Evaluating the suppression of *Hydrilla verticillata* by manual removal and planting natives for small-scale restoration efforts in a spring-fed river

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**Abstract** – *Hydrilla (Hydrilla verticillata)* is an invasive aquatic macrophyte found on every continent except Antarctica. Due to the high number of federally listed species in the San Marcos River, Texas, management options for hydilla are limited. We examined the ability of the two native macrophytes to suppress *hydilla* in plots where 0 to 100% *hydilla* was removed. In plots where 100% *hydilla* was removed, Texas wild rice exhibited equal or greater coverage to *hydrilla*. *Hydrilla* was present in 100% of the plots at the end of the study, regardless of percent removal, while Texas wild rice (*Zizania texana*) and water stargrass (*Heteranthera dubia*) were present in 50 and 42% of the plots, respectively. While the overall survival percentage in plots was 50% for Texas wild rice, the mean root and shoot dry weights were significantly greater than *hydrilla* which occurred in all plots. However, *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 disturbed sites. The study results indicate morphological differences with *hydrilla* allocating greater biomass into its shoots while Texas wild rice allocates equal amounts of biomass into its shoots and roots.

**Keywords:** *Hydrilla* / Texas wild rice / manual removal / replanting / cover

1 Introduction

One of the greatest threats to native aquatic macrophyte populations is the introduction of invasive macrophyte species which have the ability to form monocultures and outcompete native plants (Havel et al., 2015). Invasive aquatic plants alter aquatic ecosystem by increasing sedimentation rates, reducing light penetration, decreasing water velocity, altering the banks of river channels, reducing dissolved oxygen concentrations, and interfering with recreation and flood control (Dayan and Netherland, 2005; Hussner et al., 2017). Multiple management options exist for control of invasive aquatic plants (Hussner et al., 2017, Haller and Richardson 2020). Herbicide use within river systems is not recommended due to rapid dilution and dispersion due to turbulence and mixing within the water column (Getsinger et al., 1996; Mohr et al., 2007).

Spring-fed rivers represent novel challenges for managing invasive aquatic macrophyte species if endangered species are present. The clear and shallow spring-fed water, favorable water velocity, and constant water chemistry and cool temperatures make the San Marcos River in Central Texas home to multiple federally listed species but also susceptible to invasion. Lemke (1989) found that 25.8% of the 31 plant species collected in the upper San Marcos River were non-native with *hydrilla* [*Hydrilla verticillata* (L.F.) Roy] being the most abundant. The volume of floating aquatic vegetation in the San Marcos River was dominated by *hydrilla* which made up 34–64% of the aquatic vegetation collected over different seasons (Owens et al., 2001). During 2010, *hydrilla* made up 26.6% of the aquatic plant coverage in the San Marcos River (Hardy et al., 2010).

In 2013, in response to the growing number of non-native species invading the San Marcos River, the City of San Marcos with funding from 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 other native aquatic plants to increase habitat for the endangered fountain darter (*Etheostoma fonticola*). One of the primary invasive plants designated for removal was *hydrilla*.

Because of the vulnerability of Texas wild rice and other federally listed species to herbicide treatment, the Texas Parks and Wildlife Department 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. In addition, no current biocontrols are available to manage hydrilla in flowing water. Manual removal and planting of native aquatic plants are the only viable management options available in the San Marcos River. Yu et al. (2018) found that native plant diversity and coverage were a key component to prevent invasions of non-native species in aquatic systems. Planting native aquatic plants in sites with reduced or no initial competition offers a potential management alternative. Owens et al. (2008) found that Vallisneria americana was effective at preventing the establishment of invasion by hydrilla fragments. A competition study by Doyle et al. (2007) indicated that the presence of V. americana and herbivory resulted in a 30–40% reduction of total hydrilla biomass.

Hydrilla is considered one of the most invasive species of aquatic weeds in the United States (Langeland, 1996) and was first documented in the San Marcos River in 1975 (Flook, 1975). The rapid spread of hydrilla is due to its rapid growth rates, multiple modes of reproduction, and ability to adapt to disturbed conditions. Glomski and Netherland (2012) found that a 10 cm fragment can increase to >8000 cm of shoot tissue in five weeks. Hydrilla’s resilience, rapid growth rate, and multiple modes of reproduction make it a difficult species to manage in aquatic ecosystems. In addition, dioecious hydrilla, which is the dominant biotype in Texas, depends on tubers and turions for reproduction (Steward and Van 1987). Thus, hydrilla can re-invade from tubers and turions even after the physical removal of roots and shoots.

Texas wild rice is endemic to the upper 3-4 km of the San Marcos River, Hays County, Texas. Texas wild rice is a perennial, C3 grass (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). Texas wild rice has been documented to have rapid growth rates which may indicate this species is a strong competitor against non-native aquatic plants (Hutchinson, 2019). Lastly, a 716 m² area of hydrilla was documented to have rapid growth rates which may indicate this species in aquatic systems. Planting native aquatic plants in sites with reduced or no initial competition offers a potential management alternative. Owens et al. (2008) found that Vallisneria americana was effective at preventing the establishment of invasion by hydrilla fragments. A competition study by Doyle et al. (2007) indicated that the presence of V. americana and herbivory resulted in a 30–40% reduction of total hydrilla biomass.

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As part of the EARIP (2012) native aquatic plants were planted in areas where hydrilla was removed. One of the native plants, water stargrass was not selected because past research indicated it may out-compete Texas wild rice. Several studies suggested that water stargrass (Heteranthera dubia) forms canopies at the upper water surface that would give this species a competitive advantage over hydrilla and possibly other aquatic plants. However, it is unknown how water stargrass and Texas wild rice interact with each other or interact with hydrilla.

Another component of the EARIP (2012) is maintaining a minimal spring discharge in the San Marcos River of 2.8 m³ s⁻¹. This discharge is considered the minimal ecological flow required to maintain normal growth, reproduction, and survival of flora and fauna in the river. For aquatic plants, higher discharge reduces the boundary layer between the water and leaves allowing plants to take in more CO₂ (Madsen et al., 1991). Due to high heterogeneity of resources on a large scale, competition may be more important on a finer scale at the plot level (Pulzatto et al., 2019). The removal of hydrilla from small plots and discharge rates ≥ 2.8 m³ s⁻¹, planted Texas wild rice and water stargrass may provide biotic resistance by preventing the reestablishment of hydrilla in the plots through competition.

The objectives of this study were to evaluate the growth of Texas wild rice and water stargrass in areas where different percentages of hydrilla were manually removed and evaluate if either native species can suppress hydrilla growth in small plots. 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. We tested the following predictions: 1) the survival of Texas wild rice and water stargrass would be >90% in plots where hydrilla was removed, 2) the removal of hydrilla at different percentages in plots followed by the planting of Texas wild rice and water stargrass would result in greater coverage and biomass of the two natives compared to hydrilla regardless of the percent hydrilla initially removed, and 3) that discharge patterns ≥ 2.8 m³ s⁻¹ would result in greater coverage and biomass of the two native aquatic plants compared to hydrilla.

2 Methods
2.1 Study sites

The study site was located in the San Marcos River, Hays County, Texas (Fig. 1). 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. The San Marcos River is considered one of the most biologically diverse rivers in the Southwestern United States (USFWS, 1996). The upper San Marcos River is spring fed and has consistent, uniform hydrological and physiochemical conditions 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 research sites in the San Marcos River were selected that occurred a minimum of 100 m apart and comprised ≥75% hydrilla coverage. The study sites were high in silt and organic matter 10-12 cm deep. 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.

Escaping lack of independence among plots cannot be accomplished with Texas wild rice since it only occurs in the upper stretch of the San Marcos River. The area above the study plots has been treated for hydrilla with regularly occurring maintenance for removal of new hydrilla growth. The lower stretch of the river has scattered patches of hydrilla but Texas wild rice coverage is minimal and limited plans are in place to increase coverage in the lower section of the river. The limited number of plots used in the study was due to ongoing EARIP (2012) research and long-term monitoring plots. We were allocated a small stretch for the study from the City of San Marcos’s Watershed Protection Manager who
oversees the EARIP in the river. Based on the size of area in the river we were allocated, we spaced out the three areas as far apart from each other as possible to minimize for this lack of independence.

2.2 Plant propagation

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. The plants were placed at the 3600 L flow-through raceways with input of Edwards Aquifer water in the U.S. Fish and Wildlife Service’s San Marcos Aquatic Resource Center greenhouse (29° 50’ 22.34”N, 97° 58’ 32.00”W) located in San Marcos, Texas. The top portions of plants were cut back as needed to maintain heights of 75 +/- 20 cm before planting in the San Marcos River.

2.3 Experimental design

In each of the three research sites during October 2020, hydrilla was manually removed at 25, 50, 75, or 100 % by United States Fish and Wildlife Service scuba divers within each 0.25 m² plot (Fig. 2). One plot per site was left untreated (0% hydrilla removal) where hydrilla coverage was >75%. All hydrilla removed from each plot was collected, bagged, and oven-dried at 90°C for 5 days. Following drying, dry weights were recorded to the nearest 0.01 g. During plot set-up, an additional 0.2 m of hydrilla was removed around the plot to create a buffer from the surrounding hydrilla at the site.

On November 10, 2020, USFWS scuba divers removed the remaining hydrilla including roots, tubers, and any hydrilla regrowth from cleared areas in all plots. The same day, five Texas wild rice and five water stargrass, each 75 +/- 10 cm in height, were planted randomly within five of the eight 0.25 m² plots at each research site. The use of five plants per plots was
and biomass of each species in response to the percentage of means. Linear regression was used to compare trends in cover analyzed with a Kruskal-Wallis test and Dunn was used to test dry weight biomass of hydrilla, Texas wild rice, and water stargrass (SG) planted. Plots were randomly placed in each location where hydrilla coverage was > 75% coverage.

due to the endangered status of Texas wild rice which limited the use of experimental plants. Additionally, there were control plots at each site where 100% hydrilla was manually removed and no native plants were planted within the plot, and two other plots where 100% hydrilla was manually removed and five Texas wild rice or water stargrass were planted.

One replicate plot of each treatment was placed at each site for a total of three replicates per treatment for 24 total plots among the three sites. Each plot was marked by a 0.4 m rebar that was placed in each corner of the plot and covered with orange caps. A waterproof metal detector (Vibra Tector 740 Waterproof Pinpointer, Treasure Products Inc., Simi Valley, CA, 93065) was used to relocate each plot during monitoring.

Plant coverage within each site and plot was monitored monthly for eight months from November 2020 to July 2021. No monitoring occurred during February 2021 due to logistical issues due to travel restrictions. In each plot, the percent cover was estimated by counting the number of squares within 25 grids of a 0.25 m² plot based the presence of each species within each grid. On July 29, 2021, all plants within each plot were harvested by USFWS divers, separated by roots and shoots for each species, bagged, and placed in an oven dryer at 90°C for 5 days. Dry weights were recorded to nearest 0.01 g for roots and shoots.

2.4 Statistical analysis

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 tested for normality and equality of variance with the Shapiro-Wilk and Brown-Forsythe tests, respectively. If data did not meet the assumptions, the data were ln transformed (Sokal and Rohlf, 1995). 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 species. Tukey’s HSD or Dunn’s test were 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. Root to shoot ratio was analyzed with a Kruskal-Wallis test and Dunn’s test to separate means. Linear regression was used to compare trends in cover and biomass of each species in response to the percentage of hydrilla removed in each plot. Pearson correlation was used to compare daily mean discharge per month to coverage of each species. Data was 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).

3 Results

3.1 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 plant 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 survival was 50% (n = 12) and 42% (n = 10) out of 24 plots, respectively.

3.2 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) (Fig. 3). 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, and Texas wild rice was planted, Texas wild rice exhibited equal or greater to hydrilla (P < 0.05). Hydrilla coverage was significantly greater (P < 0.05) following 100% removal and planting of water stargrass. In plots where 25, 50, and 75% hydrilla removal occurred, hydrilla coverage was significantly greater (P < 0.05) at the end of the study.

3.3 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). No interactions (P > 0.05) were found and dry biomass was combined as one composite sample and analyzed with a one-way ANOVA to determine differences among plant species. 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) (Fig. 4a) and shoot (F = 12.5, df = 2, P < 0.001) (Fig. 4b) biomass 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. The root to shoot ratio was significantly different among all three plants (F = 11.80, df = 2, P < 0.001) (Fig. 4c) based on a Kruskal-Wallis test with Texas wild having a greater root to shoot ratio compared to hydrilla and water stargrass. Based on linear regression, no patterns were observed for the cover or biomass of hydrilla, water stargrass, and Texas wild rice based on the percentage of hydrilla removed.
on the percent hydrilla removed prior to planting with the exception of hydrilla cover ($R^2 = 0.805$, $F = 12.39$, df = 1, $P = 0.039$) (Fig. 5). Hydrilla exhibited a reverse trend with cover increasing in plots where the most hydrilla was initially removed.

3.4 Discharge patterns

River discharge ($m^3 s^{-1}$) varied from $3.76 m^3 s^{-1}$ (132 ft³ s⁻¹) during hydrilla removal and planting during November 2020, to a low of $2.90 m^3 s^{-1}$ (102 ft³ s⁻¹) in April 2021, but increased to $4.8 m^3 s^{-1}$ (172 cfs) during June and July of 2021 (Fig. S1). A moderate positive correlation ($r = 0.47$) was found for water stargrass coverage and discharge. Weak correlations were observed for hydrilla ($r = -0.14$) and Texas wild rice ($r = 0.21$) coverage and discharge.

4 Discussion

4.1 Aquatic plant survival

Survival rates were low ($\leq 50\%$) for Texas wild rice and water stargrass compared to 100% for hydrilla. This is in contrast to Hardy et al. (2016) who found 90% survival rates for Texas wild rice planted in Segments A and B where hydrilla was removed. 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 (Hofstra et al., 2010; Louback-Franco et al., 2020). Texas wild rice survival and growth may be facilitated in the presence of hydrilla. Non-native aquatic plants can facilitate morphologically dissimilar native plants and establishment (Thiébaut and Martinez, 2015).

In this study, hydrilla may have facilitated Texas wild rice by providing more stable substrate and protection from flow during root establishment. In plots where Texas wild rice survived, the plants were healthy and robust. Water stargrass that survived appeared stressed, exhibited chlorosis and was covered with silt. However, 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, indicating this species has the ability to resprout and grow.

The survival rate of Texas wild rice was 50% but this species allocates a significant amount of biomass to both roots and shoots compared to hydrilla. 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. However, in plots where all plants were removed, hydrilla quickly invaded and became dominant. 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 plots, the silt was >10 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.

4.2 Changes in plant coverage over the study

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...
4.3 Differences in dry biomass among species

The differences in cover, dry weight biomass, and root to shoot ratio are a reflection of the leaf morphology and the vertical or horizontal growth of macrophytes. Hydrilla has slender ascending stems and small whorled leaves while water stargrass and Texas wild rice have overlapping ribbon-like leaves. This variation in the leaf morphology likely accounted for the difference between cover and biomass among the species. Aquatic macrophytes that grow into the upper water column typically have high coverage relative to biomass (Edwards and Brown, 1960). Wood et al. (2012) found that percent cover and dry weight biomass were positively related, but the relationship varied significantly by sites and to a lesser degree by months.

Across all plots and study sites, there was a mean reduction of 69% in hydrilla biomass from 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 yr in moist sediment (Van and Steward, 1990). The density of tubers varies considerably with densities of 2000 to 9000 m² in controlled studies (Steward, 1980) and >1700 m² in lakes (Nawrocki, 2011). Until large mats of hydrilla have been cleared, hydrilla will continue to invade and colonize areas downstream from fragments.
4.4 Effects of discharge on plants during the study

The discharge range during the study is considered above the minimal discharge value of 2.8 m$^3$ s$^{-1}$ 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 documented during the study indicate conditions should have been adequate for establishment of Texas wild rice and water stargrass. 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).

Weed mats covering and blocking sunlight to submerged aquatic plants is an on-going problem in the upper San Marcos River (Power, 1996; EARIP, 2012). Study site 3 turned out to be an anomaly among the three study sites after the initial evaluation indicated the sites were similar in hydrilla coverage. Vegetation mats were present at site 3 during three non-consecutive monitoring periods which made it difficult to find the plots. The weed mats were estimated to be ca. 60-70 m$^2$ and 0.4-0.6 m thick covering all plots. The weed mats were composed primarily of hydrilla fragments (95%) but with a mixture of other aquatic plants (5%). It is unknown how long the vegetation mats remained over the plots 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, a lower number of stems, and decreased water velocity. The reduced water velocity results in increased silty sediment creating an undesirable substrate for Texas wild rice. Vegetation mats resulted in multiple variables that impact submerged macrophytes and likely the cause of the low survival of Texas wild rice and water stargrass at site 3.

The results of this study indicate that Texas wild rice can compete with hydrilla and offers an alternative management option but hydrilla will still require management. Larger areas of hydrilla must be cleared and higher planting densities of native plants are required for long-term hydrilla control. The coverage of Texas wild rice has increased significantly since the planting of Texas wild rice began in 2013 (Poole et al., 2022) indicating that the restoration work performed in the San Marcos River under the EARIP (2012) has been successful where large areas of hydrilla were removed. This study indicates that small scale restoration efforts to manage hydrilla provide general insight into plants that may be competitive with hydrilla, but it will require long-term studies and funding to fully evaluate.

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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 Permit #RES 03 21-155.

Conflicts of interest

We declare we have no conflict of interest.

Data availability statement

The data supporting this study are available from the corresponding author upon request.

Author contribution statement

Angela Maroti: methodology, field sampling, data management, statistical analysis, writing the manuscript, and reviewing manuscript revisions. Jeffrey Hutchinson: resources, conceptualization, methodology, funding acquisition, field sampling, reviewing and editing.

Supplementary Material

Fig. S1. Mean daily discharge patterns per month and mean plant coverage of hydrilla, water stargrass, and Texas wild rice (TWR) from November 2020 to July 2021 in the San Marcos River.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2024001/olm.

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