000 m^2 in 2019 during the USFWS survey (Chris Hathcock, USFWS, personal communication).

Texas wild rice has been documented to have very fast growth rates and is consequently a strong competitor against other aquatic plants. In a greenhouse study, the total blade length of Texas wild rice grown from seedlings increased from a mean leaf length of 10 cm at potting to 4,254 cm at 14 weeks (Hutchinson, 2019). Furthermore, a transect study conducted by Poole and Bowles (1999) found that hydrilla and *Egeria densa* were present in greater percentages in transects without Texas wild rice than in transects with Texas wild rice. Lastly, a large area of hydrilla was removed at Cypress Island in the San Marcos River and replanted with Texas wild rice. Currently, Texas wild rice accounts for > 75% coverage within the treated area but hydrilla is recolonizing and comprised ca. 25-30 % coverage in 2021 (Jeffrey Hutchinson, pers. observ.).

Water stargrass (*Heteranthera dubia*) is a submerged plant native to southern Canada and the United States (USDA-NRCS. 2021) with slender branching stems, ribbon-like leaves, and yellow emergent flowers which are distinctive characteristics of this species (Stutzenbaker, 1999) (Figure 4). The emergent leaves develop a waxy cuticle which aids in preventing predation and allows it to survive periods of low water (Smart et al., 2005). Additionally, water stargrass prefers mineral soils where salinities range from 0 to 0.5 ppt (Stutzenbaker, 1999). Water stargrass' distribution is listed as occasional (Lemke, 1989) making up 0.54% of the aquatic plant abundance in the San Marcos River (Hardy et al., 2010) with larger coverage occurring in the lower San Marcos River above the confluence with the Blanco River (Jeffrey Hutchinson, UTSA, personal communication). During a study to assess the volume of floating aquatic vegetation fragments in the upper San Marcos water stargrass accounted for < 0.01% of all fragments captured from March to December 2000 (Owens et al., 2001). Water stargrass has been documented to be successful in restoration projects in eutrophic lakes (Knopik and Newman, 2018) but its use in river restoration projects is unknown.

Figure 4. Emergent water stargrass, cultivated in the UTSA greenhouse, exhibiting linear blades and solitary yellow flowers.

A study conducted by Smart (1994) suggested that because of the similar growth rates of water stargrass and hydrilla and their tendencies to form canopies at the surface of the water, the removal of hydrilla would give water stargrass a competitive advantage allowing it to form a canopy that prevents hydrilla regrowth. Furthermore, a study conducted in a New York Lake found that water stargrass is more abundant when coexisting with the invasive species Eurasian watermilfoil (*Myriophyllum spicatum*) (Zhu and Georgian, 2014). The authors of this study suggested that when water stargrass is dominant, it can suppress the recruitment and reestablishment of invasive Eurasian watermilfoil and other non-natives, providing evidence that it can be a strong competitor against hydrilla. However, it is unknown how water stargrass and Texas wild rice interact with each other.

Illinois pondweed (Figure 5) is a native species that is distributed from northern Canada to Northern Mexico (USDA-NRCS, 2021). It prefers coarse to very coarse silica sediment and warm temperatures between 26-30 C° (Gosselin et al., 2018). Illinois pondweed is listed as common in the San Marcos River (Lemke, 1989) and makes up to 6.4% of the total aquatic macrophytes in the San Marcos River over different seasons (Hardy et al., 2010). Illinois pondweed is considered valuable for fish habitat and waterfowl food (Smart et al., 2005). Illinois Pondweed has been documented to have significantly higher growth rates than hydrilla when grown together (Bilbo, 2015). Illinois pondweed has similar morphology to Texas wild rice; both grow long ribbon-like leaves and have similar growth rates.

Figure 5. Illinois pondweed exhibiting thin submersed and floating elliptical shaped leaves in the San Marcos River.

The objective of the first *ex situ* greenhouse winter and summer study was to evaluate the competitive interactions of hydrilla, water stargrass, and Illinois pondweed grown at different ratios. The objective of the second *ex situ* greenhouse study was to examine the interactions of hydrilla, water stargrass, or Illinois pondweed grown together at equal ratios. Illinois pondweed was used as a surrogate species for Texas wild rice because state and federal permits were not obtained to grow Texas wild rice at the UTSA greenhouse. The objectives of the *in situ* study were to evaluate the growth of Texas wild rice and water stargrass in areas where different percentages of hydrilla have been manually removed and assess if either native species can suppress hydrilla regrowth. Evaluating the ability of Texas wild rice and water stargrass to suppress hydrilla is a unique opportunity to examine the interactions and competitive ability of an endangered species and a common native aquatic plant with a highly invasive species. In this study, we hypothesized that Illinois pondweed and water stargrass would exhibit rapid growth and suppress hydrilla when grown in a lower ratios than hydrilla in the *ex situ* study. It was further hypothesized that removal of hydrilla and planting water stargrass and Texas wild rice *in situ* would give native species a competitive advantage and result in the suppression of hydrilla.

Methods

Plant Propagation

Ex situ

The *ex situ* studies took place in the University of Texas at San Antonio's Department of Integrated Biology greenhouse under ambient conditions (Figure 6). Plants were maintained in 21 L mesocosms labeled with a number and placed on wooden tables inside of the greenhouse. An established colony of water stargrass, Illinois pondweed, and hydrilla located at the University of Texas at San Antonio's Department of Integrated Biology greenhouse were used for both of the greenhouse *ex situ* studies. Water stargrass, Illinois pondweed, and hydrilla were collected from the San Marcos River from June 2017-August 2017. Fragments 10 cm in length were inserted 5 cm into the sediment and placed in 21 L mesocosms. The water level was maintained at 18 cm deep and the plants were grown to a height of 75 +/- cm.

Figure 6. The UTSA Department of Integrated Biology greenhouse in San Antonio, Texas.

In situ

Texas wild rice was grown from seed and water stargrass was propagated from 10 cm fragments and planted in 0.5 L plastic pots filled with locally purchased topsoil that were composed of compost, topsoil, and cedar flakes (Hutchinson, 2017). The plants were placed at the 3600 L flow-through raceways in a greenhouse at the U.S. Fish and Wildlife Service's San Marcos Aquatic Resource Center (29° 50' 22.34"N, 97° 58' 32.00"W; Figure 7). The Texas wild rice and water stargrass were grown to a height of 75 +/- 20 cm before planting in the San Marcos River. No hydrilla was planted in the *in situ* study, instead, existing populations of established hydrilla in the San Marcos River were used for the *in situ* study.

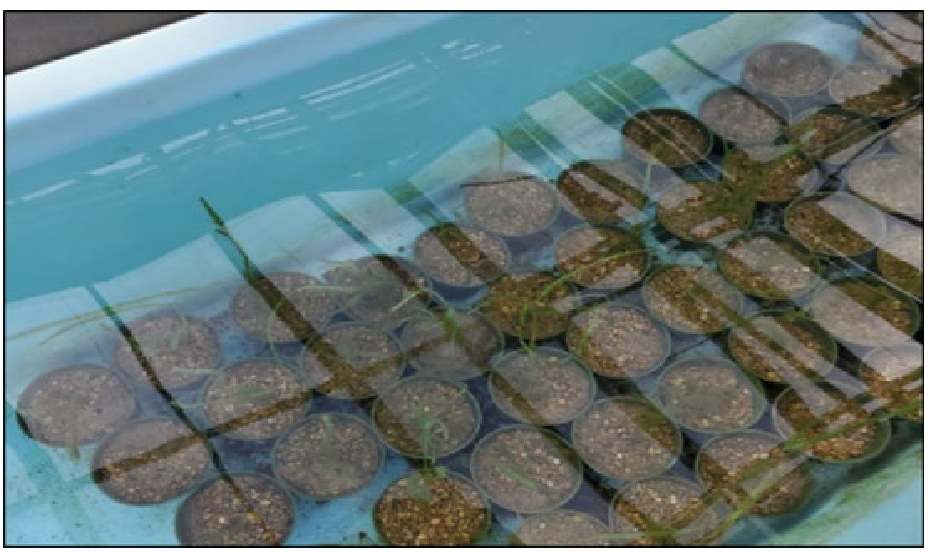


Figure 7. Texas Wild rice seedlings grown from seed at the USFWS San Marcos Aquatic Resource Center, San Marcos, Texas

Study Sites

Ex situ

The *ex situ* study site was located at the University of Texas at San Antonio Department of Integrated Biology greenhouse (Figure 6; 29° 34’ 45.00”N, 98° 37’ 45.41”W). The greenhouse is not climate controlled and subjected to ambient temperatures.

In situ

The *in situ* study site was located in the San Marcos River, Hays County, Texas. The San Marcos River originates from the groundwater supplied by the Edwards aquifer and flows 5.1 km to the confluence of the Blanco River before emptying into the Guadalupe River (Saunders, et al., 2001.) The San Marcos River is considered one of the most biologically diverse rivers in the Southwestern United States and provides habitat to eight endangered species (USFWS, 1996). The San Marcos River displays the consistent, uniform hydrological and physiochemical conditions of a spring fed river with a constant temperature of 22.2 °C (Groeger et al., 1997). The study site within the river is located in the upper 2.2 km of the San Marcos River. Three sites in the San Marcos River were selected that occur a minimum of 100 m apart with > 75% hydrilla coverage (Table 1, Figure 8 and 9). Daily discharge values, including flow from springs, groundwater, tributaries, and run-off, were taken from USGS gauge GS_08170500 in the San Marcos River (USGS, 2021), and water depth was recorded to the nearest cm at the time of initial planting.

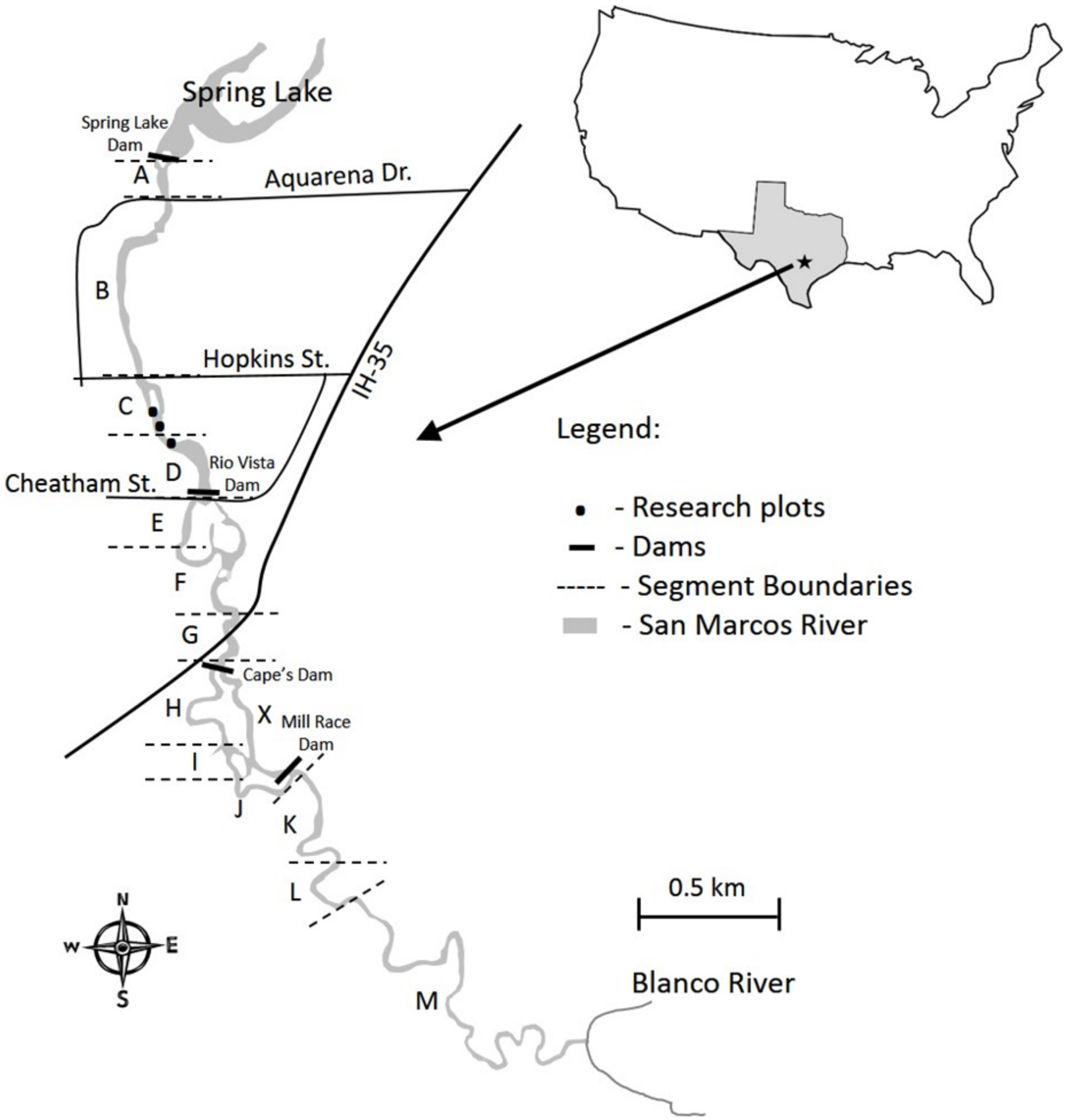


Figure 8. Map of the San Marcos River depicting dams, river segments, and research plots where hydrilla was removed in different percentages and planted with water stargrass and Texas wild rice. Research site 1 is at the north end, site 2 in the middle, and site 3 at the south end.

Figure 9. Photos of research sites with > 75% hydrilla coverage prior to removal of hydrilla.

Table 1. GPS coordinates of each research site in the San Marcos River where hydrilla was removed and planted with water stargrass and Texas wild rice.

Site Number	GPS Coordinates
Site #1	29° 52’ 53.21”N, 97 56’ 04.40”W
Site #2	29° 52’ 49.67”N, 97 56’ 01.73”W
Site #3	29° 52’ 48.98”N, 97 55’ 59.03”W

Experimental Design

Ex situ (experiment 1)

The *ex situ* examined the interactions between water stargrass and Illinois pondweed and evaluated their abilities to compete with hydrilla when grown in different ratios under static, no flow conditions. The study used an additive design in that the density of one species (hydrilla) varied while the density of the two native macrophytes (water stargrass and Illinois pondweed) remained constant (Harper, 1977). The experiment was conducted twice with a winter and summer study. Illinois pondweed was used as a surrogate species for Texas wild rice in the greenhouse study to evaluate this species’ ability to suppress hydrilla. The plant apical tips were planted at ratios of 32:5:5, 16:5:5, 8:5:5, 4:5:5, 2:5:5, 1:5:5, 5:0:0, 0:5:0, and 0:0:5, with the first value of each ratio representing hydrilla, the second value water stargrass and the third value Illinois pondweed, respectively (Table 2).

Table 2. Treatments for *ex situ* study 1 with the number of apical tips of each species planted per treatment.

Treatment	Number of Apical Tips Planted		
	Hydrilla	Water stargrass	Illinois Pondweed
1	32	5	5
2	16	5	5
3	8	5	5
4	4	5	5
5	2	5	5
6	1	5	5
7 Hydrilla	5	0	0
8 Water stargrass	0	5	0
9 Illinois pondweed	0	0	5

For each treatment, 15 cm apical tips of each macrophyte used in this study were collected from existing greenhouse plants. The apical tips were inserted 7.5 cm into the sediment and maintained in 21 L mesocosms (Figure 10). Within each mesocosm, the water temperature (C°), pH, total dissolved solids (mg L^{-1}), and conductivity (µS cm^{-1}) were recorded three times a week.

Figure 10. Mixed polyculture of hydrilla, water stargrass, and Illinois pondweed grown in 21 L mesocosms in the UTSA greenhouse.

Mean temperature was 24.8 (SE = 3.30) for the winter and 34.1 (SE=0.07) for the summer. Mean pH for the winter was 10.06 (SE = 0.39) and 8.86 (SE = 0.08) for the summer. Mean dissolved solids for the winter was 198.4 (SE = 3.3) and 219.6 (SE = 8.4) for the summer. Mean conductivity was 396.5 (SE = 6.5) for the winter and 431.0 (SE = 18.6) for the summer. All mesocosms were watered daily.

The plants were grown for 6 weeks for the summer study and 13 weeks for the winter study, and then harvested by sorting roots and shoots (Figure 11). Roots and shoots were placed in separate labeled paper bags and dried at 40 °C for 5 days in dryers. Dry weights were recorded to nearest 0.01 g for roots and shoots.

a)
b)
c)

Figure 11. Harvested hydrilla (a), water stargrass (b), and Illinois pondweed (c) plants separated by shoots and roots for dry weight biomass.

Ex situ (experiment 2)

The second *ex situ* greenhouse study evaluated the interactions between hydrilla and water stargrass or Illinois pondweed when grown at equal ratios, and provided further insight into the first *ex situ* study. There were five apical tips of each species planted for each treatment (Table 3) with four replicates of each treatment. Apical tips (15 cm) of each macrophyte used in this study were collected from existing greenhouse plants. The plants were inserted 7.5 cm into the sediment and maintained in 21 L mesocosms. Within each mesocosm, the water temperature (°C), pH, total dissolved solids (mg L^{-1}), and conductivity (µS cm^{-1}) were recorded three times a week for the duration of the study. The mean temperature was 26.22 (SE = 0.34). The mean pH was 9.45 (SE = 0.08). The mean electrical conductivity was 502.88 (SE = 10.07). The mean total dissolved solids was 251.51 (SE = 5.07). All mesocosms were filled with water each day, and algae was removed using a small net. Following planting in the mesocosms, the plants were grown for six weeks from March 2021 to May 2021 and harvested by sorting roots and shoots of each species. Roots and shoots were placed in separate labeled paper bags and dried at 40 °C using dryers. Dry weights of each plant were recorded to nearest 0.01 g for roots and shoots.

Table 3. Treatments for *ex situ* study 2 with the number of apical tips of each species planted.

	Number of Apical Tips Planted		
Treatment	Hydrilla	Water stargrass	Illinois Pondweed
1	5	0	5
2	5	5	0
3	0	5	5
4 Hydrilla	5	0	0
5 Water stargrass	0	5	0
6 Illinois pondweed	0	0	5

In situ study

To evaluate if water stargrass and Texas wild rice can become established and compete in areas where hydrilla has been manually removed, three research sites > 100 m apart were designated in the San Marcos River where hydrilla coverage is > 75%. In October 2020, at each of the three research sites, 0, 25, 50, 75, or 100% of hydrilla was manually removed by United States Fish and Wildlife Service scuba divers within weighted 0.25 m^2 plots made with PVC pipe (Figure 12). An additional 0.2 m of hydrilla was removed around the plot to create a buffer from the surrounding hydrilla at the site (Figure 13).

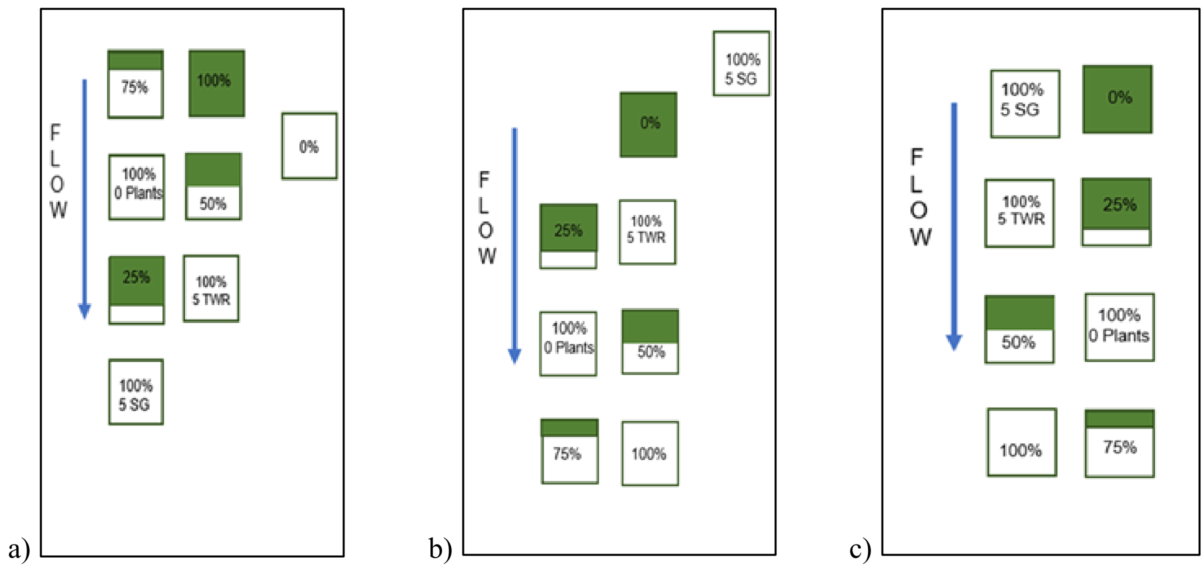


Figure 12. Experimental design for *in situ* plots at Site 1 (a), Site 2 (b), and Site 3(c) in the San Marcos River. Plots were placed in a randomized design among the three sites. Percentages indicate the percent hydrilla removed from each plot, and the green shading represents hydrilla not removed. Unless noted (i.e., 0 plants, 5 TWR, or 5 SG), five water stargrass (WG) and five Texas wild rice (TWR) were planted in each plot.

Figure 13. Photo of 0.25 m^2 research plot where 100% hydrilla was removed and a 0.2 m buffer was established in the San Marcos River.

On November 10, 2020, USFWS scuba divers removed the remaining hydrilla including roots, tubers, and any hydrilla regrowth. The same day, five water stargrass and five Texas wild rice plants, each 75 +/- cm in height, were planted randomly within five of the eight 0.25 m^2 plots in each of the three research sites. Adjustments were made when plots did not fall in areas where hydrilla coverage was < 75%. Additionally, there was a control plot at each site where 100% hydrilla was manually removed and no native plants were planted within a plot, and two other plots where 100% hydrilla was manually removed and five Texas wild rice or water stargrass were planted in separate plots (Figure 13). One replicate of each plot was placed at each site for a total of 24 plots. At each site, water depth (m) was recorded.

One replicate of each treatment was placed at each site for a total of three replicates per treatment. Each plot was marked by a 0.4 m rebar that was placed in each corner of the plot and covered with orange rebar cap. A waterproof Vibra Tector 740 Waterproof Pinpointer metal detector (Treasure Products Inc., Simi Valley, CA, 93065) was used to locate each plot during monitoring. Hydrilla removed from each plot was collected and oven-dried at 90 °C for 5 days. Following drying, dry weights were recorded to the nearest 0.01g.

Plant coverage was monitored for six months from January 2021-July 2021. No monitoring took place in December 2020 to allow for acclimation or February 2021 because of the unprecedented freeze. Percent cover was estimated by counting the number of squares in the measuring grid based on each species present within each grid (Figure 14).

Figure 14. Grid system used to estimate aquatic plant species coverage for *in situ* study.

On July 29, 2021, all plants within plots were harvested by USFWS divers for dry weights. Each species of plant was sorted by roots and shoots and placed in labeled paper bags and dried at 90 °C for 5 days using dryers. Dry weights were recorded to nearest 0.01 g for roots and shoots.

Statistical Analysis

Ex situ

Descriptive statistics (means and SE) were calculated for all variables. Relative growth rate was analyzed using the method of Hunt (1990):

$$\text{RGR}=\frac{\ln(x_2)-\ln(x_1)}{t_2-t_1}$$

Where x_1 and x_2 are mean plant dry biomass at t_1 (Day 0) and t_2 (winter study = 93 days, summer = 42 days) respectively.

Data were checked for parametric assumptions of normality and equality of variance prior to analysis. Data that did not meet the parametric assumptions underwent a transformation (Ln, square root, or arcsine-square root). For initial analysis, the winter and summer greenhouse were combined as one composite sample. Data were maintained in Excel spreadsheets, and analyzed using SigmaPlot (Version 14.0, Systat Software, Inc., San Jose CA) and PC-ORD (Version 5.10, MjM Software, Glenden Beach, OR).

The combined data (summer and winter) were analyzed for differences among treatments and by season with a non-parametric PerMANOVA at $P < 0.05$. Data were then analyzed separately by season with univariate tests (one-way ANOVA or Kruskal-Wallis test) for differences among treatment. If data did not meet assumptions of normality and equality of variance, the data were transformed as described above. If data still not meet parametric assumptions, then a non-parametric Kruskal-Wallis test was used in the analysis. If differences ($p < 0.05$) were detected with univariate test, a Tukey's or Dunn's mean separation test was used to determine differences ($P < 0.05$) among treatments.

The second *ex situ* study was analyzed using one-way ANOVA based on treatment and plant species for the shoot biomass, root biomass, root to shoot ratio, and relative growth rate (RGR). The data for all four variables in the second study met all the assumptions for parametric analysis.

In situ

Descriptive statistics (means and SE) were calculated for all variables. Survival of each plant was determined by counting the number of plots that contained each species and dividing by the total number of plots. Data were analyzed using a one-way repeated measures ANOVA to analyze coverage of plant species (hydrilla, Texas wild rice, and water stargrass) based on treatment (percent of hydrilla removed). A three-way ANOVA was used to test biomass of plant species based on site, treatment, and plant species. Data were tested for normality and equality of variance with Shapiro-Wilks and Brown-Forsythe tests, respectively. If data did not the assumptions, the data were ln transformed (Sokal and Rohlf, 1995). Tukey's HSD or Dunn's test was used to separate means. When significant interaction was present, a one-way ANOVA was used to test dry weight biomass of hydrilla, Texas wild rice, and water stargrass individually.

Results

Ex situ Study 1

At the end of both the winter and summer studies, plants had grown to and covered > 95% of the surface. No differences were detected among treatment ($F = 0.6$, $df = 5$, $P = 0.83$) but significant differences were detected between season ($F = 23.1$, $df = 5$, $P = 0.0002$) with the PerMANOVA (Table 4). No interactions were detected between treatment and season ($F = 1.03$, $df = 5$, $P = 0.41$). Data was analyzed separately by season for the winter and summer *ex situ* study.

Table 4. PerMANOVA results showing no difference (P = 0.83) among treatments but significant difference (P = 0.0002) among seasons. No interaction was detected between treatment and season (P = 0.41).

	df	MS	F statistics	P value
Treatment	5	0.07	0.60	0.83
Season	1	2.71	23.10	0.0002
Interaction	5	0.12	1.03	0.41
Residual	132	0.12		
Total	143			

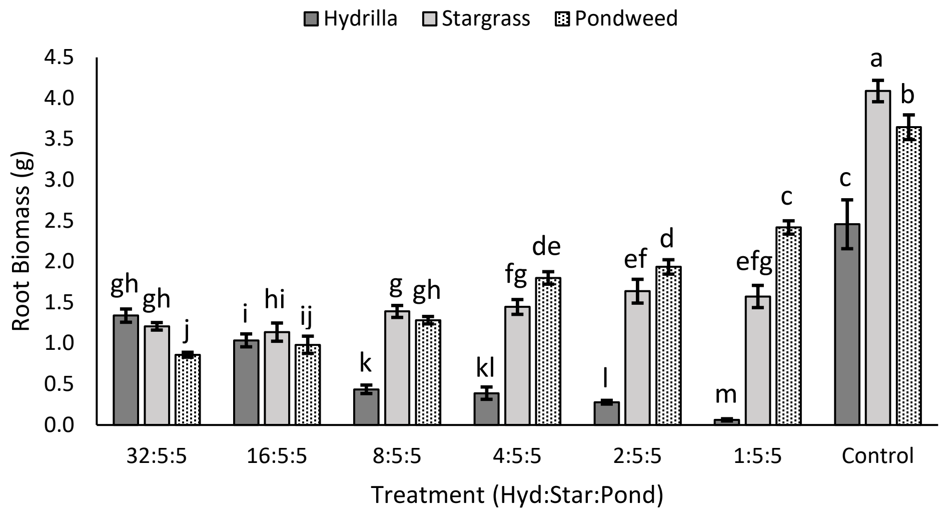
Winter study

Significant differences were found for root biomass (H = 98.9, df = 26, P < 0.001) among plant species and treatment. Illinois pondweed and water stargrass had significantly greater root biomass compared to hydrilla at the 8:5:5, 4:5:5, 2:5:5, 1:5:5 ratios and controls (Figure 15A). Significant differences (P < 0.05) were found among controls with water stargrass having greater root biomass than Illinois pondweed and hydrilla, but Illinois pondweed having greater biomass than hydrilla.

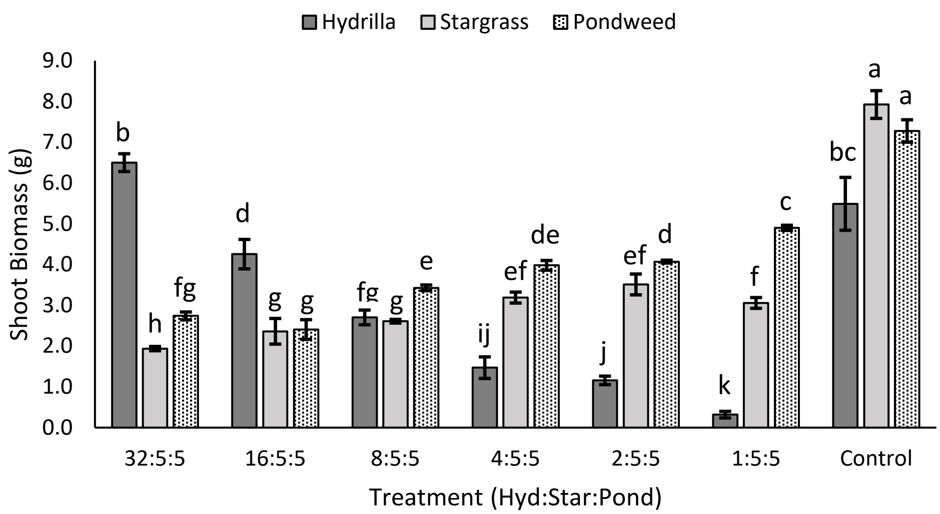
For shoot biomass, significant differences were detected among plant species and treatment (F = 30.7, df = 26, P < 0.0001) (Fig 15B). Hydrilla exhibited significantly (P < 0.05) greater shoot biomass compared to water stargrass and Illinois pondweed when planted at higher ratios of 32:5:5 and 16:5:5, but shoot biomass decreased at the 8:5:5 ratio. At treatment ratios of 4:5:5, 2:5:5, and 1:5:5, water stargrass and Illinois pondweed shoot biomass was significantly greater than hydrilla.

Water stargrass and Illinois pondweed allocated greater biomass into their roots than hydrilla based on root to shoot ratios (F = 3.7, df = 26, P < 0.0001)(Fig. 15C) for all treatments except controls. In controls, hydrilla allocated greater biomass into it roots compared to shoots compared to all other treatments.

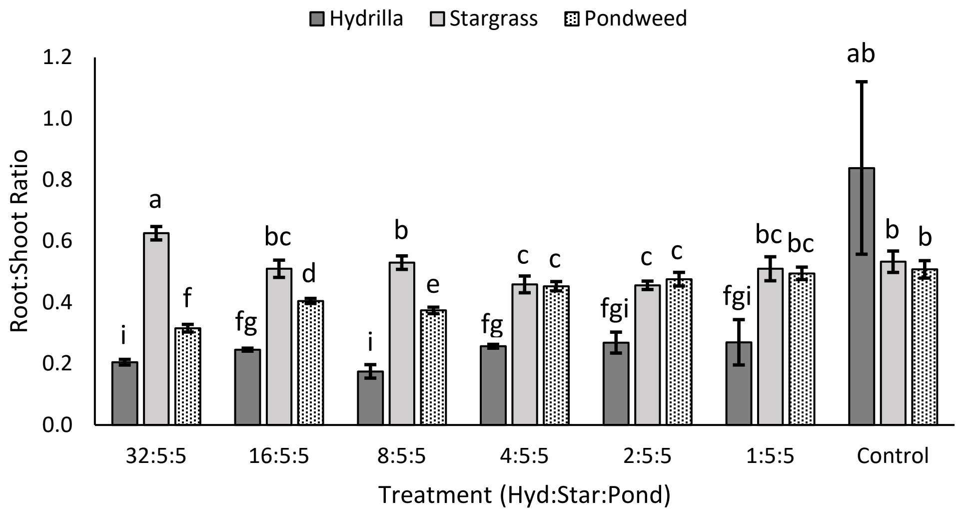
Relative growth rates were significantly different among plant species and treatment (H = 110.3, df - 26, P < 0.0001) for water stargrass grown alone compared to all treatments (Fig. 15D). The RGR for hydrilla exhibited a significant decline (P < 0.05) at a planting ratio of 4:5:5 with native plants and at higher ratios. The RGR of water stargrass and Illinois pondweed were significantly different for ratios of 4:5:5, 2:5:5, and 1:5:5 compared to hydrilla. Most noticeably, the RGR of native plants remained relatively consistent among all treatments while hydrilla exhibited a decline.


Hydrilla
Stargrass
Pondweed
Root Biomass (g)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
gh
gh
j
i
hi
ij
k
g
gh
kl
fg
de
l
ef
d
m
efg
c
c
a
b
32:5:5
16:5:5
8:5:5
4:5:5
2:5:5
1:5:5
Control
Treatment (Hyd:Star:Pond)

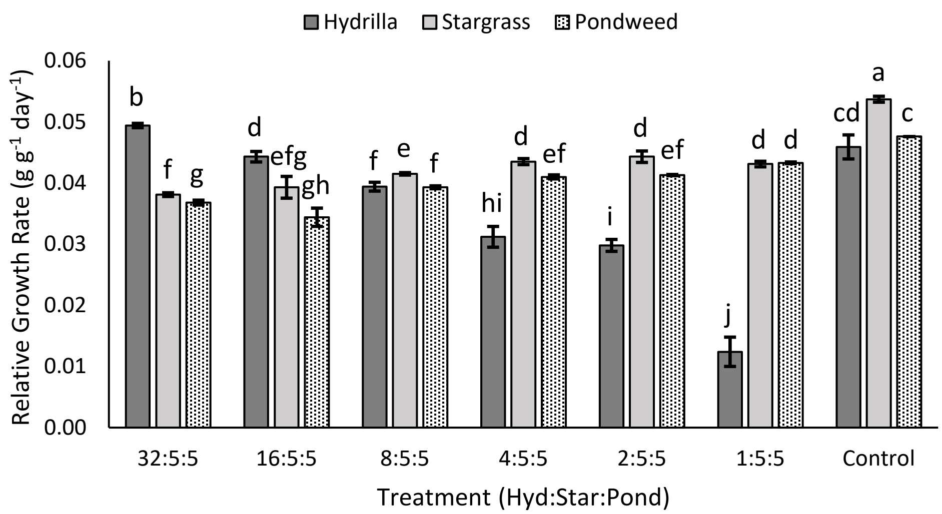
A)


Hydrilla
Stargrass
Pondweed
Shoot Biomass (g)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
b
h
fg
d
g
g
fg
g
e
ij
ef
de
j
ef
d
k
f
c
bc
a
a
32:5:5
16:5:5
8:5:5
4:5:5
2:5:5
1:5:5
Control
Treatment (Hyd:Star:Pond)

B)



C)



D)

Figure 15. Mean dry weights (g) for A) roots ($H = 98.9$, $df = 26$, $p < 0.001$), B) shoots ($F = 30.7$, $df = 26$, $p < 0.001$), C) root to shoot ratio ($F = 3.7$, $df = 26$, $p < 0.001$), and D) relative growth rate ($H = 110.3$, $df = 26$, $p < 0.001$) of hydrilla (Hyd), water stargrass (Star), and Illinois pondweed (Pond) planted at different ratios for 13 weeks during the winter (October 2019 to January 2020). Bars represent standard error and different letters represent significant differences based on a one-way ANOVA or Kruskal-Wallis test and Tukey's or Dunn's mean separation test ($p < 0.05$).

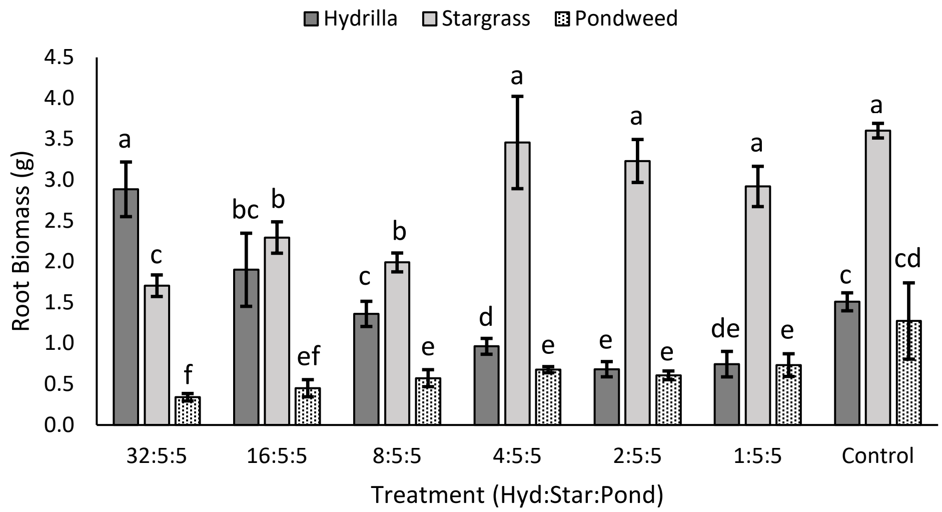
Summer study

Significant differences were found for root biomass (F = 8.79, df = 26, P < 0.001) among plant species and treatment. Water stargrass had significantly greater differences (P < 0.05) for root biomass at all ratios compared to hydrilla, except at the 16:5:5 ratio. Illinois pondweed had significantly greater differences (P < 0.05) for root biomass at ratios 4:5:5, 2:5:5, 1:5:5 and controls compared to hydrilla (Figure 16A). At the treatment ratio of 16:5:5 water stargrass surpassed the mean hydrilla biomass. The results for the root biomass indicate that Illinois pondweed had the lowest overall mean biomass.

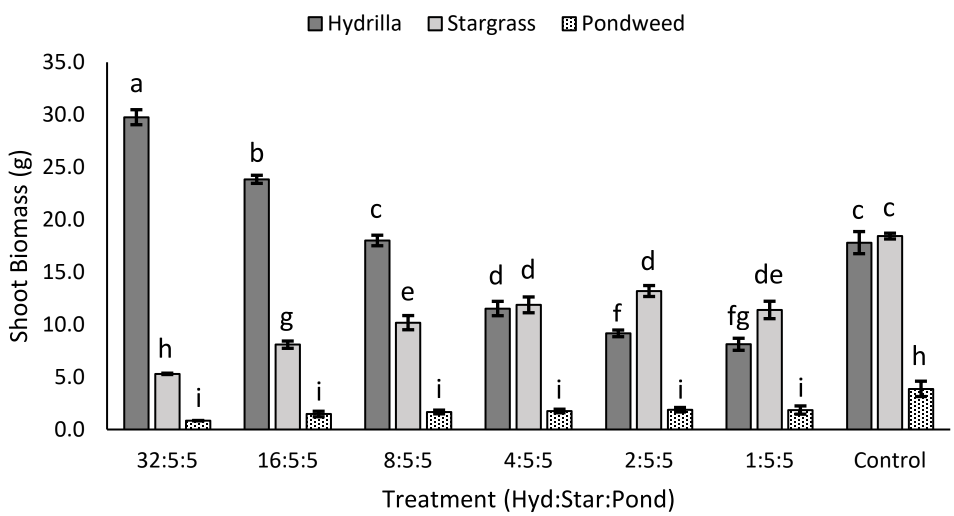
Hydrilla exhibited significantly (P < 0.05) greater shoot biomass compared to water stargrass and Illinois pondweed at treatment ratios 32:5:5, 16:5:5, and 8:5:5 (Figure 16B). At the treatment ratio of 4:5:5, water stargrass had a similar biomass to hydrilla, and was significantly greater than hydrilla at Treatment 2:5:5 and 1:5:5. For shoot biomass, Illinois pondweed produced significantly less biomass than hydrilla and water stargrass for every treatment including the control.

The root to shoot ratios were significantly greater (P < 0.05) for water stargrass and Illinois pondweed compared to hydrilla at all ratios indicating the two native species were allocating more biomass into their roots (Figure 16C). Hydrilla allocated greater biomass into it shoots which accounted for ca. 70-80% of its total biomass while water stargrass and Illinois pondweed allocated more than 20% of their biomass into the shoots for every treatment. No trends were observed among treatments for root to shoot ratio.

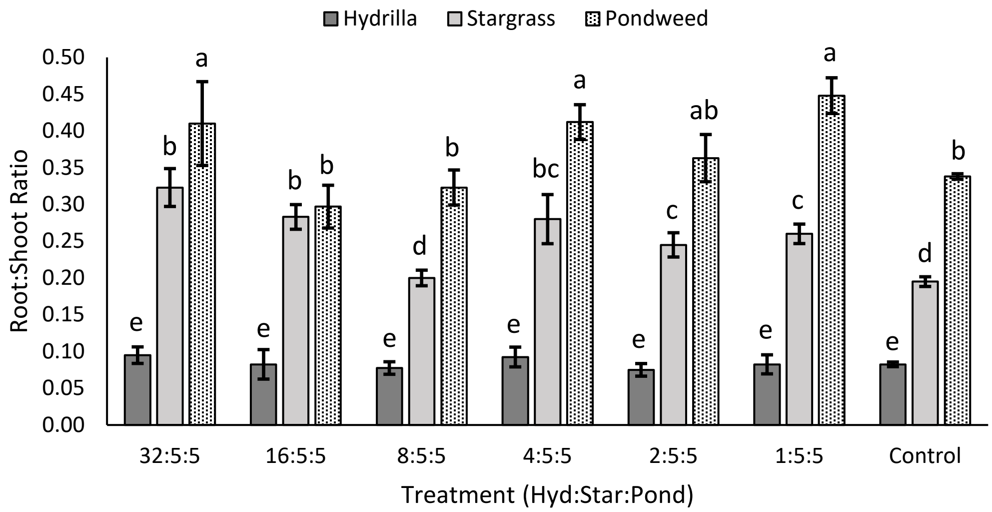
The RGR indicated that hydrilla and water stargrass were significantly greater (P < 0.05) for all treatment ratios compared to Illinois pondweed (Figure 16D). As with shoot biomass, hydrilla exhibited significantly (P < 0.05) greater RGR compared to water stargrass and Illinois pondweed at treatment ratios 32:5:5, 16:5:5, and 8:5:5. At treatment ratios of 4:5:5, 2:5:5, 1:5:5, and controls, water stargrass had greater (P < 0.05) RGR than hydrilla and Illinois pondweed. Illinois pondweed has the overall lowest RGR for every treatment. The RGR for water stargrass showed a subtle negative correlation with the number of hydrilla, and hydrilla demonstrated a relatively positive correlation with the number of hydrilla planted.


Hydrilla
Stargrass
Pondweed
Root Biomass (g)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
c
f
bc
b
ef
c
b
e
d
a
e
e
a
e
de
a
e
c
a
cd
32:5:5
16:5:5
8:5:5
4:5:5
2:5:5
1:5:5
Control
Treatment (Hyd:Star:Pond)

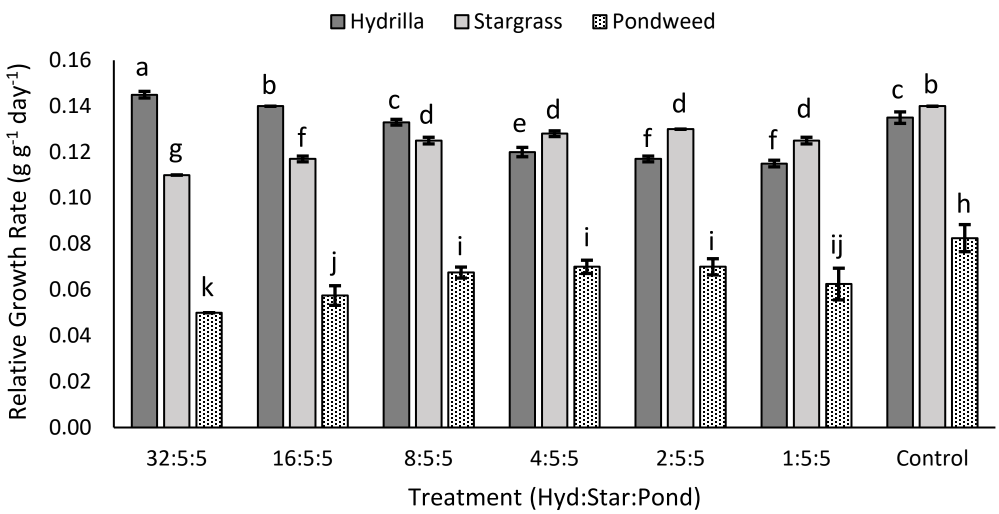
A)


Hydrilla
Stargrass
Pondweed
Shoot Biomass (g)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
a
h
i
b
g
i
c
e
i
d
d
i
f
d
i
fg
de
i
c
c
h
32:5:5
16:5:5
8:5:5
4:5:5
2:5:5
1:5:5
Control
Treatment (Hyd:Star:Pond)

B)



C)

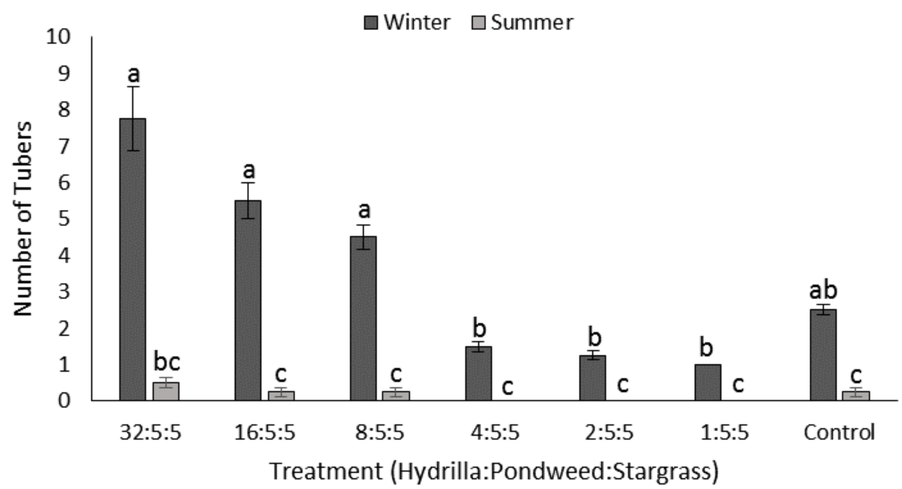


D)

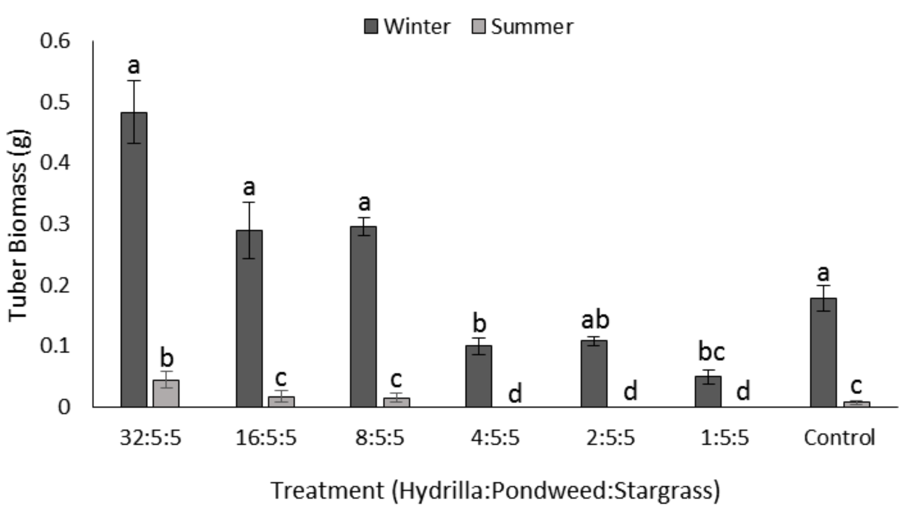
Figure 16. Mean dry weights (g) for A) roots ($F = 8.79$, $df = 26$, $p < 0.001$), B) shoots ($H = 102.7$, $df = 26$, $p < 0.001$), C) root to shoot ratio ($H = 97.3$, $df = 26$, $p < 0.001$), and D) relative growth rate ($H = 103.2$, $df = 26$, $p < 0.001$) of hydrilla (Hyd), water stargrass (Star), and Illinois pondweed (Pond) planted at different ratios for 6 weeks during the summer (June to July 2020). Bars represent standard error and different letters represent significant differences based on a one-way ANOVA or Kruskal-Wallis test and Tukey's or Dunn's mean separation test ($p < 0.05$).

Tubers

At all treatment ratios, hydrilla produced significantly more tubers (F = 13.66, df = 13, P < 0.001) and greater total tuber biomass (F = 14.40, df = 13, p < 0.001) in the winter compared to the summer study (Figure 17). The treatments during winter months affected the number of tubers produced. When more hydrilla was planted than native species at ratios of 32:5:5, 16:5:5, 8:5:5, the number of tubers produced and tuber biomass were significantly greater than when there were more native species planted at ratios of 4:5:5, 2:5:5, and 1:5:5. For the summer, tuber production was limited and no tubers were produced in the 4:5:5, 2:5:5, and 1:5:5 treatment ratios.



A)



B)

Figure 17. Mean number of hydrilla tubers for A) the summer and winter study (df = 13, F = 13.66, $p < 0.001$), and B) mean tuber biomass for the summer and winter study (df = 13, F = 14.40, $p < 0.001$) planted at different ratios for 13 weeks during the winter (October 2019 to January 2020) and 6 weeks during the summer (June 2020 to July 2020). Bars represent standard error and different letters represent significant differences based on a one-way ANOVA and Tukey's test ($p < 0.05$).

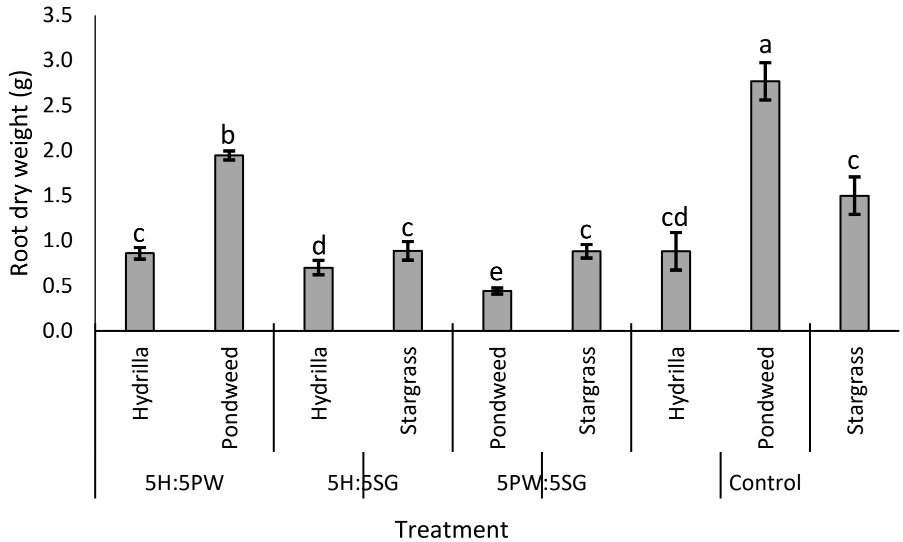
Ex situ study 2

Illinois pondweed controls had significantly greater root biomass among all treatments and species (F = 22.4, df = 8, $P < 0.001$)(Figure 18A). Illinois pondweed had greater root biomass when grown in equal ratios with hydrilla ($P < 0.05$), but significantly less root biomass when grown in equal ratios with water stargrass ($P < 0.05$). The root biomass of hydrilla and water stargrass roots were significantly different ($P < 0.05$) when alone than when grown with other species.

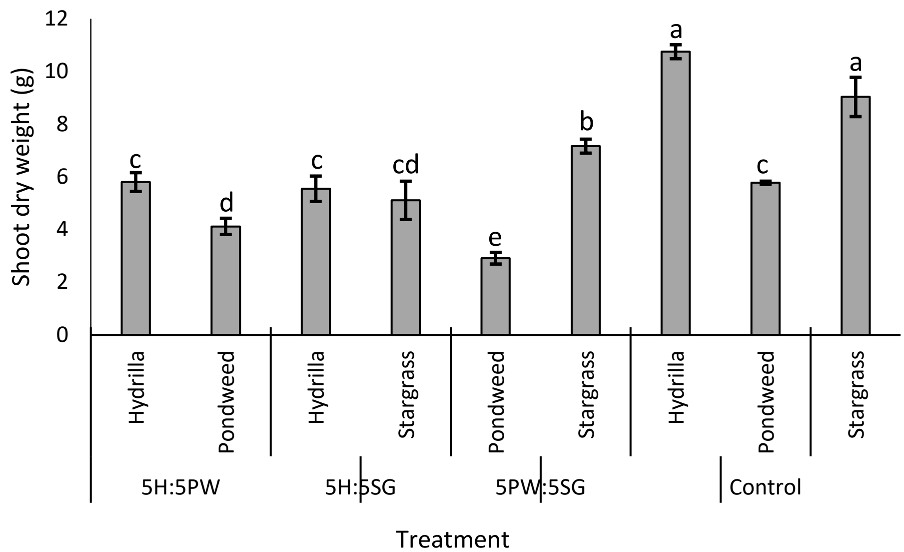
Hydrilla and water stargrass shoot biomass were significantly different when grown alone compared to all other treatments (F = 7.8, df = 8, $P < 0.001$)(Figure 18B). Compared to controls, there was a significant reduction ($P < 0.05$) in hydrilla shoot growth when grown in equal ratios with water stargrass or Illinois pondweed compared to hydrilla grown alone. However, hydrilla shoot biomass was significantly greater than Illinois pondweed when grown in equal ratios ($P < 0.05$), but no difference was detected in shoot biomass for hydrilla and water stargrass grown together in equal ratios. Water stargrass had a significantly greater shoot biomass than Illinois pondweed when grown together. Overall, Illinois pondweed sequestered less biomass into its shoots when grown alone and in equal ratios with hydrilla and water stargrass.

Illinois pondweed had significantly greater root to shoot ratio compared to water stargrass and hydrilla when grown alone and at equal ratios with hydrilla (F = 11.1, df = 8, $P < 0.001$)(Figure 18C). Water stargrass and hydrilla exhibited low root to shoot ratios and allocated greater biomass into their shoots compared to their roots. The treatments had no significant effect ($P > 0.05$) on the root to shoot ratio for hydrilla compared to the controls.

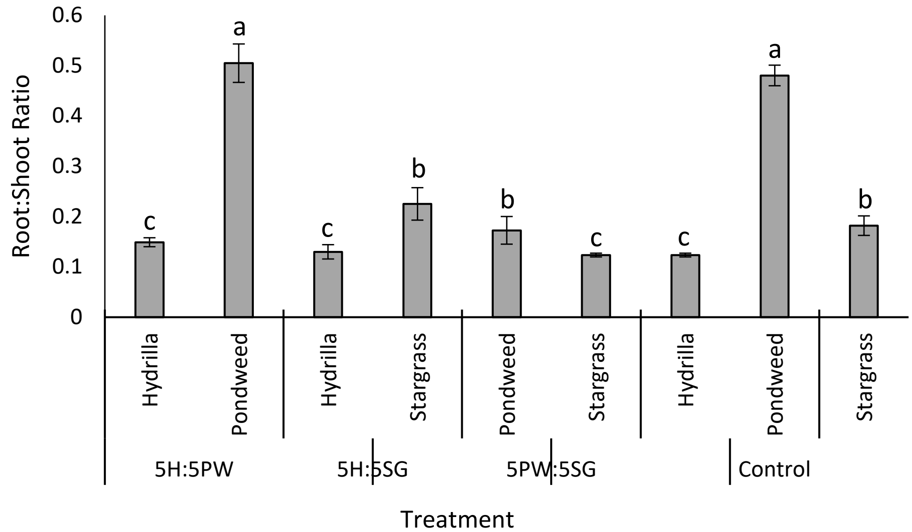
Hydrilla and water stargrass when grown alone had the highest RGR compared to all other treatments (F = 15.9, df = 8, $P < 0.001$)(Figure 18D). There was a significant difference ($P < 0.05$) in the RGR for hydrilla when grown in equal ratios with Illinois pondweed, but no difference ($P < 0.05$) was detected between hydrilla and water stargrass when grown in equal rations. Illinois pondweed had a significantly lower RGR compared to water stargrass and hydrilla for the controls and both treatments ($P < 0.05$).


Root dry weight (g)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
c
b
d
c
e
c
cd
a
c
Hydrilla
Pondweed
Hydrilla
Stargrass
Pondweed
Stargrass
Hydrilla
Pondweed
Stargrass
5H:5PW
5H:5SG
5PW:5SG
Control
Treatment

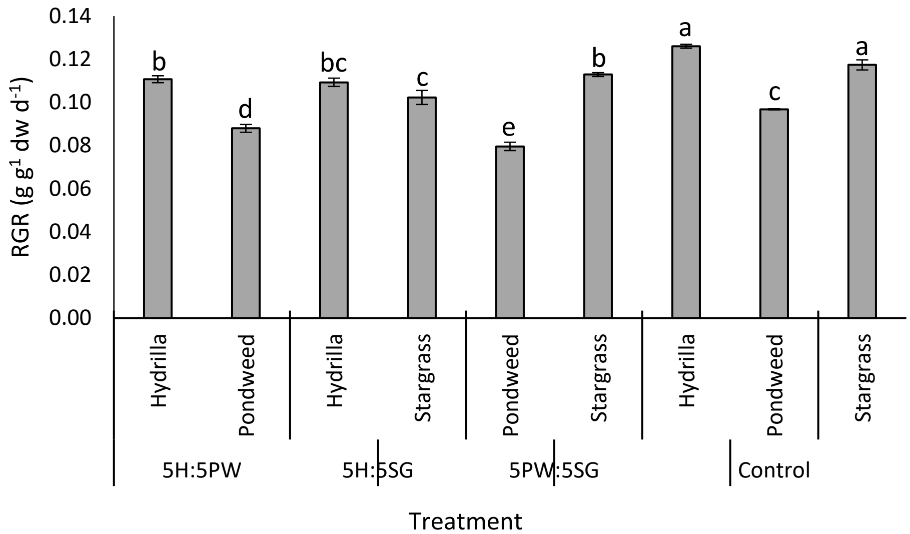
A)


Shoot dry weight (g)
12
10
8
6
4
2
0
c
d
c
cd
e
b
a
c
a
Hydrilla
Pondweed
Hydrilla
Stargrass
Pondweed
Stargrass
Hydrilla
Pondweed
Stargrass
5H:5PW
5H:5SG
5PW:5SG
Control
Treatment

B)



C)



D)

Figure 18. Mean dry weights (g) for A) roots (df = 8, F = 22.38, $p < 0.001$), B) shoots (df = 8, F = 7.75, $p < 0.001$), C) root to shoot ratio (df = 8, F = 11.1, $p < 0.001$), and D) relative growth rate (df = 8, F = 15.85, $p < 0.001$) for hydrilla, water stargrass (Stargrass), and Illinois pondweed (Pondweed) planted at equal ratios for 6 weeks from March-May 2021. Bars represent standard error and different letters represent significant differences based on a one-way ANOVA and Tukey's mean separation test ($p < 0.05$).

In situ Study

River Discharge

River discharge ($m^3 s^{-1}$) varied from 3.76 $m^3 s^{-1}$ (132 $ft^3 s^{-1}$) during hydrilla removal and planting during November 2020, to a low of 2.9 $m^3 s^{-1}$ (102 cfs) in April 2021, but increased to 4.8 $m^3 s^{-1}$ (172 cfs) during June and July of 2021 (Figure 19). A slight positive correlation ($r = 0.47$) was found for water stargrass, and weak correlations were observed for hydrilla ($r = -0.14$) and Texas wild rice ($r = 0.21$) with discharge.

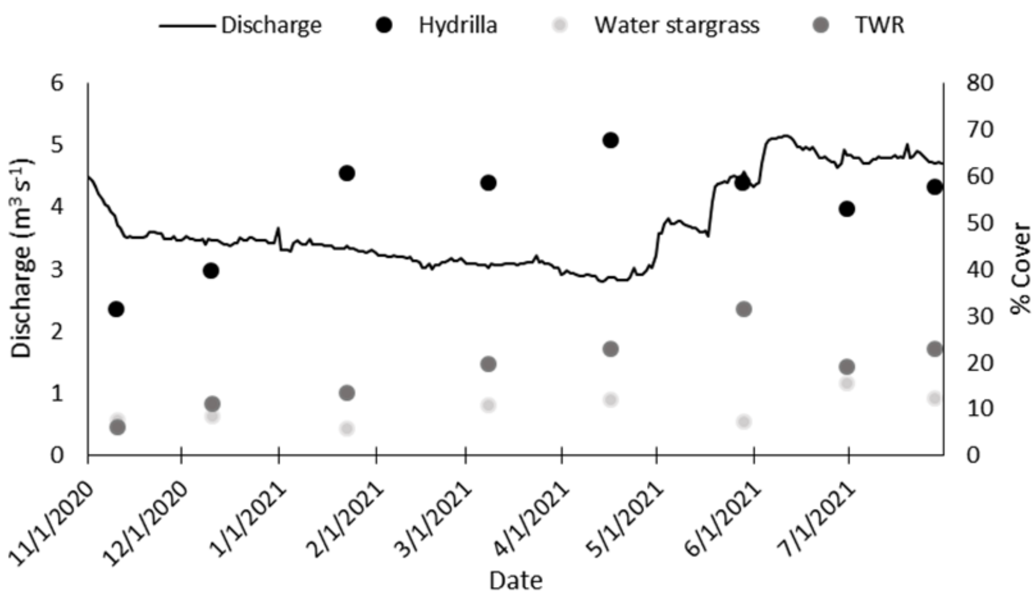


Figure 19. Mean daily discharge patterns and plant coverage of hydrilla, water stargrass, and Texas wild rice (TWR) from November 2020 to July 2021 in the San Marcos River.

Survival

Hydrilla was present in all plots (n = 24) at the end of the study regardless of percent removal at the beginning of the study. Hydrilla quickly invaded plots in which 100% of the plot was initially cleared of hydrilla. The survival of native plants was lower than hydrilla at the end of the study. Texas wild rice and water stargrass were present in 50% (n = 12) and 42% (n = 10) of 24 plots, respectively.

Percent Cover

The mean percent cover of hydrilla at the end of the study was significantly influenced by the percentage of hydrilla at the start of the study in each plot and if native plants were planted ($F = 15.98$, $df = 23$, $P < 0.001$)(Figure 20). Hydrilla coverage increased in plots where 100 to 0 % removal occurred and Texas wild rice and water stargrass were planted. In plots where 100% of the hydrilla was removed at the start of the study and no plants planted, hydrilla reestablished with a mean coverage of 70% at the end of the study. In plots where 100% of the hydrilla was removed, only Texas wild rice exhibited greater or equal cover to hydrilla where Texas wild rice was planted. Hydrilla coverage was significantly greater ($P < 0.05$) following 100% removal and planting of water stargrass.

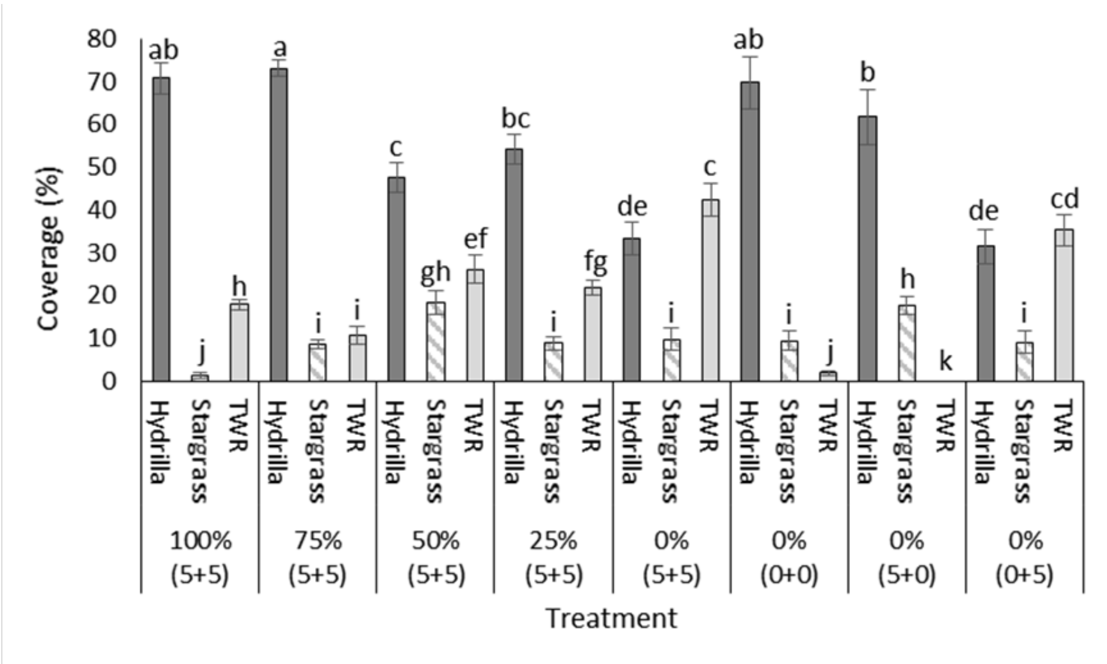


Figure 20. Mean percent coverage and standard error bars of hydrilla, water stargrass (stargrass), and Texas wild rice (TWR) for eight treatments where hydrilla was removed from 0.5 m^2 plots ($n = 3$ plots per treatment) in the San Marcos River. Treatments indicate the percent coverage of hydrilla at the time of planting and the numbers in parentheses indicate the number of water stargrass and Texas wild rice planted, respectively, in each 0.5 m^2 plot. Difference letters indicate significant difference based on a one-way repeated measures ANOVA ($F = 15.98$, df = 23, $P < 0.001$) and Tukey's mean separation test ($P < 0.05$).

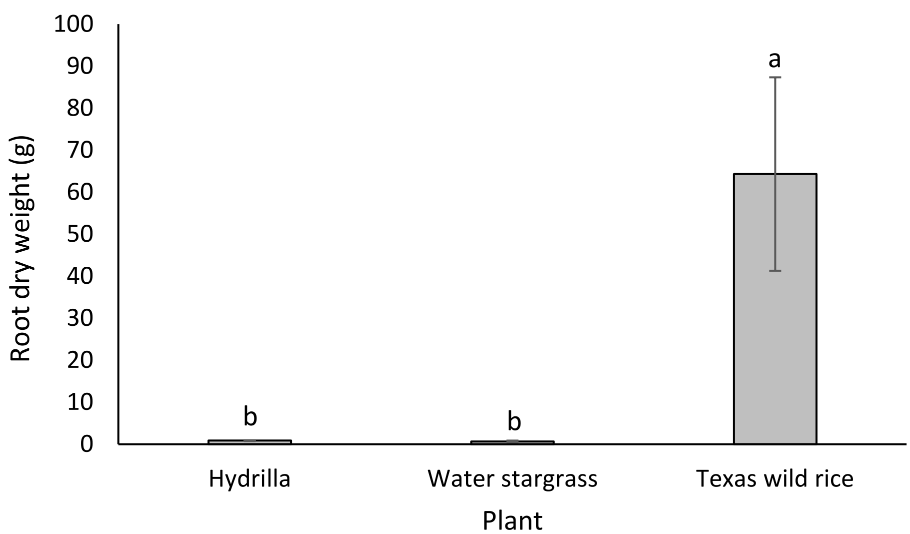
Dry Biomass

Analysis of dry biomass with a three-way ANOVA found significant differences among plant species ($F = 11.8$, df = 2, $P < 0.001$) but no significant differences among plots ($F = 0.68$, df = 7, $P = 0.69$) and sites ($F = 0.02$, df = 2, $P = 0.99$)(Table 5). No interactions ($P > 0.05$) were found and dry biomass was combined as one composite sample and analyzed with a one-way ANOVA for differences among plant species.

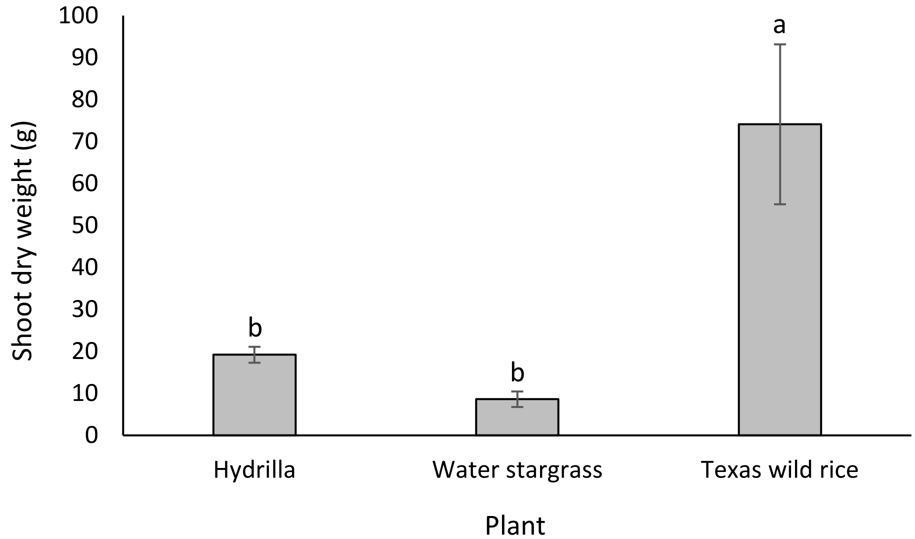
Table 5. Three-way ANOVA results showing significant difference among the plant species (P = 0.04) but no significant difference among the Plot (p=0.692) and Site (p=0.985) in the *in situ* study.

	df	MS	F statistics	P value
Plant	2	220.2	11.8	< 0.001
Plot (trt)	7	12.6	0.68	0.69
Site	2	0.28	0.02	0.99
Residual	60	18.73		
Total	71			

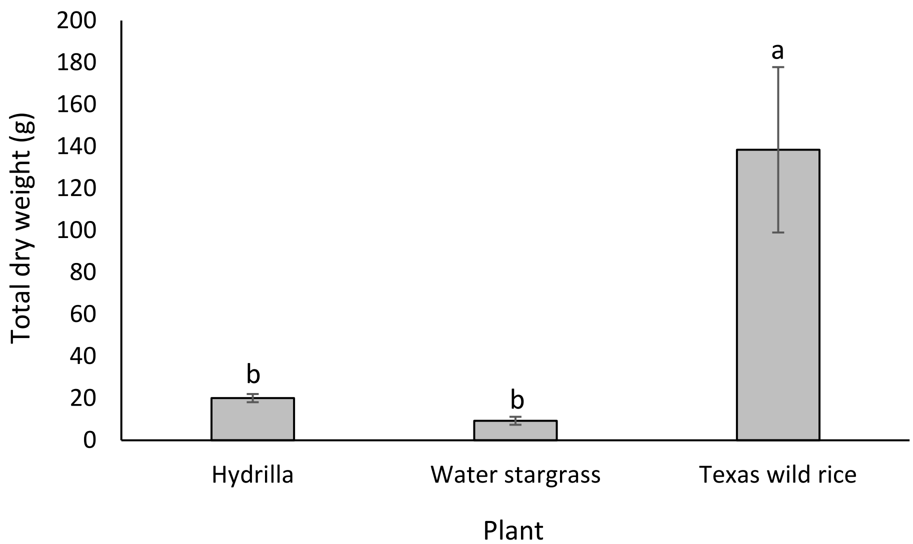
Texas wild rice survival in plots was 50%, but dry weight biomass was significantly greater in plots where it survived compared to hydrilla and water stargrass. The dry biomass was significantly different for Texas wild rice root (F = 3.39, df = 2, P = 0.04)(Figure 21A), shoot (F = 12.5, df = 2, P < 0.001)(Figure 21B), and total biomass (F = 11.80, df = 2, P < 0.001)(Figure 21C) based on a one-way ANOVA compared to hydrilla and water stargrass. No differences (P > 0.05) were detected in dry biomass between hydrilla and water stargrass.



a)



b)



c)

Figure 21. Mean dry weight of hydrilla, water stargrass, and Texas wild rice per plot for A) root biomass ($F = 3.39$, $df = 2$, $P = 0.04$), B) shoot biomass ($F = 12.5$, $df = 2$, $P < 0.001$), and C) total biomass ($F = 11.80$, $df = 2$, $P < 0.001$) based on a one-way ANOVA and Tukey's mean separation test ($P < 0.05$). Different letters represent significant differences among biomass and bars represent standard error.

Discussion

Ex situ study 1

In partial agreement with the hypothesis for the *ex situ* study, water stargrass and Illinois pondweed allocated greater biomass into their roots and shoots compared to hydrilla during the winter study. Breakpoints in the statistical analysis occurred with greater native plant biomass at the 8:5:5 ratio for roots and the 4:5:5 ratio for shoots, respectively, but the root and shoot biomass for hydrilla was equal or greater at higher planting ratios compared to the two native plants. During the winter and summer study, both water stargrass and Illinois pondweed allocated a significantly greater proportion of biomass into their roots compared to their shoots. Wang et al. (2008) found contrasting results with *Myriophyllum spicatum* growing with hydrilla in which *M. spicatum* allocated greater biomass to its shoots due to reduced light from shading. In contradiction to the hypothesis, Illinois pondweed root and shoot biomass were significantly lower than hydrilla during the winter study, with Illinois pondweed allocating about 90% of its biomass to its roots. Gosselin et al. (2019) found that Illinois pondweed reached in maximum growth in the summer months when water temperatures ranged from 26-30 °C. In the study, the summer temperatures and interspecific competition may have resulted in the lower growth rates of Illinois pondweed.

In this study, hydrilla produced significantly more tubers in the winter compared to the summer study. The combination of a shorter photoperiod and lower temperatures during the fall and winter months cause hydrilla to allocate more energy into tuber and roots compared to summer months (Van et al., 1978). The low root to shoot biomass exhibited by hydrilla in the summer and winter study may indicate that hydrilla allocated more to energy to its shoots to gain a competitive advantage due to interspecific competition. In addition, hydrilla allocated a greater amount of biomass to tuber production than its roots.

McFarland and Barko (1999) found that as temperatures increase hydrilla allocates greater biomass to shoots, but as temperatures decrease this species allocates greater biomass into roots and tubers. The greater amount of biomass allocated to hydrilla tubers during the winter may be a survival strategy that allows resprouting as photoperiod increases and temperature warm in the spring. Hydrilla allocated ca. 80% of its biomass into the roots when grown alone during the winter study, but allocated ca. 9% of its biomass into roots during the summer study. This suggests that hydrilla allocates more energy into tubers and roots than shoot biomass during the winter with no interspecific competition. Other studies have reported similar results where hydrilla allocated less biomass into their roots compared to shoots (Wang et al., 2008; Broadbent, 2018; Louback-Franco et al., 2020). By allocating greater biomass into shoot growth, hydrilla gains a photosynthetic advantage by reaching the upper canopy of the water column where greater solar radiation is present (Wang et al., 2008) by over-topping and shading out native plants, and reducing photosynthesis rates (Broadbent, 2018; Louback-Franco et al., 2020).

Similarities between seasons were observed in the root to shoot ratio, where water stargrass and Illinois pondweed allocated more biomass in their roots than hydrilla. However, hydrilla allocated significantly more biomass into its shoots than its roots. Introduced plants are theorized to reallocate greater energy into growth and less in defense from herbivory due to a lack of predators (Blossey and Notzold, 1995).

Ex situ study 2

The second *ex situ* study produced variable results for root and shoot biomass, root to shoot ratio, and RGR. The hypothesis that Illinois pondweed would exhibit rapid growth and suppress hydrilla was not proven in this study. However, the root and shoot biomass, root to shoot ratio, and RGR of water stargrass were equal or greater than hydrilla indicating that when planting occurs in equal ratios water stargrass can compete with and is not suppressed by hydrilla in controlled greenhouse conditions.

Illinois pondweed allocated greater biomass to roots when planted at equal ratios with hydrilla and planted alone compared to all other treatments. However, Gosselin et al. (2018) found that Illinois pondweed allocated greater biomass to its shoots compared to roots regardless of sediment type, particle size, and flow rate. Hydrilla allocated equal amounts of biomass into its roots when grown alone and with Illinois pondweed indicating there was no below ground competition between these two species. However, hydrilla allocated ca. 40% more biomass into it shoots when grown alone than with Illinois pondweed indicating there was interspecific competition. The rapid shoot growth of hydrilla has been documented in other studies where it forms dense mats in the upper water column reducing usurping sunlight and reducing light penetration to plants in the lower water column (Wang et al., 2008; Silveira et al., 2018).

Shoot biomass was greater for all three species when grown alone than in equal ratios with another plant indicating interspecific competition. A positive interaction is suggested to exist between hydrilla roots and shoots in which hydrilla allocates more biomass into it shoots to reach the upper water column with increasing interspecific competition (Wang et al., 2008). Similar to the first *ex situ* study, the root to shoot ratios of water stargrass and Illinois indicted these native species allocated more biomass in their roots than shoots compared to hydrilla. The root to shoot ratio was greatest for Illinois pondweed grown alone and in equal ratios with hydrilla indicating no interspecific or intraspecific competition between Illinois pondweed and hydrilla roots.

The RGR were highest in the controls where plants were grown alone indicating interspecific competition reduces growth rates when plants are grown together. In this study, the relative growth rates of Illinois pondweed were significantly less when grown with hydrilla or water stargrass indicating Illinois pondweed is a poor competitor and out-competed by hydrilla. Water stargrass exhibited similar relative growth rates as hydrilla and higher growth rates than Illinois pondweed when grown at equal ratios. This indicates that water stargrass is competitive with hydrilla, but may be more competitive if water stargrass was grown at greater rations of 5, 10, or 20 plants.

There are several factors that may have affected the outcome of the *ex situ* studies. During the summer study, Illinois pondweed accumulated more algae on the leaves than water stargrass and hydrilla, possibly impeding photosynthesis and growth. The short temporal periods of each study may have limited increased growth over 3-4 months from established roots of water stargrass and Illinois pondweed as observed in the winter study. The unregulated temperatures in the greenhouse where mesocosms containing plants were exposed to temperatures > 37.8 C may have inhibited growth of Illinois pondweed. However all three plants were exposed to the same ambient temperature. Moreover, while Illinois pondweed has a large latitudinal range in North America, Gosselin et al. (2018) found Illinois pondweed produces greater biomass at warm temperatures and high water temperatures do not reduce Illinois pondweed growth rate.

In situ Study

The discharge range during the study is considered above the minimal discharge values needed to maintain biological flows for aquatic plants in the San Marcos River (EARIP, 2012). No over bank flooding occurred during the study based on river discharge in which plants can be damaged or scoured out of the sediment. The discharge patterns document during the study indicate conditions should have been adequate for establishment of water stargrass and Texas wild rice. High discharge events and floods have been documented to scour out ca. 10% of planted Texas wild rice and other native plants (Hardy et al., 2016).

No evidence was found in this study that water stargrass and Texas wild rice gain a competitive advantage over hydrilla in plots where hydrilla was removed at percentages between 0-75% in small plots. Texas wild rice exhibited equal or greater coverage and greater dry weight biomass in plots where 100% of hydrilla was removed. Moreover, there were contrasting results observed for the percentage cover and final dry weight biomass of each macrophyte in this study.

The mean percent coverage was greater for hydrilla in all plots except where 100% removal of hydrilla occurred and Texas wild rice was planted. While the overall survival percentage in plots was 50% for Texas wild rice, the mean root and shoot mean dry weights was greater than hydrilla which occurred in all plots. While water stargrass coverage was low in all plots and only documented in 42% of the plots at the end of the study, there was no difference in the root and shoot dry weights of water stargrass and hydrilla. Wood et al. (2012) found that percent cover and dry weigh biomass were positively related, but the relationship varied significantly by sites and to a lesser degree by months.

The coverage of hydrilla was among the highest overall at 65-70% in plots where 100% of the hydrilla was removed and no plants planted, and when 100% of the hydrilla was removed and five water stargrass planted. This invasion of the most disturbed plots can be attributed to the large amount of hydrilla fragments in the vicinity of the treatment plots, which are capable of colonizing a site and developing roots. In a mesocosm study, greater than 95% of hydrilla fragments that were placed in the mesocosms successfully established (Louback-Franco et al., 2020). Once rooted in the sediment, hydrilla has fast vertical and horizontal growth rates of ca. 1600 cm per day when both stems and branches were measured (Glomski and Netherland, 2012). Another study found that hydrilla doubled in biomass at 19.8 days under controlled conditions (Bianchini et al., 2010). In the San Marcos River, hydrilla fragments represented the largest number of plant fragments documented throughout the year (Owens et al., 2001). The key mechanism in hydrilla's ability to colonize a site is due to the high number of propagules the plant produces from stem fragments (Li et al., 2015). The small size of the plots and their close proximity to hydrilla likely explains why plots cleared of hydrilla were colonized by hydrilla at the end of the study.

The low survival of Texas wild rice and water stargrass in the study plots was unexpected but agrees with previous studies indicating hydrilla easily invades aquatic habitat and can suppress native macrophytes (Langeland, 1996; Hofstra et al., 2010; Louback-Franco et al., 2021). In plots where 100% of the hydrilla was removed, only Texas wild rice had greater or equal biomass compared to hydrilla. Texas wild rice survival and growth may be facilitated in the presence of hydrilla. Native and non-native macrophytes can facilitate other non-native macrophyte colonization and establishment (Simberloff and Von Holle, 1999; Thiébaut and Martinez, 1995). In this study, hydrilla may have facilitated Texas wild rice by providing more stable substrate and protection from flow during root establishment.

The low survival of Texas wild rice and water stargrass was unexpected. In plots where Texas wild rice survived, the plants were very healthy and robust. Water stargrass that survived looked stressed and their leaves were covered with silt. Based on the dry weight biomass, water stargrass biomass was not significantly different to hydrilla despite being present in less than 50% of the plots at the end of the study.

Across all plots and study sites, there was a mean reduction of 69% in hydrilla biomass from at the start of the study to the end. This is an indication that clearing hydrilla from an area and planting native macrophytes reduces hydrilla's growth but has no impact on hydrilla's ability to recolonize a site and form canopy cover in the upper water column. Long-term management for control of hydrilla will be required once the upper reach of the San Marcos River is cleared of hydrilla due to re-sprouting from tubers. Tubers are known to remain viable for > 4 years in sediment (Van and Steward, 1990) but lose their viability in several days out of water or moist conditions (Basiouny et al., 1978). The density of tubers varies considerably with densities of 2000 to 9000 m^2 in controlled studies (Steward, 1980) and > 1700 m^2 in lakes (Nawrocki, 2011). Until large mats of hydrilla have been cleared, hydrilla will continue to invade and colonize areas downstream from fragments.

Water stargrass is not an approved native macrophyte for planting in the San Marcos River under the EARIP (2013). The possibility of water stargrass outcompeting Texas wild rice was a concern in the EARIP. In this study, water stargrass growth and coverage was minimal compared to Texas wild rice. Water stargrass should be considered by management agencies as a macrophyte for restoration efforts in the San Marcos River in areas not designated for planting Texas wild rice in areas with static or low water velocities; habitat that is less suitable for Texas wild rice (Poole and Bowles, 1999).

Weed mats covering and blocking sunlight to submerged aquatic plants is an on-going problem in the San Marcos River (Power, 1996; EARIP, 2013). Study site 3 turned out to be an anomaly among the three study sites after initial evaluation indicated the sites were similar in hydrilla coverage on November 2020. Vegetation mats were present at site 3 during three non-consecutive monitoring periods which made it difficult to find the plots (Figure 22). The weed mats were estimated to be ca. 60-70 m^2 and 0.4-0.6 m thick covering all plots in site 3. The mats were composed primarily of hydrilla fragments (95%) and a mixture of other plants (5%) that included Texas wild rice, water stargrass, East India hygrophila (*Hygrophila polysperma*), delta arrowhead (*Sagittaria platyphylla*), two-leaf water milfoil (*Myriophyllum heterophyllum*), water cress (*Nasturtium officinale*), hornwort (*Ceratophyllum demersum*), fanwort (*Cabomba caroliniana*), parrot-feather (*Myriophyllum aquaticum*), water sprite (*Ceratopteis thalictroides*), Eastern mosquito fern (*Azolla caroliniana*), floating liverwort (*Riccia fluitans*), and filamentous algae (primarily *Rhizoclonium* spp.). It is unknown how long the vegetation mats remained over the plots in site 3 before and after monitoring. Power (1996) found that vegetation mats over Texas wild rice resulted in damaged and chlorotic leaves, reduced photosynthetically active radiation below the mats, lower number of stems, and decreased water velocity. The reduced water velocity results in increased silty sediment (Madsen et al., 2001) creating an undesirable substrate for Texas wild rice. Vegetation mats result in multiple variables that impact submerged macrophytes and the main cause of the low survival of Texas wild rice and water stargrass at site 3.

Figure 22. Vegetation mat over research plots at Site Three in the San Marcos River in January 2021.

An additional factor in the contrasting results between cover and dry weight biomass in this study may be the grid system used to estimate plant coverage. If a plant part occurred in any grid, it counted as 4% of the total coverage. As suggested by Maceina and Shireman (1980) recording macrophyte coverage at the water surface only accounts for coverage on a horizontal plane but does not account for the vertical plane. The differences in cover and dry weight biomass are a reflection of the leaf morphology and the vertical or horizontal growth of macrophytes (Duartes, 1996). Hydrilla has slender ascending stems and small whorled leaves while water stargrass and Texas wild rice have over-lapping ribbon-like leaves. This difference in the leaf morphology may account for the difference found for cover and biomass among the species. Aquatic macrophytes that ascend to the upper water column typically have high coverage relative to biomass (Edwards and Brown, 1960). Based on hydrilla's rapid growth rate, it would be expected that greater numbers of hydrilla stems and shoots would reach the upper water column while more sparse stems with fewer leaves occur in the lower portions of the water column. An additional factor is the mean coverage of each macrophyte was determined as the average of each plot over 3 sites. The survival of Texas wild rice was 50% in all plots which results in a skewed mean for total Texas wild rice coverage.

Conclusion

The results of the *ex situ* studies found that water stargrass and Illinois pondweed collectively or alone cannot suppress or outcompete hydrilla when planted in smaller ratios than hydrilla, but instead must be planted in higher densities than hydrilla to gain a competitive advantage. How the results of the *ex situ* study applies to flowing conditions in the San Marcos River are unknown. Based on the greenhouse results, planting of water stargrass and Illinois pondweed need to be planted in higher densities to compete with hydrilla in static no flow conditions during the winter. Multiple areas of static or low flow exists in the upper San Marcos River where native plants such as water stargrass, Illinois pondweed, and other natives can be planted for restoration efforts in areas where hydrilla and other non-natives have been removed. Based on the results of the *ex situ* study, it is hypothesized that water stargrass and Illinois pondweed planted during the winter months will allow these species time to accumulate biomass at a faster rate than in the summer and give them an advantage in the summer if hydrilla resprouts or colonizes by fragments. The advantage gained in the winter will allow water stargrass and Illinois pondweed to spread into the upper water column and potentially suppress the growth of hydrilla. Water stargrass, Illinois pondweed, and other native species should be considered for restoration efforts in shallow and low-flow areas in the San Marcos River. Increased native coverage and biodiversity have been suggested to be an effective method of preventing non-native species invasions (Yu et al., 2018).

The results of the *in situ* experiment using 0.25 m^2 plots indicate the Texas wild rice survival rate was 50% but this species allocated a significant amount of biomass to both roots and shoots. In plots where Texas wild rice survived and 100% of the hydrilla was removed, Texas wild rice cover and biomass were equal or greater than hydrilla. The low survival rate of Texas wild rice may be due to the high amount of silt and organic matter in the plots. In some areas, the silt was > 15 cm deep. The high amount of silt may have resulted in native plants being washed out by water velocity before they could become stabilized and develop roots deeper into the sediment.

Finally, the results of this study indicate that Texas wild rice can compete with hydrilla and offers an alternative management option but hydrilla will still be the dominant macrophyte. Management using 0.25 m^2 plots cleared of hydrilla and planted with water stargrass and Texas wild rice is not effective as a management option. Larger areas of hydrilla must be cleared and higher planting densities of native plants are required for long-term hydrilla control.

Based on the large amount of biomass produced by Texas wild rice in this study, it is hypothesized that established stands of Texas wild rice will provide biotic resistance against invasion of hydrilla. In areas with high silt, alternative methods are needed to increase the survival of native plants. Long-term management of hydrilla in the San Marcos River is dependent on the availability of funding and labor to remove large areas of hydrilla, propagating and planting high densities of Texas wild rice and other native plants, removing floating weed mats off stands of native plants within 1-2 days, and quarterly or bi-annual weeding of hydrilla.

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Permits

All studies were performed in accordance with the USFWS Scientific Research Permit #TE676811-0, USFWS TPWD Scientific Research Permit #SPR-0616-153, UTSA TPWD Permit to Introduce Fish, Shellfish, or Aquatic Plants into Public Waters #INT 21 03-01, and UTSA TPWD Exotic Species Research #RES 03 21-155.

Covid Limitations

The project was delayed due to agency shutdowns due to Covid from April to October of 2020, and the San Marcos River was closed to the public during the summer of 2020. At UTSA, we were only shut down for 3 months and lab staff and students were allowed to return to research in June 2020 which allowed us to complete the greenhouse studies. The USFWS divers were also unable to assist with the initial hydrilla removal and replanting until November 2020 until they completed extra training due to Covid. Due to these delays, we were not able to finish the second part of the field study. The second part of the study evaluating planting higher densities of Texas wild rice and other native plants is expected to be completed when an incoming graduate student is found to assist with the study.

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