## DISTRIBUTION AND PHYSICAL HABITAT REQUIREMENTS OF *HYGROPHILA* POLYSPERMA IN THE SAN MARCOS RIVER, HAYS COUNTY, TEXAS

THESIS

Presented to the Graduate Council of Texas State University-San Marcos in Partial Fulfillment of the Requirements

for the Degree

Master of SCIENCE

by

Casey R. Williams B.S.

San Marcos, Texas August 2013

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#### CHAPTER I

#### INTRODUCTION

#### Study Area and Scope of Work

The San Marcos River, beginning in Hays County, Texas, emerges from the Edwards Aquifer/Balcones fault zone in the form of an artesian spring system consisting of several hundred spring openings. The San Marcos Springs are the second largest spring system in Texas (Brune,1981) and were impounded in 1849 forming Spring Lake. Outflows from Spring Lake form the San Marcos River which flows 7.4 km to the confluence with its first major tributary, the Blanco River, and then another 115 km to its confluence with the Guadalupe River (Figure 1). Spring Lake and the San Marcos River are characterized by exceptional water quality including low turbidity, stable water temperatures, and low nitrates reflective of this groundwater source (Groeger et al., 1997). In addition several endemic and endangered species are found in this system. These include the San Marcos salamander (*Eurycea nana*), Texas blind salamander (*Eurycea rathbuni*), fountain darter (*Etheostoma fonticola*) and Texas wild rice (*Zizania texana*). Water quality conditions support a diverse aquatic plant community consisting of native and introduced species. Introduced species have potentially displaced several native aquatic macrophytes including the endangered Texas wild rice (*Zizania texana*). Alterations to the upper San Marcos River, including channelization, construction of diversion channels and low head dams have decreased run and riffle habitats and increased backwater type habitats in the (Saunders et al., 2001). These areas are more suitable habitat for *Hydrilla verticillata*, *Hygrophila polysperma*, *Eichhornia crassipes*, *Pistia stratiotes*, and other exotic species.

Although *Hygrophila polysperma* has been present in the state of Texas for decades, little information regarding the biology, distribution, or growth characteristics of the species in Texas has been published. It is my intention to make others aware of the permanent presence of the species in the San Marcos River and to provide information that may lead to better management plans for the species in the future.

This study covers a wide range of techniques and methods used to answer several questions regarding the presence of *Hygrophila polysperma* along the San Marcos River. *In situ* measurements, geospatial analysis and modeled data were used to determine the distribution and physical habitat characteristics for the species.

Additionally, morphological observations were used to identify the various growth forms of *Hygrophila polysperma* to distinguish it from similar species.

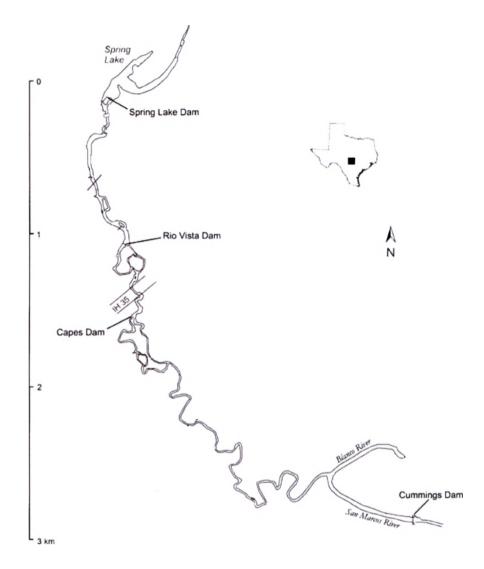


Figure 1. Map of the upper San Marcos River, Hays County, Texas adapted from Poole and Bowles, 1999.

#### History of Hygrophila polysperma

*Hygrophila polysperma* (Roxb.) T. Anderson (Acanthaceae), commonly referred to as hygrophila, is an Old World species historically ranging through India (Rataj and Horman, 1977; Mukherjee, 2011), Malaysia (Angerstein and Lemke, 1994) and Sri Lanka (Senaratna, 1945). Originally introduced into Ohio through the aquarium trade around 1942 as a species of *Ludwgia* (Innes, 1947), the first naturalized population was observed in 1965 in Florida (Schmitz et al., 1988). However, that population was thought to be a species of *Dyschoriste* until 1977 when it was properly identified by Dieter C. Wasshausen of the Smithsonian Institute (Les and Wunderlin, 1981). Currently hygrophila is considered naturalized in the states of Florida and Texas. Although Virginia populations have been observed, they are reportedly susceptible to freezes (Sutton, 1995). Mora-Olivo et al. (2008) also reported the species to occur in the state of Tamaulipas, Mexico.

While hygrophila has a well documented history in Florida, its naturalization in Texas is less well understood. Its current distribution within the state is in Hays, Comal and Val Verde (Figure 2) counties although Turner et al. (2003) mistakenly limit the distribution to only Hays and Comal counties. Angerstein and Lemke (1994) were the first to correctly identify the species as occurring in the San Marcos (Hays County) and Comal (Comal County) rivers.

Previous authors had misidentified the plant as the native species *Hygrophila lacustris* (Lemke, 1989) or *Ludwigia repens* (Staton, 1992). Most authors attribute the presence of the plant to aquarium releases (Angerstein and Lemke, 1994; Bowles and Bowles, 2001;). However, the local San Marcos Daily Record news articles indicate the plant was introduced purposely by aquatic plant nurseries to the San Marcos and Comal rivers to be propagated and collected for the aquarium trade (San Marcos Daily Record, 1962; Boxall, 1976). The actual date of introduction is unknown. Angerstein and Lemke (1994) indicate the plant was probably present at least 25 years before its identification and Bowles and Bowles (2001) label the introduction around 1940. Articles from the San Marcos Daily Record (San Marcos Daily Record, 1962; Boxall, 1976) suggest introduction around 1955. This date would be more consistent with the species' availability to the U.S. aquarium trade than the date Bowles and Bowles suggest. The presence of hygrophila in Val Verde County is not published, but confirmed by this author.

While hygrophila is not currently on the list of prohibited aquatic species for the state of Texas, it is prohibited or regulated in eight states and listed on the federal noxious weed list (Schmitz et al., 1988; USDA-APHIS, 2006).

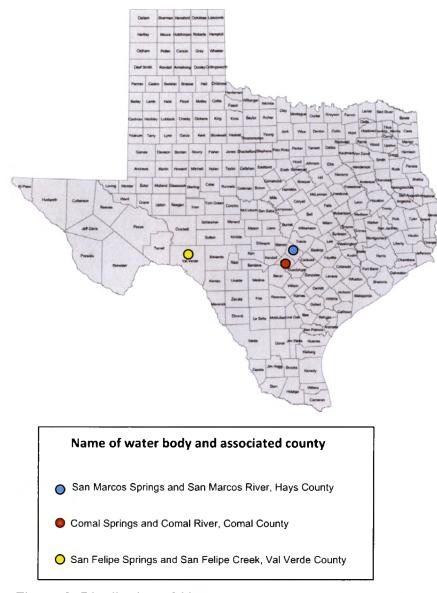


Figure 2. Distribution of Hygrophila polysperma in Texas.

#### Identification and Physical Characteristics of Hygrophila polysperma

The genus *Hygrophila* is included in the family Acanthaceae which is commonly recognized as consisting of usually herbaceous plants with square stems, opposite leaves and cystoliths in the epidermis (Godfrey and Wooten, 1981). Well known aquatic genera in the family include *Justicia*, *Dyschoriste* and *Ruellia*. *Hygrophila* is mostly limited to the Old World tropics with one species, *Hygrophila lacustris*, native to North America.

*Hygrophila polysperma* looks similar to other species causing issues with mis-identification. Since its introduction, the plant has been confused with other members of the family Acanthaceae including *Dyschoriste, Justicia* and the congeneric *Hygrophila lacustris*. These misidentifications can be attributed to a lack of knowledge regarding the presence of hygrophila in the area and collections of sterile material (Angerstein and Lemke, 1994). However, due to hygrophila's variable growth forms and simple opposite leaves it can also be confused with other species in different families. The morphology of submerged hygrophila is similar to that of submerged *Ludwigia repens* and both species also share similar growth characteristics (Doyle et al., 2003). The terrestrial form of hygrophila can be confused with species such as *Calyptocarpus vialis*, *Eclipta prostrata* or emergent *Ludwigia repens* which are commonly found in moist, muddy areas along the edge of waterways.

*Hygrophila polysperma* (Figure 3) can be distinguished from all other species by several methods. In the field hygrophila can be identified as an

herbaceous perennial with opposite simple leaves that are broadly elliptic with acute tips.

Color, size and shape of the leaves can vary depending on light conditions (Reams, 1953) but often the upper leaf surface is reddish or brownish with glabrous undersides appearing a silver or white color. Stems are rhizomatous and are usually square and brittle. Roots are fibrous and shallow spreading in the soil. Adventitious roots commonly occur at the nodes along main stems (Cuda and Sutton, 2000). Flowers are autogamous, solitary and occur at emergent tips within leaf axils. Corollas are purple to bluish-white (Les and Wunderlin, 1981). In Texas, flowering tends to be rare and has been reported to occur September to October (Angerstein and Lemke, 1994). Only a few flowering specimens have been collected along the San Marcos River. The earliest specimen was collected in May of 2010 by the author. Other flowering specimens were collected in Val Verde County in November of 2010 by Dr. David Lemke and the author.

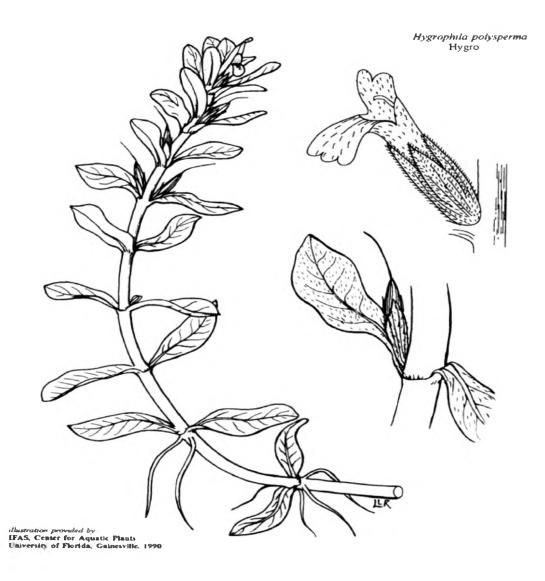


Figure 3. *Hygrophila polysperma*. (left) The distal portion of a submersed stem showing adventitious roots (upper right) flower (lower right) fruit.

A major flowering event occurred along the San Marcos River in October 2011. During this time the river discharge reached a low of 2.55 m<sup>3</sup>/s. Large stands of emergent hygrophila, which are submersed during an average discharge of 4.81 m<sup>3</sup>/s, were observed and collected in flower and fruit in areas along Sewell Park and City Park. Specimens with fruit were collected as were living specimens that were further reared in a greenhouse where fruits reached maturity indicating production of viable seed may be possible (Figure 4). Voucher specimens for these collections have been deposited in the herbarium of Texas State University-San Marcos.

While hygrophila is predominantly a submersed species it does show a wide variety of growth forms (Botts et al., 1990) but does not exhibit heterophylly (Sutton, 1995). The terrestrial form can be described as a short, spreading ground cover with very short internodes, and smaller, green leaves. Submersed growth may also produce emergent tips which have been observed blooming. The presence of cystoliths in the leaf and stem material is a unique characteristic of Acanthaceae that can separate the species from other species that are similar in appearance. In addition Angerstein and Lemke (1994) note nodal morphology (Figure 5) of hygrophila can easily separate it from *Ludwigia repens* and other morphologically similar species. This technique works well regardless of growth form.



Figure 4. *Hygrophila polysperma* with dehisced seed capsule. Specimen collected at Sewell Park in October of 2011.

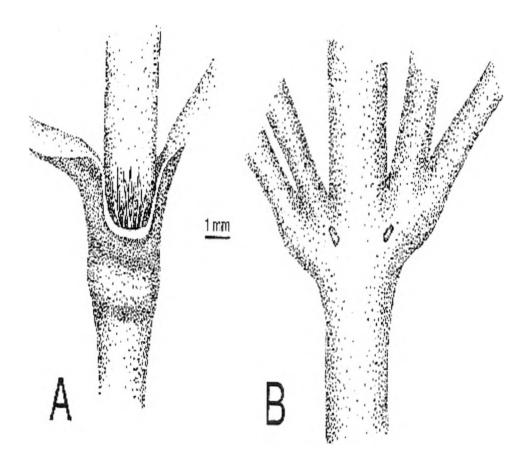


Figure 5. Comparison of nodal morphology in A. *Hygrophila polysperma* and B. *Ludwigia repens*. From Angerstein and Lemke, 1994.

The various growth characteristics and overall hardiness of hygrophila, such as reported tolerance to a wide range of light and water quality conditions, of hygrophila contribute to its success as an invasive weed. Adventitious rooting and ease of fragmentation are two main characteristics which allow for the species' spread (Cuda and Sutton, 2000). Other factors such as the ability of detached leaves to produce roots (Rataj and Horeman, 1977), amphibious growth forms, and potential for high biomass production (Spencer and Bowes, 1985), add to the weediness of hygrophila.

In Florida, hygrophila most often occupies flowing water habitats such as drainage canals (Vandiver, 1980), irrigation ditches, and rivers (Schmitz et al., 1988) with pH ranges between 5 and 7 (Spencer and Bowes, 1985). Van Dijk et al. (1986) concluded that the plant produced an overall higher biomass in moving waters compared to static waters. Hygrophila continues to grow throughout the year in Florida but produces its highest biomass from June to October (Sutton and Dingler, 2000; Cuda and Sutton; 2000). The species' low light compensation points allow for shade adaptability and tolerance and its photosynthetic rate is less influenced by temperature than that of other tropical aquatic species (Spencer and Bowes, 1985).

#### **Objectives**

The purpose for this study was to meet the following objectives:

# 1. Map the current distribution of *Hygrophila polysperma* along the upper San Marcos River.

This objective is aimed at developing a spatially explicit field based vegetation map of aquatic vegetation and the distribution of hygrophila within the San Marcos River.

## 2. Determine a habitat profile based on the following factors; light, soil, water depth, water velocity and associated vegetation.

This objective is intended to determine the physical and biological characteristics associated with the distribution of hygrophila within the San Marcos River.

### 3. Determine areas of suitable habitat based on depth and velocity.

The purpose of this objective is to demonstrate quantitative relationships between depth and velocity, and the spatial distribution of existing and potential hygrophila habitat within the San Marcos River.

#### CHAPTER II

#### MATERIALS AND METHODS

#### Mapping

Mapping of hygrophila in the San Marcos River was conducted as part of a larger aquatic vegetation mapping project and plant inventory for the upper San Marcos River, extending from Spring Lake to Interstate Highway 35, conducted by the Meadows Center for Water and the Environment (formally the River Systems Institute) at Texas State University-San Marcos in support of the Edwards Aquifer Recovery Implementation Program (EARIP) (Williams et al., 2010). Inventory and mapping were conducted between September 2009 and January 2010. The mapping portion of this project was conducted using Trimble Geo XH Geoexplorer 2008 series GPS units (Trimble, US).

Vegetation patches greater than one meter in diameter were delineated using several methods including wading, kayaking and snorkeling. Polygons were produced by tracing the perimeter of plant stands with the GPS unit. Plants were identified to species in the field and herbarium vouchers were collected and deposited in the Texas State University-San Marcos Herbarium and the herbarium of the Botanical Research Institute of Texas. Percent cover per species was visually estimated for mixed patches. Substrate types and percentages within patches were also recorded. Mapping was begun during low flow conditions when the San Marcos River discharge reached a low of 2.70 m<sup>3</sup>/s in August of 2009 followed by an increase to 4.25 m<sup>3</sup>/s in September and further increased to 5.10 m<sup>3</sup>/s by January of 2010.

In order to facilitate a more efficient method of mapping, no patches of vegetation less than an estimated one meter in diameter were recorded with the GPS unit. Attributes for each polygon consisted of the top 4 dominant species; the percent cover for each of those species; and the top 3 substrate types and percentages of each type. In instances where the vegetation was continuous with no specific boundary, plant patches were delineated based on the presence of a dominant species. At this point an area with an observed dominant species was delineated, and the perimeter of the area covered by the dominant species traced producing a polygon vector, and the data fields recorded. In areas shallow enough to be reached by foot, patches were delineated by wading around the perimeter of the patch. In deeper areas patches were delineated by kayak and with the aid of a snorkeler. This allowed for more precise plant identification and estimation of attribute values.

Once these primary data were collected it was post processed. Post processing included projecting the primary vegetation data into the Geographic Coordinate System and datum of WGS 1984. The shape files produced from the primary data were then used for various geospatial analyses in ARCmap 9.2 by ESRI Inc. (Redlands, CA). The U.S. Army Corps of Engineers (Owens et al., 2010) provided vegetation shape files for sections of the San Marcos not mapped during this project, specifically from I-35 to the Blanco River confluence.

Once a single aquatic vegetation map was produced a hygrophila distribution map was created. This distribution map distinguishes between areas where hygrophila was found to be the dominant species (highest percent cover within a polygon) and areas where hygrophila was considered sub-dominant thus representing the widespread distribution of hygrophila along the San Marcos River. Two hundred five dominant hygrophila stands were identified and a separate shape file for dominant hygrophila was produced to use in modeling and analysis.

#### Determining a Habitat Profile

To determine a habitat profile, physical and chemical data were collected. The study area used to determine the habitat profile for hygrophila was limited to the area of the San Marcos upstream of the Interstate 35 bridge (Figure 5 ). This was done for several reasons. First, this area has a high abundance of hygrophila and second, this area allowed for much easier access for data gathering. This study area was further divided into four river segments. The Sewell Park to City Park segment; City Park to Snake Island segment; Snake Island to Rio Vista segment and Rio Vista to I-35 segment.

Standard survey equipment and Trimble XH GPS units were used to collect the physical data throughout each river segment. Physical data were gathered using a "systematic irregular sampling strategy that targets capturing all available spatial heterogeneity within the river," and reported by Hardy et al. (2011). Data including latitude (x), longitude (y), depth, and substrate type were recorded for surveyed points. The raw data was converted into three dimensional orthogonal rectilinear grid cells of approximately 0.25 meter resolution. Each grid cell of data gathered in these segments was later used to produce an array of hydraulic and habitat suitability models, in GIS, for multiple species in support of analyses for the Edwards Aquifer Recovery Implementation Plan (Hardy et al., 2011).

The shape files including dominant hygrophila stands was overlaid onto each of the four hydraulic modeling grids produced from the irregular sampling technique conducted by Hardy et al. (2011) as well as the associated hydraulic model results for depth and velocity in each grid at a discharge of 6.00 m<sup>3</sup>/s. This discharge represents an approximation to the discharge rate when a majority of the mapping and data collection took place and is also near the historical mean annual discharge recorded at the USGS gage # 08170500 from the period of 1956 to 2012. The grid areas in the model not encompassed by a hygrophila polygon were removed. The presence of hygrophila within the sub meter grid cells, which are the basic unit of the models, was used to calculate the frequency distribution for two components, depth and velocity.



San Marcos River Segments and Hygrophila Study Sites

Casey R. Williams 2011 Aerial Photo courtesy of CAPCOG.org

Figure 6. Study area used for habitat profiling with each river segment (rectangles) representing a grid area.

This frequency distribution was then used to infer habitat suitability with a high frequency of occurrence within a data range relating to a high suitability value Suitability values for each component ranged from 0.0 to 1.0 with 0.0 indicating low suitability and 1.0 indicating optimal conditions. This value was then used to produce habitat suitability curves for occupied habitat.

Riparian canopy density data were collected along the entire length of the upper San Marcos River with the use of a hand held densitometer and was measured from the river surface. The canopy outline was traced with a Trimble GPS unit to produce canopy polygons with density measurements attributed to each canopy polygon. These canopy polygons were then placed over the hygrophila polygons located in the four river segments. Riparian canopy cover was divided into shaded (canopy cover  $\geq$  50 %) and unshaded (canopy cover < 50%). Area coverage of hygrophila polygons located underneath shaded canopy cover was then calculated to produce a *"Hygrophila polysperma* in shade estimate."

In addition, data on physical and chemical data velocity, depth, soil type and photosynthetically active radiation, dissolved oxygen, temperature and pH were collected by the author within four hygrophila stands located within each river segment (Figure 5). These data were collected along five transects set through each hygrophila stand and parallel to river flow. Water velocity (m/sec) was measured at the stream bottom, 20, 40, 80% of depth and surface of the water column with a Flo-mate<sup>TM</sup> velocity meter producing a velocity profile. These data were then combined to produce a mean value for velocity at each point

along the transect, similar to the methodology of Sand-Jensen and Peterson (1999). Total depth (m) was also measured at each point.

Soil samples were collected with a soil corer to a total depth of 23 cm. The soil types were visually identified and classified into sand, clay, gravel and organic types. To get an idea of the amount of light reaching the water, photosynthetically active radiation (PAR) measurements were taken just below the water surface with a Li-Cor 660 spherical quantum sensor. These were then compiled into an average PAR reading and assigned to each of the four field study sites.

Chemical data were collected using an YSI 2000<sup>™</sup> multiparameter sonde. The parameters collected were pH and dissolved oxygen and temperature. Chemical data were collected at approximately mid-depth at each point. These were then compiled into an average PAR reading and assigned to each of the four field study sites.

Determination of the associated aquatic macrophytes was carried out by recording the number of additional species present in hygrophila stands and the coverage area of these species within hygrophila stands.

#### Determining Areas of Suitable Habitat

In order to determine geographical locations that meet the habitat suitability for hygrophila based on the habitat suitability curves, habitat suitability criteria (HSC) for depth and velocity were produced using the- combined suitability formula below provided by Kristina Towers Tolman at the Meadows

Center for Water and the Environment which combined depth suitability (dS) and current velocity suitability (cvS). The HYGRO combined suitability values ranged on a scale from 0.0 to 1.0.

#### HYGRO Combined Suitability= (HYGROdS \* HYGROcvS)<sup>1/2</sup>

This formula produced a HYGRO combined suitability value for each grid cell in each of the four sections over three preselected modeled river discharges: 3.40 m<sup>3</sup>/s, 5.70 m<sup>3</sup>/s and 7.40 m<sup>3</sup>/s. These modeled discharges were selected to represent discharge in drought conditions, average conditions and flood conditions respectively and were chosen from an array of modeled discharges that were produced for the Edwards Aquifer Recovery Implementation Plan (Hardy et al., 2010). The cells were then manipulated in GIS to produce contour maps for each river section over the selected discharges showing areas of suitable habitat for hygrophila based on combined depth and velocity. It should be noted that these are predicted areas of suitability and do not include other factors that may affect suitability such as sediment type or presence of other plant species.

In addition, Weighted Usable Area was calculated for the above discharges by combining the suitability for depth and velocity for each cell and multiplying this combined suitability by the area of the cell. The areas within all cells containing hygrophila were totaled for each discharge to produce the final Weighted Usable Area in m<sup>2</sup>.

#### CHAPTER III

#### RESULTS

#### **General Distribution**

The distribution of hygrophila is widespread along the upper San Marcos River (see distribution map in Appendix I). The species occurs within Spring Lake and continues to the Blanco River confluence with no rooted submersed plants found below this point. Hygrophila is most abundant between Spring Lake and the I-35 crossover with a noticeable decrease in the abundance of the plant beginning to occur below the I-35 crossover. Total surface area coverage for the species for the upper San Marcos River, including Spring Lake, was estimated at 13,282 m<sup>2</sup>. When removing Spring Lake the surface area coverage is reduced to 12,001 m<sup>2</sup> making hygrophila the third most common aquatic plant in the San Marcos River (Figure 7). Coverage in the stretch below Spring Lake to the I-35 crossover equaled 8,654 m<sup>2</sup>, accounting for 65% of the total coverage (13, 282 m<sup>2</sup>). Table 1 summarizes these coverages.

Table 1. Surface area coverage in square meters of hygrophila along the upper San Marcos River

River Areas	M <sup>2</sup>	% of total	
Spring Lake	1,282	9	
Below Spring Lake to I-35	8,654	65	
I-35 to Blanco confluence	3,346	25	
Total	13,282	100	

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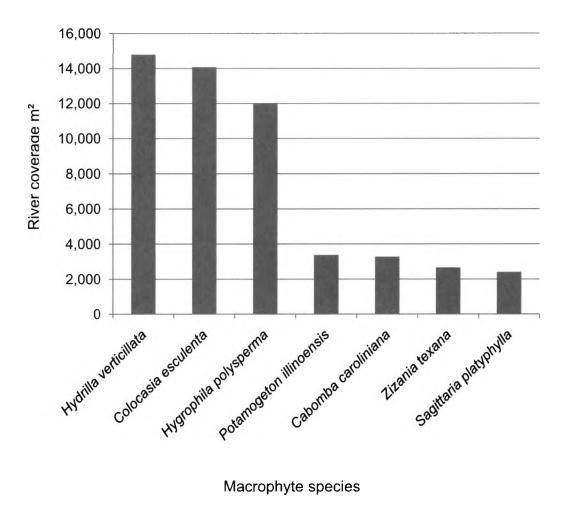


Figure 7. Area coverage of aquatic macrophyte species along the upper San Marcos River, not including Spring Lake.

#### Habitat profile

Depth and velocity

Habitat suitability graphs were derived from modeled data within the four modeled river segments. When looking at depth suitability values for hygrophila stands, habitat suitability values for modeled depths were highest between 0.30 and 0.70 meters with depth around 0.40 meters reaching a suitability value of 1.0 (Figure 8). The suitability values for hygrophila in areas shallower than 0.30 and deeper than 0.70 meters decreased dramatically. Depth values among the four field sites ranged from a minimum of 0.21 m to a maximum of 1.07 m (Figure 9).

Habitat suitability values were highest near velocities of 0.05 m/sec. The habitat suitability decreased substantially in areas with velocities slower than 0.03 m/sec or faster than 0.07 m/sec. (Figure 10). Among the four field sites velocity tended to increase moving downstream. Velocities among these field sites ranged from 0.01 to 0.25 m/sec. Field data were collected at an average river discharge rate of 6.23 m<sup>3</sup>/sec (Figure 11).

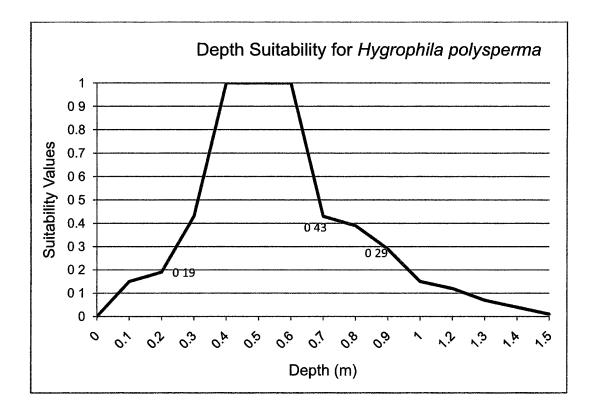


Figure 8. Hygrophila depth suitability at 5.66 m/sec within total number of grid cells (n=39445). Higher suitability values positively correlate to higher frequency of occurrence.

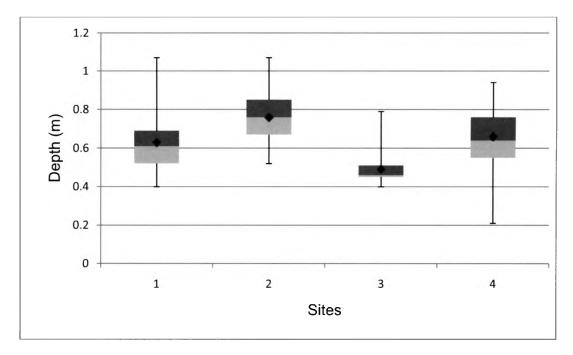


Figure 9. Depth measured within field study sites where site 1 is northern most site and site 4 is southern most site. Bars represent maximums and minimums, dark gray represents third quartiles, light gray represents the median and diamonds represent the average.

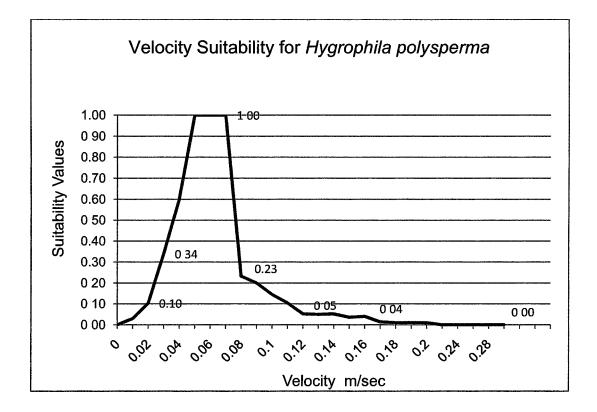


Figure 10. Hygrophila velocity suitability at 5.66 m/sec. within total number of grid cells (n=39445). Higher suitability values positively correlate to higher frequency of occurrence.

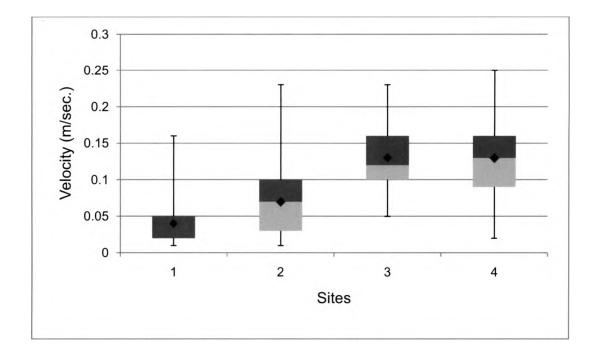


Figure 11. Velocity measured within field study sites where site 1 is most northern site and site 4 is most southern site. Bars represent maximums and minimums, dark gray represents third quartiles, light gray represents the median and diamonds represent the average.

## Aquatic vegetation

Although hygrophila was observed growing most frequently in monospecific stands, covering 9,111 m<sup>2</sup>, twenty other aquatic macrophyte species were observed and mapped growing with hygrophila. These included native and non native species such as *Zizania texana*, *Hydrilla verticillata*, *Cabomba caroliniana* and *Vallisneria spiralis*. The species occurrence table (Table 2) shows that *Hydrilla verticillata* and *Colocasia esculenta* were two of the more commonly identified aquatic plant species in dominant hygrophila stands. *Hydrilla verticillata* was present in 35, or 17%, of the 205 dominant hygrophila stands making it the most common species associated within hygrophila stands along the total stretch of the upper San Marcos River.

When looking at the amount of area associated species constitute within the dominant hygrophila stands (Table 3) the native macrophyte *Cabomba caroliniana* covered the most area at 892 m<sup>2</sup>. *Hydrilla verticillata* covered 453 m<sup>2</sup> and the native macrophyte *Sagittaria platyphylla* covered 292 m<sup>2</sup>.

Species	# of stands present	% of stands
Hygrophila polysperma*	205	100.00
Hydrilla verticillata*	35	17.50
Colocasia esculenta*	22	11.00
Cabomba caroliniana	20	10.00
Zizania texana	20	10.00
Sagittaria platyphylla	19	9.30
Potamogeton illinoensis	17	8.30
Nasturtium officinale*	13	6.34
<i>Cyperus</i> sp.	9	4.40
Heteranthera dubia	8	4.00
Hydrocotyle verticillata	8	4.00
Ceratophyllum demersum	6	3.00
Polygonum sp.	5	2.43,
Ludwigia repens	5	2.40
Grass	4	2.00
Nuphar advena	4	2.00
Eichhornia crassipes*	3	1.50
Vallisneria spiralis*	3	1.50
Justicia americana	2	1.00
Myriophyllum heterophyllum	2	1.00
Limnophila sessiliflora*	2	1.00
Algae	2	1.00

Table 2. Percent occurrence of macrophyte species within the number of dominant hygrophila stands (n=205) with asterisk indicating non-native species.

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Table 3. Total amount of area composition of macrophyte species within dominant hygrophila stands (n=205) with asterisk indicating non-native species.

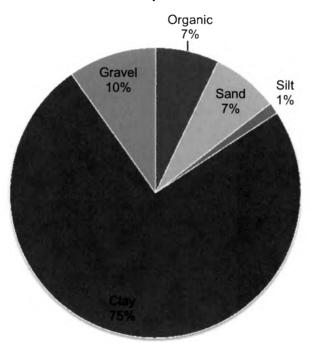
Species	m²
Hygrophila polysperma*	9,111
Cabomba caroliniana	892
Hydrilla verticillata*	453
Sagittaria platyphylla	293
Polygonum sp.	286
Cyperus sp.	215
Grass	195
Nasturtium officinale*	180
Potamogeton illinoensis	156
Justicia americana	155
Zizania texana	150
Heteranthera dubia	144
Eichhornia crassipes*	144
Colocasia esculenta*	142
Algae	107
Hydrocotyle verticillata	95
Ceratophyllum demersum	55
Nuphar advena	47
Vallisneria spıralis*	27
Ludwigia repens	19
Myrophyllum heterophyllum	14
Limnophila sessiliflora*	7

#### Substrate

Substrate collected within the four hygrophila field study sites shown in Figure 9 was overwhelmingly composed of clay (75%), while other substrate types were sparsely present. Organic detritus (7%) was mostly composed of leaves, wood chips or vegetation fragments. Gravel substrate (10%) was composed of small gravel in the 4-8 mm range.

## Light

Based on canopy densitometer readings taken from Spring Lake to the I-35 crossover 3,253 m<sup>2</sup> of hygrophila (38%) were found underneath riparian canopies with a density reading of 50% or more (shaded conditions). Figure 13 illustrates hygrophila distribution in relation to riparian canopy cover. Photosynthetically Active Radiation readings collected at each field were variable. Readings ranged from 30  $\mu$ E/m<sup>2</sup>/sec (deep shade) to 17000  $\mu$ E/m<sup>2</sup>/sec (full sun). PAR values varied greatly among sites. Values for sites 1 to 3 indicated these sites received mostly full sunlight while site 4 was more shaded overall and received a variety of light intensities (Figure 14).



# Percent Composition of Substrate

Figure 12. Substrate composition within field study sites.

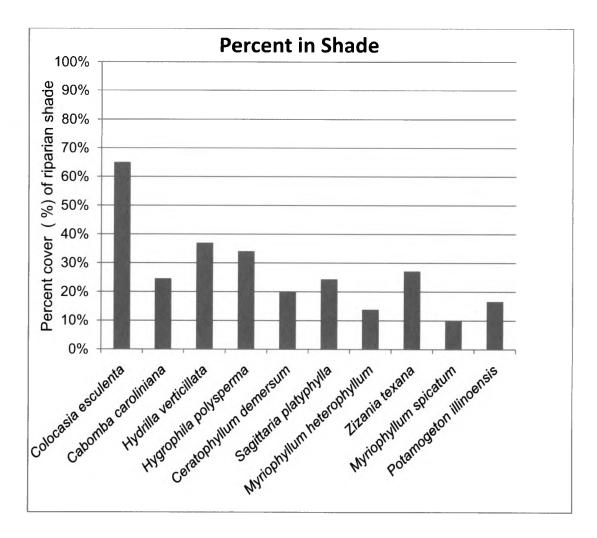


Figure 13. Percent cover of macrophyte species found within riparian shade.

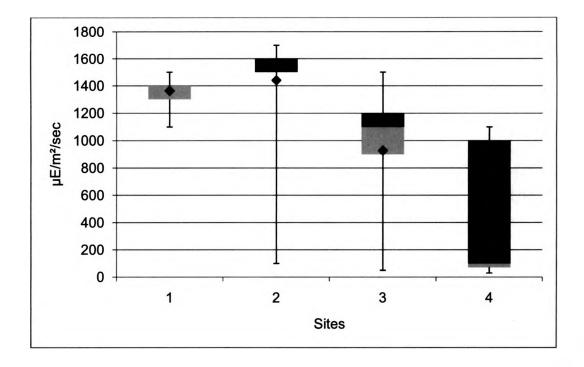


Figure 14. PAR values measured within field study sites. Site 1 is most northern site and site 4 is most southern site. Bars represent maximums and minimums, dark gray represents third quartiles, light gray represents the median and diamonds represent the average.

Physical and chemical parameters

Due to the strong influence of spring flows, water quality data was expected to be uniform, yet differences did occur among field sites. Dissolved oxygen values ranged from a minimum of 6.86 mg/L to a maximum of 9.23 mg/L across all sites. Dissolved oxygen tended to decrease slightly while moving downstream. Dissolved oxygen data for site 4 was removed due to malfuncition of the probe. The waters of the upper San Marcos are considered well mixed and dissolved gases are expected to be maintained near an atmospheric equilibrium (Hannan and Doris, 1970). The pH levels remained stable across the four sites. Values for pH across all sites ranged from a minimum of 7.09 to 7.92. Water temperature at the springs average approximately 22°C with temperature variability increasing with distance from the springs (Saunders et al., 2001). Variation in temperatures among field sites can be attributed to increasing distance from the springs as well as the influences of depth, velocity and plant density on water temperatures, even though the spring fed waters of the San Marcos are noted for their consistent temperature. Figure 15 summarizes data collected within the field study sites.

#### Habitat suitability

Appendix I illustrates the areas of suitability for hygrophila, based on depths and velocities, across river discharge rates of 3.40 m<sup>3</sup>/sec, 5.70 m<sup>3</sup>/sec, and 7.40 m<sup>3</sup>/sec. Location and total area of suitability changes as discharge rates change. This can be seen when looking at weighted usable area values (Figure 16). At a discharge rate approaching drought conditions (3.40 m<sup>3</sup>/sec) a total of 61,487 m<sup>2</sup> are considered at suitable depth and velocity for hygrophila. That number decreases to 54,690 m<sup>2</sup> when looking at an average monthly discharge rate of 5.70 m<sup>3</sup>/s. At a higher discharge rate of 7.40 m<sup>3</sup>/sec suitable area for hygrophila rises to 57,728 m<sup>2</sup>.

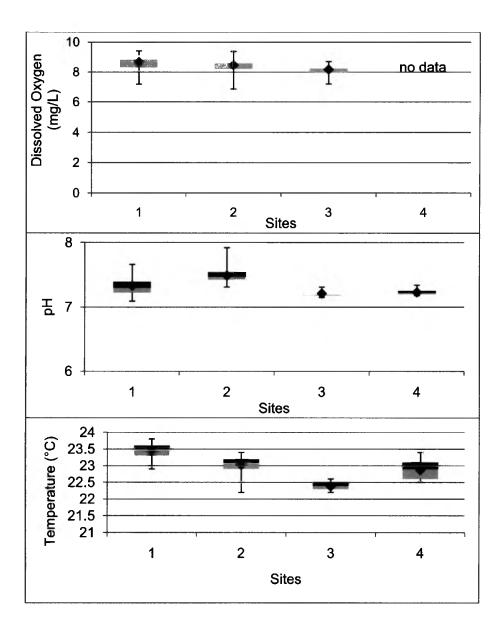
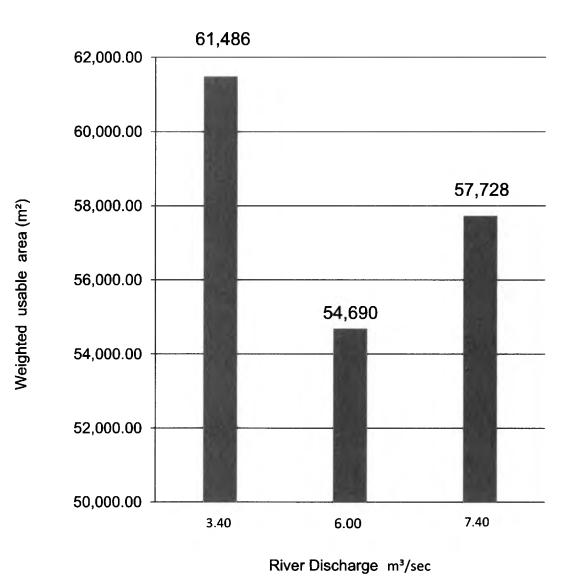


Figure 15. Physical and chemical parameters measured within field study sites where site 1 is northernmost site and site 4 is most southern site. Bars represent maximums and minimums, dark gray represents third quartiles, light gray represents the median and diamonds represent the average.



Weighted Usable Area

Figure 16. Weighted Usable Area for hygrophila across three river discharges.

#### CHAPTER IV

#### DISCUSSION

Since hygrophila was first identified in 1994 as occurring in the San Marcos River historical accounts of its distribution are few. Hygrophila has been misidentified in previous vegetation surveys of both the river and Spring Lake, making it difficult to discern the species' distribution (Espey, Huston and Associates, 1975; Staton, 1992). Lemke and Angerstein (1994) gave no account of distribution in their research although it seems the species was common. In a study conducted by Owens et al. (2001) a high percentage of hygrophila fragments were collected along their entire study site from Spring Lake to Capes Dam. Towns (2002) reported hygrophila present as present in Spring Lake in small amounts. Recent aquatic macrophyte surveys (Saunders et al. 2001; Doyle, 2002) showed that hygrophila was persistent throughout the upper San Marcos River, although each study combined hygrophila into complexes with other macrophyte species making it difficult to narrow down locations of hygrophila stands. In their mapping study Owens et al. (2009) provided area coverage of aquatic macrophyte species in which hygrophila distribution and coverage was similar to that reported in this study.

My results suggest suitable habitat for hygrophila occurs in areas with low to moderate water velocities. For field collected data maximum depths remained below 1.2 meters and velocities remained below a 0.25 m/sec. (Figure 7).

In 2001 Saunders at al. measured depth and velocity within plant stands and produced corresponding habitat suitability curves for a variety of plant species including hygrophila (Figures 17 and 18). Suitability curves produced in my study indicate a narrow velocity suitability, 0.03-0.06 m/sec, while Saunders et al. showed a wider optimal suitability, from 0.00 to 0.15 m/sec. Such a wide suitability determination could possibly be an artifact of Saunders' sampling regime in which velocity measurements were taken within plant stands. Existing vegetation is known to strongly modify water flow (Sand-Jenson and Pederson, 1999). Differences can also be seen for the depth suitability curves in which my results indicated generally lower suitability values in deeper waters than Saunders et al. These differences may be attributed to the temporal component of the sampling regime, in which Saunders et al. collected depth and velocity data at the center of 10m<sup>2</sup> x 10m<sup>2</sup> bio-grids (N=815 for hygrophila) over a span of five months and varying discharge rates (2.61 to 3.06 m³/sec). While these suitability curves cannot be directly compared to mine it is important to note that optimal suitability from my analysis falls within the range of optimal suitability determined by Saunders et al. for both velocity and depth. This study used multiple modeled depth and velocity data per dominant hygrophila polygon (N=39,445 for hygrophila) at a standardized discharge rate of 5.66 m<sup>3</sup>/s thus

removing the variability in river discharge as well removing the influences of existing vegetation and increasing sample size.

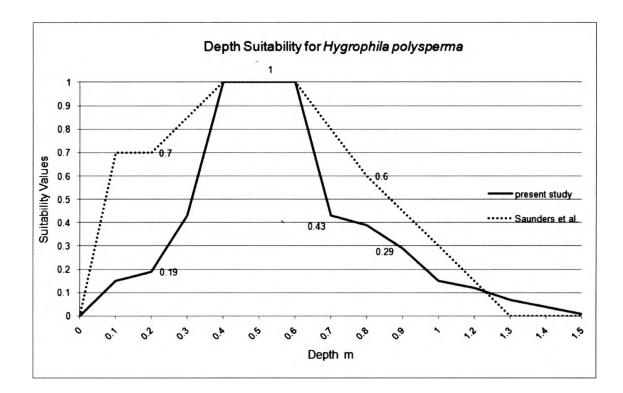


Figure 17. Overlay of depth suitability curves of the present study and Saunders et al. (2001). Note, this study and Saunders et al. cannot be directly compared due to differences in sampling regime.

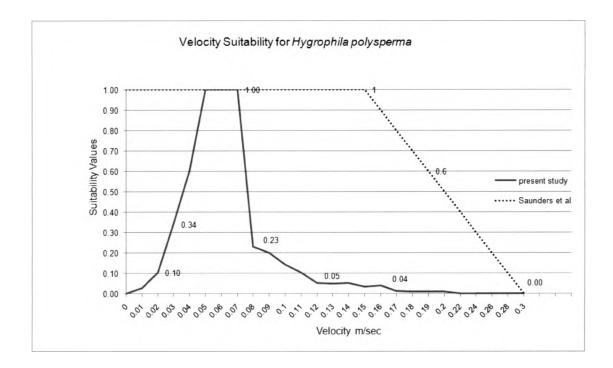


Figure 18. Overlay of velocity suitability curves of the present study and Saunders et al.(2001). Note, this study and Saunders et al. cannot be directly compared due to differences in sampling regime.

These conclusions can be bolstered when looking at hygrophila's general distribution across the U.S. It is most commonly present in areas with shallow, flowing waters including irrigation ditches, small rivers and spring runs. In one study by van Dijk et al. (1986) it was found that hygrophila produced as much as five times greater biomass under flowing water conditions than in lentic conditions. The plant showed an increase in shoot length in flowing water as well. In their study Fast et al. (2008) concluded that hygrophila produced higher root biomass and larger leaves in treatments simulating flowing waters. Interestingly, Fast et al. found hygrophila produced the highest root and shoot biomass when the plant was grown in terrestrial but sub irrigated conditions as compared to static submersed and emergent conditions. These results led Fast et al. to conclude hygrophila was better suited as a terrestrial plant and subsequently spreads into shallow flowing waters of rivers and canals and was less likely to grow submersed or emersed in static waters.

Along the San Marcos River, hygrophila has been observed in all growth forms. The presence of low head dams and slow backwaters provide ample habitat for submersed hygrophila, however in conditions where water levels are low the submersed form can shift to emergent and terrestrial growth forms. This was seen in September of 2011 when large areas of river bed became dewatered due to severe drought. In areas where hygrophila normally grows submersed the plant was found growing, and blooming, in its terrestrial form in sub irrigated soils. While hygrophila can be found in relatively fast flowing waters

its frequency there should be limited as a result of its large leaf size and brittle stems.

Although this study did not produce a suitability curve for sediment, results from Saunders et al. (2001) showed a greater variability in sediment type when compared to this study. Larger substrate size, cobble and stratified bedrock, were labeled with high suitability along with silt and sand. This contradicts my results in which clay composed the majority of the sediment types. While the sediment data set is limited in this study to the four field sites (N=154) when compared to the sediment data set for Saunders et al. (N=815) it attempts to be more thorough by collecting sediment within the root zone (upper 23 cm) of hygrophila. While hygrophila has been observed growing in gravel the poorly developed, fibrous root structure may limit its ability to grow in coarse media. It is possible Saunders et al. mistakenly attributed a morphologically similar plant, *Ludwigia repens*, as hygrophila growing in cobble or stratified bedrock as Poole and Bowles (1999) did.

While both native and non native macrophyte species were found growing within dominant hygrophila stands, the stands were typically monospecific. Several studies suggest that hygrophila has the ability to outcompete both native and other non-native species. Doyle et al. (2003) indicated in their study that hygrophila overwhelmingly out-competed the native macrophyte *Ludwigia repens* due to its ability to produce a thicker plant canopy. Floating mat formation from excessive fragmentation of hygrophila and other species has also been indicated as a cause for the decline in the Federally Endangered Texas wild rice, *Zizania* 

*texana* (Beaty, 1975; Power, 1996; Owens et al., 2001). Poole and Bowles (1999) indicated the habitat of Texas wild rice to be very different from the habitat characterization I propose for hygrophila. Only 10 percent of dominant hygrophila stands contained *Zizania texana*. However, in several instances large clumps of *Zizania texana* were observed growing within hygrophila stands. Since Texas wild rice is generally a long lived, deep rooted macrophyte these clumps could be remnants of larger historical stands of wild rice that have been surrounded by hygrophila as certain conditions have changed. These conditions may include habitat alterations such as sediment accretion and flow divergence, which may have provided hygrophila an advantage in expanding its range while negating the physical requirements needed by Texas wild rice. Also sedimentation may play a role in the availability of nutrients necessary for hygrophila growth.

Sutton and Dingler (2000) suggested hygrophila growth was highly responsive to ammonia nitrogen, sodium, and magnesium contents in sediments. Interestingly, Poole and Bowles (1999) found that sediment collected in non Texas wild rice transects in the San Marcos River were higher in amounts of sodium and significantly higher in amounts of magnesium.

The rapid growth rate and establishment for hygrophila provides a clear advantage over the slower growth rate and longer establishment period for Texas wild rice. Experimental studies by Spencer and Bowes (1985) and Fast et al. (2008) show hygrophila is capable of out-competing other fast growing species such as *Limnophila sessiliflora* and *Hydrilla verticillata*.

As a result of light attenuation in water, submersed aquatic plants are generally better adapted to the low light conditions of underwater habitats and can generally be categorized as shade tolerant (Bowes, 1987). In submersed form hygrophila is known to have low light compensation and saturation points for photosynthesis providing for high shade adaptability (Schmitzs and Nall, 1984). However, emersed leaves of the plant are less shade adapted and yet do not exhibit heterophylly as would be expected in amphibious type plants (Spencer and Bowes, 1985). Such a variation in light requirements allows hygrophila to sustain itself in a wide range of conditions, as seen in Figure 10, from dense riparian canopy cover (50  $\mu$ E/m<sup>2</sup>/sec ) to full sun exposure (1700  $\mu$ E/m<sup>2</sup>/sec ). In this study only 38% of hygrophila stands were located under riparian canopy cover of 50% or more (Figure 11).

The most notable effect light has on hygrophila involves leaf morphology, and color. Reams (1952) showed that in experimental low daylight conditions hygrophila produced oblong-lanceolate leaves. Under 40 watt fluorescent lighting, simulating daylight, the apical leaves begin to acquire red tinted margins. Finally when growing in full sunlight or incandescent lighting the leaves became smaller and more ovate-oblong with much shorter internode lengths. These traits have been observed in the field.

Studies of suitable water quality parameters for hygrophila are few. suggested that hygrophila grows best at a pH of 5 to 7. Our results showed the pH within the upper limit of this range (Figure 9). Typically, pH for the upper San Marcos River remains in the 6.5 to 7.5 range until the river combines with the

Blanco River where pH levels begin to increase gradually above 7.5 (Groeger et al., 1997).

Environmental temperature limits of hygrophila are not known. Reams (1952) reported the plant can sustain freezing temperatures and had become naturalized in lakes around the Richmond, Virginia, area, however extreme winter events in the 1970's may have killed the population (USGS, 2012) since the current Virginia plant atlas lists the plant as not naturalized (Virginia Botanical Associates, 2012). While winter low air temperatures for San Marcos can reach below freezing, water temperatures of the San Marcos River remain quite constant (approximately 22° C) until the river's confluence with the Blanco (Groeger et al., 1997). This consistency ensures that submersed forms of the plant can persist regardless of ambient air temperatures.

Adequate dissolved oxygen values are important for submersed aquatic plants because oxygen is consumed during plant respiration (Reimer, 1984). Waters of the upper San Marcos are well mixed and it is unlikely that dissolved oxygen plays a limiting factor in hygrophila growth. Plant canopy formation, decay of organic matter and floating plant mats are known to alter dissolved oxygen levels, as well as other water quality parameters (Carpenter and Lodge, 1986) and may play an important role in limiting the growth of more sensitive native species such as Texas wild rice (Power, 1996).

The habitat suitability models (Appendix I) are meant to be used in locating potential areas of suitable habitat for the submersed form of hygrophila. Generally these models indicate that greater amounts of suitable habitat for

hygrophila exist than is being utilized. However, these models are only based on two criteria, depth and velocity, and do not take into account other factors such as soil type or the presence and competition of other aquatic macrophyte species. It is important to note that while such areas may be highly suitable in one criterion they may be poorly suitable in the other thus lowering the overall suitability values.

As expected, areas of suitability are dynamic and change in relation to differing discharge rates. At a lower discharge rate, 3.40 m<sup>3</sup>/s, areas previously non suitable may become more suitable because of lowered velocity and decreased depths while previously suitable areas may become dewatered and therefore not suitable for submersed hygrophila. A higher river discharge rate, 7.40 m<sup>3</sup>/s, may flood normally dry areas or produce water flow in slough and backwaters thus making these usually non suitable habitats suitable for the plant.

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### CHAPTER V

#### CONLUSIONS

Vandiver (1980) suggested hygrophila was limited to southern Florida because it could not survive the colder temperatures of central and northern Florida. Currently the species is recorded as naturalized throughout Florida including the panhandle of the state. Additionally, as of 2012, hygrophila is recorded in one location in South Carolina, Alabama and several locations in the state of Tamaulipas, Mexico (Mora-Olivo et al. 2008; U.S. Geologic Survey, 2012; Kral et al., 2012). The species is no longer listed as naturalized in the state of Virginia. Microsatellite data collected by Mukherjee (2011) indicate that hygrophila introductions in North America originated from a single source.

These recent discoveries suggest that expansion of the range of hygrophila is continuing. The species seems well suited to climates other than the semi tropics of southern Florida as originally proposed by Schmitz and Nall (1984) and may pose a significant threat within at least the southern tier states. With hygrophila already well established in three major spring systems in Texas there is reason to believe the plant may spread downstream from these headwaters. The Guadalupe River, to which the San Marcos is a major tributary, is an important source of irrigation water for Texas rice farmers and provides large quantities of water for transfer into other basins (Texas Water Development

Board, 2000). With this in mind there is no reason to dispute the possibility for hygrophila fragments to move downstream and establish in irrigation canals, clog flood control structures or spread into other river basins (Schmitz and Nall, 1984). New introductions of hygrophila may threaten similar spring systems in Texas as well.

With the environmental impact hygrophila poses, management options for the species are limited. Before any management options are carried out on the upper San Marcos River two questions must be addressed fully. First, does hygrophila serve an ecological benefit to the system by providing habitat or other services? Second, would management of the species have a more negative impact than the species itself?

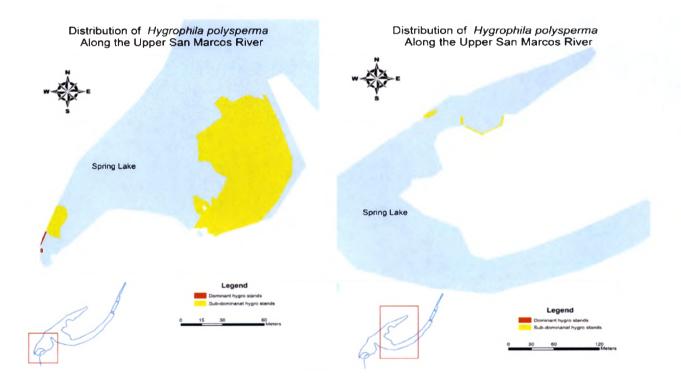
In their assessment of hygrophila Cuda and Sutton (2000) found the plant to score high as a suitable target for herbivorous biological control. However, Neisch (pers. com.) found hygrophila to be unpalatable to grass carp (*Ctenopharyngodon idella*), and other biological control agents are lacking (Mukherjee, 2011). Currently research is being conducted to locate potential biocontrol agents from hygrophila's native range (Mukherjee and Cuda, 2012). Locally, several herbivorous insect species have been lab reared from hygrophila collected in the San Marcos River at the U.S. Army Corps of Engineers Environmental Research Development Center in Vicksburg MS. These include *Ussingeriessa onyxalis* and *Parapoynx sp.* (*P. allionealis* Walker or *P. obscuralis* Grote). The potential for these to be reared en mass is uncertain. *Parapoynx sp.* was noted to have considerable defoliation in the lab (Nathan Harms, pers. comm.). Mukherjee et al. (2012) showed that plant growth responded negatively to simulated defoliation by bio control agents indicating that defoliating agents may prove effective.

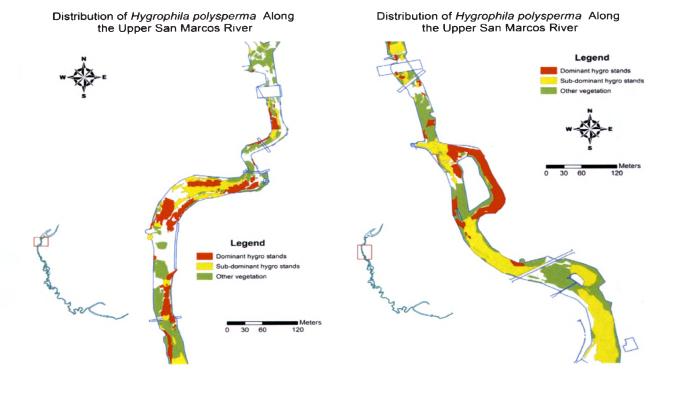
In my survey of hygrophila along the San Marcos River submerged dodder was found in abundance within several stands of hygrophila. Dodder (*Cuscuta* sp.) is a euparasitic angiosperm that is commonly found along stream margins in Central Texas. The effects of this parasitic plant on the health of hygrophila are unknown and the potential for *Cuscuta* sp. as a bio control agent is minimal at best.

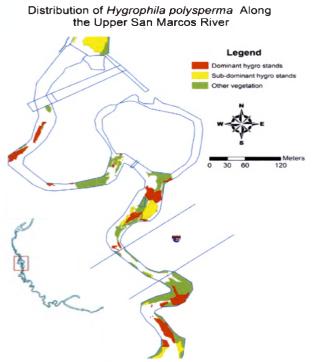
Chemical control techniques for hygrophila are also minimal. Vandiver (1980) indicated that even at maximum label rates endothall plus copper, a commonly used aquatic herbicide, was ineffective against submersed hygrophila. Fast et al. (2009) tested ten herbicide treatments commonly used in the control of many other aquatic weeds. Their results indicated these measures were inconsistent with controlling emergent hygrophila and that even at higher application rates sufficient biomass reduction did not occur. Granulated Flumioxazin has recently been found to be effective in controlling submersed hygrophila in irrigation canals within a 24 hour contact time and a target application rate of 200 ppb (Jim Petta 2012, pers. comm.). No studies have been completed to test the effects of this chemical on emergent hygrophila. Even if acceptable herbicide treatments are found it would be difficult to treat such a fast flowing water body as the upper San Marcos and potential for environmental impact would be high. Mechanical control of hygrophila has proven problematic as well. Although the plant does not have a dense root structure, like that of hydrilla, its nodal rooting, ease of fragmentation and ability to root from detached leaves provides difficulties when mechanical control is attempted and can aid in spreading the plant (Sutton, 1995). The plant is also able to survive desiccation for up to 24 hours (Williams, unpublished data).

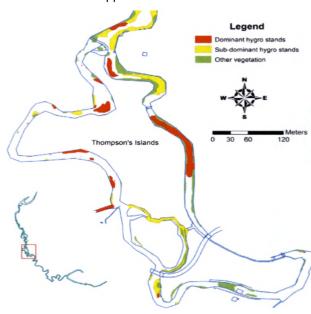
Since removal of this species is of high priority for the Army Corps of Engineers Section 206 restoration project in the San Marcos River (U.S. Army Corps of Engineers, 2008) and Hardy et al. (2011) indicated removal of hygrophila with hydrilla could provide an additional 1000 m<sup>2</sup> of habitat for Zizania texana, methods must be determined for mechanical removal of the plant and efficiently collecting fragments to prevent further spreading. A proposed control method includes placing a netted boom for fragment collection around hygrophila stands that are to be removed, clipping the above ground portion of the plant, dredging the remaining below ground portions and replanting the bare area with native species that grow in similar conditions, such as Cabomba caroliniana or Ludwigia repens. Similar methods have been utilized to remove Hydrilla verticillata from Spring Lake and Cryptocoryne beckettii from portions of the upper San Marcos River. Terrestrial hygrophila must also be managed so it can not establish new submersed stands. Since hygrophila is considered semi suitable fountain darter habitat (Bio-West, 2011) remediation must be provided while hygrophila removal is in progress.

Due to its affinity for slow moving lotic habitats, wide tolerances to water quality and light parameters, ease in reproduction and rapid growth rate and limited control options, hygrophila poses a continuing threat to the San Marcos River and other similar water bodies of the State of Texas. Although not currently listed by Texas Parks and Wildlife as a prohibited exotic plant state authorities should consider the current impacts on sensitive environmental areas such as the San Marcos River as well as potential impacts on other waterways, specifically irrigation, and flood control canals, if the plant were to spread. Appendix

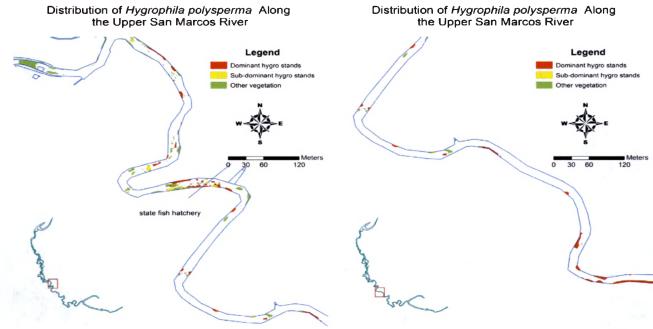


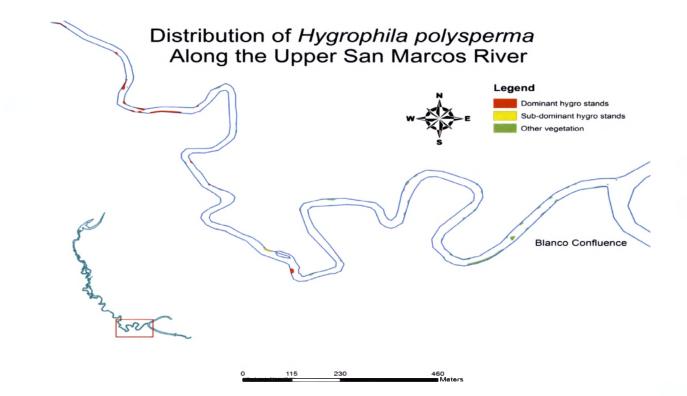


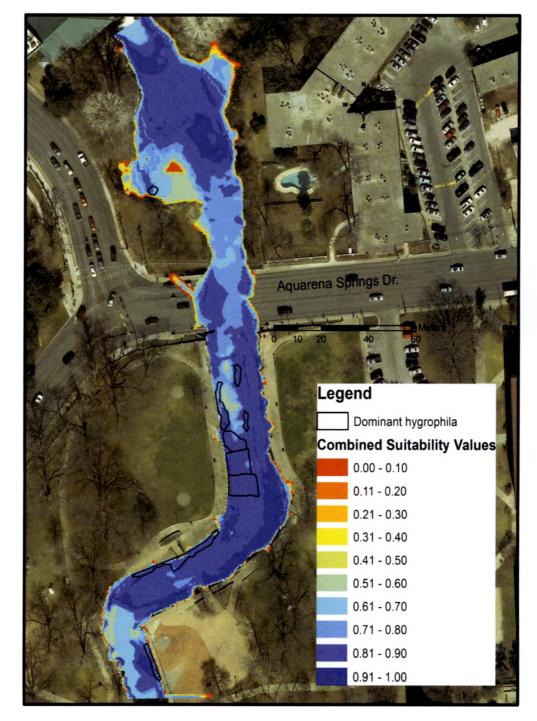




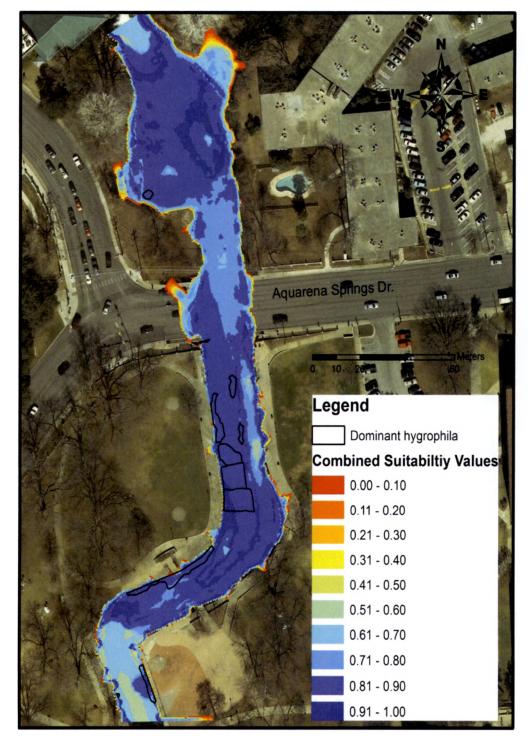
Distribution of *Hygrophila polysperma* Along the Upper San Marcos River





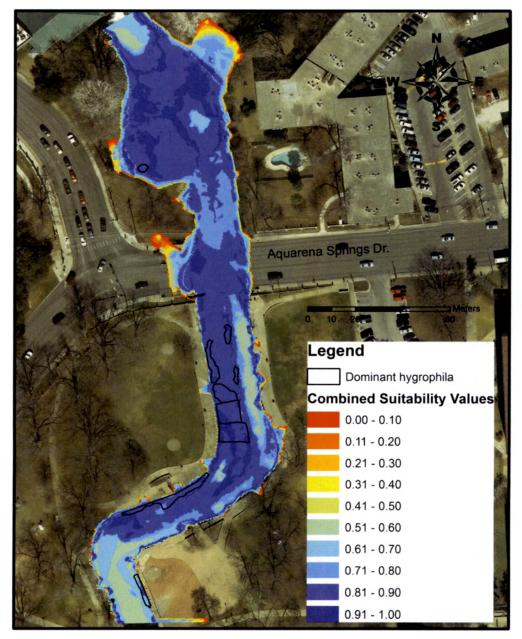


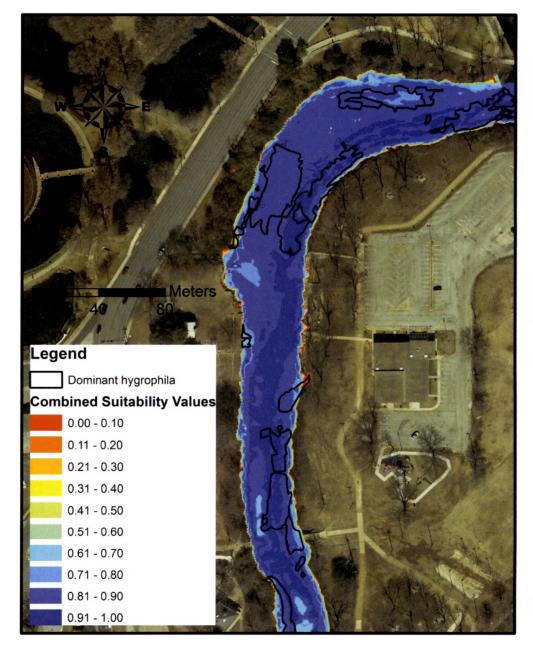
Hygrophila Suitability at 3.40 m<sup>3</sup>/s Salt Grass to Sewell Park



Hygrophila Suitability at 5.66 m<sup>3</sup>/s Salt Grass to Sewell Park

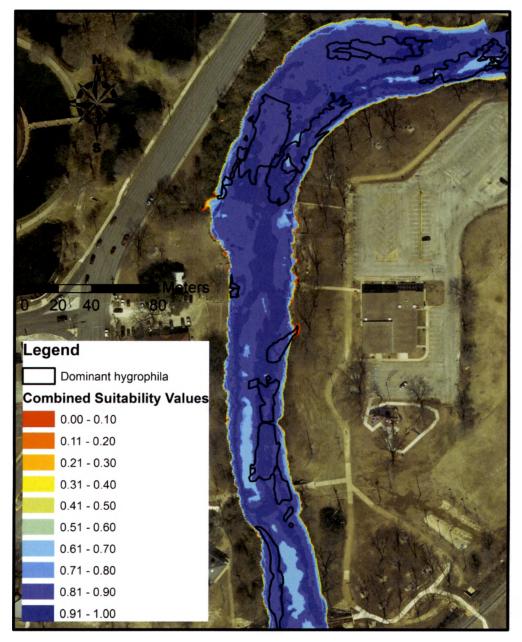
Hygrophila Suitability at  $m 7.40 \ m^{3/s}$  Salt Grass to Sewell Park

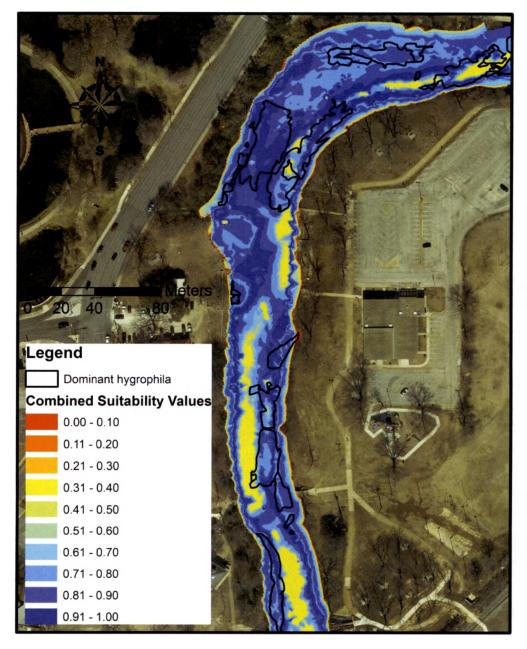




Hygrophila Suitability at 3.40 m<sup>3</sup>/s Sewell Park to Snake Island A

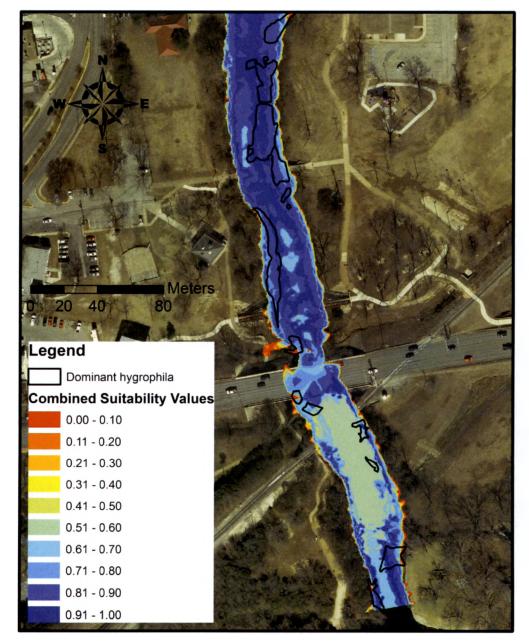
Hygrophila Suitability at  $5.66 \text{ m}^{3/s}$ Sewell Park to Snake Island A

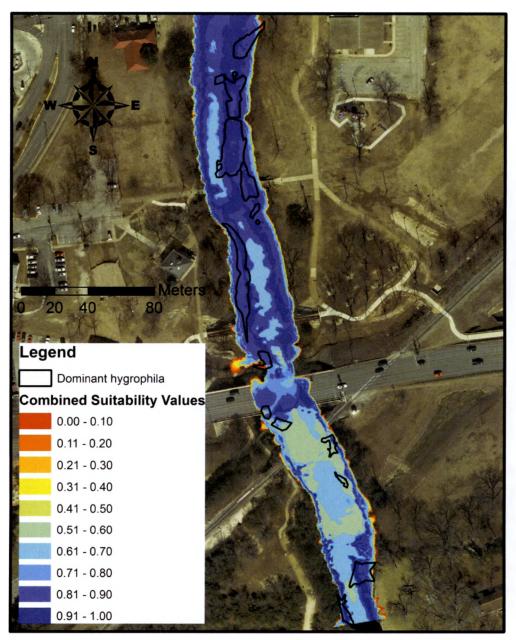




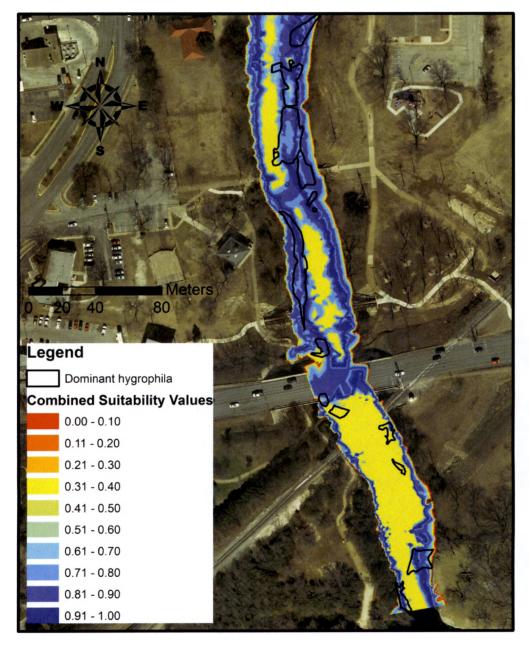
Hygrophila Suitability at 7.40 m<sup>3</sup>/s Sewell Park to Snake Island A

Hygrophila Suitability at 3.40 m<sup>3</sup>/s Sewell Park to Snake Island B

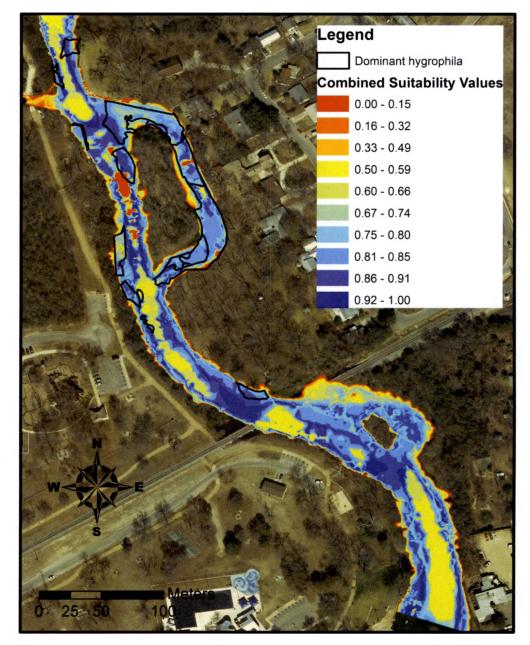




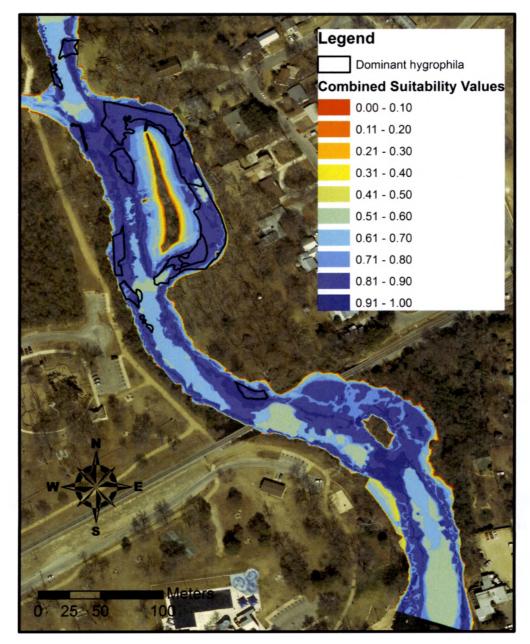
Hygrophila Suitability at  $5.66 \text{ m}^{3/s}$ Sewell Park to Snake Island B



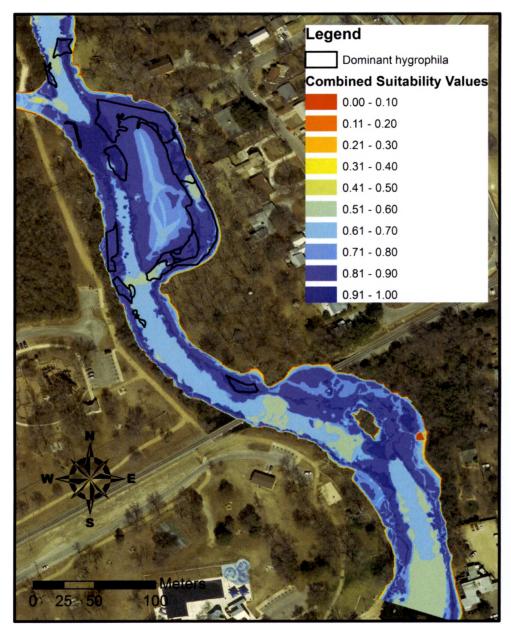
Hygrophila Suitability at 7.40 m³/s Sewell Park to Snake Island B



Hygrophila Suitability at  $3.40 \text{ m}^{3/s}$ Snake Island to Rio Vista Park

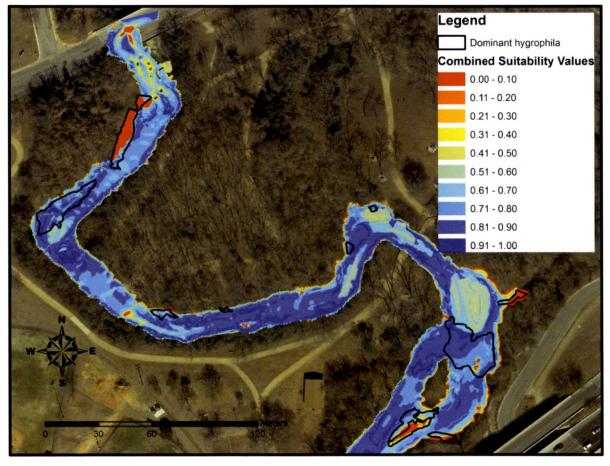


Hygrophila Suitability at 5.60 m<sup>3</sup>/s Snake Island to Rio Vista Park

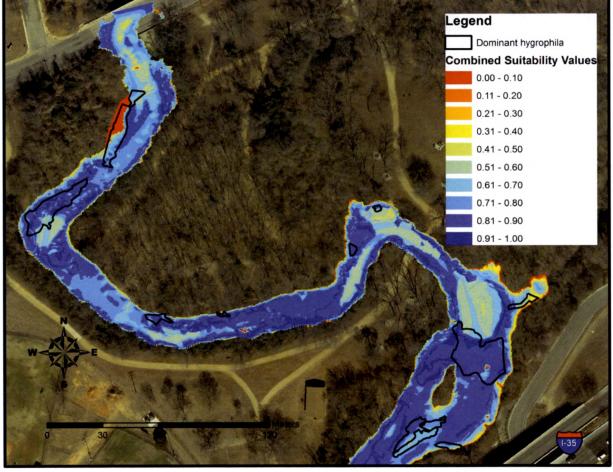


Hygrophila Suitability at 7.40 m<sup>3</sup>/s Snake Island to Rio Vista Park

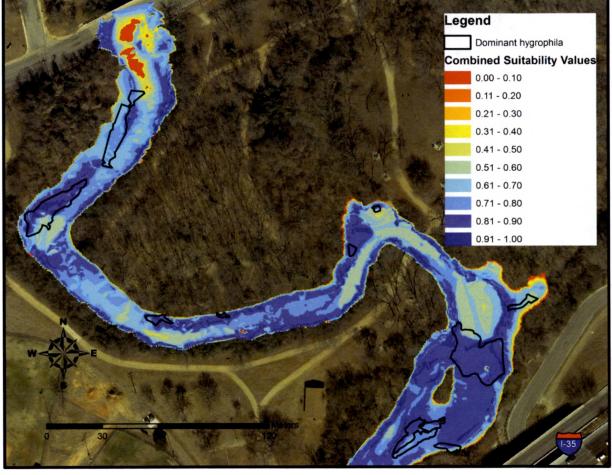
## Hygrophila Suitability at 3.40 m<sup>3</sup>/s Rio Vista Park to I-35



## Hygrophila Suitability at 5.66 m<sup>3</sup>/s Rio Vista Park to I-35



## Hygrophila Suitability at 7.40 m<sup>3</sup>/s Rio Vista Park to I-35



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Vita

Casey R. Williams was born in Dallas, Texas but raised in the farm country near Peaster, Texas where his parents taught at Peaster Independent Schools. He graduated from Peaster High School in 2000 and enrolled in Weatherford College where he was active in the Weatherford Science Club. He also began working at a local plant nursery where his love for Texas native plants was fostered. After two years of classes at Weatherford College Casey applied to then Southwest Texas State University and was accepted into the Aquatic Biology program. During his tenure as an undergraduate Casey was able to gain valuable work experience as a student worker at the Xiphophorous Genteic Stock Center and the A.E. Wood State Fish Hatchery a founding member of the Texas State Aquatic Biology Club. He graduated from Texas State University-San Marcos with a B.S. in Aquatic Biology in 2005. After graduation Casey continued his work experience at the A.E. Wood State Fish Hatchery and then moved to the Dominican Republic to work with research on sustainable shrimp farming. Casey returned to Texas in 2007 to work at the Possum Kingdom State Fish Hatchery and finally decided to return to graduate school at Texas State University-San Marcos. While initially being interested in fisheries his research interests turned to aquatic plant ecology. In 2008 Casey began working with Aguarena Center and the Meadows Center for Water and the Environment, formerly River Systems Institute, on the Edwards Aguifer Habitat Conservation

Plan. This work involved mapping and vouchering all obligate aquatic plants in both the San Marcos and Comal Rivers. While continuing his research Casey was able to spend time as a student worker at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, Texas where he was involved in propagating native aquatic plants for restoration projects across the United States. Casey has presented his research at conferences of the Aquatic Plant Management Society, Texas Invasive Plant and Pest Council and Southwestern Association of Naturalists. In his spare time Casey enjoys being involved with his family's Percheron horses and miniature Hereford cattle

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