Final Report for *Ludwigia repens* Competition Study

Edwards Aquifer Authority Contract #14-727L

PREPARED BY

Center for Reservoir and Aquatic Systems Research
Baylor University
Waco, Texas 76798

BIO-WEST, Inc.
1812 Central Commerce Court
Round Rock, Texas 78664

This page left blank intentionally

TABLE OF CONTENTS

List of Figures

LIST OF TABLES

1.0 Introduction

The San Marcos and Comal Rivers have unique aquatic plant communities that support a wide variety of native and endemic wildlife including several listed species. In 2013 the Edwards Aquifer Habitat Conservation Plan (EAHCP) was enacted to enhance and expand habitat for covered species including the fountain darter (*Etheostoma fonticola*). Part of this long-term plan includes removal of the non-native aquatic plant species *Hydrilla verticillata* and *Hygrophila polysperma* and reintroduction of native aquatic plants such as *Ludwigia repens* – all of which will be referred to by their genus name throughout this report. Hydrilla and Hygrophila are becoming increasingly abundant in these systems (Lemke, 1989; Bowles and Bowles, 2001) and tend to support fewer numbers of fountain darters than certain species of native aquatic plants (BIO-WEST, 2015). The persistence and expansion of Hydrilla and Hygrophila pose a threat to efforts in re-establishing beneficial native aquatic vegetation for *E. fonticola* (Bormann, 2012). Predicting the long-term success of revegetation efforts and which species, native or non-native, dominate is vital in the development of a submerged aquatic vegetation module for the EAHCP Ecological model.

Interspecific competition, or the success of a particular plant species relative to another, is a potentially important factor in determining the complex structure of aquatic plant communities. Abiotic factors like substrate and water quality (Szosszkiewicz et al, 2014) as well as differences in species-specific characteristics such as growth rate, plant architecture, reproductive vigor and susceptibility to herbivory (Spencer and Bowes, 1985), phenological plasticity (Garbey et al, 2004; Thouvenot et al, 2013) and, in certain cases, chemical defenses (Gopal, 1993; Gross, 2003) all play a role in the distribution and abundance of species within the plant community. While competitive pressure among naturally co-existing species may appear to be low (Chambers and Prepas, 1990), various studies suggest that these communities do display spatiotemporal variability based on interactions between competitive ability and environmental gradients (McCreary, 1991; Barrat-Segretain, 1996). Non-native species may possess traits that confer a competitive advantage over native species, decreasing species richness, facilitating shifts in community composition and precipitating negative effects throughout the ecosystem (Santos et al, 2011).

Invasive aquatic plant species are well known for their ability to spread rapidly via fragmentation of stems, basal rooting structures, such as stolons, tubers or corms, or specialized structures, such as turions, which can detach and move downstream or float on currents into new locations colonizing in rapid fashion (Sculthorpe, 1967; Langeland and Sutton, 1980). Typically aquatic plants reproduce asexually (Arbor, 1920; Haynes, 1988) and vegetative structures are primed for growth upon settling into new habitat with root structures or leaves still attached (Sutton, 1996). As a consequence, in many cases, invasion of an aquatic species into new areas can take very little time (Santamaria, 2002). For example Eurasion watermilfoil, *Myriophyllum spicatum*, a widespread problematic submersed aquatic plant has been documented to establish and dominate littoral zones of lakes within two to three years after introduction (Aiken et al., 1979; Newroth, 1985) and is known to suppress growth of a native species (Agami and Waisel, 1985). A North American native *Elodea nuttaalii* has spread rapidly in Japan's largest lake covering the lake bottom within a few years after introduction there (Kadono, 2004). Closer to home in the San Marcos system the exotic plant *Cryptocoryne becketti* was documented to quickly establish and spread within 2 years after initial discovery with a recorded expansion rate of 80% a year (Doyle, 2001) and annual mapping by BIO-WEST has shown the dramatic expansion of Hygrophila in the Old Channel Study Reach of the Comal River (BIO-WEST, 2015). Some invasive aquatic plants not only colonize rapidly but they can displace native aquatic plants by producing a dense canopy structure limiting light availability to other submersed species.

With recent documented expansions of invasive aquatic plants within the San Marcos and Comal systems data is needed to predict how native plants may respond. Few studies regarding native versus non-native aquatic plant competition have been conducted with regard to either of these systems. In one particular study, Doyle et al. (2003) conducted a study in a static container (35 gallon barrels) within an outdoor raceway to evaluate the competitive ability of *Ludwigia repens* against *Hygrophila polysperma*. Our experiment expanded upon that of Doyle et al. (2003) to help further understand the competitive outcome under more realistic environmental flow and ambient light conditions and to additionally investigate the competition between Ludwigia and Hydrilla.

Ludwigia repens (Forester), red ludwigia, is a perennial obligate aquatic plant native to the Comal and San Marcos rivers with common distribution throughout Texas. Ludwigia is an amphibious plant that produces both submersed and emergent growth and can grow terrestrially as well. The architecture of Ludwigia is characterized as caulescent and multi-branched. Submersed growth is typically upright within the water column and nodal rooting is common while terrestrial growth is typically low growing and prostrate. Ludwigia is considered prime habitat for the fountain darter (*Etheostoma fonticola*) and is being utilized in the restoration of darter habitat in both systems.

Hygrophila polysperma (Roxb.) T. Anderson is a non-native plant introduced from Asia. *Hygrophila polysperma* is morphologically similar to Ludwigia in many ways and has been confused with Ludwigia in some instances. Hygrophila is common within the Comal and San Marcos rivers but is not a common invasive plant in Texas as its known distribution is limited to Comal and San Marcos Rivers and San Felipe creek in Val Verde County (Williams, 2013). Like Ludwigia, Hygrophila is also amphibious exhibiting both completely submersed forms, emergent forms and terrestrial growth.

Hydrilla verticillata (L. f.) Royle is another non-native submersed plant introduced from Africa and Eurasia. Hydrilla is a widespread and common invasive aquatic plant with widespread distribution in the United States. It too is an obligate aquatic plant, but does not produce emergent or terrestrial growth forms. Hydrilla only exists as a submersed aquatic plant typically producing dense growth in upright fashion towards the water surface producing a thick canopy. Absent in the Comal River, Hydrilla is common in the San Marcos River but has been successfully controlled in Spring Lake where it was once the dominant aquatic plant species (Williams, et al. 2011).

The data reported here provide information on the short-term (10 week) early establishment and growth period of viable sprigs of Ludwigia, Hygrophila, and Hydrilla under three levels of competition from the other species. Additionally, it evaluated the short term (10 week) impact(s) of sprig invasion from a competing species on the continued growth and development of established plants. These experiments were conducted at various locations within the Comal and San Marcos Rivers to provide more realistic environmental conditions than was possible with the static tank experiments previously conducted by Doyle et al. (2003).

2.0 Materials and Methods

Two separate studies were conducted to compare the competitive interactions of Ludwigia with Hygrophila and Hydrilla. The site of the Ludwigia X Hygrophila study took place within the Comal River. Since Hydrilla does not occur in the Comal system the Ludwigia X Hydrilla study was conducted separately in the San Marcos River located approximately 12 km north of the Comal River. Both rivers are spring-fed systems fed by the Edwards Aquifer and have similar water quality and general biological characteristics.

2.1 Study Design

Two separate but related two-factor factorial experiments for each species pair (Ludwigia X Hygrophila and Ludwigia X Hydrilla) comprised the studies (Tables 1 and 2). In each experiment the impact of competition (C) and location (L) was evaluated separately for each species.

The first experiment of each study (Table 1A, Table 2A) was designed to document initial establishment and growth of colonizing sprigs of each species in three competitive environments. Two sprigs of each species were planted into pots with no competition (empty pots without a competitor species) moderate competition (pots with 50:50 ratio Ludwigia: competitor sprigs) and high competition (pots with established plants of the competitor species). A second experiment evaluated the continued growth of established plants of Ludwigia or the non-native species without competition with those “invaded” by sprigs of the competing species (Table 1B, Table 2B). Experimental design and analysis followed that of Doyle et al., 2003. The combined experiments resulted in seven different treatments (Table 3).

Table 1. **Comal River Ludwigia X Hygrophila competition study designs. A) Top. 3x4 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hygrophila and Hygrophila X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of four locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.**

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
4X Locations	Landa Lake, High Light	8X	8X	8X
	Landa Lake, Low Light	8X	8X	8X
	Upper Spring Run	8X	8X	8X
	Old Channel	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
4X Locations	Landa Lake, High Light	4X	8X
	Landa Lake, Low Light	4X	8X
	Upper Spring Run	4X	8X
	Old Channel	4X	8X

Each of the two competition experiments were replicated at multiple locations: four locations on the Comal for the Hygrophila study (Table 1), and two locations on the San Marcos for the Hydrilla study (Table 2).

Table 2. **San Marcos River Ludwigia X Hydrilla competition study designs. A) Top. 3x2 Two-Factor Factorial Design (Competition X Location) for the Ludwigia X Hydrilla and Hydrilla X Ludwigia sprig competition experiments. Eight replicate plantings of sprigs of each species into three competitive environments were made at each of two locations. B) Bottom. 2X4 Two-Factor Factorial Design (Competition X Location) for established plants with and without invasion by sprigs of the other species. Invasion treatment was replicated eight times at each location, while the non-invaded treatment was replicated only four times at each location.**

A. Sprig Experiments		3X Level of Competition		
		No Competition	Moderate Competition	High Competition
2X Locations	City Park	8X	8X	8X
	I 35	8X	8X	8X

B. Established Plant Experiments		2X Level of Competition	
		Not Invaded	Invaded by 2 sprigs
	City Park	4X	8X
	I35	4X	8X

For the Ludwigia X Hygrophila or Ludwigia X Hydrilla experiments seven treatments were included (Table 3). The same treatments were used at all study locations. Our treatment nomenclature utilizes lower case letters to designate sprigs of a species and capital letters to designate established plants. The first three treatments utilize only plant sprigs planted into previously empty pots of sediment. These include freshly collected Ludwigia sprigs planted in monoculture into empty pots (ll), Hygrophila (or Hydrilla) sprigs planted in monoculture into empty pots (hh), a 50/50 mix of Ludwigia sprigs and Hygrophila (or Hydrilla) sprigs (llhh, 2 sprigs of each species). The use of newly sprigged fragments in empty pots provides information on the colonization potential of both species when free of competitive pressures (ll and hh). The 50:50 sprig mixture (llhh) provides information on the competitive outcome of “equal start” moderate-competition environments. The high-competition environment was obtained by planting sprigs of each species into pots of established plants of the other species (hhLL and llHH).

Table 3. Treatments for Ludwigia vs. Hygrophila (or Hydrilla) competition experiments.

Symbol	Treatment	Count
ll	Ludwigia sprigs into empty pot (No competition)	8
hh	Hygrophila (or Hydrilla) sprigs into empty pot (No competition)	8
ll hh	50 : 50 mix Ludwigia and Hygrophila (or Hydrilla) sprigs into empty pots (Moderate competition)	8
ll HH	Ludwigia sprigs planted into pots of established Hygrophila (or Hydrilla) (High competition for the sprigs; invasion scenario for established plant))	8
hh LL	Hygrophila (or Hydrilla) sprigs planted into pots of established Ludwigia (High competition for the sprigs; invasion scenario for established plant)	8
HH	Growth of established Hygrophila (or Hydrilla) plants (no competition from invading sprigs)	4
LL	Growth of established Ludwigia plants (no competition from invading sprigs)	4

Four treatments utilized established plants of the native or the competitor species (Figure 1). Sprigs of Ludwigia or the competitor species were planted into the pots containing established plants (llHH, hhLL) while other pots containing only established plants (HH, LL) were used to track the continued plant growth without any competitive pressure from invading fragments of the other species. All individual pots were secured within Mobile Underwater Plant Propagation Trays (MUPPT) developed and used for EAHCP restoration and applied research projects (Figure 1).

Note that the llHH and hhLL pots serve dual purpose. The sprig growth in these pots represents the growth of plant sprigs in high-competition environments (Experiment 1A or 2A). The continued growth of the established plant following invasion from the sprigs is the invaded scenario of the established plant experiments (Experiments 1B and 2B).

Figure 1. **Example of Experimental layout of treatments within a MUPPT (left) and MUPPT deployed in the San Marcos River (right). Examples of pots of several of the treatments are highlighted.**

2.2 Initial Setup and Sampling

Seven experimental treatments (Table 3) were randomly assigned and simultaneously placed into paired MUPPTs similar to the arrangement diagrammed in Figure 2. A total of 48 pots contained 8 replicates of 5 treatments – only Ludwigia sprigs (ll), only Hygrophila or Hydrilla sprigs (hh), a combination of sprigs (llhh), established plants with sprigs of the opposite species (LLhh and hhLL) – and 4 replicates of established plants for both species (LL and HH). Adjacent spaces were left empty to minimize interaction between pots, resulting in two MUPPTs being needed at each location.

Pre-established plants and sprigs were planted in 600mL quart-sized nursery pots filled with native silty/clay sediment collected from the respective rivers in which the study was carried out. Native sediment was collected in areas with no plant growth and further screened for plant propagules to prevent extraneous plant growth in treatments. Established plants were obtained by pre-culturing plants for three weeks in MUPPTs near the Landa Lake High Light location (Comal study) or at the experimental location used on the San Marcos (City Park) to allow robust initial establishment and growth. Healthy plants of uniform size were selected for the experiment as well as to obtain initial biometric measurements. Stem cuttings were collected from healthy, established plants and inspected to ensure they had no visible signs of herbivory or disease. Sprigs 20cm in length were selected for experimental use and harvested for initial biomass.

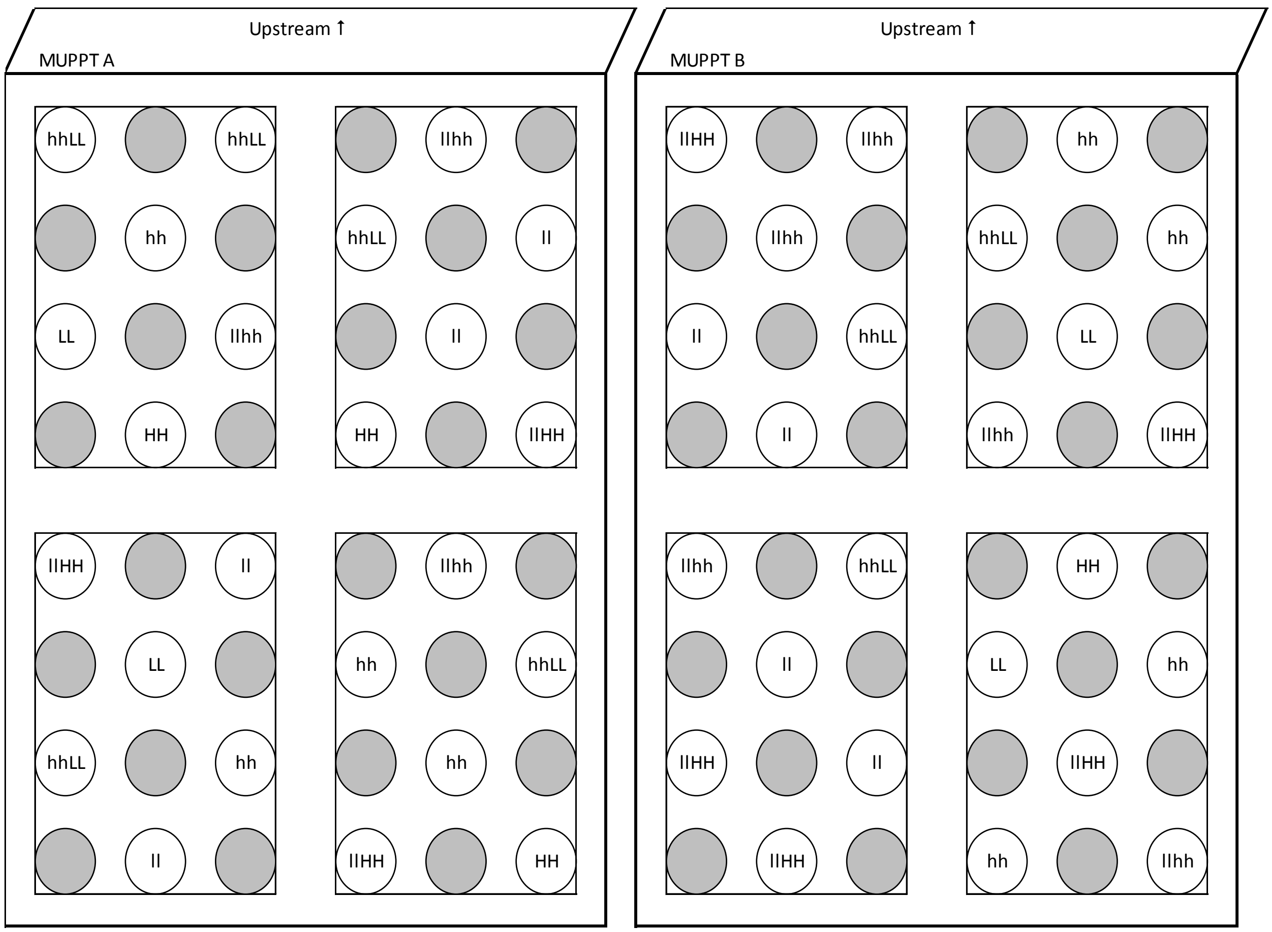

Figure 2. **Illustrated arrangement of alternating experimental pot placement within two MUPPTS anchored at each location. Open circles display the 7 possible experimental combinations (Table 3), and gray circles represent empty spaces.**

Four locations were selected on the Comal River to represent the variability of environmental conditions found within this system. Locations were selected within the Upper Spring Run (USR), Landa Lake in a shaded location (Landa Lake Low Light, LLLL), Landa Lake in a full sun exposure location (Landa Lake High Light, LLHL), and the Old Channel (OC; Figure 3). The Landa Lake High Light location was adjacent to the MUPPT culture station for restoration plantings while the Landa Lake Low Light location was along the western shoreline under the shade of an overhanging live oak tree. All four of these locations were initially planted on May 13, 2015 and harvested on July 27, 2015. In the San Marcos River two locations were chosen.

One location (1) above Rio Vista falls at City Park (CP) and another location (2A) below Rio Vista falls (Figure 3).

Rio Vista falls provides a distinctive dissection in the velocity characteristics of the San Marcos with river velocities below this point typically faster than velocities above the falls. The San Marcos study was initiated on April 23, 2015. Unfortunately, the significant flood event of May 2015 scoured out and destroyed the portion of the experiment at the downstream location (2A). The City Park location was minimally impacted, and continued until it was harvested on June 30, 2015. In order to provide information from the lower portion of the river, another site near the I35 crossing was selected (location 2B or I35, Figure 3) and plantings were initiated on July 6, 2015. The plants at this downstream location were harvested on September 11, 2015.

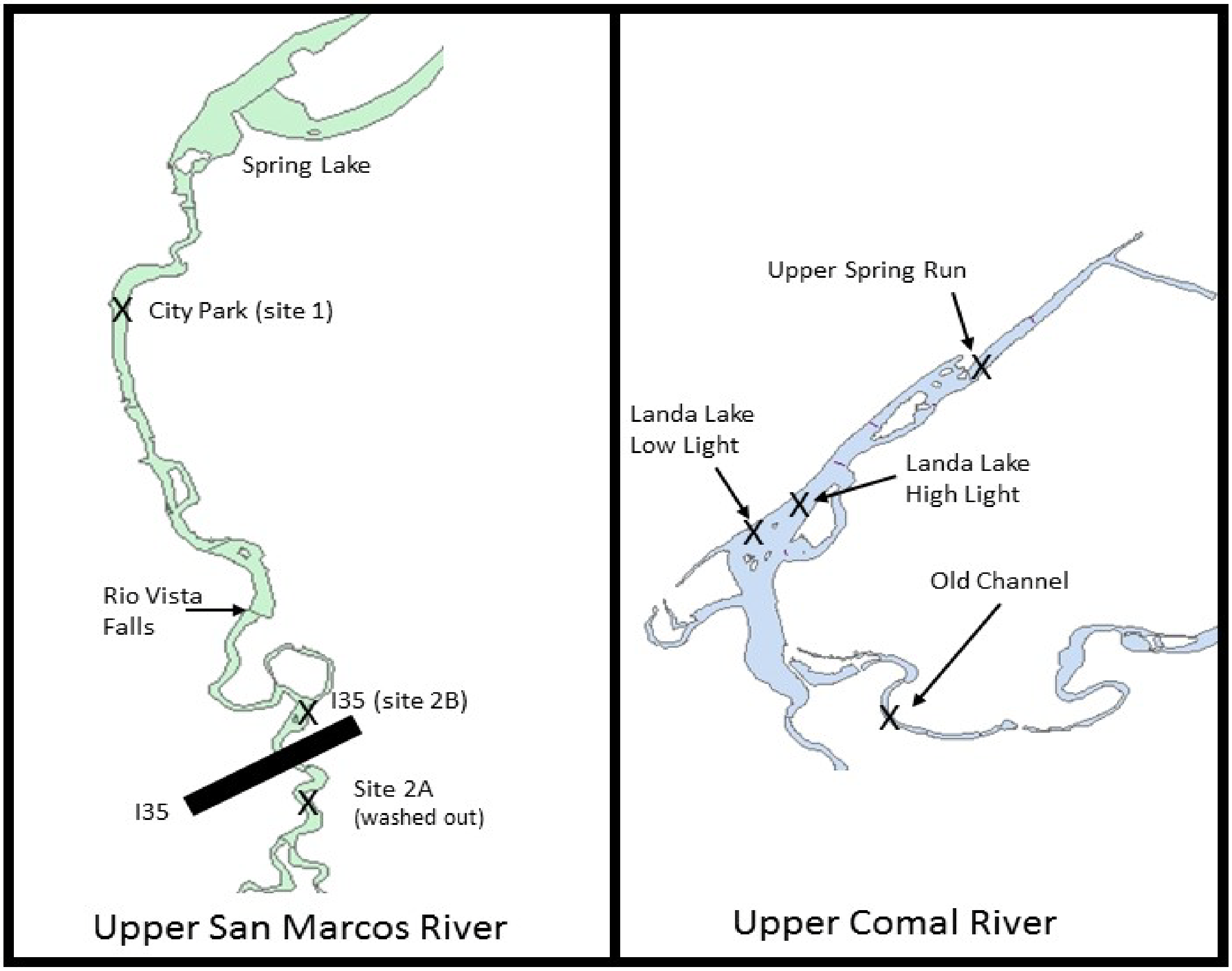

Figure 3. **Maps of the upper San Marcos and upper Comal Rivers showing locations of MUPPT deployment for competition experiments.**

After plantings were made, monitoring of growth and environmental characteristics (total depth, velocity at 80% and 20% of depth, temperature, DO and pH) occurred once per week. Photosynthetically active radiation or PAR was measured intermittently at each location over the course of several days using the Odyssey™ deployable waterproof sensor. Each experimental location, maximum stem length per species was recorded on two randomly selected individuals per treatment. Velocity and water depth were measured weekly with a Marsh-Mcbirney flo-mate while pH, temperature and dissolved oxygen (DO) were recorded at each location with a YSI™ multiparameter sonde.

Plants were harvested after 10 weeks of growth. Morphometric characteristics (stem counts and lengths) were recorded, then samples were separated into above-and-below ground tissues and dried at 60 °C for >72 hours then weighed to the nearest 0.1 mg at Baylor University.

3.0 Results

3.1 Initial measurements and Environmental conditions

Sprigs and established plants of both species were harvested to provide initial biomass estimates for each experiment. These average initial dry-weight biomass values (g/pot) ± SE, (n) were:

Comal River, Ludwigia pair of sprigs (g/pot), 0.47 ± 0.05 (16)

Comal River, Hygrophila pair of sprigs (g/pot), 0.27 ± 0.07 (16)

Comal River, established Ludwigia (g/pot), 4.15 ± 0.61 (6)

Comal River, established Hygrophila (g/pot), 2.17 ± 0.29 (6)

San Marcos (1) CP, Ludwigia pair of sprigs (g/pot), 0.48 ± 0.03 (25)

San Marcos (1) CP, Hydrilla pair of sprigs (g/pot), 0.23 ± 0.01 (30)

San Marcos (1) CP, established Ludwigia (g/pot), 4.79 ± 0.49 (6)

San Marcos (1) CP, established Hydrilla (g/pot), 2.65 ± 0.59 (6)

San Marcos (2B) I35, Ludwigia pair of sprigs (g/pot), 0.38 ± 0.03 (13)

San Marcos (2B) I35, Hydrilla pair of sprigs (g/pot), 0.54 ± 0.02 (16)

San Marcos (2B) I35, established Ludwigia (g/pot), 6.28 ± 0.30 (6)

San Marcos (2B) I35, established Hydrilla (g/pot), 2.63 ± 1.24 (6)

Environmental factors at each experimental location are summarized in Table 4. The recorded PAR maximums for each location were LLHL: 876 E/m² ; LLLL: 620 E/m² ; OC: 699 E/m² day. ; CP: 620 E/m² day. Average daily PAR at the LLHL location were 26% higher than average daily PAR measurements at the LLLL location. Data from USR and I35 were not recoverable.

Table 4. Summary of environmental parameters (± SE) for locations selected for the competition experiments. Depth and Velocity were measured in U.S. and converted to metric.

Location	Depth (cm)	Temp (°C)	DO (mgL^{-1})	pH	Vel. at 80% ($msec^{-1}$)	Vel. at 20% ($msec^{-1}$)
Comal River						
USR	98 ± 1	24.3 ± .2	4.67 ± .18	7.62 ± .04	0.08 ± .01	0.2 ± .01
LLHL	95 ± 2	24.1 ± .1	4.71 ± .18	7.46 ± .10	0.09 ± .02	0.23 ± .02
LLLL	120 ± 1	23.9 ± .1	4.61 ± .14	7.63 ± .07	0.09 ± .02	0.27 ± .02
OC	92 ± 1	23.9 ± .1	4.88 ± .08	7.62 ± .03	0.05 ± .03	0.56 ± .02
San Marcos River						
I35 (2B)	79 ± 1	22.2 ± .2	5.02 ± .20	7.55 ± .05	0.32 ± .07	1.03 ± .06
CP (1)	95 ± 4	22.1 ± .1	5.99 ± .29	7.42 ± .07	0.32 ± .12	0.6 ± .05

3.2 Plant growth over study period.

Figures 4 and 5 show the average growth of plant sprigs and established plants in the Comal (Ludwigia and Hygrophila) and the San Marcos (Ludwigia and Hydrilla). These data show that growth of Ludwigia was relatively robust at all locations. Growth of Hygrophila and Hydrilla was much more variable, and in general much less robust than the growth of Ludwigia.

Ludwigia sprigs (red bars, Figures 4 and 5) showed good establishment and growth in all experiments, although maximum stem length remained relatively modest as the plants appear to have mostly grown laterally. Established plants of Ludwigia showed very consistent data through time. Because the plants were in relatively high light environments, the plants tended to "bush out" rather than grow in length, a common adaptation for high-light growth environments. This effect is evident from the observation of the plants at San Marcos City Park (Site 1) at the end of the growth period (Figure 6). The MUPPT is very full of robust Ludwigia plants, although it is evident that the plants are "bushy" rather than elongated. Hygrophila sprigs in the Comal showed growth similar to that of Ludwigia sprigs at USR and OC, but lower growth in the two locations within Landa Lake. Hygrophila sprigs required repeated sprigging within the first week as many initial sprigs did not remain in their pots. In the San Marcos, Hydrilla sprigs tended to decline towards the end of the experimental growth periods. Established Hydrilla grew well at City Park (Site 1), but declined through time at the I35 (Site 2B) location.

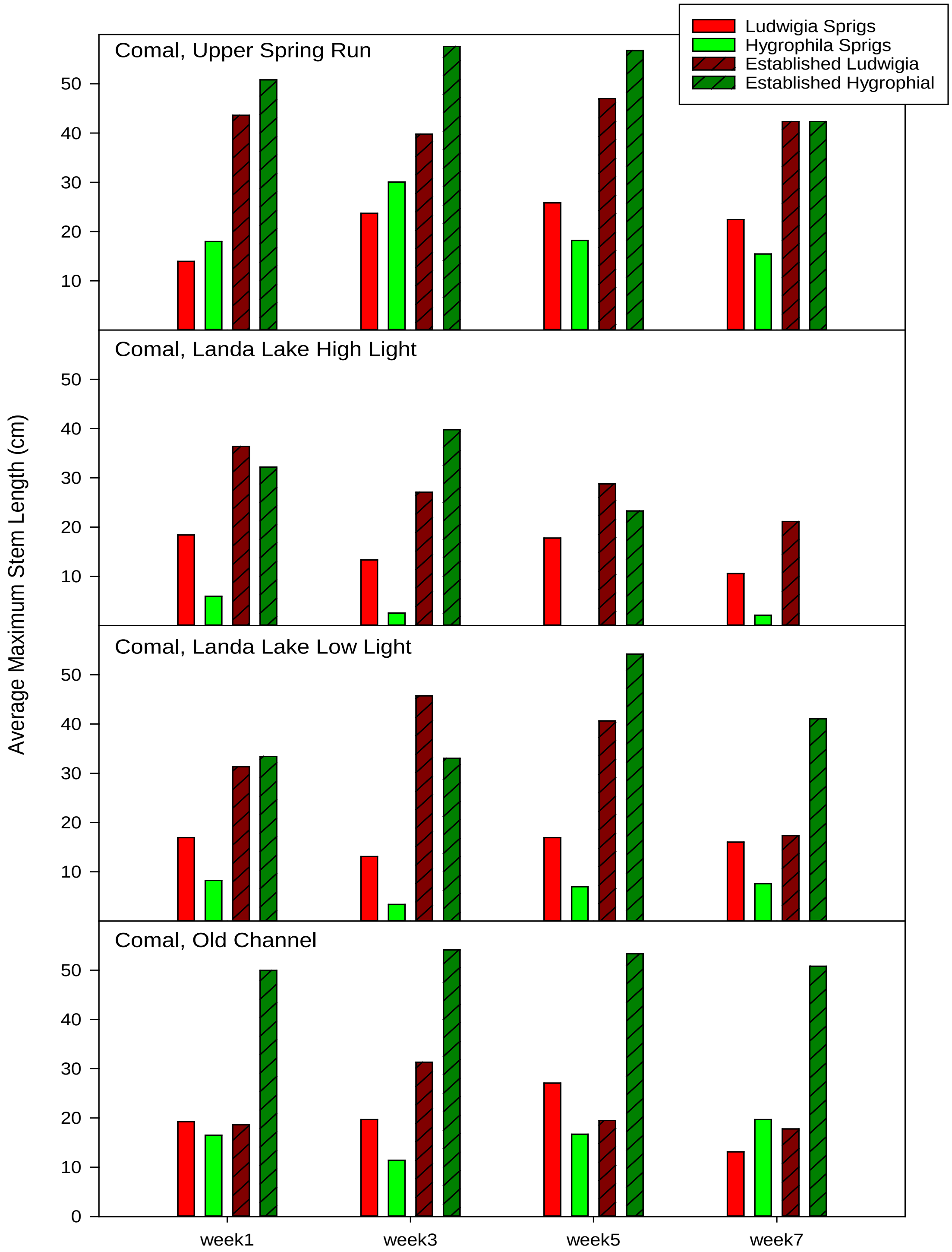

Figure 4. **Average maximum stem length of plants at each of the four experimental locations on the Comal River. Data is shown for sprigs of Ludwigia (red) and Hygrophila (green) as well as established Ludwigia (dark red, hatched) and Hygrophila (dark green, hatched).**

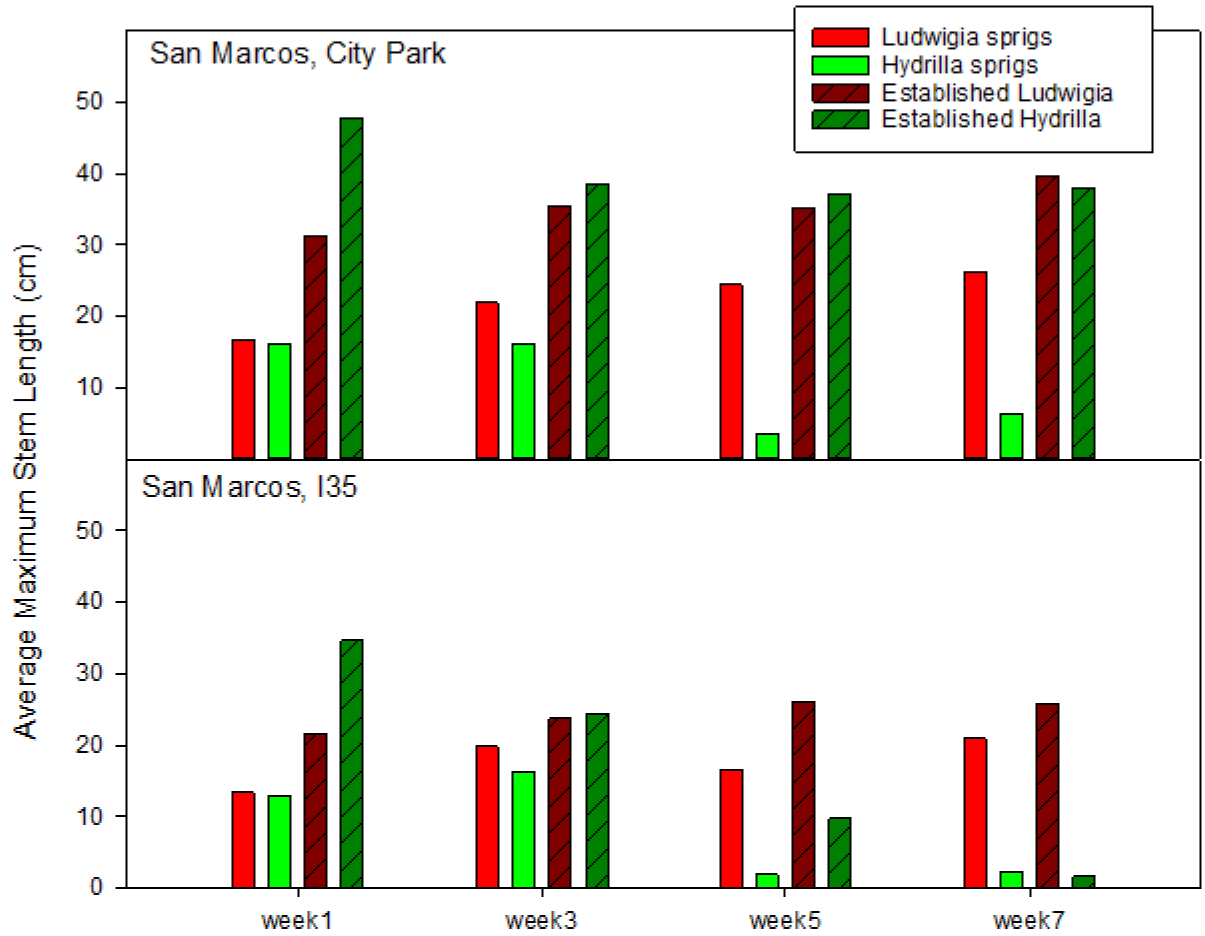

Figure 5. **Average maximum stem length of plants at each of the two experimental locations on the San Marcos River. Data is shown for sprigs of Ludwigia (red) and Hydrilla (green) as well as established Ludwigia (dark red, hatched) and Hydrilla (dark green, hatched).**

Figure 6. **MUPPT at final harvest at San Marcos City Park (Site 1) location. Ludwigia plants (red) showed very robust growth. Hydrilla plants (green) showed variable success, although some plants were clearly very healthy.**

3.3 Ludwigia X Hygrophila Sprig Competition Experiments.

Table 5 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hygrophila. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across all four planting locations and vice versa, the impacts of location were consistent regardless of level of competition.

Competition was not significant ($p>0.05$) for all growth parameters measured for both species. Even though the competition factor was not significant at the 0.05 level, Ludwigia total mass and total number of shoots showed a tendency toward lower values when the sprigs were planted into established Hygrophila ($P=0.07$, Table 5, Figure 7, white bars). However, there was no indication of lowered growth when the Ludwigia sprigs were planted with Hygrophila sprigs. The average maximum length at harvest and allocation of tissues to above ground versus below ground tissues of Ludwigia sprigs were not impacted by competition (Table 5, $P=0.30$, 0.62, respectively).

When planted in monoculture (two sprigs in empty pots), the biomass of Ludwigia at the end of the growth period exceeded that of Hygrophila by about 3.5x (Table 5, Figure 7). This result differs from that of Doyle et al. (2003) where the plants in monoculture had virtually identical growth.

Table 5 shows strong location effects on the growth of both species, indicating that the planting location had strong impacts on growth at all levels of competition. The location effect is significant for Ludwigia total mass and number of shoots. The biomass and number of shoots of Ludwigia was consistently 2-3x higher at the Landa Lake high light location (LLHL) than at the Landa Lake Low Light (LLLL) and the Old Channel (OC) locations. The impacts of location were much more severe for Hygrophila, where the plants were virtually eliminated at LLHL (possibly by herbivory) but was much higher at the OC location. Only in the OC was the growth of Hygrophila higher than the growth of Ludwigia.

Table 5. **Final mean and standard error (SE) for growth parameters of Ludwigia or Hygrophila sprigs grown under varying levels of competition (none, sprigs, established) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)			Locations (L)*				Two-way ANOVA		
	None	Sprigs	Est.	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia										
Total Mass (g)	1.89^{a} (0.36)	1.90^{a} (0.49)	0.86^{a} (0.20)	2.47^{b} (0.60)	0.96^{a} (0.28)	1.75^{ab} (0.45)	1.01^{a} (0.25)	0.39	0.07	0.04
# shoots	2.59^{a} (0.50)	2.25^{a} (0.46)	1.28^{a} (0.30)	3.21^{b} (0.64)	1.04^{a} (0.23)	2.25^{ab} (0.56)	1.67^{a} (0.41)	0.22	0.07	0.01
Max Lgth (cm)	20.4^{a} (2.6)	20.7^{a} (3.1)	15.1^{a} (2.9)	13.0^{a} (1.6)	16.6^{a} (3.3)	23.4^{a} (3.6)	21.8^{a} (3.9)	0.94	0.30	0.11
AG:BG	4.09^{a} (0.61)	4.31^{a} (0.51)	3.28^{a} (0.97)	3.23^{a} (0.43)	4.60^{a} (1.01)	3.74^{a} (0.82)	4.25^{a} (0.82)	0.47	0.62	0.73
Hygrophila										
Total Mass (g)	0.54^{a} (0.23)	0.89^{a} (0.28)	0.38^{a} (0.12)	0.02^{a} (0.01)	0.09^{ab} (0.05)	0.95^{bc} (0.21)	1.35^{c} (0.42)	0.33	0.19	0.00
# shoots	0.94^{a} (0.36)	1.18^{a} (0.26)	0.72^{a} (0.18)	0.13^{a} (0.07)	0.21^{a} (0.08)	1.63^{b} (0.35)	1.83^{b} (0.42)	0.48	0.40	0.00
Max Lgth (cm)	8.8^{a} (3.17)	16.3^{a} (4.0)	7.6^{a} (2.6)	0.3^{a} (0.2)	5.0^{a} (2.8)	19.1^{b} (4.4)	18.6^{b} (4.6)	0.19	0.08	0.00
AG:BG	4.66^{a} (1.63)	5.86^{a} (1.20)	2.02^{a} (0.58)	0.50^{a} (1.41)	2.88^{a} (1.38)	3.81^{a} (0.81)	5.49^{a} (1.35)	0.34	0.08	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

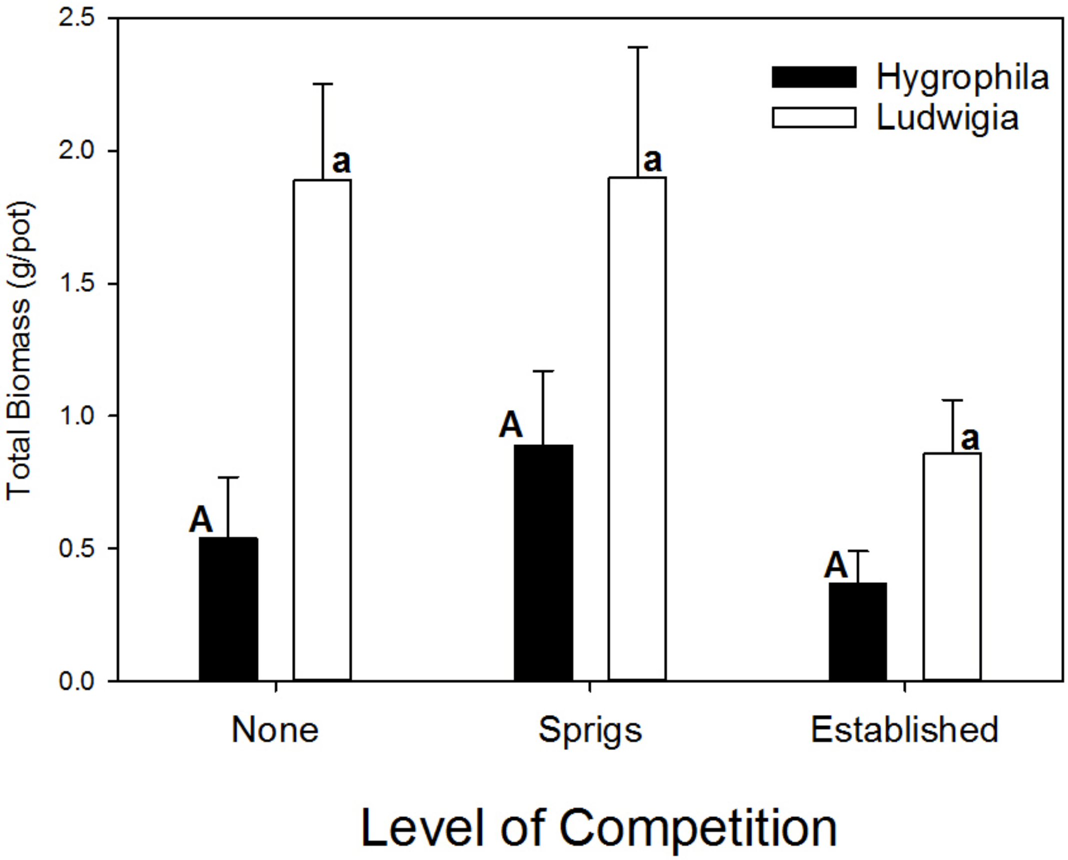

Figure 7. **Final total biomass of plants of Hygrophila (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor was not significant for either species (P=0.19 Hygrophila, P=0.07 Ludwigia).**

3.4 Ludwigia X Hygrophila Continued Growth of Established Plants With and Without Invasion.

Table 6 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across all four planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was not impacted by invasion with Hygrophila sprigs. The averages of plants grown without competitive pressure and those invaded by sprigs of Hygrophila were virtually identical (Table 6, Figure 8). This result differs strongly from that of Doyle et al. (2003), where invasion of sprigs suppressed the continued growth of Ludwigia by 35%.

Surprisingly, the growth of Hygrophila was somewhat impacted by invasion by Ludwigia sprigs (Table 6). Hygrophila shoot number was significantly reduced ($P=0.03$) while total biomass showed a tendency to be reduced by about 30% ($P=0.10$, Figure 8) and plants tended to have lower proportional growth of above ground tissues ($P=0.06$). This comparison was not made by Doyle et al. 2003.

The continued growth of established Ludwigia and Hygrophila plants was also strongly impacted by planting location (Table 6, $P<0.00$ for all parameters measured). For example, the total biomass of Ludwigia at USR was 6.5X higher than that in the OC. The location impact was even larger for Hygrophila where total biomass at the OC site exceeded that at LLHL by more than 15X.

Table 6. Final mean and standard error (SE) for growth parameters of established Ludwigia or Hygrophila grown without competitive pressure (none) or after invaded by two sprigs of the other species (invaded) at four locations in the Comal River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.

	Competition Treatments (C)		Locations (L)*				Two-way ANOVA		
	None	Invaded	LLHL	LLLL	USR	OC	C X L	C	L
Ludwigia									
Total Mass (g)	5.60^{a} (1.21)	5.49^{a} (0.97)	6.24^{b} (1.17)	2.42^{a} (0.75)	11.67^{c} (1.40)	1.78^{a} (0.40)	0.61	0.91	0.00
# shoots	7.94^{a} (1.42)	4.94^{a} (0.88)	5.08^{a} (0.80)	1.92^{a} (0.50)	11.75^{b} (1.63)	2.33^{a} (0.68)	0.88	0.37	0.00
Max Lgth (cm)	26.3^{a} (4.9)	24.7^{a} (2.8)	17.2^{a} (1.7)	20.7^{a} (5.1)	45.1^{b} (2.0)	18.0^{a} (4.9)	0.40	0.67	0.00
AG:BG	1.81^{a} (0.39)	1.84^{a} (0.38)	1.09^{a} (0.12)	1.19^{a} (0.44)	4.06^{b} (0.58)	0.72^{a} (0.18)	0.16	0.99	0.00
Hygrophila									
Total Mass (g)	7.27^{a} (1.87)	5.08^{a} (0.85)	0.74^{a} (0.24)	3.88^{ab} (0.95)	7.31^{bc} (0.93)	11.32^{c} (2.17)	0.29	0.10	0.00
# shoots	6.00^{b} (1.38)	4.04^{a} (0.60)	0.92^{a} (0.29)	3.08^{ab} (0.82)	5.67^{b} (0.86)	9.17^{c} (1.23)	0.06	0.03	0.00
Max Lgth (cm)	38.1^{a} (6.4)	31.9^{a} (4.2)	4.0^{a} (1.1)	34.5^{b} (7.0)	41.9^{bc} (4.4)	55.4^{c} (3.8)	0.63	0.21	0.00
AG:BG	4.32^{a} (1.18)	2.54^{a} (0.52)	0.46^{a} (1.07)	2.87^{a} (0.94)	3.95^{ab} (0.86)	5.71^{b} (0.72)	0.14	0.06	0.00

*Locations: Landa Lake High Light (LLHL), Landa Lake Low Light (LLLL), Upper Spring Run (USR), Old Channel (OC)

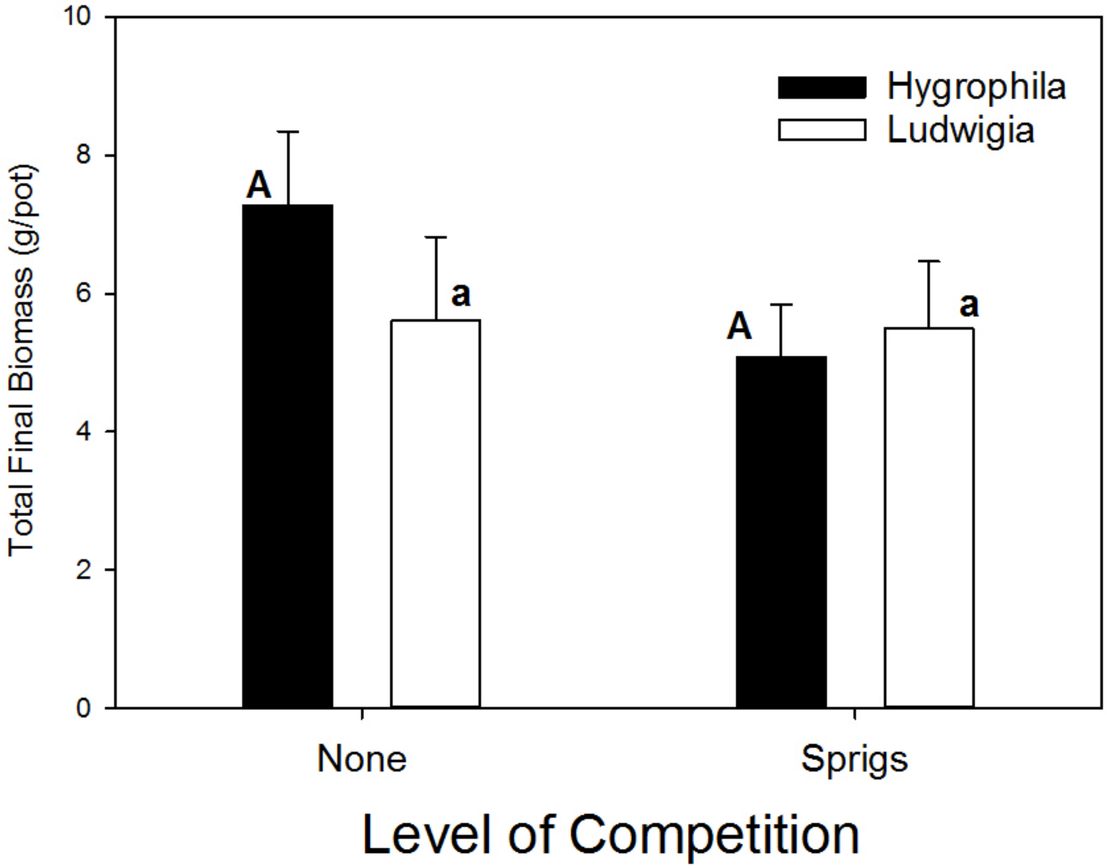

Figure 8. **Final total biomass of established plants of Hygrophila (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The treatment factor (shown) was not significant for either species (P=0.10 Hygrophila, P=0.91 Ludwigia).**

3.5 Ludwigia X Hydrilla Sprig Experiments.

Table 7 reports the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the growth of establishing sprigs of Ludwigia and Hydrilla in the San Marcos River. Notably, the lack of significant interaction between the two factors (C X L) allows evaluation of the C and L main effects. This lack of a significant interaction effect confirms that the pattern of competition impacts on the plant growth was consistent across each planting location and vice versa, the impacts of location were consistent regardless of level of competition. This finding is particularly significant in light of the fact that the experiments at the two locations on the San Marcos did not occur simultaneously. As described earlier, the initial downstream location planted on April 23 was completely scoured by flooding prior to harvest. This downstream site was re-planted at I35 (Site 2B) in early July. Hence, the "location" factor for the San Marcos also contains a "season" factor imbedded in it.

The very poor survival and growth of Hydrilla sprigs when grown without competition was a very surprising outcome (Table 7, Figure 9). In fact, by the end of the experiment, most pots planted with Hydrilla sprigs failed to survive at all. Importantly, this identical same result was found for Hydrilla sprigs at both locations, which include the upstream planting made in April and the downstream planting in July. Ludwigia survival and growth when planted into empty pots was vigorous, and much higher than that of Hydrilla (Figure 9).

Ludwigia biomass accumulation over the experimental growth period was negatively impacted by Hydrilla competition, despite the poor growth of the Hydrilla sprigs. Ludwigia sprigs competing with Hydrilla sprigs or with established plants of Hydrilla showed significant declines of 25% and 64% respectively compared to Ludwigia sprigs grown alone (Figure 9). Additionally, all Ludwigia growth parameters measured showed a significant negative response to Hydrilla competition. In addition to biomass, shoot number, maximum length, and proportional investment in above ground tissues were all significantly lower for sprigs planted into pots with established Hydrilla (Table 7).

The level of Ludwigia competition was not a significant factor in Hydrilla growth. Hydrilla sprig growth was statistically similar at all levels of Ludwigia competition. However, the overall very poor growth of Hydrilla sprigs likely masks any possible competitive impact Ludwigia may have had.

The location factor was significant for Ludwigia total mass and number of shoots, with higher values for plants grown at the I35 location. In contrast, location was not a significant factor for Hydrilla biomass or stem number, likely due to the overall poor growth of Hydrilla sprigs at both locations.

Table 7. **Final mean and standard error (SE) for growth parameters of Ludwigia or Hydrilla sprigs grown under varying levels of competition (none, sprigs, established) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences among competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)			Locations (L)*		Two-way ANOVA		
	None	Sprig	Est.	CP	I35	C X L	C	L
Ludwigia								
Total Mass (g)	6.57^{c} (0.61)	4.96^{b} (0.61)	2.39^{a} (0.57)	3.78^{a} (0.41)	5.87^{b} (0.63)	0.32	*0.00*	*0.00*
# shoots	5.44^{b} (0.80)	4.19^{ab} (0.39)	3.19^{a} (0.52)	3.04^{a} (0.29)	5.50^{b} (0.56)	0.20	*0.01*	*0.00*
Max Lgth (cm)	34.5^{b} (1.4)	29.6^{ab} (1.5)	26.3^{a} (2.2)	30.8^{a} (1.2)	29.46^{a} (1.8)	0.56	*0.02*	0.50
AG:BG	7.01^{b} (0.74)	6.14^{ab} (0.83)	4.69^{a} (0.58)	7.54^{b} (0.66)	4.36^{a} (0.32)	0.24	*0.03*	*0.00*
Hydrilla								
Total Mass (g)	0.06^{a} (0.02)	0.22^{a} (0.14)	0.13^{a} (0.03)	0.17^{a} (0.10)	0.11^{a} (0.02)	0.26	0.42	0.55
# shoots	0.38^{a} (0.18)	0.94^{a} (0.27)	1.06^{a} (0.25)	0.88^{a} (0.21)	0.71^{a} (0.19)	0.12	0.09	0.53
Max Lgth (cm)	2.5^{a} (1.3)	6.2^{a} (3.7)	5.1^{a} (1.8)	7.2^{a} (2.7)	1.6^{a} (0.7)	0.37	0.54	0.07
AG:BG	1.00^{a} (0.58)	2.15^{a} (1.35)	2.07^{a} (1.60)	4.07^{b} (1.74)	0.23^{a} (0.08)	0.52	0.45	*0.02*

*Locations: City Park (CP), Interstate I35 crossing (I35)

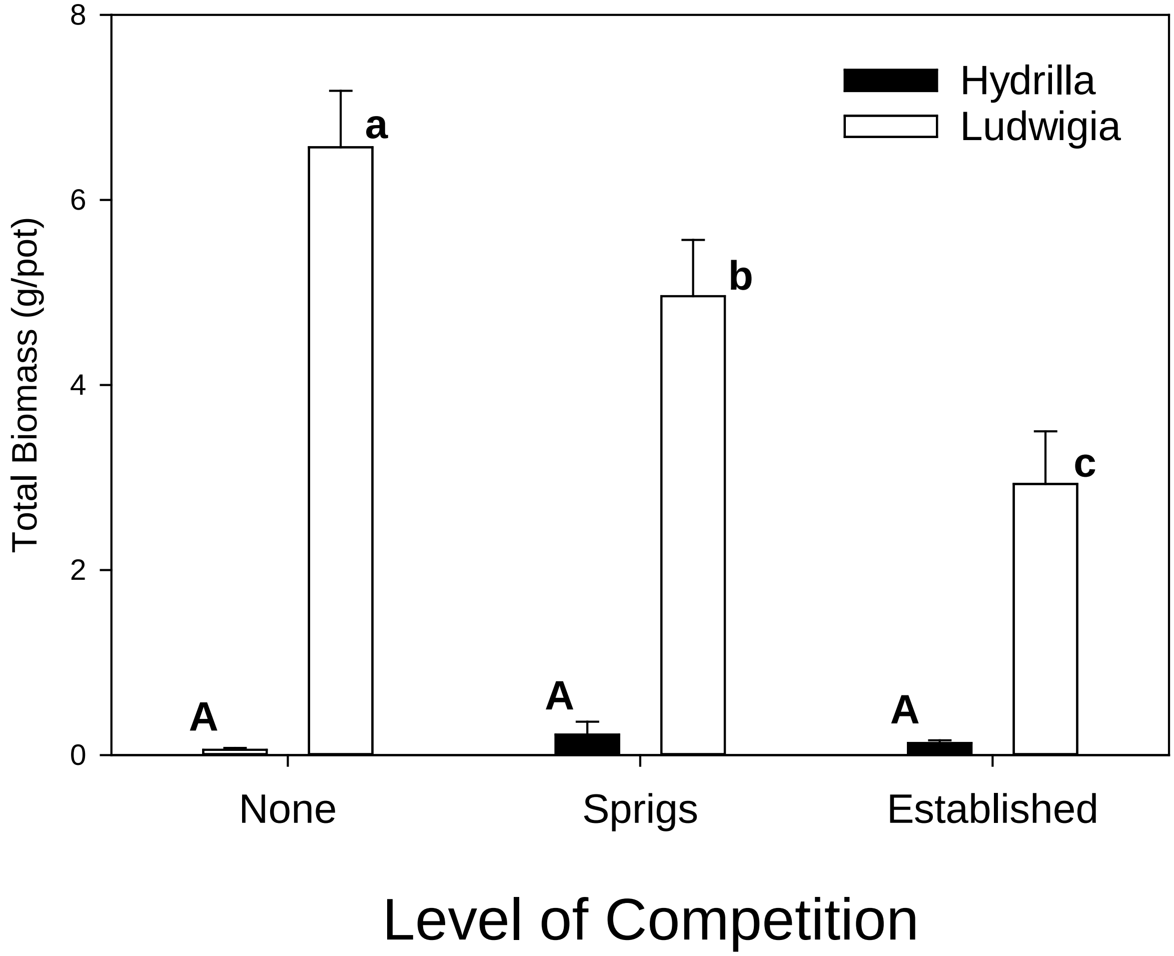

Figure 9. **Final total biomass of plants of Hydrilla (black bars) or Ludwigia (white bars) grown from two planted sprigs under three levels of competition (no competitor, two sprigs of competitor, established competitor). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor was significant for Ludwigia (P=0.00) with declining total biomass as level of competition increased. The competition factor was not significant for Hydrilla (P=0.42) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

3.6 Ludwigia X Hydrilla Continued Growth of Established Plants With and Without Invasion. Table 8 shows the outcome of the two-way ANOVA investigating the impact of competition (C) and location (L) on the continued growth of established Ludwigia and Hydrilla plants with and without invasion by sprigs of the other species. For both species, the lack of a significant interaction effect (C X L) for most parameters allows the evaluation of the main effects (C and L) on the growth of the plants. Again, this fact confirms that the pattern of competition impact on the plant growth was consistent across both planting locations and vice versa, the impact of location was consistent regardless of level of competition.

The continued growth of established Ludwigia plants was impacted by invasion with Hydrilla sprigs (Figure 10). The biomass of established Ludwigia plants invaded by Hydrilla was significantly reduced by 17% relative to plants continuing to grow without invasion. This invasion impact is particularly notable given the overall poor growth of the Hydrilla sprigs. Possibly, under conditions with higher Hydrilla growth, the impact on the Ludwigia may be higher.

The continued growth of established Hydrilla plants was not impacted by Ludwigia competition (P=0.32). There was no statistically significant difference in any of the growth parameters measured for Hydrilla plants invaded by Ludwigia relative to uninvaded plants.

The continued growth of established Ludwigia and Hydrilla plants was significantly impacted by planting location. The total biomass and number of shoots of established Ludwigia plants at the end of the experimental growth period were significantly higher at I35 relative to that at City Park, while the opposite was true for Hydrilla (Table 8).

However, the overall growth of the two species was strikingly different. Overall, established Ludwigia plants growing without competitive pressure was more than 15X higher than that of established Hydrilla growing alone (Figure 10).

Table 8. **Final mean and standard error (SE) for growth parameters of established Ludwigia or Hydrilla grown without competition (none) or after invaded by two sprigs of the other species (invaded) at two locations in the San Marcos River. Also shown is the significance level of the two-way ANOVA testing effect of competition levels and location. Differences between competition levels or among locations determined by HSD-Tukey post hoc comparisons if interaction term was not significant and indicated by different letter superscripts.**

	Competition Treatments (C)		Locations (L)*		Two-way ANOVA		
	None	Invaded	CP	I35	C X L	C	L
Ludwigia							
Total Mass (g)	23.98[b] (1.29)	19.79[a] (1.48)	18.16[a] (1.43)	24.20[b] (1.32)	0.52	<u>*0.04*</u>	<u>*0.01*</u>
# shoots	11.88[a] (1.32)	11.69[a] (0.69)	9.75[a] (0.62)	13.75[a] (0.71)	0.22	0.86	<u>*0.00*</u>
Max Lgth (cm)	40.4 (2.7)	40.9 (1.3)	43.8 (1.2)	37.7 (1.8)	<u>*0.01*</u>	0.82	0.00
AG:BG	4.20[a] (0.33)	4.33[a] (0.27)	4.38[a] (0.31)	4.19[a] (0.29)	0.38	0.78	0.49
Hydrilla							
Total Mass (g)	1.59[a] (0.43)	2.71[a] (0.87)	3.86[b] (1.03)	0.81[a] (0.14)	0.26	0.32	<u>*0.03*</u>
# shoots	5.50[a] (1.02)	4.63[a] (0.68)	5.08[a] (0.75)	4.75[a] (0.86)	0.43	0.49	1.00
Max Lgth (cm)	25.7[a] (9.14)	24.7[a] (6.9)	46.0[b] (6.4)	4.2[a] (0.8)	0.99	0.89	<u>*0.00*</u>
AG:BG	1.31[a] (0.49)	2.00[a] (0.65)	3.29[b] (0.68)	0.26[a] (006)	0.34	0.35	<u>*0.00*</u>

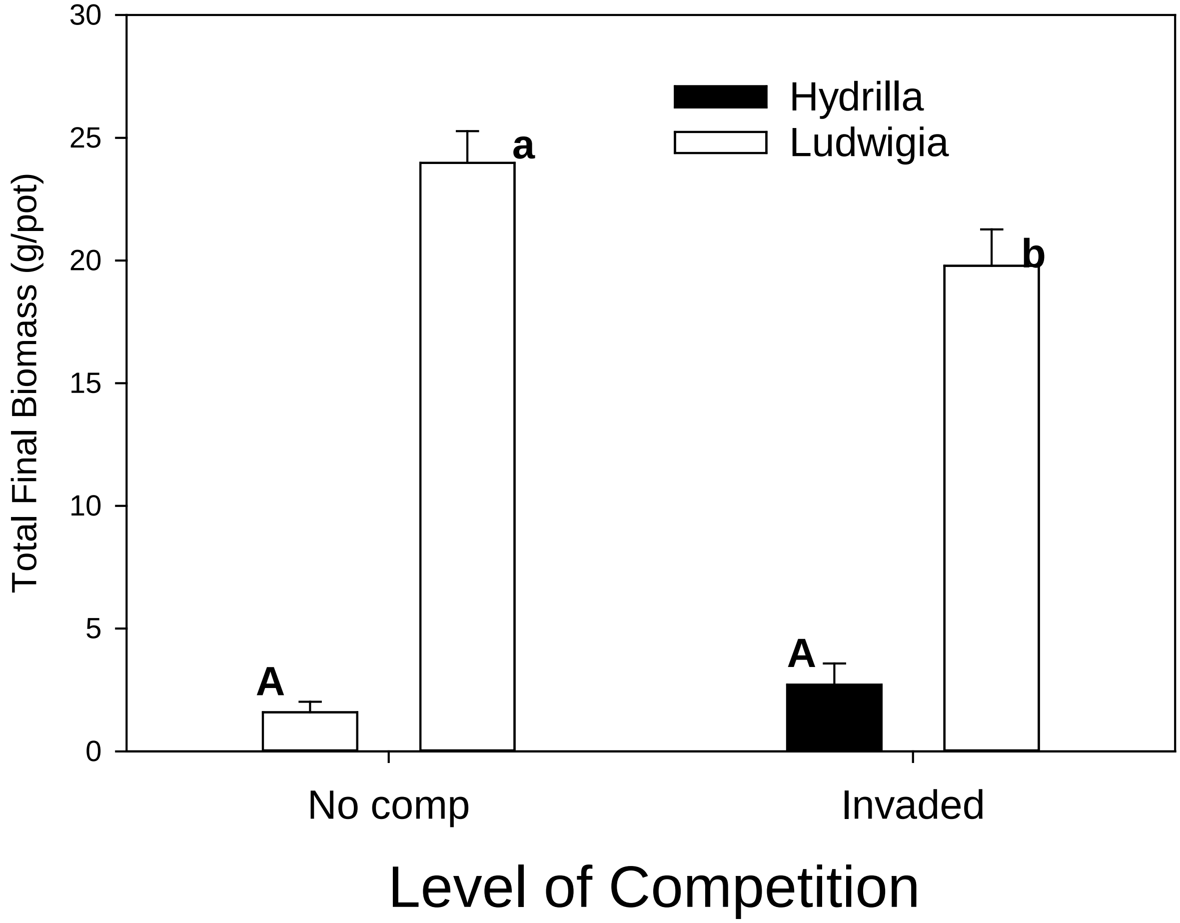

Figure 10. **Final total biomass of established plants of Hydrilla (black bars) or Ludwigia (white bars) grown with no competitive pressure (none) or invaded by two sprigs of the other species (sprigs). Shown are mean +/- SE. Two Way ANOVA (Location X Treatment) analysis showed no significant interaction between terms. The competition factor (shown) was significant for Ludwigia (P=0.04) with lower biomass levels in pots invaded by Hydrilla sprigs. The competition factor was not significant for Hydrilla (P=.32) although these results appear to be highly impacted by heavy herbivory and biomass loss.**

4.0 Discussion

Ludwigia is a native plant that appears to face competitive pressure from Hygrophila and Hydrilla, two widely distributed non-native species in the Comal (Hygrophila) and San Marcos (Hygrophila and Hydrilla) Rivers. All of these species share a similar branching growth form and are capable of asexual reproduction via establishments of viable sprigs. However, Ludwigia provides better habitat for the endangered fountain darters, and is currently being widely used in native plant restoration efforts in both rivers.

4.1 Growth of all species without competition

The results of the short-term competition experiments are generally good news for the continued use of Ludwigia in habitat restoration/enhancement efforts. The growth of Ludwigia sprigs (ll treatment) under no-competition conditions exceeded that of Hygrophila and Hydrilla (hh treatments). In fact, the establishment and growth of sprigs of the native species was more than 3X higher than Hygrophila (Table 5) and more that 10x higher than Hydrilla (Table 7) in our 10-week growth experiments. Both Hygrophila and Hydrilla sprigs appear to have suffered high mortality and poor growth under the experimental conditions tested. Likewise, the total biomass of established Ludwigia plants growing without competition (LL) was similar to that of Hygrophila (HH) (Table 6, Figure 8) and much higher than that observed for Hydrilla (HH) (Table 8, Figure 10).

These in-situ experiments include effects other than competitive interactions between the plants. Notably, we believe that herbivory negatively affected all experimental plants and proved particularly detrimental to the establishment of Hygrophila and Hydrilla sprigs. During routine monitoring we observed that the Hygrophila and Hydrilla sprigs often appeared damaged, and in some cases were entirely missing from the planted pots. In the Comal study red swamp crayfish (*Procambarus clarkii*) were observed burrowing into soil within pots and final harvest and clipped stems of some plants, especially those growing in the Landa Lake High Light location, were evident. For the established Hygrophila and Hydrilla plants, a potential explanation for the loss or zero net gain in biomass could be due to the brittle or easily fragmenting nature of the stems – a potential trade-off which might be advantageous for dispersal and colonizing new habitats.

The strong growth of Ludwigia under “no competition” conditions confirm the experience of restoration efforts in the Comal River that Ludwigia establishment and short-term growth is excellent.

4.2 Impacts of Hygrophila Competition and Location on Ludwigia Growth.

The growth of Ludwigia sprigs was not impacted by Hygrophila competition under the conditions tested in the Comal River. These results differ sharply from those of Doyle et al. (2003) that found that Ludwigia sprig relative growth rate was strongly impacted by competition from Hygrophila sprigs (-40%) and profoundly suppressed by the presence of established Hygrophila plants (-80%).

The continued growth of established Ludwigia was likewise not impacted by competition from invading sprigs of Hygrophila (Table 6). These results also differ from those of Doyle et al. (2003) that found that total biomass of Ludwigia invaded by Hygrophila sprigs to be only 65% of that of uninvaded plants.

Ludwigia growth showed a strong location effect in the Comal River (Tables 5 and 6). The final biomass of the Ludwigia plants that developed from the sprigs varied significantly (2.4X) among the four locations, with higher values at Landa Lake High Light and lower values in the Old Channel and Landa Lake Low Light. Likewise, the final biomass of the established Ludwigia plants at the end of the experimental growth period varied by a factor of 6.5X with the highest values observed at the USR site and the lowest values seen in the OC. It is not surprising that Ludwigia showed strong location impacts, as we deliberately selected locations with variability in the factors known to impact plant growth, especially flow and light conditions. The overall growth of Ludwigia sprigs at Landa high light was more than 2.5 X higher than Landa low light (Table 5, 2.47 g versus 0.96 g) while overall growth of established Ludwigia was also greater than 2.5X at the high light location than at the low light location (Table 6, 6.24 g versus 2.42 g). While light did dramatically impact biomass accumulation, it did not necessarily impact the outcome of competition between Ludwigia and Hygrophila species. The mechanisms regulating the location effect, however, were not clear from these experiments and may warrant additional study to tease out impacts of light or velocity on the competitive interactions between plant species.

4.3 Impacts of Hydrilla Competition and Location on Ludwigia Growth.

In the San Marcos River, Ludwigia sprig growth was impacted by both Hydrilla competition and location. Ludwigia sprigs planted with Hydrilla sprigs or into pots of established Hydrilla showed significant suppression of 25% and 64%, respectively relative to pots growing without any Hydrilla competitor (Table 7). Likewise, established pots of Ludwigia showed significant (17%) suppression of growth when invaded by Hydrilla sprigs. These impacts are particularly notable given the overall poor growth of Hydrilla. For reasons we have not identified, the overall growth of Hydrilla at both locations was much lower than Ludwigia and much lower than expected based on previous experience with Hydrilla. Hydrilla is a widely distributed and successful invasive species that has been shown to be a very strong competitor, especially in "equal start" competition experiments (Smart et al., 1994, Van et al., 1999). However, the results of a New Zealand study which paired Hydrilla with various aquatic species indicate that its growth varies depending on the species with which it is planted and, subsequently, has variable impacts on the resultant biomass of that species (Hofstra et al., 1999).

Ludwigia also showed a significant location effect. Both sprigs and established Ludwigia plants showed significantly higher growth at the I35 site than at the City Park site.

4.4 Summary Evaluation

Overall, these data indicate positive short-term establishment and growth characteristics for Ludwigia, and supports the continued use of the species for restoration efforts. Ludwigia used in restoration efforts is likely to effectively establish and quickly colonize unvegetated areas of the rivers. In fact, the growth of Ludwigia sprigs was higher over the 10-week growth periods than either Hygrophila or Hydrilla. Although both non-native species appear to have suffered from herbivory impacts, there is no reason to believe that the experimental conditions used do not reflect actual levels of herbivory impacts in these systems. Therefore, Ludwigia planted into currently unvegetated areas or areas where the non-native plants have been removed are likely to grow very well.

Furthermore, Ludwigia may be less susceptible to competition impacts than previously documented. Under our experimental growth conditions, Ludwigia sprigs or established Ludwigia plants were not impacted by Hygrophila competition. Ludwigia sprigs and established plants were negatively impacted by Hydrilla, but even there all treatment levels showed significant positive growth.

While a common outcome of invasive versus native plant competition is that the invasive plant wins (hence the term “invasive”) our data show that experiments conducted in situ may show a different outcome. While the biotic growth potential of a species is often linked to invasive species success, the outcome can depend on other factors too. Soil fertility, selective grazing pressures, propagule pre-emption and water velocity as well as other stressors are all factors which may promote the success of a native species and the depression of an introduced species or vice versa. Several studies have investigated the ability of *Vallisneria americana* to dominate over *Hydrilla verticillata* (Van et al., 1999, Smart et al., 1994) but soil fertility seems to determine the outcome. In our study Hydrilla continued to exert impacts upon Ludwigia despite a reduction in top growth biomass. *Hydrilla verticillata* is known to produce dense below ground biomass and propagules which may continue to compete with neighboring plant species despite its loss of stems and leaves. Also, although the Hydrilla plants were not present in some pots at the time of the final harvest, earlier growth in the season may have slowed the growth of the native plant.

The pre-emption of propagule establishment from mature native plant communities can play a preventative role in invasive plant success (Chadwell and Englehardt, 2008). In our study invasion of Hygrophila sprigs had virtually no impact upon established Ludwigia plants. As shown in studies with other invasive aquatic plants the establishment and dominance of the invasive may depend on the degree of intact native plant cover in the area of introduction. If a well-developed native plant community exists at the site of introduction then the opportunity for invasion may substantially decrease (Bickel and Perrett, 2014).

Preferential grazing can heavily impact both native and introduced plants (Parker and Hay, 2005) and evidence suggests that this may be determined by the nutrient content, phenolic compounds or chemical or physical defenses of individual plant species (Lodge, 1990). We witnessed what was believed to be heavy herbivore grazing on Hygrophila and Ludwigia at both Landa Lake sites. While this factor probably does not fully explain our findings, we believe the effect of herbivory warrants further investigation (see below).

Finally, physical characteristics can greatly influence growth of aquatic macrophytes. As witnessed in our study, where location played a significant factor for all three species, exposure to gradients in velocity, depth and light can have significant impacts on plant growth and success. Stream velocities can provide positive conditions for plant growth yet aquatic plant biomass can be greatly reduced once a threshold is surpassed (French and Chambers, 1996) (Madson and Douglas, 2001). However certain species show phenotypic plasticity towards velocity and light gradients and can maintain vigorous growth compared to less adaptable species. A recent competition study conducted by Bilbo (2015) between *Hydrilla verticillata* and *Potamogeton illinoensis* also carried out in the San Marcos River bolsters our findings which indicate Hydrilla growth is not as vigorous when subjected to velocities above a certain threshold and several local studies have been conducted regarding occupancy of aquatic plant species along velocity gradients (Saunders et al., 2001) (Williams, 2013)

In conclusion, our study has shown that in-situ testing of competition between native and non-native aquatic vegetation species in the Comal and San Marcos systems provides differing results than when tested in a no-flow laboratory environment (Doyle et al., 2003). This updated information may be extremely valuable to the development of the EAHCP Ecological model and will be provided directly to that project team for consideration. The study also emphasizes that the successful establishment of aquatic plants is strongly location dependent and furthermore depends on a variety of factors and stressors and that the origin of the plant (native or non-native) does not automatically dictate the success of establishment or the competitive outcome.

5.0 Future Study Considerations

As is common with many studies the outcome of the data tends to ask more questions than provide answers. As such below are a few study questions instigated by the current study which may warrant further investigation.

1. What is the quantity and viability of aquatic plant propagules in the San Marcos and Comal Rivers?

The success of native and non-native aquatic plant establishment relies heavily on propagule production and distribution. In 2000 the distribution and dispersal of propagules of native and nonnative species was investigated in the San Marcos River (Owens et al., 2001). One indication garnered from this study was that propagules of non-native species dominated across all study locations while propagules of native species were poorly represented and many not viable. Unfortunately, this study was not repeated in the Comal River. With on-going large scale removal of invasive plant species and re- introduction of native species a current understanding of propagule loading rates and viability would be important to help determine the future sustainability and outcome of the restoration projects in both systems.

2. What is the nutrient availability and how does nutrient partitioning influence growth of aquatic plants in the San Marcos and Comal Rivers?

As discussed previously several factors affect the recruitment, growth, persistence and expansion of aquatic plants in river systems. Nutrient stoichiometry—the ways in which aquatic plants use and partition nutrients—is an important process which either limits or drives the productivity of aquatic plants, but species respond to and use nutrients differently (Barko et al., 1991). Elevated levels of sediment nitrogen can limit the productivity of aquatic plant species or increase productivity in other species and uptake mechanisms of nutrients varies greatly by species (Fang et al., 2007). In essence, one factor which contributes to the growth and health of aquatic plants within these systems is sediment nutrients which have yet to be researched in-depth in either the Comal or San Marcos systems. A study to investigate the fertility of the sediment and how native and introduced plant species use or partition those nutrients would be an important step towards understanding and predicting the prolonged composition of the aquatic plant community in both systems.

3. What role does herbivory play in the establishment, growth and expansion of aquatic plants in the San Marcos and Comal Rivers?

Another observation often noted during active restoration and experimentation efforts is the impact of herbivory on plant establishment and continued growth. Defoliation pressures on the native and non-native species in this system are not well understood as they are imposed by a wide array of herbivorous vertebrate and invertebrate species. Many insect species are known to have specialized, co-evolved relationships with aquatic host plants, affecting not only floating or emergent leaf tissue but submerged anatomical features as well (Harms and Grodowitz, 2009). Recent documentation details the destructive impacts of a moth species' aquatic larvae on the native aquatic plant nurseries at the San Marcos Aquatic Resources Center (Hutchinson et al., 2015). Destruction of plant growth by aquatic caterpillars has been observed in the field as well. The invasive giant rams-horn snail (*Marisa cornuarietis*) - known to have a voracious appetite - and other herbivorous mollusks have been observed and documented feeding on the local vegetation (Grantham et al., 1995; Horne et al., 1992; Karatayev et al., 2009). Other common species with aquatic plant-dominated diets include crayfish, turtles, tilapia and water fowl. Observational and reported data suggest that the sustainability of restoration efforts could benefit from a deeper analysis of herbivore pressures.

6.0 Literature Reviewed and Literature Cited

Agami, M., Waisel, Y., 1985. Inter-relationships between *Najas marina* L.and three other species of aquatic macrophytes. Hydrobiologia, 126:169-173.

Aiken, S. G., P. R. Newroth and I. Wile. 1979. The biology of Candaian weeds. 34. *Myriophyllum spicatum* L. Can. J. Plant Sci., 59:201-215.

Angerstein, A. B., and D. E. Lemke. 1994. First records of the aquatic weed *Hygrophila polysperma* (Acanthaceae) from Texas. Sida, 16:365-371.

Barrat-Segretain, M.H. 1996. Strategies of reproduction, dispersion and competition in river plants: A review. Vegetation, 123:13-37.

Bickel, T.O. and C. Perrett. 2014. Competitive performance of *Cabomba caroliniana*. In: 19th Australasian Weeds Conference, September 2014, Tasmanian Weed Society, Hobart, Tasmania.

Bilbo, J. N. 2015. Competitive interactions between native and invasive macrophyte species in a spring fed river. Master's thesis. Texas State University, San Marcos, TX. 39p

BIO-WEST. 2015. Habitat Conservation Plan Biological Monitoring Program: Comal Springs / River Aquatic Ecosystem. 2014 Annual Report. Technical report to the Edwards Aquifer Authority. 94p plus appendices.

Bormann, R. L. 2012. Native macrophyte restoration in a spring fed river system. master's thesis. Baylor University, Waco, TX. 90p

Bowles, D.E., and B. D. Bowles. 2001. A review of the exotic species inhabiting the upper San Marcos River, Texas, USA. Texas Parks and Wildlife Department, Austin, TX, 30 p.

Bowes, G., T. K. Van, L. A., Garrard, W. T. and Hailer. 1977. Adaptation to low light levels by Hydrilla, Journal of Aquatic. Plant Management, 15:32-35.

Chadwell, T. B., and K. Engelhardt. 2008. Effects of pre-existing submersed vegetation and propagule pressure on the invasion success of *Hydrilla verticillata*. Journal of Applied Ecology, 45.2:515-523.

Chambers, P.A., Prepas, E.E. 1990. Competition and coexistence in submerged aquatic plant communities: the effects of species interactions versus abiotic factors. Freshwater Biol., 23:541-550.

Dibble, E. D., K. J. Killgore, and G. O. Dick. 1996. Measurement of plant architecture in seven aquatic plants. Journal of Freshwater Ecology, 11:311-318.

Doyle, R.D. 2001. Expansion of the exotic aquatic plant *Cryptocoryne beckettii* (Araceae) in the San Marcos River, Texas. Sida, 19:1027-1038.

Doyle, R.D., M. D. Francis, and R. M. Smart. 2003. Interference competition between *Ludwigia repens* and *Hygrophila polysperma*: two morphologically similar aquatic plant species. Aquat. Bot., 77:223-234.

Fang, Y. Y., O. Babourina, Z. Rengel, X. E. Yang, and P. M. Pu. 2007. Spatial distribution of ammonium and nitrate fluxes along roots of wetland plants. Plant Science, 173:240-246.

Fast. B.J., C. J. Gray, J. A. Ferrell, G. E. Macdonald and F. M. Fishel. 2008. Water regime and depth effect on Hygrophila growth and establishment. 46:97-99.

French, T. D. & P. A. Chambers, 1996. Habitat partitioning in riverine macrophyte communities. Freshwater Biology. 36:509–520.

Garbey C, Thiebaut G, Muller S. 2004. Morphological plasticity of a spreading aquatic macrophyte, *Ranunculus peltatus*, in response to environmental variables. Plant Ecology 173: 125–137.

Gopal B. and Goel U. 1993. Competition and allelopathy in aquatic plant communities. Botanical Review, 59:155-210.

Grantham, O.K., Moorehead, D.L. and Willig, M.R. 1995. Foraging strategy of Giant Ramshorn snail, *Marisa cornuarietis*: an interpretive model. Oikos, 72:333-342

Gross, E.M. 2003. Critical Reviews in Plant Science, 22:313-339.

Harms, N.E., M. J. Grodowitz, 2009. Insect herbivores of aquatic and wetland plants in the United States: a checklist from literature. Journal of Aquatic Plant Management. 47:73-96.

Haynes, R. R. 1988. Reproductive biology of selected aquatic plants. Ann. Missouri Bot. Gard. 75:805-810.

Hofstra DE, Clayton J, Green JD, Auger M. 1999. Competitive performance of *Hydrilla verticillata* in New Zealand. Aquatic Botany, 63:305-324.

Horne F.R., Arsuffi, T.L. and Neck, R.W. 1992. Recent introduction and potential botanical impact of the Giant Rams-horn Snail, *Maris cornuarietis* (Pilidae), in the Comal Springs ecosystem of Central Texas. The Southwestern Naturalist 37:194-214.

Hutchinson, J. T., Huston, D. C., and Gibson, J. R. 2015. Defoliation of cultured creeping primrose willow (*Ludwigia repens*) and other aquatic plants by *Parapoynx obscuralis* (Lepidoptera: Crambidae). The Southwestern Entomologist, 40:227-232.

Kadono, Y., 2004. Alien aquatic plants naturalized in Japan: history and present status. Global Environ. Research, 8:163-169.

Kartesz, J.T., The Biota of North America Program (BONAP). 2014. North American Plant Atlas. (http://bonap.net/napa). Chapel Hill, N.C. [maps generated from Kartesz, J.T.

2014. Floristic Synthesis of North America, Version 1.0. Biota of North America Program (BONAP). (in press)].

Karatayev, A. Y., L. E. Burlakova, V. A. Karatayev and D. K. Padilla. 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. Hydrobiologia, 619:181–194.

Lemke, D. E. 1989. Aquatic macrophytes of the upper San Marcos River, Hays Co., Texas. The Southwestern Naturalist, 289-291.

Lodge, D. M. 1991. Herbivory on freshwater macrophytes. Aquatic botany, 41:195-224.

Madsen, J. D., P. A., Chambers, W. F., James, E. W., Koch, and D. F., Westlake. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia, 444:71-84.

Madsen, J. D., J. W. Sutherland, J. A. Bloomfield, L. W. Eichler and C. W. Boylen. 1991. The decline of native vegetation under dense Eurasian watermilfoil canopies, J. Aquat. Plant Management, 29:94-99.

Madsen, J.D. and D. Smith. 1999. Vegetative spread of dioecious Hydrilla colonies in experimental ponds. J. Aquat. Plant Management, 37:25-29.

McFarland, D. G., and J. W. Barko. 1987. Effects of temperature and sediment type on growth and morphology of monoecious and dioecious Hydrilla. Journal of Freshwater Ecology. 4:245-252.

McCreary, N. 1991. Competition as a mechanism of submersed macrophyte community structure. Aquatic Botany, 41:177-193

Mora-Oliva, M., T. F. Daniel and M. Martinez. 2008. First record in the Mexican Flora of *Hygrophila polysperma* (Acanthaceae), an aquatic weed. Revista Mexicana de Biodeversidad, 79:265-269.

Newroth, P.R., 1985. A review of Eurasian water milfoil impacts and management in British Columbia. Proc. First Int. Symp, on watermilfoil (*Myriophyllum spicatum*) and related Haloragaceae species. July 23-34, Vancouver, BC, Canada. 139-153.

Owens, C.S., R. M. Smart and G. O. Dick. 2012. Tuber and turion dynamics in monoecious and dioecious hydrilla (Hydrilla verticillata). J. Aquat. Plant Manage. 50:58-62.

Parker, J. D., and M. E. Hay. 2005. Biotic resistance to plant invasions? Native herbivores prefer non-native plants. Ecology Letters 8.9:959-967.

Santamaria, L. 2002. Why are most aquatic plants widely distributed? Dispersal, clonal growth and small scale heterogeneity in a stressful environment. Aceta Oecologica 23:137-154

Santos, M. J., L. W. Anderson, S. L. Ustin. 2011. Effects of invasive species on plant communities: an example using submersed aquatic plants at the regional scale. Biol Inv., 13: 443-457.

Sculthorpe CD. 1967. The biology of aquatic vascular plants. London UK: Edward Arnold Ltd.

Smart, R., J. W. Barko and D. G. McFarland. 1994. Competition between *Hydrilla verticillata* and *Vallisneria americana* under different environmental conditons. Technical Report A-94-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Spencer, W., and G. Bowes. 1985. Limnophila and Hygrophila: A review and physiological assessment of their weed potential in Florida. J. Aquat. Plant Manage., 23:7-16.

Sutton, D. L., 1995. Hygrophila is replacing Hydrilla in South Florida. Aquatics, 17:4-10.

Sutton, D. L., 1996. Depletion of turions and tubers of *Hydrilla verticillata* in the North New River Canal, Florida. Aquat. Bot., 53:121-130.

Sutton, D. L., R. C. Littell and K. A. Langeland. 1980. Intraspecific competition of *Hydrilla verticillata*. Weed Science., 425-428.

Sutton, D.L. and P. M. Dingler. 2000. Influence of sediment nutrients on growth of emergent Hygrophila. J. Aquat. Plant Manage., 38:55-61.

Szoszkiewicz K. H., A. Ciecierska, S. C. Kolada, M. Schneider, J. Szwabinska. 2014. Parameters structuring macrophyte communities in rivers and lakes – results from a case study in North-Central Poland. Knowledge and Management of Aquatic Ecosystems, 415, 08.

Thouvenot L, Haury J, Thiebaut G. 2013. Seasonal plasticity of *Ludwigia grandiflora* under light and water depth gradients: an outdoor mesocosm experiment. Flora, 208:430-437.

Van Dijk, G.M., D. D. Thayer, W. T. Haller. 1986. Growth of Hygrophila and Hydrilla in flowing water. J. Aquat. Plant Manage., 24:85-87.

Van, T.K. and K. K. Steward. 1990. Longevity of monoecious Hydrilla propagules. J. of Aquat. Plant Manage., 28:74-76.

Van, T.K., G. S. Wheeler, and T. D. Center. 1999. Competition between *Hydrilla verticillata* and *Vallisneria americana* as influenced by soil fertility. Aquat. Bot., 62:225-233.

Williams, C.R., K. Tower and T. Hardy. 2010. San Marcos River Aquatic Vegetation Survey and Inventory. River Systems Institute. San Marcos, TX. 21p.

Williams, C.R., K. Tower and T. Hardy. 2011. Spring Lake Aquatic Vegetation Mapping Project and Historical Assessment. River Systems Institute. San Marcos, TX. 10p.