ABSTRACT

Native Macrophyte Restoration in a Spring-Fed River Ecosystem

Rachel L. Bormann, M.S.

Mentor: Robert D. Doyle, Ph.D.

Restoration of native macrophytes is considered a high-priority objective in the San Marcos River in San Marcos, Texas. This study examines the effects of various factors on the short and long-term survival of seeded and transplanted native macrophytes. Neighboring invasive plants had a significantly negative effect on *S. platyphylla*, *H. dubia*, and *L. repens* transplant short-term survival. Radiation/canopy cover, depth, velocity, and substrate had mixed effects on transplant short-term survival among these three species. Rapid expansion of transplants to large, colony size macrophyte beds had a significantly positive effect on the long-term survival of *S. platyphylla* and *Vallisneria sp*. Regarding methods of planting the endangered *Z. texana*, tillers and whole plants provided higher short-term survival than seed packs. Deeper depths and presence of neighboring plants negatively affected *Z. texana* whole plant short-term survival and larger initial basal area positively affected tiller and whole plant short-term survival.

Native Macrophyte Restoration in a Spring-Fed River Ecosystem

by

Rachel L. Bormann, B.S.

A Thesis

Approved by the Department of Biology

Robert D. Doyle, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

Approved by the Thesis Committee

Robert D. Doyle, Ph.D., Chairperson

Joseph D. White, Ph.D.

Joe C. Yelderman, Ph.D.

Accepted by the Graduate School
August 2012

J. Larry Lyon, Ph.D., Dean

Page bearing signatures is kept on file in the Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

Many people have made the completion of this thesis possible. I would first like to thank Dr. Robert Doyle for accepting me into the Baylor University biology program, for his invaluable assistance in the field, for his financial support of my research and studies, for reading and reviewing countless versions of this work, and for his mentorship and leadership throughout the process. I also would like to thank my other committee members, Dr. Joseph White and Dr. Joe Yelderman, for their reviews and support in completing this work. Thanks to Dr. Mara Alexander at U.S. Fish and Wildlife for reviewing the Texas Wild-rice proposal and for her assistance in the field and thanks to U.S. Fish and Wildlife for the use of their greenhouse facilities. I am grateful for the significant help I received in the field from Melissa Mullins, Sara Seagraves, Derek Hagy, Cindy Contreras, and U.S. Fish and Wildlife personnel Patricia Grant, Matt Johnson, and Todd Schnakenberg. I would also like to thank the U.S. Fish and Wildlife Service for the partial funding of my work. Finally, thanks to my family and friends for their support in this journey.

CHAPTER ONE

Introduction and Background

River Restoration and Restoring Aquatic Macrophytes

Rivers and streams provide many essential ecological services, including clean water and food, diverse habitats for a large range of plant and animal species, and aesthetic and recreational services (Bernhardt and others 2005; Giller 2005). Providing these services requires properly functioning ecosystems. However, the number and magnitude of stressors threatening the functioning of rivers and streams are growing rapidly (Giller 2005; Malmqvist and Rundle 2002). Threats include direct pollution and other alterations to water chemistry, geomorphic engineering of the river channel, sedimentation, species removal and addition, and land-use changes in the catchment (Giller 2005; Malmqvist and Rundle 2002). As a result, river restoration is increasing in importance as we attempt to address the problems that have arisen from our use and misuse of freshwater habitats and resources (Giller 2005).

Many river restoration projects include the restoration of their native aquatic plants. Native aquatic vascular plants (macrophytes) provide a number of ecological functions. These include providing shelter and food for invertebrates and fish, providing oviposition sites, stabilizing substrate (Larned and others 2006), providing a substrate for epiphytic algae, and modifying stream flow, sediment size, and water chemistry (Gregg and Rose 1982; Larned and others 2006; Riis 2008). Moreover, diversity in macrophytes is important – a reduction in the diversity of aquatic plants results in less variation in water depths and current velocities and a reduction in the range of invertebrate and fish habitats (Baattrup-Pedersen and Riis 1999). In degraded rivers, plant populations may be reduced overall, or the diversity of plant populations may be reduced by the introduction and spread of non-native or invasive macrophytes. Non-native plants are not necessarily always invasive. A species is considered invasive if it establishes a rapidly growing population and proliferates in an ecosystem where it was previously not present (Greipsson 2011). Traits of non-native plant species that promote invasiveness may include rapid growth, high dispersal ability, and ability to survive under a wide range of environmental conditions (e.g., occupies a large variety of habitat types or has lower light or nutrient requirements than native plants) (Greipsson 2011; Kolar and Lodge 2001). Invasive macrophytes can modify the structure of plant communities and affect ecosystem functioning by reducing biodiversity, altering soil chemistry, inducing soil erosion along river banks, hybridizing with native plants (further reducing native biodiversity), and introducing diseases (Greipsson 2011). Invasive plants compete (and sometimes outcompete, for reasons listed above) with native plants for available space, light, and nutrients (Greipsson 2011). If invasive plants are a problem in a river or stream, restoration of native aquatic plant communities involves removal of invasive plants, planting native macrophytes to occupy niches and potentially prevent occupation of invasive plants, or both.

Study Objectives and Thesis Organization

This document examines native macrophyte restoration methods, factors related to transplant success, and macrophyte habitat suitability criteria. I have divided this thesis into four chapters. The current chapter contains introductory and background material. Chapter two details a native macrophyte transplant experiment examining short-term survival factors and possible facilitative interactions with existing plants. Chapter three examines the current status of macrophyte transplants made a decade or more ago and possible factors influencing their long-term survival. Chapter four examines transplant methods for an endangered endemic macrophyte (*Zizania texana*) and transplant survival factors.

Study Area

The San Marcos River (29°53'31"N, 97°55'58"W) originates from springs emanating from the Edwards Aquifer in several large fissures and numerous small openings in San Marcos, Hays County, Texas (Puente 1976), is Texas' second largest spring system, and flows approximately 110 kilometers (km) primarily southeastward until its confluence with the Guadalupe River near Gonzales, Texas (U.S. Fish and Wildlife Service 1996). The upper portion refers to the first 6.4 km of the river, until its confluence with the Blanco River (Figure 1.1) (U.S. Fish and Wildlife Service 1996). Although fluctuations in the water level of the Edwards Aquifer cause the flow rate to vary, the spring flows have never ceased during measured time periods since 1894 (Bowles and Arsuffi 1993; Puente 1976) and are the dominant source of water for the river other than local runoff and several small creeks. The long-term median spring flow through 1998 at San Marcos Springs is 4.45 cubic meters per second (m^3/s), the record measured low spring flow is 1.30 m^3/s during the drought of 1956, and the record high is 12.77 m^3/s (Saunders and others 2001). Since the upper portion of the river has little flow input other than groundwater springs, it is defined by the aquifer's properties, with nearly constant year-round cool water temperature of 22°C (Guyton, W. F and Associates 1979) and a pH ranging from 7 to 8 (Ogden, Spinelli, Horton 1985; Slattery and Fahlquist

1997). Downstream of the Blanco River confluence, the San Marcos River has much greater fluctuations in temperature and other physical and chemical parameters. Partly because of the constancy of the water in temperature and flow, the San Marcos River has one of the highest diversities of organisms of any aquatic ecosystem in the southwestern United States (U.S. Fish and Wildlife Service 1996). The spring-fed and relatively isolated river system provides unique habitats for flora and fauna and has high endemism, including several federal threatened and endangered species such as the San Marcos salamander (*Eurycea nana*), the San Marcos gambusia (*Gambusia georgei*), the fountain darter (*Etheostoma fonticola*), and Texas wild-rice (*Zizania texana* Hitchc.) (U.S. Fish and Wildlife Service 1996).

The portion of the upper San Marcos upstream of I-35 is highly urbanized, being located in the city of San Marcos and running through the Texas State University campus and several city parks. This part of the river is a popular recreational venue, including wading, swimming, tubing, canoeing, kayaking, and fishing. Pet dogs are also frequently in the river. This portion generally has a more open tree canopy cover than downstream of I-35 and contains Spring Lake Dam and Rio Vista Dam. The part of the river downstream of I-35 is more rural, running through several private properties used for farming and grazing. It also runs past the state fish hatchery, which withdraws from and discharges used water into the river, and the San Marcos wastewater treatment plant, which discharges treated wastewater into the river. This lower portion has higher canopy cover and contains Capes Dam. It generally becomes slower and deeper as you become closer to the Blanco River due to Cummings Dam, which is located just downstream of the confluence between the Blanco and San Marcos rivers.

The San Marcos River ecosystem and its endangered species are threatened by decreased springflows, impacts resulting from increased urbanization near the rivers, recreational use, pollution, alterations of the rivers, introduction of nonnative species, and other concerns (U.S. Fish and Wildlife Service 1996).

The river is threatened by flow reductions primarily due to water withdrawals from the Edwards Aquifer. The Edwards Aquifer is the primary source of drinking water for more than 2 million people in south central Texas, including the city of San Antonio (RECON Environmental and others 2011). Other users include agricultural and industrial interests in the surrounding area. It is also the source for the largest spring-fed river in Texas, the Comal River. Spring systems fed by the Edwards Aquifer (of which the Comal and San Marcos are the major systems) account for approximately 55% of the water leaving the aquifer, but pumping removes the remaining 45% (Saunders and others 2001). There is precedence for concern regarding reduced flows: 65 of 281 major springs in Texas have dried up (Saunders and others 2001). Recharge rates for the Edwards Aquifer have at times fallen below withdrawal rates and it is feared pumping from the aquifer may soon exceed average annual recharge (Saunders and others 2001). Projections given current population growth offer a chance the San Marcos springs could stop flowing as soon as 2020 without conservation measures (Saunders and others 2001). Lower aquifer levels and springflows may also decrease water quality because of a decreased dilution ability, which would be compounded during drought (U.S. Fish and Wildlife Service 1996).

Humans have impacted or potentially impact the river in many ways. In addition to the dams discussed above, peak flows in the river have been altered by five floodwater

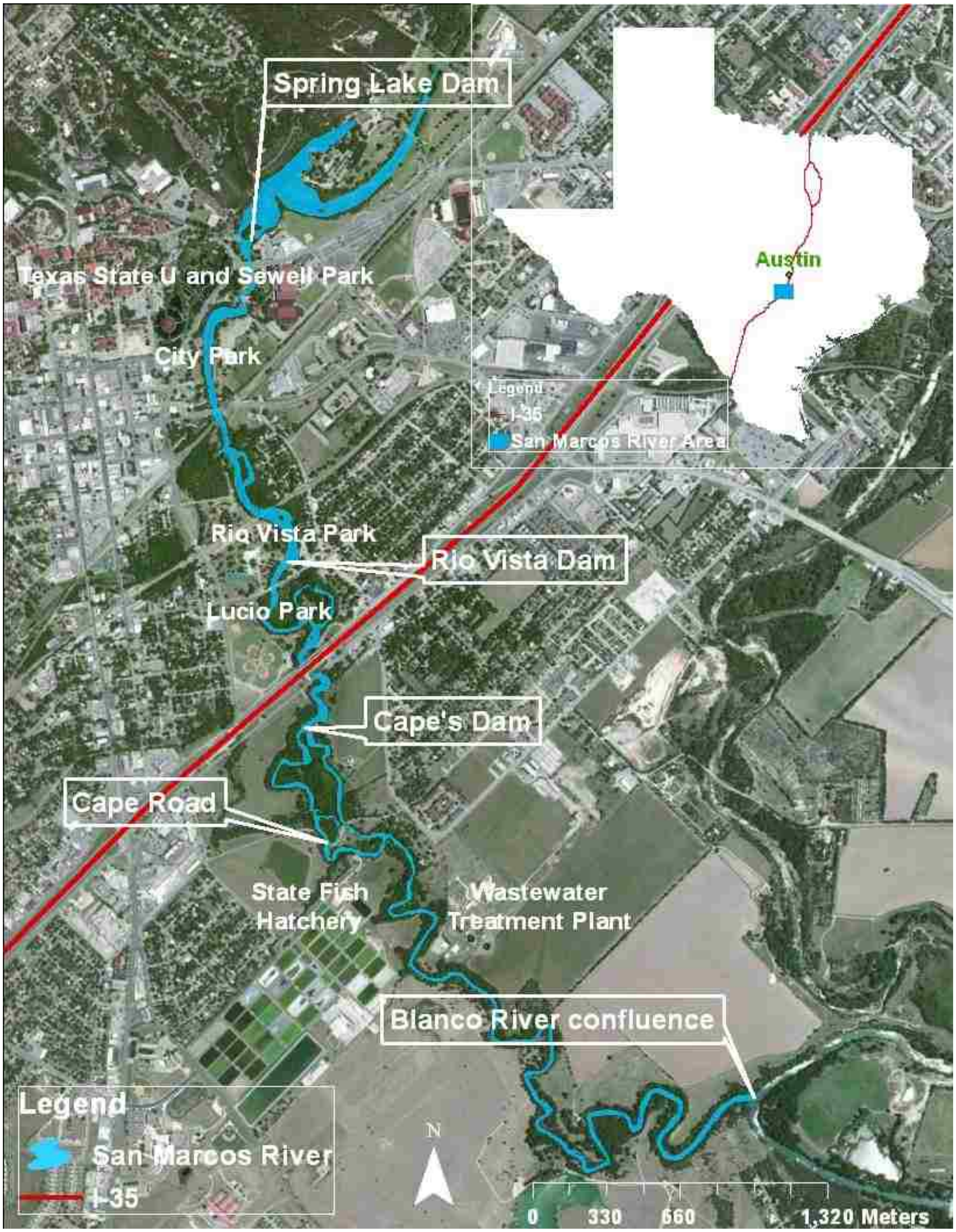

Figure 1.1 Map of the upper San Marcos River

retention dams built on two creeks which feed the river, reducing natural flood events (Saunders and others 2001). Flooding can remove accumulated silt, create openings in vegetation, and potentially reduce abundances of nonnative fish, all of which may benefit native species (U.S. Fish and Wildlife Service 1996). Another human effect, urbanization, can bring increased pollution, erosion to river banks, and siltation in the river channel (U.S. Fish and Wildlife Service 1996). Recreational activity may directly damage stream substrate, plants, and animals in the river, and indirectly by erosion of stream banks, litter, pollution, and runoff from parking areas and support facilities (U.S. Fish and Wildlife Service 1996).

Invasive species pose a significant threat to threatened and endangered species in the San Marcos River (U.S. Fish and Wildlife Service 1996). Invasive plants such as *Hydrilla verticillata* (L.f.) Royle, *Hygrophila polysperma* (Nees. T. Anderson), and *Colocasia esculenta* (L.) Schott (a riparian plant), and invasive animals such as giant ramshorn snails (*Marisa cornuarietis)*, nutria (*Myocaster coypus)* and various fish compete with native species for habitat and resources, negatively modify habitat, introduce disease and parasites, and even directly prey on native and endangered species (U.S. Fish and Wildlife Service 1996).

All of these threats are targets of current restoration and species recovery plans for the San Marcos River and set the background for the issues addressed in this study.

CHAPTER TWO

Restoring Native Aquatic Macrophytes in the San Marcos River

Introduction

Degradation of rivers often includes a reduction in native macrophyte populations, as a result of the negative impacts of invasive plants or other factors. Reductions in macrophyte populations create cascading effects that affect their ability to recover their populations without human aid. Riis and others (2009) discussed one potential effect, the lack of retention of plant propagules (seed and plant shoots). Retention depends on river roughness elements (banks, stones, woody debris, plant beds) to trap the propagules (Riis and Sand-Jensen 2006; Riis and others 2009). Riis and Sand-Jensen (2006) found low stream roughness results in low retention of plant shoots and that the most important retention agents in small rivers are macrophytes. Reduced plant cover overall could therefore result in a circular negative feedback of less retention for new population growth (lower populations leads to lower retention, which leads to even lower populations, etc.). Vegetation surveys of the San Marcos River in 2001 (R. Doyle 2001, Baylor University, Waco, TX, unpublished data) and 2009 (Owens and others 2010) indicate the overall macrophyte areal coverage has ranged in recent times from approximately 42% in 2001 to 33% in 2009, with a notably lower coverage in the portion of the river downstream of I-35 of approximately 16% in 2009. Therefore, retention agents may be an issue in some reaches in this system, particularly downstream of I-35. Riis and Sand-Jensen (2006) noted another potential bottleneck is that where no upstream plant populations are present, colonization of a particular species depends on dispersal from other freshwater systems due to a lack of seed and propagules instream, and natural colonization of previously common but now rare plant species can be very slow. In the San Marcos River, a large decline in native plant populations in the last eighty years has likely reduced both the number of seeds and the number of propagules in the river available to expand or maintain their individual populations. In 2000, Owens and others (2001) surveyed propagules at five sites from Sewall Park to Cape's Dam over four dates during the year. They found invasive species propagules consisted of 67% to 82% of the total, illustrating the relative lack of native species propagules. Supplemental plantings may be necessary to not only increase current native populations, but also to potentially increase seed and propagule production so the populations can be self-sustaining.

In addition to the lack of upstream production of propagules and their poor retention rates, Riis (2008) listed four other possible bottlenecks to the development of a viable aquatic plant population: dispersal of propagules, primary colonization of propagules (establishing attached roots in the sediment), net colonization of propagules (primary colonizations less lost colonizations due to physical conditions such as high flow), and survival of perennial populations during frequent disturbances or suboptimal growth conditions (such as winter conditions). Of those bottlenecks, Riis (2008) found primary colonization to be the main bottleneck as in her study only 0.034% of dispersed shoots and 0.004% of dispersed seeds successfully colonized a 100 m reach in a growing season. In my study, transplants were intended to assist with upstream production of native propagules and eliminate the problem of retention. The propagation technique we used allowed the plants to establish roots in sediment prior to transplanting them with roots and sediment into the river to overcome the primary colonization bottleneck. The anchoring technique used was designed to reduce losses due to high flow or other disturbances.

Another potential issue when transplanting macrophytes is the interaction between the new transplants and existing plants. There has long been evidence for positive, or facilitative, interactions among plants, or the "nurse plant" effect. Facilitative interactions may include protection or amelioration of harsh conditions (shade protection from high temperatures for terrestrial plants is a common example), alteration of substrate characteristics, or protection from herbivores (Callaway 1995). These interactions may be particularly important during the vulnerable seedling stages (Callaway 1995). Of course, interactions can be negative also, including competition for limiting resources (such as food, light, or space) or increased rates of detection by predators (Stachowicz 2001). Most likely, both types of interactions take place; the net interaction will determine if the overall effect is beneficial or harmful (Stachowicz 2001). Strong benefits such as increased survival rate can override even intense negative competitive effects (Stachowicz 2001).

Rivers and other running water environments have been little studied for facilitative interactions among plants. For example, two recent meta-analyses examined studies on facilitative effects. Flores and Jurado (2003) analyzed 296 papers; none were in running water environments. Gomez-Aparicio (2009) analyzed 87 papers; again, none were in running water environments. Possible facilitative interactions in rivers could include a reduction in scouring effects on newly establishing seedlings or transplants, allowing them to establish a stabilizing root system, or protection from herbivores. It has been found that plants in both terrestrial and marine environments that are preferred by herbivores grow faster and suffer less herbivory when associated with unpalatable plants than when growing alone (Callaway 1995; Stachowicz 2001). Herbivory has been shown to be a hindrance to initial establishment for small seedlings in aquatic environments, particularly in otherwise unvegetated areas (Smart, Dick, Doyle 1998). Specifically, one of the native plants used in this study, *Sagittaria platyphylla* (Engelm.) J.G. Smith, has been observed to suffer from herbivory (Doyle 2010, Baylor University, Waco, TX, personal communication) and may benefit from being planted within beds of the invasives *H. verticillata* or *H. polysperma*.

Another of the native plants used in this study, *Ludwigia repens* Forst., was the subject of a competition study with *H. polysperma* (Doyle, Francis, Smart 2003). Doyle and others (2003) compared each plant's growth in a monoculture and with low and high competition from the other plant in concrete raceway conditions. In the presence of competition, *L. repens* had a significantly lower growth rate, lower biomass, and lower allocation of biomass to above-ground tissues than when in monoculture (Doyle, Francis, Smart 2003). My study will further examine negative competitive versus positive nurse plant interactions for *L. repens* and other native plants in river conditions.

The relatively constant year-round conditions in the upper San Marcos River have long supported a diverse native macrophyte population. This aquatic plant distribution reflects the habitat differences upstream and downstream of I-35 discussed in chapter one. With higher riparian cover, less incident radiation available, and deeper and slower water, there is less vegetative cover in the section downstream of I-35, as discussed further below.

In the first attempted complete listing of aquatic macrophytes in the upper river, Lemke (1989) found thirty-one species, with *Potamogeton illinoensis* Morong and *S. platyphylla* reported as the most abundant native plants. The native macrophyte *L. repens* was also listed as "common (found in relatively large numbers throughout the area)". Native macrophytes *Heteranthera dubia* (Jacq.) MacMill. (identified by Lemke as *H. liebmannii*) and *Z. texana* were listed as "occasional (found in relatively small numbers throughout the area)". Of the thirty-one species, Lemke found eight invasive macrophytes, including several listed as common (*Myriophyllum brasiliense* Camb., *Egeria densa* Planch., and *H. verticillata*). It has been theorized that the aquarium industry is a likely source of many of the invasive plants in the San Marcos River as commercial sellers of aquaria plants had previously used the river as a place to grow their inventory, and also possible careless dumping by aquarists (Angerstein and Lemke 1994; Emery 1967).

Two additional invasive macrophytes have had significant impacts on the San Marcos River in the last two decades that were not found or identified at the time of Lemke's 1989 survey. *H. polysperma* is now the third most abundant macrophyte in the river and is the dominant non-riparian macrophyte in the portion of the river downstream of I-35 (Owens and others 2010). Angerstein and Lemke (1994) noted that Lemke's 1989 survey had misidentified *H. polysperma* as *Hygrophila lacustris* (Schlecht. et Cham.) Nees, a similar native plant, and another study by Staton (1992) misidentified it as *L. repens*, another similar looking native plant listed as common by Lemke. Lemke had listed *H. lacustris* as "uncommon (restricted to one or a few locations)", so the prolific spread of *H. polysperma* has likely taken place since 1989. A more recent invasion has been by *Cryptocoryne beckettii* Thw. ex R. Trim. First discovered in the river around 1996 (Rosen 2000), surveys found its areal coverage increased from 171 m^2 in 1998 to 646 m^2 in 2000 (Doyle 2001). In addition to its aggressive expansion rate, the presence of *C. beckettii* was of concern due to its invasion of a portion of the endangered *Zizania texana*'s critical habitat and its preference for the same habitat conditions (depth and flow) as *Z. texana* (Alexander, Doyle, Power 2008). In response to this threat, the U.S. Fish and Wildlife Service (USFWS) used a hand-operated suction dredge to remove some *C. beckettii* and its root tissue from the river. From August 2002 to March 2004, they removed 537 m^2 of *C. beckettii* growing in a 610 meter (m) segment of the river that was within the critical habitat (Alexander, Doyle, Power 2008). However, by April 2005, *C. beckettii* still covered 1,951 m^2 beyond the previously cleared area (Alexander, Doyle, Power 2008). A decision was made to utilize a larger diameter dredge and a follow up diver to eradicate *C. beckettii* and its roots from the entire infested stretch of the river (Alexander, Doyle, Power 2008). Figure 2.1 shows the expansion of *C. beckettii* at its greatest extent prior to this removal. This major removal work was done in the spring of 2006. Surveys since that time have found only a few individual plants, which have been subsequently removed (USFWS, San Marcos, TX, unpublished data). After this work, only scattered clumps of vegetation (mostly *H. polysperma*) remained in the dredge area (Owens and others 2010). Given the apparent suitability of *C. beckettii* for this particular area and the popularity of the species within the aquarium trade, the likelihood of its re-introduction is high (Doyle 2001). Even if *C. beckettii* is not re-introduced, expansion of *H. polysperma* in this area seems likely, given its expansion downstream of I-35 in the past two decades.

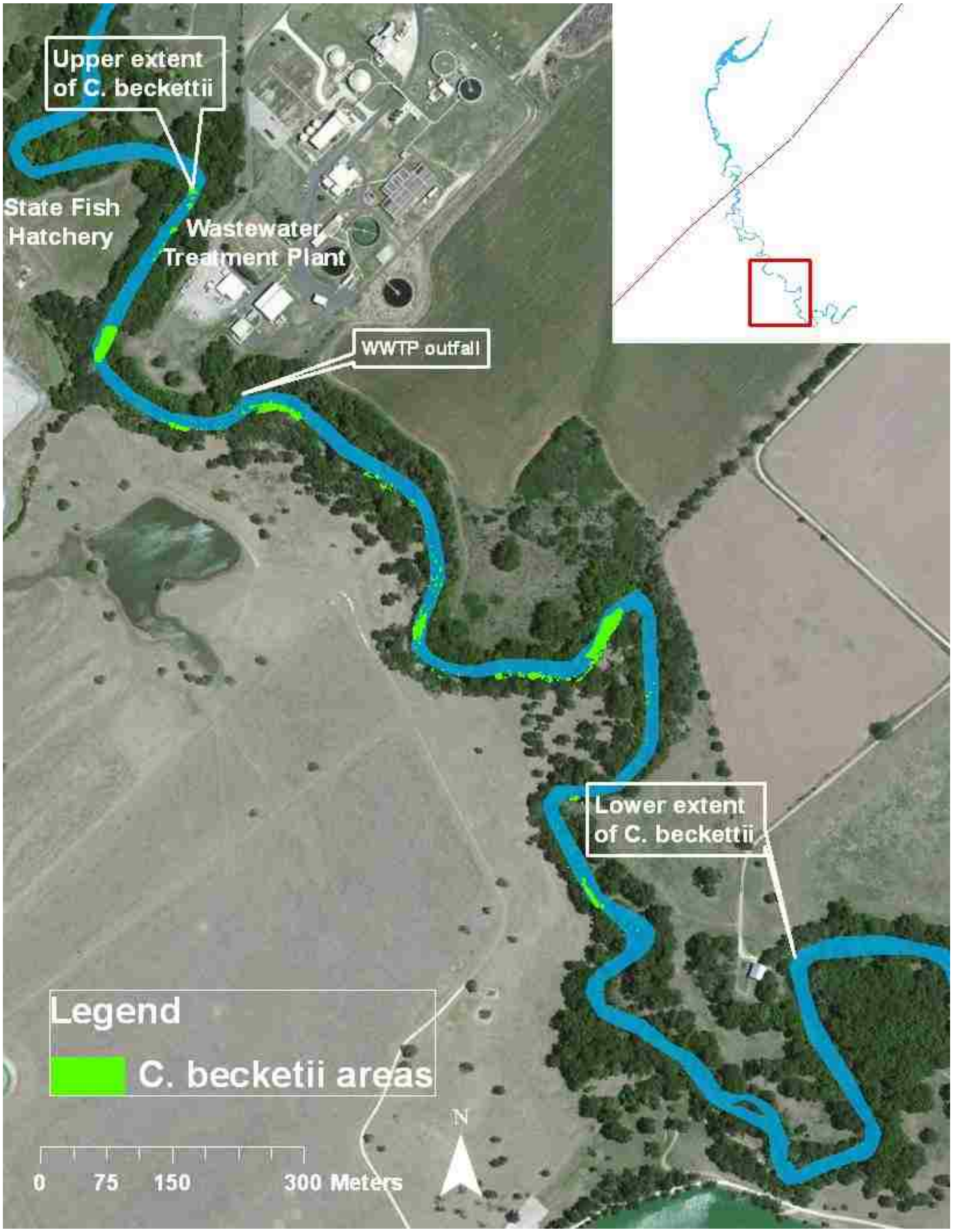

Figure 2.1 Map of *C. beckettii* at its greatest extent. *C. beckettii* data provided by the U.S. Fish and Wildlife Service.

A 2009 survey provided updated data on the vegetation in the upper San Marcos River (Owens and others 2010). They found twenty-four macrophyte species with a total plant cover of approximately 54,200 m^2 (out of a total riverbed area of approximately 166,000 m^2). Ten species of invasive macrophytes comprised 75% of the total vegetation cover. A small amount of a new invasive plant, alligatorweed, was noted, with a total area of 92 m^2, but no *C. beckettii* was found. *H. verticillata*, *H. polysperma*, and *C. esculenta* made up greater than 95% of the total invasive coverage. The largest native plant populations were *Potamogeton illinoensis* Morong. and *S. platyphylla*, with coverage of about 3,500 m^2 each. The differences in vegetation cover discussed in chapter one between the sections upstream and downstream of I-35 are evident in this study, with 38,800 m^2 found upstream from I-35 (2.6 km) and 15,300 m^2 downstream from I-35 (3.8 km). In addition, vegetation covers only approximately 4,800 m^2 in the 2.8 km downstream of Cape Road, the area of this portion of my study.

Study Objectives and Data Analysis

This portion of my thesis looks to build upon earlier native plant reintroductions, focusing on the portion of the river starting just below Cape Road to near the Blanco River confluence (see Figure 1.1), which includes the stretch of the river where *C. beckettii* was dredged. This part of the research is funded by a cooperative agreement between Baylor and the USFWS to develop restoration techniques for native plant re-establishment in the lower portion of the upper San Marcos River. I cultured propagules of *H. dubia*, *L. repens*, and *S. platyphylla* (Figure 2.2) and transplanted them into the river.

Figure 2.2 *Sagittaria platyphylla* (top left), *Heteranthera dubia* (top right), and *Ludwigia repens* (bottom)

These species were chosen for several reasons. As discussed above, *S. platyphylla* and *H. dubia* have been successful to some extent in prior transplants in the San Marcos River. *H. dubia* occupies more mesohabitat types than any other native aquatic macrophyte taxa in the San Marcos River (Saunders and others 2001) (identified as *H. liebmannii* in that study). This indicates that the plant has a relatively broad range of habitat preference in terms of depth, current and substrate type, a useful characteristic for restoration purposes. *S. platyphylla* has been found to be one of the most common native plants in the river (Lemke 1989; Owens and others 2010). *L. repens* had also been found as a common native plant in the past (Lemke 1989). Utilization of these three widely distributed species was thought to provide the highest probability of initial success. Planting species that are more specialized could be considered in future restoration efforts. In addition to supplementing the native macrophyte populations, I examined two primary factors relating to plant survival.

First, I examined the impact of immediate neighbors on transplant success. I planted the macrophytes in pairs within existing plant beds and outside of any currently existing plant beds (on bare substrate) to examine if there is a possible immediate positive "nurse plant" or a negative competition effect that may increase or decrease the survival probability of newly established plants in the San Marcos river. The hypotheses regarding the effect of neighboring plants are:

H_0: There is no difference in the survival percentage of plantings based on existence of neighboring plants.

H_A: There is a difference in the survival percentage of plantings based on existence of neighboring plants.

Second, I examined the effect of the amount of radiation received by the plants on survival rates. Even before the dredge activity, that area of the river had a much lower density and diversity of macrophytes when compared to more upstream areas (Owens and others 2010; Saunders and others 2001). The differences in radiation received by the plants in this area of the river compared to areas upstream may account for lower macrophyte populations. I examined the radiation effect using two methods, by measuring canopy cover and by modeling incident radiation received by the plants. For future reintroductions in this section of the river, it would be useful to know if canopy cover or incident radiation is related to short-term survival of transplants. The hypotheses regarding the effect of radiation are:

> H_0: There is no difference in the survival percentage of plantings based on either the average instantaneous daily rate incident radiation received by the plants or canopy cover.
>
> H_A: There is a difference in the survival percentage of plantings based on either the average instantaneous daily rate incident radiation received by the plants or canopy cover.

In addition to these primary factors, I examined the impact of depth, velocity, and substrate by splitting the distributions in this experiment into groups based on variability in these factors. However, because I selected sites where these factors fell within known (or assumed) suitability ranges, this is not an evaluation of these factors over the entire range actually available in the river.

Statistical analyses were performed using R (R Development Core Team 2011). I used a chi-square analysis of contingency tables to analyze survival of native plantings versus presence of neighboring plants, average instantaneous daily rate incident radiation, canopy cover, and other site physical factors. No continuity correction was applied in the chi-square analysis as recommended by Zar (2010) for two by two contingency tables with one fixed margin. I used an alpha (α) level of 0.10 to determine significance because of the rather small dataset available.

Experimental Design and Methods

In June and July 2010, I propagated 192 *S. platyphylla*, 528 *H. dubia*, and 474 *L. repens* plants in the USFWS' San Marcos National Fish Hatchery (San Marcos, Texas) greenhouse tanks, using separate tanks for each species. These plants grew in the tanks for 10 to 12 weeks before transplanting into the river. Following procedures previously used successfully by Dr. Robert Doyle's lab (Doyle 2010, Baylor University, Waco, TX, personal communication), we separated *S. platyphylla* daughter plants from existing *S. platyphylla* plants cultured in USFWS greenhouse tanks. We cut off stems about 15 to 20 centimeters (cm) from the root mass and transplanted them with roots into 4.5 inch peat pots contained in one quart round plastic pots, using a sediment mix provided by the USFWS. The USFWS sediment mix is one-third pea gravel and two-thirds four-way soil, which consists of compost, top soil, orange sand, and cedar flakes. We cut *H. dubia* and *L. repens* shoots containing an apical meristem of about 10 to 15 cm from existing plants cultured in USFWS greenhouse tanks and transplanted three shoots each into 3 inch peat pots contained in 3.5 inch plastic pots, filling the pots with about one-third to one-half USFWS sediment mix and topping with decorative garden pebbles to prevent the propagules from dislodging and floating to the surface of the water. I filled *S. platyphylla* tanks with standing water pumped in by the USFWS from shallow groundwater with water quality conditions virtually identical to those of the San Marcos River. I filled *H. dubia* and *L. repens* tanks with the same water and used a circulating system to simulate river flows. I did not use running water for *S. platyphylla* as the USFWS had been successfully cultivating *S. platyphylla* in standing water. No pots of *S.*

platyphylla or *H. dubia* were lost (died) during propagation. Ten pots of propagated *L. repens* were lost prior to transplanting in the river.

We transplanted the plants into the San Marcos River over four weeks from September 23 to October 14, 2010, after the plants had established viable roots. We dug a hole in the sediment using a planting dibble, placed the peat pot in it, and anchored it with a 25 cm piece of 3/8 inch thick rebar bent in the shape of a hook. I attached a small plastic plant tag with a zip tie to use for individual plant identification (Figure 2.3). When we were not able to insert the rebar completely into rocky substrate, we placed a large rock on top of the root mass and rebar as an additional anchor.

Figure 2.3 Anchor and plant tag used for *S. platyphylla*, *H. dubia*, *L. repens*, and *Z. texana* whole plants (left), anchor and plant tag used for *Z. texana* tillers and seed packs (right)

Planting sites are in the stretches of the river where *C. beckettii* was removed (see Figure 2.1) and in areas approximately 1.1 km above this (Figure 2.4). I selected sites so that river depth, current velocity, and substrate could be kept roughly within a range for each species as listed in Table 2.1, following the suitability criteria contained in Saunders (2001). However, some individual plantings fell outside of these criteria. *H. polysperma* criteria was used as a proxy for *L. repens* as it is believed to occupy the same habitats and no suitability criteria was available for *L. repens*.

Between October 27 to November 12, 2010 (2 to 4 week survey) and between January 4 to 29, 2011 (3 to 4 month survey), sites were surveyed for survival by noting if a plant of the same species existed in the location of the anchor and tag. A final survey was conducted in May 2011 (7 to 8 month survey). Anchors and tags were removed after the final survey.

Table 2.1 Transplant site criteria by species[a]

Species	Depth (m)	Velocity (m/s)	Substrate[b]
S. platyphylla	0.675-1.575	0.03-0.30	Silt, Sand, Fgrv, Mgrv, Scob
H. dubia	0.450-1.350	0.30-0.75	Sgrv, Cgrv, Lgrv, Scob
L. repens	0.030-1.350	0.00-0.30	Silt, Sand, Scob, Sbdr

[a] Source: Adapted from Appendix III in Saunders and others (2001).

[b] Substrate codes: Fgrv = fine gravel, Sgrv = small gravel, Mgrv = medium gravel, Cgrv = course gravel, Lgrv = large gravel, Scob = small cobble, Sbdr = small boulder.

To test for the "nurse plant"/competition effect, I planted macrophytes in pairs by species, one pot located within a bed of other plants and one pot nearby in an area without other plants (that is, on bare substrate, with at least a 0.1 to 0.2 meter buffer between any other plants). I chose the exact bare area spot in order to minimize differences in depth or velocity between the two pots.

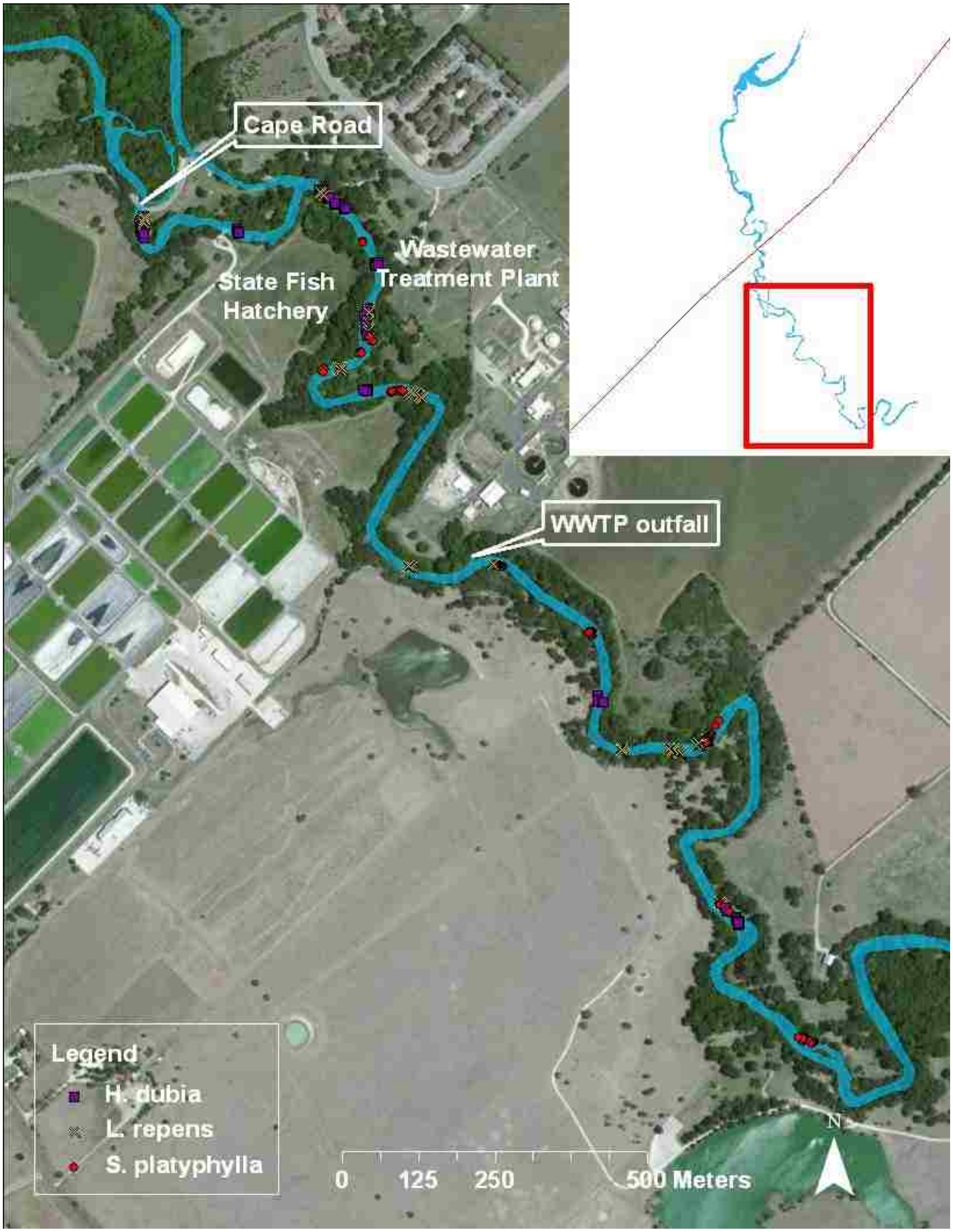

Figure 2.4 Map of native planting locations in this study

At planting, I recorded the location of each planting pair with a Trimble GeoXH handheld GPS unit (post-processing resolution of 30 cm). We also measured river depth, velocity, type of neighboring plants, substrate, and percent canopy cover for each pair. We measured velocity with a Marsh-McBirney Flo-Mate, setting the top-setting-wading rod to measure flow at 60% of river depth. We measured percent canopy cover as densiometer readings with a Forestry Suppliers, Inc. convex spherical densitometer, taking readings in the directions north, south, east, and west and averaging these readings.

To test if the amount of radiation affects the short-term survival of plants in the San Marcos River system, I estimated the incident radiation in different areas of the river using the MT-CLIM for Excel simulation model (Glassy and Running 1994; Hungerford and others 1989; Running, Nemani, Hungerford 2003; Running, Nemani, Hungerford 1987; Thornton and Running 1999; Thornton, Hasenauer, White 2000). This model extrapolates meteorological variables from a point of measurement (referred to as the "base" station) to the study site of interest, making corrections for differences in elevation, slope, and aspect between the base station and the site, with one of the outputs being daily total incident shortwave radiant flux density (Watts (W) per m^2) for site locations (Hungerford and others 1989). MT-CLIM models radiation from sunrise to sunset, but truncates the daily direct beam solar irradiance by east and west horizons (Hungerford and others 1989).

I modeled radiation over the time period of this study (9/1/10 to 5/31/11). Inputs for the model included base station weather data (daily high and low temperature and precipitation) for San Marcos (National Climatic Data Center (NCDC), U.S. Department of Commerce 2012) for the time period of this study, base station isohyet ([SRCC] Southern Regional Climate Center 1997), and base and site elevation from 10 meter resolution National Elevation Data (NED) ([USGS] United States Geological Survey 2009). Site slope and aspect were calculated in ArcMap 10.0 (ESRI Inc. 2010) using the NED. I estimated the angles to the east and west horizons for each site by measuring the horizontal distance to the nearest, tallest trees in the east and west directions, assuming a height of 15 meters for these tallest trees, and calculating the angle using the arctan function. I measured the horizontal distance using one meter resolution Digital Ortho-rectified Quarter Quad (DOQQ) imagery ([USDA] United States Department of Agriculture Aerial Photography Field Office 2010). Radiation was not modeled for all 258 pairs planted, but rather a calculation was done for groups of plants, with groupings determined by differences in aspect at the site or differences in distance to the nearest, tallest trees. As a result of the groupings, I modeled a total of forty-three sites distributed over the reach of the river where my plantings were made.

Once I modeled the incident radiation at each site, I adjusted the radiation using the Beer-Lambert Law (Campbell 1977) to account for local canopy cover from surrounding trees as follows:

$$I_L = I_0 * e^{-k1*LAI},$$

where I_0 and I_L are the radiation above the canopy (modeled incoming radiation) and the radiation at the surface of the river (W/m^2), respectively, k1 is the extinction coefficient for the canopy, and LAI is a dimensionless parameter representing the area of leaf surface over unit area of ground. I used an extinction coefficient of 0.6 and an average LAI of 4.0 during the months of March through November (leaf-on period) and 1.0 for the months of December through February (leaf-off period) per Maass and others (1995) as they calculated for a deciduous canopy. The LAI during the leaf-on period was adjusted downward for the average canopy cover for all plantings in that grouping (LAI * average percent canopy cover).

Next, I used the Beer-Lambert Law to adjust the modeled incoming radiation to the radiation received by the plants underwater:

$I_U = I_L * e^{-k2*z}$,

where I_L and I_U are the radiation at the surface of the river (after canopy adjustment) and radiation underwater, respectively, k2 is the extinction coefficient in water (in m^{-1}), and z is the depth of the river at the site (in meters). I calculated an average extinction coefficient for the San Marcos River by taking an instantaneous radiation reading using a handheld spherical quantum sensor (LI-COR LI-1400) at twelve locations dispersed throughout the river (Figure 2.5). We measured incoming radiation using a clear sky and no canopy cover. These readings were taken twice at each of two depths, just below the surface of water and at a depth of 0.34-0.75 below the surface. The extinction coefficient was calculated using the methodology in Lind (1985) and the results of these calculations are shown in Table 2.2. I used the average of the extinction coefficients over my study area (downstream of Cape Road, sample sites 7 through 12), for the k2 extinction coefficient in the Beer-Lambert formula. I used the average depth of all plantings within that grouping for use in the Beer-Lambert formula.

In this study, reported light radiation is the average instantaneous radiation reaching the sediment surface during the daylight period after correction for east and west

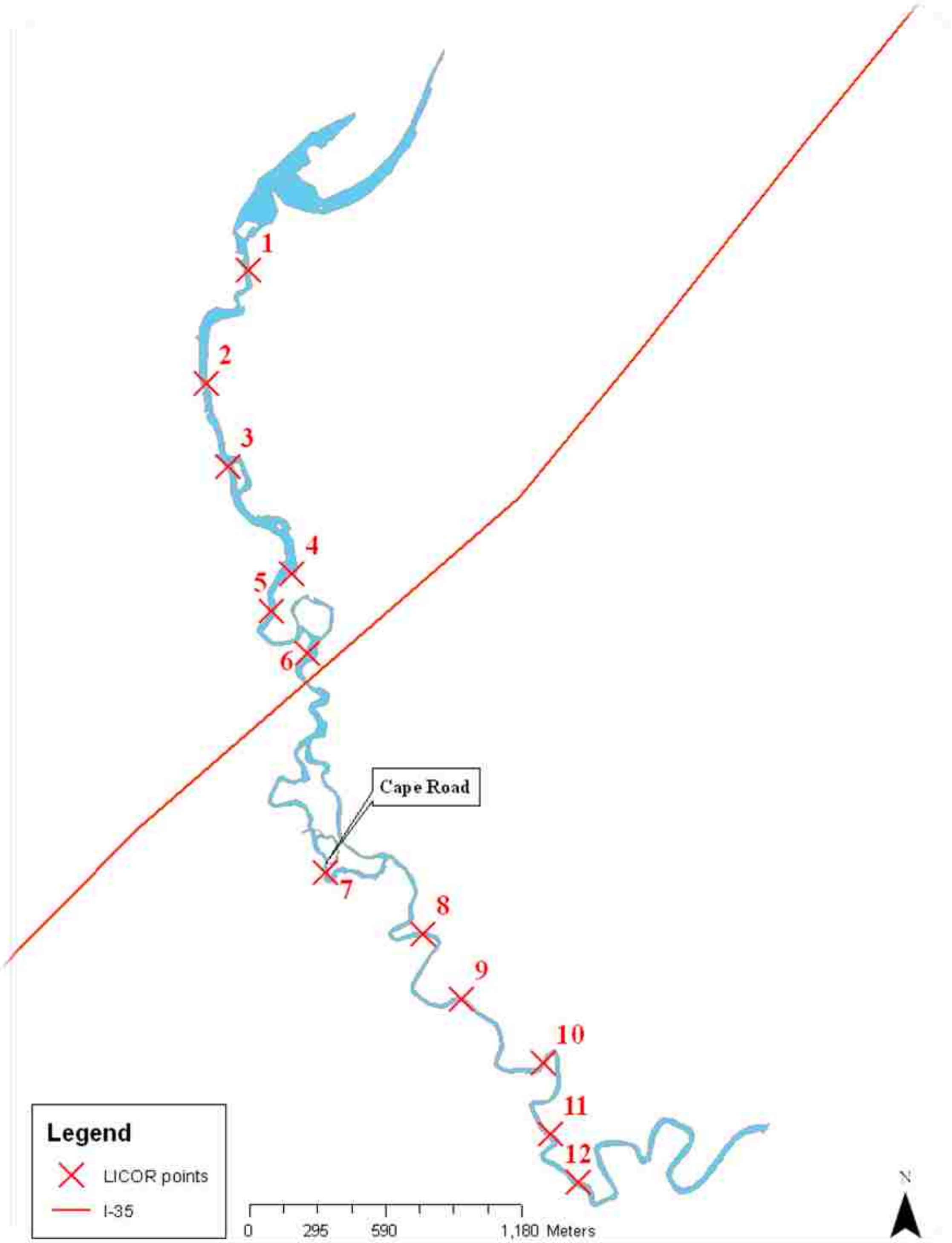

Figure 2.5 Points for LI-COR radiation measurements on San Marcos River

horizons (tallest trees along east and west horizons), local canopy cover, and attenuation due to water turbidity.

Table 2.2 Extinction coefficients in the San Marcos River

Sampling site	Extinction coefficient (k2)	Sampling site	Extinction coefficient (k2)
1	0.27	7	0.42
2	0.25	8	0.64
3	0.35	9	0.25
4	0.29	10	0.53
5	0.70	11	0.58
6	0.46	12	0.54
Average upstream of I-35 (sites 1-6)	0.38	Ave downstream of Cape Road (sites 7-12)	0.49
		Average for all sites	0.44

Results

The number surviving and percentage survival of the three native transplanted macrophytes are shown in Tables 2.3, 2.4, and 2.5. Table 2.3 shows the survival of all macrophytes after two to four weeks (October-November 2010 survey), after three to four months (January 2011 survey), and after seven to eight months (May 2011 survey). Tables 2.4 and 2.5 show the same for the macrophytes planted with neighboring plants and those planted without neighboring plants, respectively. *S. platyphylla* had higher survival than *H. dubia* or *L. repens*, with and without neighboring plants, particularly in the earlier surveys. *L. repens* had the lowest survival.

Survival was significantly different with regard to neighboring plants for all three species at the time of the May 2011 survey (*S. platyphylla*, $\chi^2 = 8.35$, $p < 0.01$; *H. dubia*, $\chi^2 = 18.29$, $p < 0.01$; *L. repens*, $\chi^2 = 11.94$, $p < 0.01$). For all three species, survival was higher than randomly expected for those without neighboring plants, supporting the

Table 2.3 All transplanted macrophytes and survival at subsequent surveys

	Sept-Oct 2010	Oct-Nov 2010		Jan 2011		May 2011	
	Planted	2-4 weeks		3-4 months		7-8 months	
Species		Alive	Percent	Alive	Percent	Alive	Percent
S. platyphylla	192	191	99.48%	178	92.71%	100	52.08%
H. dubia	176	119	67.61%	89	50.57%	61	34.66%
L. repens	148	109	73.65%	68	45.95%	26	17.57%
Totals	516	419	81.20%	335	64.92%	187	36.24%

Table 2.4 Macrophytes transplanted with neighboring plants and survival at subsequent surveys

	Sept-Oct 2010	Oct-Nov 2010		Jan 2011		May 2011	
	Planted	2-4 weeks		3-4 months		7-8 months	
Species		Alive	Percent	Alive	Percent	Alive	Percent
S. platyphylla	96	96	100.00%	88	91.67%	40	41.67%
H. dubia	88	52	59.09%	27	30.68%	17	19.32%
L. repens	74	53	71.62%	30	40.54%	5	6.76%
Totals	258	201	77.91%	145	56.20%	62	24.03%

Table 2.5 Macrophytes transplanted without neighboring plants and survival at subsequent surveys

	Sept-Oct 2010	Oct-Nov 2010		Jan 2011		May 2011	
	Planted	2-4 weeks		3-4 months		7-8 months	
Species		Alive	Percent	Alive	Percent	Alive	Percent
S. platyphylla	96	95	98.96%	90	93.75%	60	62.50%
H. dubia	88	67	76.14%	62	70.45%	44	50.00%
L. repens	74	56	75.68%	38	51.35%	21	28.38%
Totals	258	218	84.50%	190	73.64%	125	48.45%

alternative hypothesis that there is a difference in the survival percentage of plantings based on existence of neighboring plants for these species. Survival was also significantly different with regard to neighboring plants for *H. dubia* for both the October-November and January surveys (October-November, $\chi^2 = 5.84$, $p = 0.02$, January, $\chi^2 = 18.29$, $p < 0.01$), but was independent with regard to neighboring plants for

S. platyphylla (October-November, $\chi^2 = 1.01$, $p = 0.32$, January, $\chi^2 = 0.31$, $p = 0.58$) and *L. repens* (October-November, $\chi^2 = 0.31$, $p = 0.58$, January, $\chi^2 = 1.74$, $p = 0.19$). Like the May survey, survival in the earlier surveys was higher than randomly expected for *H. dubia* without neighboring plants.

Table 2.6 shows the results of the MT-CLIM radiation model, after adjustment for extinction through canopy and water. Radiation received by plants ranged from an average of 21 W/m^2 to 96 W/m^2. Figure 2.6 shows the relationship between modeled radiation and canopy cover for these sites. The r-squared of 0.79 indicates a strong relationship between the two variables. Unfortunately, the heavily shaded reach of the river where I worked does not provide a robust dataset from which to evaluate the canopy/radiation effects since the sites are heavily weighted towards highly shaded sites. Only four modeled sites have 40% or less local canopy cover. Therefore, my conclusions related to light impacts must be evaluated in this context.

Figures 2.7a, 2.7b, and 2.7c show the incident radiation compared to the May survival of all plants and all plants with and without neighbors. Figures 2.7d, 2.7e, and 2.7f show the canopy cover compared to the May survival of all plants and all plants with and without neighbors. My plantings had canopy cover ranging from 9.88% to 100% when planted, although the average cover of 74.39% reflects the relatively heavy riparian vegetation present in this section of the river. The thick, red vertical lines indicate a visual "threshold" above or below which survival distribution differs. For modeled radiation, survival for all plants was 35% at 45 W/m^2 or lower (average of points to the left of the red line in Figure 2.7(a)) and 45% at greater than 45 W/m^2 (to the right of the red line). For canopy cover, survival for all plants was 50% at 63% cover or lower

Table 2.6 Modeled average instantaneous incident radiation, as adjusted for canopy and water (9/1/10 – 5/31/11)

Site	Incident radiation (W/m^2)	Site	Incident radiation (W/m^2)	Site	Incident radiation (W/m^2)	Site	Incident radiation (W/m^2)
1	88	12	25	23	38	34	27
2	96	13	32	24	41	35	39
3	51	14	24	25	22	36	50
4	50	15	26	26	24	37	42
5	81	16	25	27	29	38	37
6	50	17	32	28	30	39	35
7	47	18	44	29	26	40	61
8	40	19	24	30	24	41	34
9	50	20	21	31	23	42	25
10	28	21	79	32	23	43	34
11	21	22	87	33	27		

(average of points to the left of the red line in Figure 2.7(d)) and 34% at greater than 63% cover (to the right of the red line).

Table 2.7 shows a summary of the modeled instantaneous incident radiation and the average canopy cover measured at the time of planting for each of the modeled sites. For the purpose of performing chi-square analyses on this data, I divided these factors into three categories, using natural breaks and distribution of the data. These categories are also shown in Table 2.7. Breakouts of survival rates for each species using these categories are shown in Tables 2.8 and 2.9.

Survival was significantly different with regard to modeled radiation for *S. platyphylla* for the January ($\chi^2 = 6.42$, $p = 0.04$) survey, for *H. dubia* for the October-November and January surveys (October-November, $\chi^2 = 15.47$, $p < 0.001$, January, $\chi^2 = 14.44$, $p < 0.001$), and for *L. repens* for the May survey ($\chi^2 = 5.79$, $p = 0.06$). *S. platyphylla* survival was higher than randomly expected for lower radiation levels and

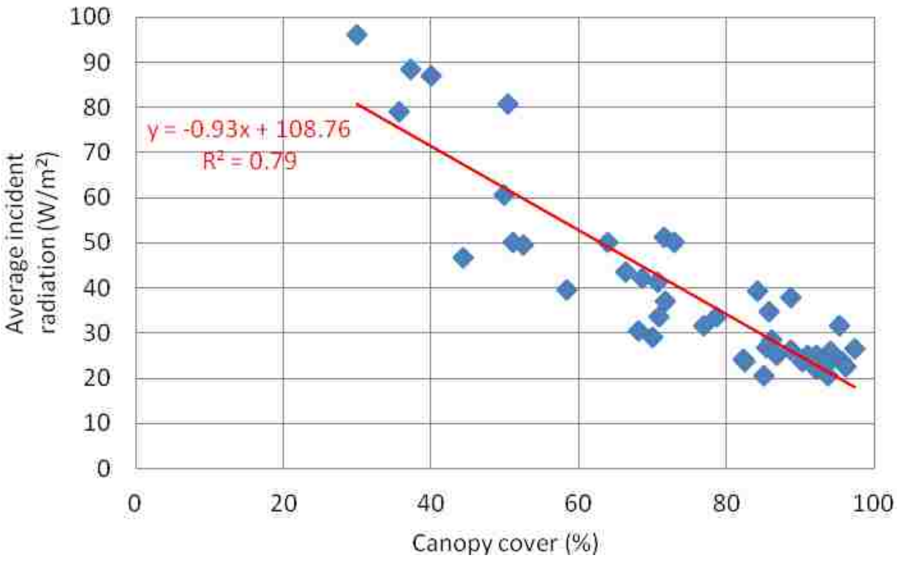

Figure 2.6 Relationship between modeled radiation and canopy cover

lower than expected for intermediate levels. *H. dubia* survival was higher than randomly expected for lower and higher radiation levels and lower than expected for intermediate levels for both the October-November and January surveys. *L. repens* survival was lower than randomly expected for lower radiation levels and higher than expected for intermediate levels. The alternative hypothesis that there is a difference in the survival percentage of plantings based on incident radiation is supported for *L. repens* for the May survey and *H. dubia* for the earlier surveys.

Survival was significantly different with regard to canopy cover for *S. platyphylla* for the May survey ($\chi^2 = 4.83$, $p = 0.09$) and for *L. repens* for the October-November and January surveys (October-November, $\chi^2 = 8.83$, $p = 0.01$, January, $\chi^2 = 8.01$, $p = 0.01$). *S. platyphylla* survival was higher than randomly expected for lower canopy cover and lower than expected for intermediate and higher canopy cover. *L. repens* survival was lower than randomly expected for intermediate canopy cover and higher than expected

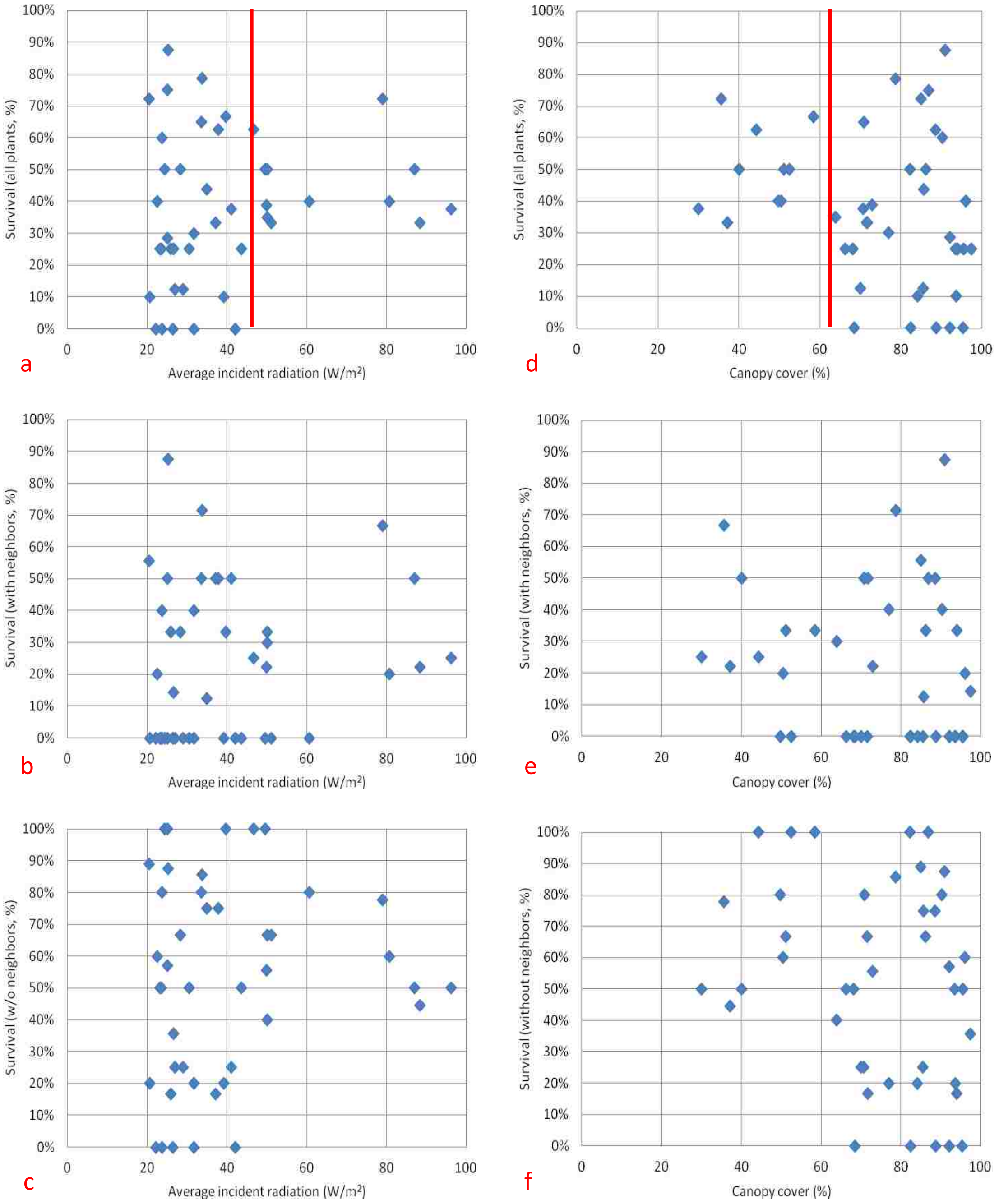

Figure 2.7 Relationship between average incident radiation and 7-8 month survival at each radiation modeled site for all plantings (a), plantings with neighbors (b), and plantings without neighbors (c). Relationship between canopy cover and 7-8 month survival at each radiation modeled site for all plantings (d), plantings with neighbors (e), and plantings without neighbors (f). The red lines indicate a visual "threshold" above and below which survival distribution differs.

Table 2.7 Summary of modeled light and canopy cover.
Categories are divisions used for chi-square analyses.

Factor	Mean ± standard deviation (range)	Category 1 (*n*)	Category 2 (*n*)	Category 3 (*n*)
Modeled radiation (W/m2)				
All plants	41 ± 19	—	—	—
	(21 to 96)	—	—	—
S. platyphylla	39 ± 18	23 to 31	32 to 78	79 to 87
	(23 to 87)	(72)	(94)	(26)
H. dubia	46 ± 21	21 to 39	40 to 80	81 to 96
	(21 to 96)	(54)	(96)	(26)
L. repens	37 ± 17	22 to 37	38 to 87	88 to 96
	(22 to 96)	(82)	(56)	(10)
Canopy cover (%)				
All plants	74 ± 20	—	—	—
	(10 to 100)	—	—	—
S. platyphylla	79 ± 18	28 to 70	71 to 86	87 to 100
	(28 to 100)	(40)	(72)	(80)
H. dubia	67 ± 20	28 to 70	71 to 85	86 to 98
	(28 to 98)	(98)	(36)	(42)
L. repens	77 ± 19	10 to 70	71 to 85	86 to 97
	(10 to 97)	(32)	(56)	(60)

for higher canopy cover for the October-November survey and lower than randomly expected for lower canopy cover and higher than expected for higher canopy cover for the January survey. The alternative hypothesis that there is a difference in the survival percentage of plantings based on canopy cover is supported for *S. platyphylla* for the May survey and for *L. repens* for the earlier surveys.

Table 2.10 shows the physical factors of each of the planting pair sites, measured at the time of planting. For the purpose of performing chi-square analyses, I divided the continuous

Table 2.8 Survival rates by modeled radiation categories

	Survival % by modeled radiation categories		
Planting	Lower	Intermediate	Higher
S. platyphylla			
Oct-Nov 2010	100%	99%	100%
January 2011	99%	88%	92%
May 2011	50%	50%	65%
H. dubia			
Oct-Nov 2010	80%	55%	88%
January 2011	67%	38%	65%
May 2011	41%	29%	42%
L. repens			
Oct-Nov 2010	72%	75%	80%
January 2011	51%	41%	30%
May 2011	11%	27%	20%

Table 2.9 Survival rates by canopy cover categories

	Survival % by canopy cover categories		
Planting	Lower	Intermediate	Higher
S. platyphylla			
Oct-Nov 2010	98%	100%	100%
January 2011	90%	90%	96%
May 2011	68%	49%	48%
H. dubia			
Oct-Nov 2010	65%	69%	71%
January 2011	44%	61%	57%
May 2011	34%	42%	31%
L. repens			
Oct-Nov 2010	66%	64%	87%
January 2011	28%	43%	58%
May 2011	19%	21%	13%

factors into two or three categories, using natural breaks and distribution of the data. These categories are also shown in Table 2.10. Breakouts of survival rates for each species using these categories are shown in Tables 2.11, 2.12, and 2.13.

Using these categories, I performed chi-square analyses to test if survival was independent of those factors (Table 2.14). Depth and velocity were significant factors for *S. platyphylla* survival for the January survey (survival lower than randomly expected at highest depths and higher than randomly expected at lowest and highest velocities). Substrate was a significant factor for *S. platyphylla* survival for the May survey (survival higher than expected on coarse substrates). Depth, velocity, and substrate were significant factors for *H. dubia* survival for the October-November and January surveys and velocity was a significant factor for the May survey. Survival was higher than randomly expected in shallower depths and lower than randomly expected in higher depths. Survival was lower than randomly expected at slower velocities and higher than randomly expected at faster velocities. Survival was higher than randomly expected for coarse substrates. Finally, depth was the only significant factor for *L. repens* survival, and for the January survey only. Survival was higher than randomly expected for shallower depths.

Discussion

Macrophytes have a number of bottlenecks to overcome to establish new populations (Riis 2008). This portion of study attempted to overcome some of those initial bottlenecks (low propagule production and retention, establishing root systems in the sediment, and loss of seedlings due to high flow) by transplantation, propagation technique, and anchoring. Herbivory may be an early bottleneck in some systems, but

Table 2.10 Summary of data collected for each pair at time of planting. Categories are divisions used for chi-square analyses.

Factor	Mean ± standard deviation (range)	Category 1 (*n*)	Category 2 (*n*)	Category 3 (*n*)
Depth (m)				
All plants	0.63 ± 0.25	—	—	—
	(0.14 to 1.40)	—	—	—
S. platyphylla	0.63 ± 0.22	0.23 to 0.50	0.51 to 0.74	0.75 to 1.35
	(0.23 to 1.35)	(72)	(72)	(48)
H. dubia	0.63 ± 0.23	0.27 to 0.49	0.50 to 0.79	0.80 to 1.18
	(0.27 to 1.18)	(58)	(74)	(44)
L. repens	0.64 ± 0.31	0.14 to 0.49	0.50 to 0.79	0.80 to 1.40
	(0.14 to 1.40)	(50)	(50)	(48)
Velocity (m/s)				
All plants	0.11 ± 0.08	—	—	—
	(0.00 to 0.34)	—	—	—
S. platyphylla	0.07 ± 0.05	0.00 to 0.04	0.05 to 0.09	0.10 to 0.20
	(0.00 to 0.20)	(60)	(72)	(60)
H. dubia	0.17 ± 0.07	0.02 to 0.14	0.15 to 0.20	0.21 to 0.34
	(0.02 to 0.34)	(66)	(48)	(62)
L. repens	0.09 ± 0.06	0.00 to 0.05	0.06 to 0.12	0.13 to 0.24
	(0.00 to 0.24)	(50)	(54)	(44)
Substrate[a]				
S. platyphylla	—	Fine	Coarse	—
	—	(168)	(24)	—
H. dubia	—	Fine	Coarse	—
	—	(96)	(80)	—
L. repens	—	Fine	Coarse	—
	—	(122)	(26)	—

[a] Fine = silt or sand, Coarse = gravel or cobble

Table 2.11 Survival rates by depth categories

	Survival % by depth categories		
Planting	Shallower	Intermediate	Deeper
S. platyphylla			
Oct-Nov 2010	99%	100%	100%
January 2011	94%	96%	85%
May 2011	46%	56%	56%
H. dubia			
Oct-Nov 2010	79%	66%	55%
January 2011	60%	53%	34%
May 2011	31%	42%	27%
L. repens			
Oct-Nov 2010	84%	68%	69%
January 2011	58%	42%	38%
May 2011	16%	22%	15%

Table 2.12 Survival rates by velocity categories

	Survival % by velocity categories		
Planting	Slower	Intermediate	Faster
S. platyphylla			
Oct-Nov 2010	98%	100%	100%
January 2011	98%	83%	98%
May 2011	48%	47%	62%
H. dubia			
Oct-Nov 2010	39%	77%	90%
January 2011	21%	60%	74%
May 2011	11%	52%	47%
L. repens			
Oct-Nov 2010	66%	80%	75%
January 2011	36%	52%	50%
May 2011	20%	13%	20%

Table 2.13 Survival rates by substrate categories

Planting	Survival % by substrate Coarse	Fine
S. platyphylla		
Oct-Nov 2010	100%	99%
January 2011	100%	92%
May 2011	83%	48%
H. dubia		
Oct-Nov 2010	79%	58%
January 2011	65%	39%
May 2011	44%	27%
L. repens		
Oct-Nov 2010	69%	75%
January 2011	42%	47%
May 2011	19%	17%

Table 2.14 Chi-square (χ^2) tests for independence of survival vs. other factors for each survey date

Factor (degrees of freedom)	χ^2 Oct-Nov 2010	Jan 2011	May 2011
Depth (2)			
S. platyphylla	1.68[b]	5.14[a,b]	1.81
H. dubia	7.12[a]	7.13[a]	3.12
L. repens	4.18	4.62[a]	1.06
Velocity (2)			
S. platyphylla	2.21[b]	14.98[a,b]	3.23
H. dubia	40.57[a]	38.46[a]	27.31[a]
L. repens	2.54	3.04	1.25
Substrate (1)			
S. platyphylla	0.14[b]	2.16[b]	10.73[a]
H. dubia	8.31[a]	12.22[a]	5.35
L. repens	0.32	0.17	0.06[b]

[a] $p < 0.10$

[b] Contains some expected contingency cell values of < 5

was only observed on a handful of *S. platyphylla* in this study and likely is not a large factor in this portion of the river.

The overall survival results for my *S. platyphylla* transplants were excellent after one and four months (99% and 93%, respectively). These results are favorable compared to other *S. platyphylla* plantings on the San Marcos River over similar periods. May 1999 transplants in the San Marcos River had a survival rate of 93% after one month (see Chapter 3 for detail) (Doyle 2002), March 2008 plantings had a survival rate of >75% and 46% after six weeks and 3.5 months, respectively, and November 2008 plantings had a survival rate of >50% after five months (Doyle, Mullins, Bormann 2011). Survival for my plantings dropped to 52% after eight months (but 62.5% without neighboring plants, discussed further below), lower than the 79% after six months and 66% after one year for the May 1999 transplants (Doyle 2002), but higher than the 20% after eight months for the November 2008 transplants (Doyle, Mullins, Bormann 2011). It should be noted that both 2008 plantings were within the reaches of the river of my plantings, while the 1999 transplants had sites in the upper portions of the river that appear to be more favorable to macrophytes. Counting only transplants within the reaches of the river for my plantings, the May 1999 plantings had survival rates of 75% and 62.5% after six months and one year, respectively. Comparing these to my survival rates, my propagation and anchoring techniques for this species appear effective and the survival rate was reasonably good, particularly without neighbors.

Overall survival for my *H. dubia* transplants were lower than *S. platyphylla* at 68% overall after one month, 51% after four months, and 35% after eight months (but 76%, 70%, and 50% without neighbors, respectively). These rates compare to >80% and

25% after six weeks and 3.5 months, respectively for San Marcos River March 2008 plantings and >75% and 34% after five months and eight months, respectively, for November 2008 plantings (Doyle, Mullins, Bormann 2011). Like the corresponding *S. platyphylla* plantings, these 2008 *H. dubia* plantings were in the same reaches as my transplants. My survival rates without neighbors are favorable compared to the prior plantings and the propagation and anchoring techniques for this species appear effective.

L. repens had the poorest survival of my transplants, with rates of 74%, 46%, and 18% after one, four, and eight months, respectively, with rates only slightly improved when only considering those planted without neighbors at 76%, 51%, and 28%, respectively. Only one other major planting of L. repens had been done in the past on the San Marcos River – in 2004, >250 L. repens were planted in my section of the river, but none were found two years later. My plants had grown extremely well in culture and considering the anchoring did not seem to be a problem for the other two species, it is unlikely the poor survival could be attributed to anchoring technique.

Beyond propagation and anchoring, "nurse plants" offered the potential for protection during the early stages of establishment from herbivory and harsh conditions such as high flow. Results of this study indicate the negative competitive effects of neighboring plants surrounding new transplants eventually outweighed any positive benefits they may have provided as all three species had significantly higher survival without neighboring plants after eight months. *L. repens* had particularly dismal survival with neighboring plants (7% after eight months); this corroborates Doyle, Francis, and Smart's (2003) findings that *L. repens* was a poor competitor with *H. polysperma*. It should be noted that with the exception of four *L. repens* and one *S. platyphylla* planted within natural *H. dubia* plant beds, all of the transplants with neighboring plants in this study were planted within invasive *H. verticillata* or *H. polysperma* plant beds as those species were what was available for neighboring plants in the sections of the river planted. Both *H. verticillata* and *H. polysperma* are known to be highly competitive (Langeland 1996; Sutton 1995) and this has been evidenced in the San Marcos River by the expansion of their populations in the last few decades. *H. polysperma* and *H. dubia* were a particularly bad combination, with only one of thirty-two *H. dubia* in *H. polysperma* surviving after four months and zero surviving after eight months (data not shown). *L. repens* performed equally poor in *H. verticillata* and *H. polysperma*. There were no *S. platyphylla* planted in *H. verticillata*. While we can conclude the two invasive macrophytes do not provide net positive "nurse plant" benefits, this may not be the case for native macrophytes.

Results for the radiation analysis were mixed and may reflect the lack of planting locations with higher light availability. Survival for *S. platyphylla* was significantly higher for low modeled radiation for the January survey but significantly higher for low canopy cover (higher radiation) for the May survey. However, survival was still high enough for *S. platyphylla* in January that some of the chi-square expected frequencies were small (less than five) and the test may not be as robust for that survey. The result for the May survey is likely stronger, indicating higher light conditions are suitable for *S. platyphylla* in the short-term.

Survival for *H. dubia* was significantly higher for low and high modeled radiation for the January survey but not significant with regards to canopy cover for any survey. The results may indicate a broad tolerance for light conditions. This is consistent with earlier findings that H. dubia occupies more mesohabitat types than any other native aquatic macrophyte taxa in the San Marcos River (Saunders and others 2001).

Survival for *L. repens* was significantly lower for low modeled radiation for the May survey but significantly lower for low canopy cover (higher radiation) and higher for high canopy cover (low radiation) for the October-November and January surveys. However, *L. repens* survival was so poor by the May survey that several cells had expected frequencies below five and the results should be interpreted with caution. The canopy cover results indicate short-term survival for *L. repens* is not limited by lower light levels.

The MT-CLIM model was intended to provide a potentially improved estimate of the relative light received by plants from site to site than canopy cover by taking into account the different aspect (slope direction) and truncated horizons for each site. The model has been shown to have high accuracy over widely varying environmental conditions and locations (Thornton and Running 1999; Thornton, Hasenauer, White 2000). The modeled radiation and canopy cover for each site were highly correlated (see Figure 2.6), indicating reasonable model results, but the results from chi-square tests on each modeled radiation and canopy cover were not consistent. Future use of the model in this system may benefit from field verification of distance to tallest trees and tree height. In addition, distribution of both the modeled radiation and canopy cover were highly skewed towards low radiation/high canopy cover in this portion of the river. The interpretation of the effect of light on survival would likely benefit from a wider distribution of light conditions for all three species.

Other than neighboring plants and radiation, we also chose suitable microhabitats (depth, velocity, substrate) for each macrophyte based on the habitat findings of previous studies. Hence, these physical factors were skewed toward these previous findings and I would not have expected the factors to have a significant impact on survival. However, there were a few exceptions. Coarse substrates had higher than expected survival for *S. platyphylla* after eight months and this was the opposite of other studies (Doyle 2002; Saunders and others 2001), which found higher survival or occurrence on fine substrates. Substrate may be more of a factor in longer-term survival. The significantly higher survival at lowest and highest velocities for *S. platyphylla* was somewhat puzzling as that was only for the January survey. However, survival was still very high at that time, limiting the chi-square analysis. My entire range appears to be suitable for longer-term survival. That deeper depths had lower than expected survival was also surprising, as Saunders and others (2001) had found *S. platyphylla* was found more often in depths of approximately 0.675 m or deeper. However, again, this was only for the January survey and my entire range appears to be suitable for longer-term survival.

Saunders and others (2001) had found *H. dubia* occurred more often at deeper depths, faster velocities, and coarser substrates. My results support this for velocity and substrate. Although survival was higher than expected at shallower depths for the one month and four month surveys, my shallow depths were still within acceptable (although not ideal) range according to Saunders and others (Saunders and others 2001). Also, this difference was no longer significant at the eight month survey, so this range appears suitable for longer-term.

Depth was the only significant factor for *L. repens*, and only for the January survey. However, survival was so poor overall, it is difficult to read much into this one result. Overall, it seems possible that the suitability criteria for *H. polysperma* (which I used as a proxy for *L. repens*) is not as good a fit for *L. repens* as we had thought. One final possible factor affecting *L. repens* survival could have been the size of the plants at planting. The plants grew so well in culture, most were a meter or more long. *L. repens* has a branching morphology with multiple apical meristems which have the advantage of being able to generate roots at each node that comes in contact with the sediment, but the branches are more prone to stem breakage in flowing waters than morphologies like rosettes. While length may be a benefit for a branching morphology in that the branches can all generate roots, the negative effect of stem breakage may have overwhelmed any benefit of length.

In this lower stretch of the upper San Marcos River, we seem to be able to get past the first few bottlenecks to higher native macrophyte populations with our propagation and anchoring techniques. Neighboring "nurse" plants should not be used, at least not *H. verticillata* or *H. polysperma*. *S. platyphylla* and *H. dubia* have shown they can have good short-term survival without neighboring plants but *L. repens* likely needs further refinement of habitat needs and possibly a shorter culture time (shorter length) if it is used in restoration plantings again.

S. platyphylla should likely be planted in lower canopy cover areas. This may be a reason *S. platyphylla* survival rates have been higher in upstream reaches of the river than downstream reaches as *S. platyphylla* has previously been found to have higher survival or occurrence in deeper depths, slower velocities, and finer substrates (Doyle

2002; Saunders and others 2001), the conditions that are found in downstream reaches as opposed to upstream. Earlier findings that *H. dubia* had higher survival or occurrence rates in higher velocity areas are corroborated by this study. Otherwise, I did not find depth, velocity, and/or substrate to have a significantly different impact on short-term survival than found in previous studies.

CHAPTER THREE

Review and Evaluation of Previous San Marcos River and Comal River Macrophyte Transplants

Introduction

Long-term survival data for macrophyte reintroductions is critically lacking in the literature. While short-term survival surveys for macrophyte restoration projects are often made, longer-term survival data (years, even decades) is rarely available but perhaps more ecologically relevant. Once plants have endured the short-term bottlenecks to survival discussed in Chapter 2, what factors may contribute to longer-term success (years to decades)? Baylor University has led several prior native macrophyte reintroduction efforts in the San Marcos and Comal Rivers, which I will re-examine to see if early expansion may contribute to the probability of long-term success.

The Comal River (29°42'52"N, 98°8'7"W) is a similar physical and biological ecosystem to the San Marcos River and is located within the city of New Braunfels, Comal County, Texas. It is Texas' largest spring-fed river, also emanates from the Edwards Aquifer, and is located about 30 km southeast of the San Marcos River, which, as mentioned in chapter one, is Texas' second largest spring-fed river. It flows approximately 5.1 km in a southeasterly direction through its impoundment, Landa Lake, and two channels from until its confluence with the Guadalupe River (Figure 3.1) (Hardy 2009). Like the San Marcos River, the Comal River's watershed is also highly urbanized and developed, has high recreational use, and supports highly diverse habitats and

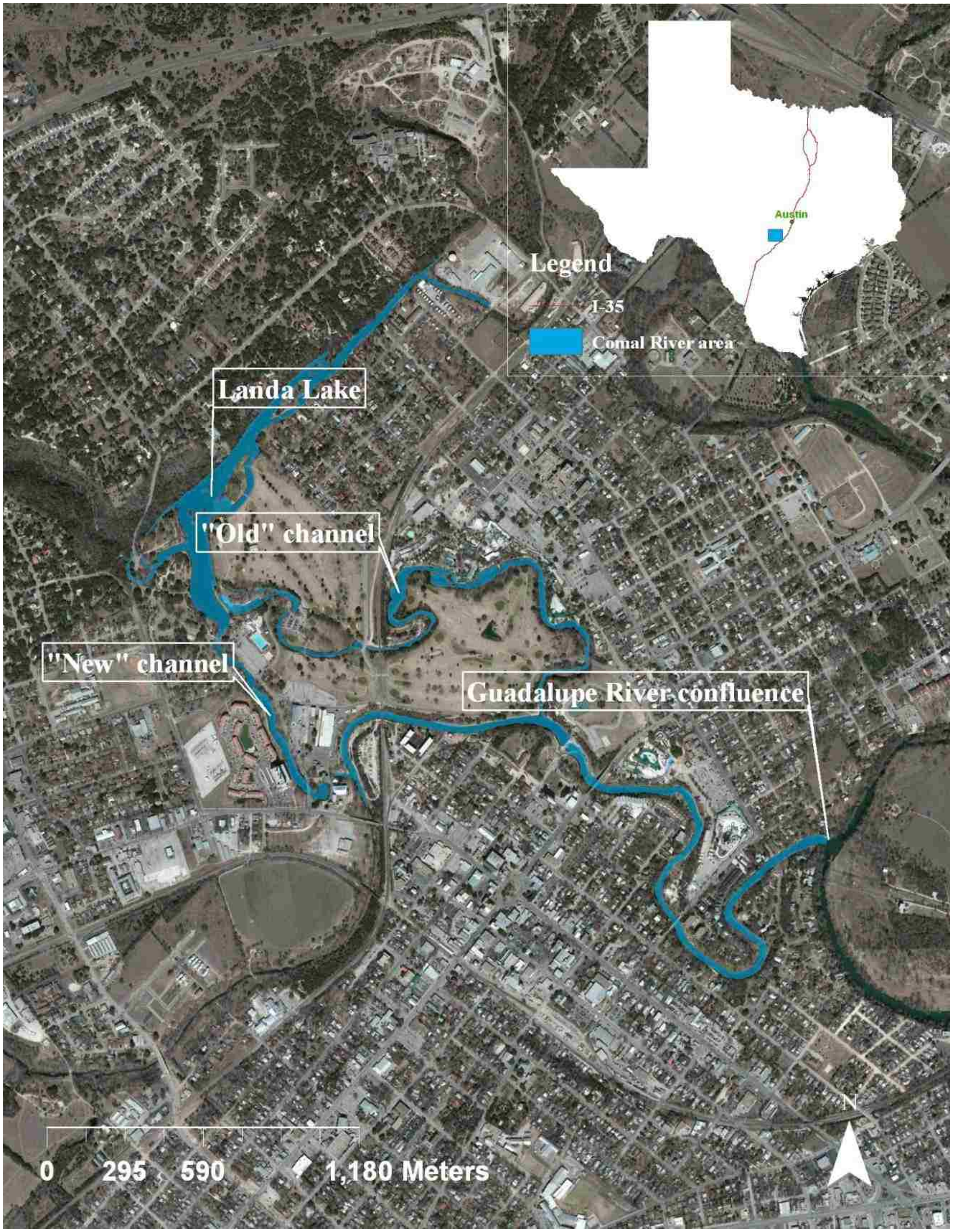

Figure 3.1 Map of the Comal River

endemism, including several threatened and endangered species (U.S. Fish and Wildlife Service 1996). Also, like the San Marcos River, the spring-fed waters of the Comal River provide excellent habitat for aquatic macrophytes. Although there have been no quantitative macrophyte surveys published for the Comal River, an unpublished survey (R. Doyle 2000, Baylor University, Waco, TX, unpublished data) showed macrophytes covered approximately 51% (~116,000 m^2) of the river, of which approximately 37% (~43,000 m^2) was invasive. *H. polysperma* comprised >98% of the invasive areal coverage. The two most abundant invasive species on the San Marcos River by area, *H. verticillata* and *C. esculenta*, only covered approximately 700 m^2 and <1 m^2, respectively. The most abundant native macrophytes were *Vallisneria sp.* (~39,500 m^2), *Cabomba caroliniana* Gray (~18,000 m^2), and *S. platyphylla* (~5,500 m^2). The *Vallisneria* species in the Comal has long been considered to be *V. americana*. However, recent molecular data casts doubt on this identity (Doyle 2012, Baylor University, Waco, TX, personal communication). Therefore, for the purposes of this study, I will refer to the species as *Vallisneria sp*.

Restoration plantings in both rivers were led from 1998-2000 by Dr. Robert Doyle of Baylor University as part of a USFWS study (Doyle 2002). Each macrophyte's location was recorded with a handheld GPS unit (sub-meter accuracy). Two plantings were done prior to October 1998 (one in each river) but were affected by a historic 500 year flood in that month, significantly affecting transplant survival. Accordingly, these plantings are not included in this study. Also, 12 L. repens were transplanted in April 1999, but none were found in September 2011. These transplants have not been included in this study either. The included plantings are as follows.

In May 1999, 144 *S. platyphylla* were transplanted on the San Marcos River between just upstream of Hopkins Street to upstream of the power lines (Figure 3.2). These plants were cultured for at least 8 weeks in raceways at Texas State University and anchored with a sediment anchor driven through the root mass (Doyle 2002). Table 3.1 summarizes the plantings and their survival rates on subsequent survey dates.

Table 3.1 Summary of San Marcos River May 1999 plantings and subsequent surveys

	S. platyphylla	
Date	# of plants	% survival
May 1999 planting	144	—
June 1999 survey	134	93%
Oct 1999 survey	114	79%
May 2000 survey	95	66%

Comal River plantings included 46 *S. platyphylla* and 51 *Vallisneria sp.* in December 1998 (Figure 3.3), 71 *S. platyphylla*, and 72 *Vallisneria sp.* in April 1999 (Figure 3.4), and 24 *S. platyphylla* and 24 *Vallisneria sp.* in May 2000 (Figure 3.5). Table 3.2 summarizes the plantings and their survival rates on subsequent survey dates.

The December 1998 transplants were cultured in greenhouses for approximately 12 weeks at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Denton, TX. One-half of the plants were protected using a plastic wire mesh anchored to the sediment and one-half were anchored with a sediment anchor driven through the root mass (Doyle 2002). The type of anchoring was not determined significant to survival, but the culture conditions may have been (Doyle 2002). The flow environment and water chemistry at LAERF (stagnant and low CO_2) were very different from the conditions on the Comal River (high flow and high CO_2). The plants were considered to be in poor

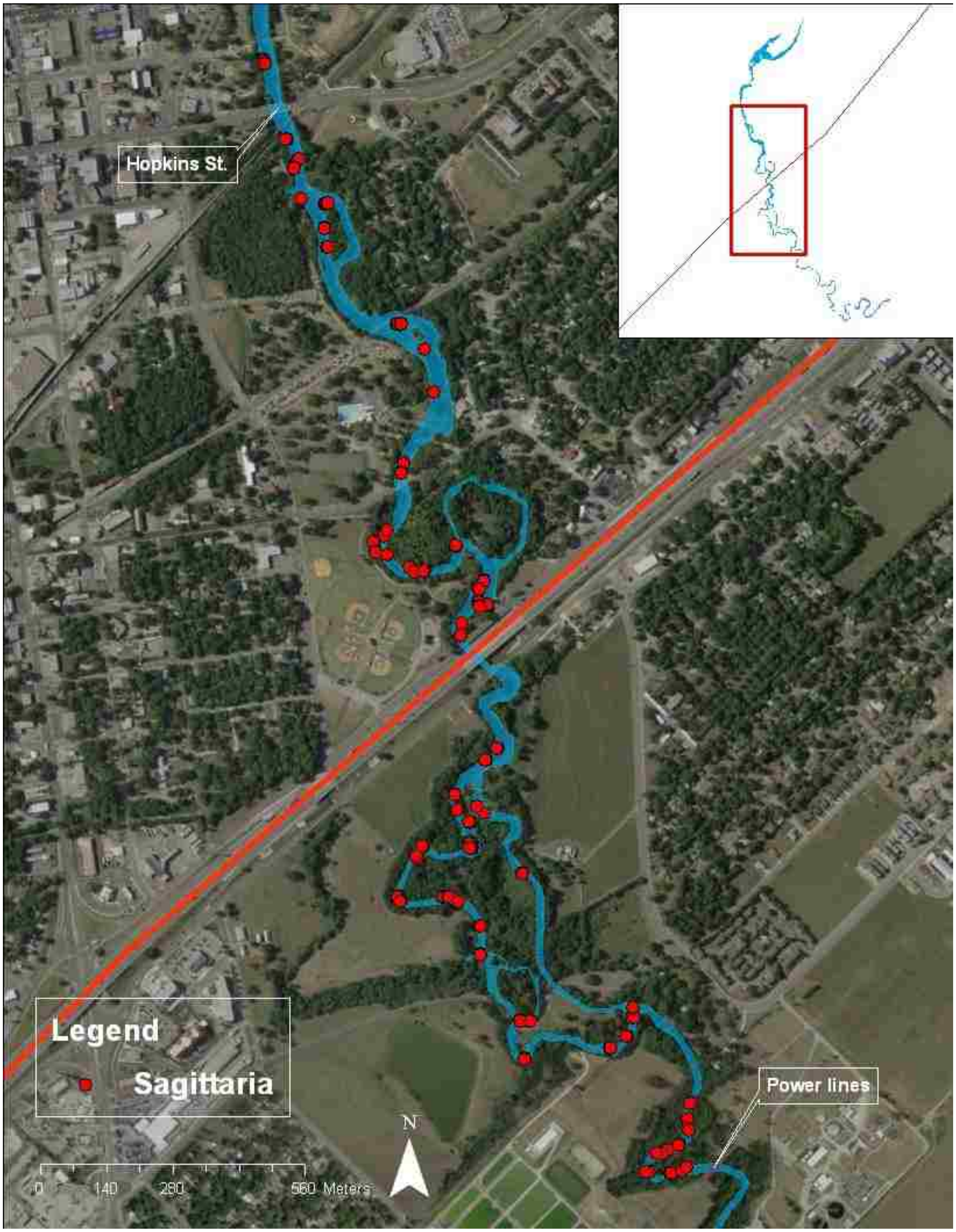

Figure 3.2 Map of the May 1999 San Marcos River restoration plantings

Figure 3.3 Map of the December 1998 Comal River restoration plantings

Figure 3.4 Map of the April 1999 Comal River restoration plantings

Figure 3.5 Map of the May 2000 Comal River restoration plantings

condition after culturing and this likely contributed to the low overall survivorship (Doyle 2002).

Table 3.2 Summary of Comal River plantings and subsequent surveys

	S. platyphylla		*Vallisneria sp.*	
Date	# of plants	% survival	# of plants	% survival
December 1998 planting	46	—	51	—
January 1999 survey	35	76%	24	47%
April 1999 survey	18	39%	9	18%
June 1999 survey	16	35%	6	12%
October 1999 survey	10	22%	2	4%
May 2000 survey	9	20%	1	2%
April 1999 planting	71	—	72	—
June 1999 survey	48	68%	49	68%
October 1999 survey	43	61%	31	43%
May 2000 survey	29	41%	21	29%
May 2000 planting	24	—	24	—
June 2000 survey	24	100%	24	100%
October 2000 survey	21	88%	12	50%
March 2001 survey	20	83%	—	—
July 2001 survey	18	75%	11	46%

The April 1999 transplants were again cultured at the LAERF greenhouses for approximately 16 weeks, but improvements were made to the flow and CO_2 conditions (Doyle 2002). Plants appeared much healthier than the December 1998 transplants (Doyle 2002). Plants were anchored with a sediment anchor driven through the root mass (Doyle 2002).

The May 2000 transplants were direct, bare root transplants harvested from one area of the river and transplanted into another area the same day (Doyle 2002). Plants were anchored with a sediment anchor driven through the root mass (Doyle 2002).

Study Objectives and Data Analysis

I will evaluate the current survival of prior restoration plantings in the San Marcos River and the Comal River relative to each macrophyte's status at its last short-term survey (May 2000, October 2000, or July 2001), which ranged from 5 months after planting to 1 1/2 years, depending on the planting. The question relates to the relationship between current status and short-term survey status. Is the current presence of the same species in the same place as one of the prior plantings independent of that plant's survival and expansion (number of plants) status at the time of the last survey? The hypotheses are:

H_0: The current presence and plant stand size of a species is independent of their status at the last survey.

H_A: The current presence and plant stand size of a species is not independent of their status at the last survey.

Statistical analyses were performed using R (R Development Core Team 2011). I used a chi-square analysis of contingency tables with no continuity correction and an alpha (α) level of 0.10 due to the small dataset. I compared the 2011 (long-term) plant survival status relative to the last available short-term (6 to 18 month) status using the chi-square analysis, running a separate analysis for each transplant date and species. A significant chi-square indicates that the long-term status was significantly related to the short-term status.

Experimental Design and Methods

Planting sites for the San Marcos and Comal Rivers restoration plantings from 1998-2000 were located using available GPS points and plants noted as present or not present and the number of plants present. The San Marcos River survey was conducted on August 16, 2011 and the Comal River survey was conducted on September 1, 2011. The existence of a particular species in a spot near (<1 m) where that species was previously transplanted (as located by GPS point) was assumed to be from the original planting. I divided short-term status at the last survey and current status into three categories – not present, present, but less than colony size, or colony size. Colony size was defined as ten or more plants for *S. platyphylla* and three or more plants for *Vallisneria sp.* based on the records of their rates of short-term expansion for all the prior plantings.

Results

Table 3.3 shows the groupings of May 1999 San Marcos River *S. platyphylla* plantings according to plant stand size as of the May 2000 survey and as of our August 2011 survey. Of the 144 total transplants, 51 (35%) had grown to colony size after one year (May 2000). Of those 51 colony sized sites, 24 (47%) were still of colony size 11 years later (August 2011). We only found two planting sites that had colony size plant stands in August 2011 where less than 10 plants had been found in May 2000. At the planting sites where there was no short-term survival, we found only four sites with plants present at low densities and none were colony sized plants. This may represent a low rate of natural spread for the species.

Table 3.3 San Marcos River May 1999 *S. platyphylla* transplants – current and May 2000 status

August 2011 status[a]	May 2000 status[b] A	B	C	Totals
1	46	41	23	110
2	4	0	4	8
3	0	2	24	26
Totals	50	43	51	144

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (10+ plants)

[b] A = not present, B = present, but less than colony, C = colony (10+ plants)

A chi-square analysis using these categories supports the alternative hypothesis that the long-term success of *S. platyphylla* transplants is not independent of their short-term ability to expand into larger colonies in the San Marcos River ($\chi^2 = 50.63$, $p < 0.001$). That is, sites showing good short-term establishment were much more likely to have plants 11 years later.

Table 3.4 shows the groupings of December 1998 Comal River *S. platyphylla* plantings according to plant stand size as of the May 2000 survey and as of our September 2011 survey. Of the 46 total transplants, 7 (15%) had grown to colony size after 17 months (May 2000). Of those seven colony sized sites, 3 (43%) were still of colony size 11 years later (September 2011). We found three planting sites that had colony size plant stands in September 2011 where no plants had been found in May 2000.

Table 3.5 shows the groupings of December 1998 Comal River *Vallisneria sp.* plantings according to plant stand size as of the May 2000 survey and as of our September 2011 survey. Of the 51 transplants, one (the lone survivor, 2%) had grown to colony size after 17 months (May 2000). That one colony sized site was still of colony size 11 years later (September 2011). We found nine planting sites that had colony size plant stands in September 2011 where no plants had been found in May 2000.

Table 3.4 Comal River December 1998 *S. platyphylla* transplants – current and May 2000 status

Sept 2011 status[a]	May 2000 status[b] A	B	C	Totals
1	32	2	4	38
2	2	0	0	2
3	3	0	3	6
Totals	37	2	7	46

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (10+ plants)

[b] A = not present, B = present, but less than colony, C = colony (10+ plants)

Table 3.5 Comal River December 1998 *Vallisneria sp.* transplants – current and May 2000 status

Sept 2011 status[a]	May 2000 status[b] A	B	C	Totals
1	41	0	0	41
2	0	0	0	0
3	9	0	1	10
Totals	50	0	1	51

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (3+ plants)

[b] A = not present, B = present, but less than colony, C = colony (3+ plants)

A chi-square analysis for *S. platyphylla* December 1998 transplants supports the null hypothesis that their long-term success of is independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 6.90$, $p = 0.14$).

A chi-square analysis for *Vallisneria sp.* December 1998 transplants supports the alternative hypothesis that their long-term success is not independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 4.18$, $p = 0.05$).

Table 3.6 shows the groupings of April 1999 Comal River *S. platyphylla* plantings according to plant stand size as of the May 2000 survey and as of our September 2011 survey. Of the 71 transplants, 22 (31%) had grown to colony size after 13 months (May 2000). Of those 22 colony sized sites, 14 (64%) were still of colony size 11 years later (September 2011). We found 12 planting sites that had colony size plant stands in September 2011 where less than 10 plants had been found in May 2000.

Table 3.6 Comal River April 1999 *S. platyphylla* transplants – current and May 2000 status

Sept 2011	May 2000 status[b]			
status[a]	A	B	C	Totals
1	31	5	8	44
2	1	0	0	1
3	7	5	14	26
Totals	39	10	22	71

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (10+ plants)

[b] A = not present, B = present, but less than colony, C = colony (10+ plants)

Table 3.7 shows the groupings of April 1999 Comal River *Vallisneria sp.* plantings according to plant stand size as of the May 2000 survey and as of our September 2011 survey. Of the 72 transplants, 15 (21%) had grown to colony size after 13 months (May 2000). Of those 15 colony sized sites, all 15 were still of colony size 11 years later (September 2011). We found three planting sites that had colony size plant stands in September 2011 where less than 10 plants had been found in May 2000.

A chi-square analysis for *S. platyphylla* April 1999 transplants supports the alternative hypothesis that their long-term success of is not independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 13.90$, $p = 0.01$).

A chi-square analysis for *Vallisneria sp.* April 1999 transplants supports the alternative hypothesis that their long-term success is not independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 59.66$, $p < 0.001$).

Table 3.7 Comal River April 1999 *Vallisneria sp.* transplants – current and May 2000 status

Sept 2011 status[a]	May 2000 status[b] A	B	C	Totals
1	50	4	0	54
2	0	0	0	0
3	1	2	15	18
Totals	51	6	15	72

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (3+ plants)

[b] A = not present, B = present, but less than colony, C = colony (3+ plants)

Table 3.8 shows the groupings of May 2000 Comal River *S. platyphylla* plantings according to plant stand size as of the July 2001 survey and as of our September 2011 survey. Of the 24 transplants, 14 (58%) had grown to colony size after 14 months (July 2001). Of those 14 colony sized sites, 10 (71%) were still of colony size 10 years later (September 2011). We found one planting site that had a colony size plant stand in September 2011 where less than 10 plants had been found in July 2001.

Table 3.9 shows the groupings of May 2000 Comal River *Vallisneria sp.* plantings according to plant stand size as of the October 2000 survey and as of our September 2011 survey. Of the 24 transplants, two (8%) had grown to colony size after 6 months (October 2000). Of those 2 colony sized sites, none were still of colony size 11 years later (September 2011). We found 5 planting sites that had colony size plant stands in September 2011 where less than 10 plants had been found in October 2000.

Table 3.8 Comal River May 2000 *S. platyphylla* transplants – current and July 2001 status

Sept 2011	July 2001 status[b]			
status[a]	A	B	C	Totals
1	6	3	3	12
2	0	0	1	1
3	0	1	10	11
Totals	6	4	14	24

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (10+ plants)

[b] A = not present, B = present, but less than colony, C = colony (10+ plants)

Table 3.9 Comal River May 2000 *Vallisneria sp.* transplants – current and October 2000 status

Sept 2011	October 2000 status[b]			
status[a]	A	B	C	Totals
1	11	6	2	19
2	0	0	0	0
3	1	4	0	5
Totals	12	10	2	24

[a] 1 = not present, 2 = present, but less than colony, 3 = colony (3+ plants)

[b] A = not present, B = present, but less than colony, C = colony (3+ plants)

A chi-square analysis for *S. platyphylla* May 2000 transplants supports the alternative hypothesis that their long-term success of is not independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 11.63$, $p = 0.02$).

A chi-square analysis for *Vallisneria sp.* May 2000 transplants supports the null hypothesis that their long-term success is independent of their short-term ability to expand into larger colonies in the Comal River ($\chi^2 = 3.89$, $p = 0.14$).

Discussion

Once a macrophyte has survived the short-term bottlenecks, it must thrive in the long-term, facing additional challenges such as competing for nutrients and light and surviving suboptimal conditions such as floods, freezes, etc. (Riis 2008). This portion of my study examined whether early (6 months to 1.5 years) expansion to colony size was related to their ability to persist long-term in the San Marcos and Comal rivers. I found a significant relationship between short-term expansion to colony size and long-term survival for five out of seven plantings I examined, which included three out of four *S. platyphylla* plantings and two out of three *Vallisneria sp.* plantings. These results indicate that the current presence of these plants at these locations is not a random event and that restoration plantings have increased the probability of their long-term presence at those sites.

Future work on this topic will examine questions about what factors are related to whether or not these plantings expanded to colony size in a short-term timeframe, such as the depth, velocity, substrate, canopy cover, and neighboring plant factors I examined in Chapter 2.

CHAPTER FOUR

Transplant Methods for Zizania texana (Texas Wild-Rice)

Introduction

Zizania texana Ecology

Zizania texana is of particular interest in San Marcos River restoration plans due to its status as a federally endangered species, as listed in 1978 (U.S. Fish and Wildlife Service 1978). Originally identified by G. C. Nealley in 1892 as *Zizania aquatica*, *Z. texana* was first recognized as a species by W. A. Silveus (Silveus 1933) and named by A. S. Hitchcock (Hitchcock 1933). *Z. texana* is a perennial aquatic macrophyte in the grass family Poaceae with submersed, thin, flat, elongate blades up to four meters long and emergent wind-pollinated panicles (Figure 4.1) (Gould 1975; Oxley and others 2008; Silveus 1933; Terrell, Emery, Beaty 1978). It is endemic to the upper San Marcos River and is currently limited to the upper 5 km of the river. *Z. texana* has two distinct phenotypes: a long-lived evergreen perennial submersed form and an emergent short-lived annual form, with the emergent annual form observed in greenhouse or low-flow raceway culture conditions, but rarely found in the San Marcos River (Doyle, Power, Kennedy 2000; Power and Doyle 2004; Terrell, Emery, Beaty 1978).

At the time Silveus observed *Z. texana*, it was abundant in Spring Lake, in irrigation ditches, and in the river below the lake (Silveus 1933). Devall (1940) also noted *Z. texana* was by far the dominant macrophyte above Spring Lake Dam. By 1976, when comprehensive quantitative surveys began, Emery (1977) found the plant had an areal coverage of only 1,131 m^2 in the river, with none located in Spring Lake. A number of reasons were suggested for the population decline of *Z. texana* in the previous decades. Emery (1967) noted issues with floating debris, bottom plowing, plant collection, and pollution. At the time, Spring Lake's vegetation was regularly mowed for aesthetic reasons, sending masses of floating debris downstream, preventing *Z. texana* plants from exserting an inflorescence from the water and thus possibly preventing pollination. The river below Spring Lake Dam was plowed along the bottom to remove vegetation, preventing the plant from occurring in this area. Commercial sellers of home aquaria plants regularly pulled out native plants and replaced them with saleable species. Finally, there had been instances of raw sewage discharge into the river. All of these possibly contributed to the large population decline occurring in the 30+ years prior to Emery's observations (Emery 1967). Emery (1977) later noted all of these factors had significantly abated and the rate of decline in both amount and distribution of *Z. texana* had become less rapid.

Figure 4.1 *Zizania texana* (Texas Wild-Rice)

Current USFWS policy does not support introduction of listed species outside their designated critical habitat. *Z. texana*'s critical habitat is described as "Spring Lake and its outflow, the San Marcos River, downstream to its confluence with the Blanco River" (U.S. Fish and Wildlife Service 1996). It is not known to have ever naturally existed other than in the upper San Marcos River, however, before *Z. texana* was listed, there were some early transplant efforts outside of this zone. Beaty attempted to grow plants in Salado Creek in nearby Bell County. The plants established and produced inflorescences, but local recreational activities plus periodic removal of aquatic vegetation from the stream destroyed all plants (Beaty 1976). Emery transplanted more than 100 clones of *Z. texana* into various central Texas sites, including the Comal River in New Braunfels. However, flooding washed the plants away before they could become established (Emery 1977).

Currently, *Z. texana* mostly grows in small (<5 m^2), fragmented, widely dispersed stands throughout its limited range (Poole 2002). The Texas Parks and Wildlife Department (TPWD) began annual surveys of *Z. texana* areal coverage in 1989, when they found a total areal coverage of 1,004 m^2. When determining areal coverage, length and width is measured for the plants, and percent coverage is estimated within the resulting rectangle. Areal cover is equal to length * width * percent cover (Poole 2002; U.S. Fish and Wildlife Service 1996). Distance and azimuth from *Z. texana* land survey monuments are also recorded (Poole 2002). In their *Z. texana* surveys, TPWD divides the river into lettered segments "A" through "M", with Segment A starting at Spring Lake Dam and Segment M ending at the Blanco River confluence (Figure 4.2). I have used these lettered segment designations in this chapter.

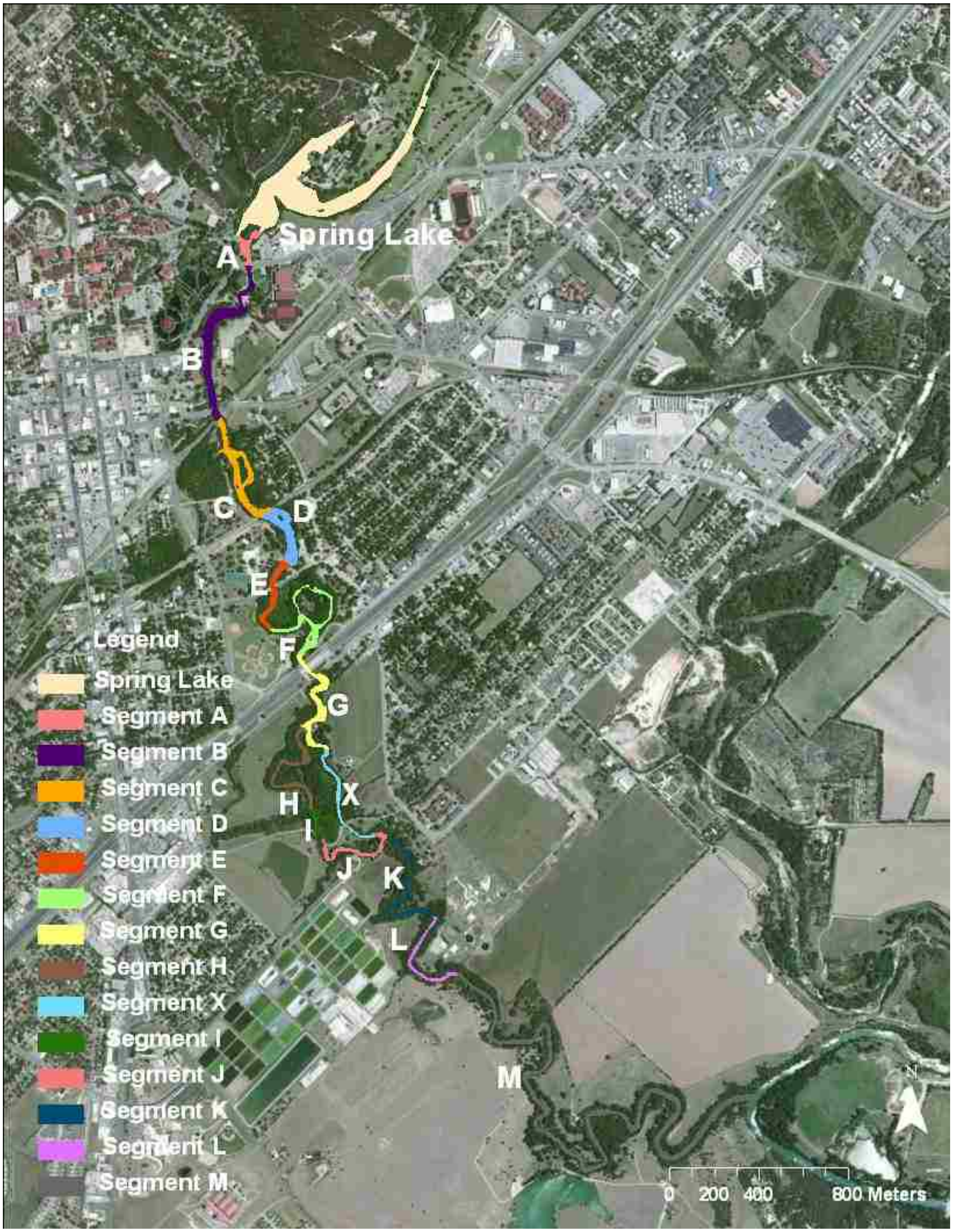

Figure 4.2 Map of the upper San Marcos River with TPWD segments

By 2010, areal coverage had increased to 4,854 m^2, an improvement, but still well short of an unofficial goal of approximately 12,000 m^2 (TPWD, Austin, TX, unpublished data). This recent increase is likely as a result of protection provided by the federal listing, which prevents dredging and cutting of the plant, and some USFWS and TPWD reintroductions (Jackie Poole 2010, TPWD, Austin, TX, personal communication). In addition to its limited population, the areal coverage of *Z. texana* is unevenly distributed throughout its critical range. As of 2010, over 61% of *Z. texana* occurs in Segment B and over 86% occurs in Segments A, B, and C, or approximately the first 1.5 km of the upper San Marcos River.

Current threats to the *Z. texana* population include possible long-term reductions in spring flows, recreational and other anthropogenic impacts (Saunders and others 2001), variable hydrology, habitat alteration, introduction of non-native species, and herbivory (Bowles and Arsuffi 1993; Poole and Bowles 1999; U.S. Fish and Wildlife Service 1996). Poole and Bowles (1999) noted that due to these factors, reintroduction may be necessary for full recovery of this species. The effects of these items are discussed separately in further detail below.

The possibility of long-term flow reductions in the San Marcos River due to water withdrawals from the Edwards Aquifer was discussed in chapter one. Reduced flows reduce potential *Z. texana* habitat by reducing river depths and river velocity, altering substrates, and possibly increasing the impacts of recreation.

Variable hydrology (droughts and floods) impacts *Z. texana* by either exposing plants to desiccation and less than optimal river depths or by scouring the plants from their base. Effects of these events are evident in the annual areal coverage survey data from TPWD. While areal coverage has generally increased year over year in the surveys (decreasing in only 5 out of 20 surveys), annual declines in this time period can often be tied to years with drought or flood events. For example, a significant drought occurred in the summer of 1996; coverage decreased by 4.11% between 1996 and 1997. Drought events (defined by extended low river flow) may become more frequent as aquifer levels are reduced. A historic flood occurred in October 1998 and widespread losses of *Z. texana* were immediately noted (Doyle, Power, Kennedy 2000). Consequently, the largest recorded year over year decrease in coverage (-15.62%) was recorded between the 1998 and 1999 surveys.

Humans have altered the San Marcos River environment in many direct and indirect ways. There are three dams present in the upper San Marcos River. Poole and Bowles (1999) noted that *Z. texana* does not grow in areas immediately behind the dams where lentic conditions are approached, thus, the dams have eliminated once available habitat. Potential indirect human impacts such as nutrient enrichment (leading to dense epiphyte coatings on submersed leaves), herbicides (resulting in direct mortality), and increased sediment runoff (reducing water clarity; altering river depth) are also likely of importance (Doyle, Power, Kennedy 2000). While the impact of these factors on *Z. texana* has not been directly evaluated in this river, there is ample evidence from other rivers that these are likely to be major concerns (Doyle, Power, Kennedy 2000).

As mentioned above, the San Marcos River is a popular recreational venue due to its urban location, relatively clear, cool water, presence of parks, and generally slow flow within city and university limits (Saunders and others 2001). Tens of thousands of people visit the river each year (Bradsby 1994; Saunders and others 2001). Human recreation has an impact on the river's macrophyte population. People may trample submersed aquatic plants. They may tear or uproot plants with their paddles, arms and feet (swimmers and tubers), or fishing lines (Bradsby 1994). Dogs have been observed damaging *Z. texana* plants (Bradsby 1994; Breslin 1997). Low flow conditions may exacerbate all of these impacts on *Z. texana* as leaves are closer to the surface and lower water levels open up more shallow areas for human activity, both of which promote more contact with the plant (Saunders and others 2001). Power (1997) also noted that recreational users may submerse emergent *Z. texana* reproductive panicles, potentially reducing the possibility of sexual reproduction.

The increase in and general negative impacts of non-native macrophyte species on native vegetation in the San Marcos River was discussed in chapters one and two. Specifically related to *Z. texana*, Poole and Bowles (1999) found that *Z. texana* appeared to be more commonly associated with other native species rather than non-native species. Doyle et al. (2000) noted observations where small *H. verticillata* populations start to grow in open spaces among *Z. texana* clumps, slowly engulfing downstream clumps of *Z. texana* and yellowing the *Z. texana* plants beneath them.

Finally, herbivory of *Z. texana* has been noted in several studies as a concern. Waterfowl and nutria have been observed feeding on emergent *Z. texana* culms, potentially reducing sexual reproduction in addition to damaging or killing the plants (Power 1995; Power 1997; Tolley-Jordan and Power 2007; U.S. Fish and Wildlife Service 1996).

Z. texana expands current stands by stoloniferous runners and produces new stands via asexual tillers, clones formed by the growth of adventitious roots at one or more nodes, which can dislodge from an existing plant and establish at a new location if it is retained and roots in the system. *Z. texana* also produces seed via sexual reproduction. However, despite a study finding sexual reproduction was possible in culture conditions (Emery and Guy 1979), Emery (1977) noted that sexual reproduction had not been observed in the wild. In fact, absence of sexual reproduction was listed as a contributing factor to listing the species as endangered (U.S. Fish and Wildlife Service 1978) and has long been a major concern, as without sexual reproduction, genetic diversity is lost and the ability to establish new populations is reduced. Some possible reasons for the repression of sexual reproduction are listed above, also, it was theorized that since the plants cannot self-pollinate, the population may have become too low and too widely dispersed to allow effective sexual reproduction (Doyle, Power, Kennedy 2000; Power 1997). However, a recent study appears to provide good news on this front. Richards et al. (2007) found high heterozygosity and few duplicate genotypes throughout the river. They concluded that the previous presumption that stands had arisen predominantly from asexual reproduction must be rejected, and that sexual reproduction occurs more often than had been assumed.

In 1999, Poole and Bowles examined habitat characterizations of *Z. texana* in the San Marcos River. This and similar studies provide useful guidelines for site selection in reintroduction efforts. They noted that most previous habitat parameter studies were done under artificial conditions outside *Z. texana* currently occupied habitat or under modified conditions. This study compared *Z. texana* occupied and non-*Z. texana* occupied sites using randomly selected transects in the natural habitat.

First, Poole and Bowles found water chemical conditions consistent among sites (temperature, dissolved oxygen, pH, specific conductance, turbidity, and chemical composition of substrate (organic matter)). This is expected in *Z. texana*'s current range due to the strong influence of spring flows on river conditions and is consistent with previous water quality studies on the river (Groeger and others 1997; Poole and Bowles 1999). Tolley-Jordan and Power (2007) found that *Z. texana* maintained a balance between vegetative and reproductive activity and its natural growth cycle at 22.5°C versus colder (15.5°C) or warmer (28.5°C) water temperatures, which may partially explain why *Z. texana*'s distribution has not expanded below the upper San Marcos River as the river does not maintain its near constant temperature as the distance increases from the springs, especially after combining with the non-spring fed Blanco River.

Second, Poole and Bowles found a significant difference in substrate particle size for *Z. texana* sites vs. non-*Z. texana* sites, with *Z. texana* found more often in moderately coarse to coarse sandy soils compared to moderately fine to fine clay soils. This seems to conflict with Power's recommendation that coarse sediments be avoided for *Z. texana* due to nutrient limitations (Power 1996b). However, it should be noted that Poole and Bowles took substrate samples adjacent to *Z. texana* sites, while Power classified her soils prior to planting *Z. texana*. Gregg and Rose (Gregg and Rose 1982) found aquatic plants modified the substrate in their area by decreasing current velocity at the plants' bases, thus increasing deposition of fine sediments and detritus deposition within the plant stands, with coarse substrate maintained surrounding the stands. The location of substrate sampling may partially explain the differences between these two studies. Saunders et al. (2001) found suitable substrates to be sand, fine gravel, and small gravel.

Like Poole and Bowles, Saunders et al. took their substrate samples outside of plant stands.

Third, Poole and Bowles found *Z. texana* primarily in sites with higher current velocity then non-*Z. texana* sites (≥ 0.46 m/s versus ≤ 0.22 m/s). Saunders et al. (2001) found a wider range of velocity preferences for *Z. texana* of 0.06 m/s to 0.61 m/s. Other studies have shown a positive relationship between higher velocity and biomass productivity in *Z. texana* (Power 1995; Power 2002; Power 1996a). As a likely obligate CO_2 plant, submersed leaves of *Z. texana* are probably carbon limited in slower moving water. In faster flowing water, the ribbon-like submersed leaves can reduce carbon limitation by exploiting the flowing water habitat where the boundary layer surrounding leaves and diffusion distances for CO_2 are reduced (Power 2002; Power and Doyle 2004). As a result, in lower water velocities, *Z. texana* has lower net productivity and allocates more biomass to reproductive organs, producing emergent culms and leaves that would not be carbon limited because these obtain CO_2 from the atmosphere where CO_2 is more readily available (Power 2002). If one wanted to preserve both growth forms (perennial, submersed and annual, emergent), reintroductions in both low and high velocity locations may be required.

Fourth, Poole and Bowles found *Z. texana* primarily in shallower areas of the river (depth of < 1 m). Saunders et al. (2001) found similar suitable depths of 0.23 m to 0.92 m.

Finally, Poole and Bowles found *Z. texana* appears to be more commonly associated with other native species rather than non-native species, with mean percentage composition of non-native species consisting of < 29% in *Z. texana* areas, versus > 47% in non-*Z. texana* areas.

Two additional studies contain important points to keep in mind for *Z. texana* restoration efforts. Power and Fonteyn (1995) found low oxygen levels positively influenced *Z. texana* seed germination and that germination was significantly higher when planted at a depth of 1.5 cm in both clay and sand substrates, where oxygen levels would be lower, versus on the surface of these substrates. Therefore, when using seeds for reintroductions, they should be planted below, rather than on, the surface of the substrate.

Alexander (2008) found *Z. texana* seed germination success was significantly greater in inundated soil than in non-inundated soil. Therefore, all areas chosen for reintroduction plantings should be believed to be covered in water during at least average spring flows (Alexander 2008).

Recent Zizania texana Reintroduction Efforts

Within the San Marcos River, transplanting and anchoring small *Z. texana* plants has had mixed success. Power had some survival success transplanting seedlings grown from seeds over one to three years in the mid-1990s at five sites in Spring Lake, although plant density and number of leaves decreased at each site (Power 1995). Doyle and Power had good survival over several years in the early 2000's transplanting seedlings in TPWD designated segment F (Doyle 2010, Baylor University, Waco, TX, personal communication).

In early 2007, tillers from plants then located in or originally grown in TPWD designated segments A, B, C, and F were potted one to three tillers per 6 inch diameter pot. Tillers were planted in stands of three in TPWD designated Segment A. The plantings had a 20% survival rate after twelve weeks (M. Alexander 2010, USFWS, San Marcos, TX, personal communication). In this study, we will transplant whole *Z. texana* plants in addition to planting tillers.

Another study used seed packs for planting *Z. texana*. Seed packs consist of a mesh material completely enclosing seeds through which seeds can germinate. A fine mesh will ensure seeds are not washed out but may inhibit germination and growth while a larger, coarser mesh will easily allow growth of the germinated seedling but may also result in loss of some seeds prior to germination. A preliminary study performed by the USFWS in 2007 in laboratory conditions found *Z. texana* seeds had a significantly higher germination rate using surgical gauze seed packs than packs using coffee filters or onion bags (M. Alexander 2010, USFWS, San Marcos, TX, personal communication). However, when placed in the river, the surgical gauze packs quickly balled up.

In this study, we used two different sizes of mesh to make seed packs, one of bridal veil material ("fine" mesh) and one of thicker burlap-like material ("coarse" mesh) (Figure 4.3). In a preliminary lab test of both materials, similar *Zizania aquatica* seeds were able to germinate through both types of seed packs (Figure 4.4).

Study Objectives and Data Analysis

The USFWS is interested in determining optimal methods of reintroducing this endangered plant, especially when using its limited seed bank, which is hand-gathered from *Z. texana* plants they have in culture. Four planting methods are compared in this study:

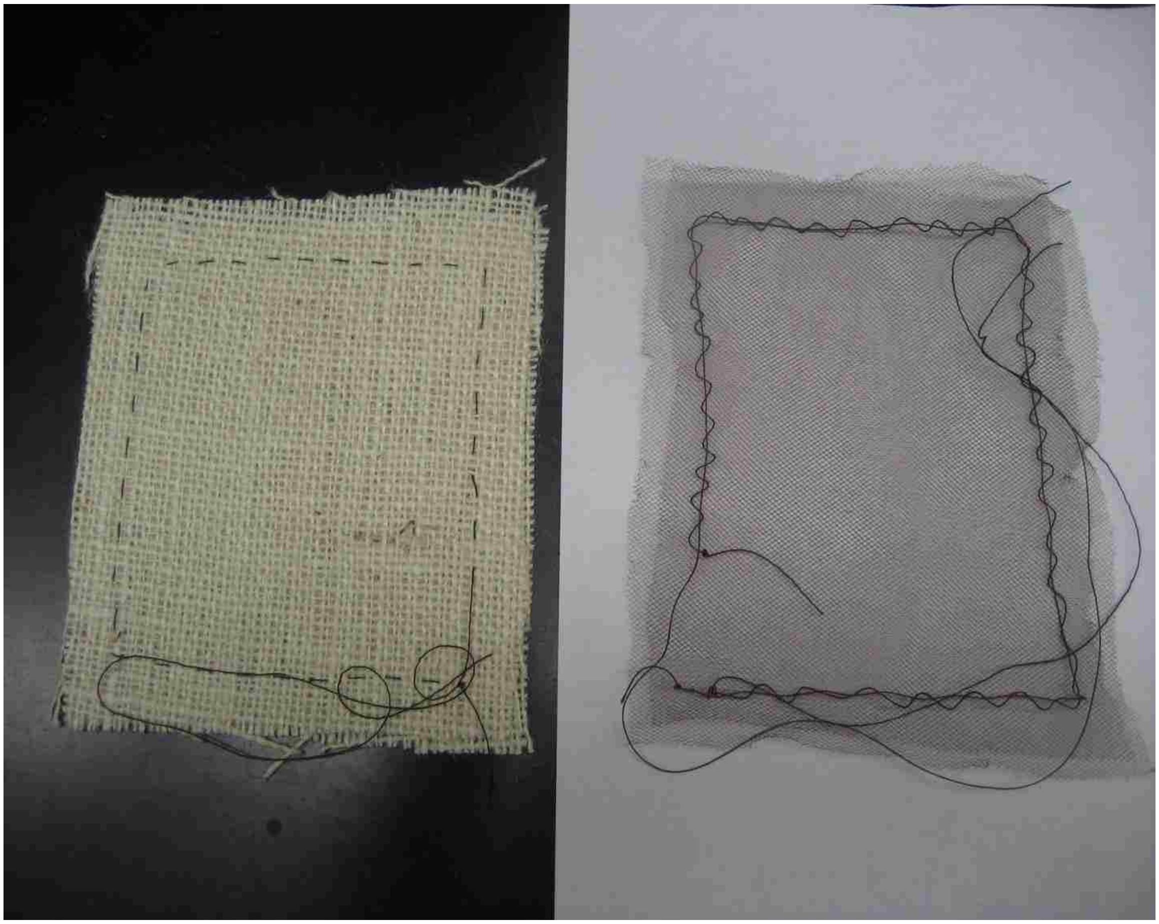

Figure 4.3 "Coarse" seed pack (left) and "fine" seed pack (right)

Figure 4.4 *Z. aquatica* seeds germinating through the "coarse" seed pack (left) and "fine" seed pack (right)

1) transplanting established whole plants grown in concrete raceways by the USFWS,
2) planting tillers in the river immediately after removing them from parent plants,
3) planting fresh seeds in coarse mesh seed packs, and
4) planting fresh seeds in fine mesh seed packs.

I examined the effectiveness of the four reintroduction methods on short-term (zero to 3 months) survival and growth of *Z. texana*. Growth was measured as the change in basal area of the plant, measured as the area of a circle formed at the base of the plant. I measured the width of the plant near its base and used one-half of this as the radius of the circle ($\pi * (\text{width at base}/2)^2$). The hypotheses regarding the effect of planting method are:

H_0: There is no difference in the survival percentage of *Z. texana* among the four methods of planting.

H_A: There is a difference in the survival percentage of *Z. texana* among the four methods of planting.

H_0: There is no difference in the growth of *Z. texana* tillers versus whole plants.

H_A: There is a difference in the growth of *Z. texana* tillers versus whole plants.

I also examined if there were any differences in survival based on location planted. I analyzed whether there was a difference between *Z. texana* planted in segments A, B, and C and those planted in segments E, F, J, K, L, and M. As discussed previously, the large majority (86%) of *Z. texana* is currently located in segments A, B, and C. The survival rate may reflect current distribution patterns.

Finally, I examined if any site characteristics (depth, current velocity, canopy cover, substrate, presence of immediate neighboring plants) or size of transplants (basal area) were related to survival.

Statistical analyses were performed using R (R Development Core Team 2011). I used a chi-square analysis of contingency tables to analyze survival and growth versus method of plantings, location of plantings, site characteristics, and size of transplants. Due to the relatively low number of sample sites, I used an alpha (α) level of 0.10 to determine significance.

Experimental Design and Methods

All research conducted with *Z. texana* was done under the direct supervision of Dr. Mara Alexander, USFWS, San Marcos, TX. Dr. Alexander is the USFWS botanist assigned to the recovery efforts for *Z. texana*, a federally listed endangered species.

I created fifty seed packets each from two types of mesh. The fine mesh is thin (similar to bridal veil material) and has openings of approximately 1 millimeter (mm) by 1 mm while the coarse mesh is thicker (similar to burlap) and has variable openings of approximately 1 mm to 2 mm by 1 mm to 2 mm wide (see Figure 4.3). The seed packets were created by sewing together two pieces of mesh approximately 13 cm by 13 cm. Ten *Z. texana* seeds were placed in each packet and the packets were sewn shut.

The seeds used in this study were collected from October to December 2010 from plants growing at the San Marcos National Fish Hatchery and Technology Center

(NFHTC) by USFWS personnel. These seeds were the most recent seeds available. As all seeds were less than five months old, they likely had very high viability (Rose and Power 2002). The most recent seeds were used first until exhausted. The plants in culture were initially collected from the San Marcos River per the recommendations of Richards et al. (2007) to best represent the genetic diversity of the wild population. Upon harvest from the plant, seeds were stored between damp brown paper towels in Ziploc bags and refrigerated at approximately 3°C until I used them in the seed packs. Packets were stored between damp brown paper towels until placed in the river, which was within one to three days after I made them. Packets were placed in the river and anchored with metal landscaping stakes (see Figure 2.3) under 2 cm of substrate if the substrate was soft enough to allow it and under about 0.5 cm if it was a harder (cobble) substrate, where it was difficult to insert the anchor in deeper. A plastic plant tag was attached to each stake with string for identification purposes.

Fifty established *Z. texana* plants under culture in the outdoor concrete raceways of the USFWS National Fish Hatchery and Technology Center in San Marcos were selected and planted in the river. Plants were growing in one to four quart pots for at least six months. Most of the plants were very large so I split them by hand at the roots into smaller plants of a reasonable size for transport to the river and planting. The basal diameter of the plants ranged from 0.01 meters to 0.30 meters and the length from 0.13 meters to 2.1 meters. They were taken from the raceways and stored inside enclosed plastic bins each morning for transport to the river until time of planting. We also collected fifty fresh tillers taken from plants in the raceways or in Segment B of the river and planted them in the river within two days. The tillers were stored in a large bucket of water until planting. Tillers from plants in the raceways were planted in Segment A and those from Segment B were planted in Segment B and downstream segments. Segment B has the highest areal coverage of *Z. texana* stands (TPWD 2010, Austin, TX, unpublished data) and has high heterozygosity within stands (Richards and others 2007), so tillers from Segment B should promote high heterozygosity when planting them in other locations downstream. For the whole plants and tillers, we used a metal planting dibble to open a hole approximately 20 cm deep in which to place the plant and packed substrate around it. I anchored the tillers with the same metal landscaping stakes used for the seed packs and the whole plants with the same 25 cm rebar stakes used in my earlier native plantings (see Figure 2.3) and a 20 to 25 cm diameter rock was placed on the root mass if we felt the anchor had a chance of coming loose due to the sediment or high current velocity. I attached a plastic identification plant tag to each stake with string.

We placed seed packets, tillers, and whole plants in the San Marcos River at fifty sites exhibiting suitable *Z. texana* habitat conditions from March 8 to 10, 2011. Specific site selection were made by Dr. Mara Alexander, USFWS, and generally followed Poole and Bowles' (1999) habitat guidelines. The distribution of sites throughout the river mixed river segments that currently have a large *Z. texana* presence and those without much current presence but with apparent potential habitat. Twenty-four sites were chosen in the upper segments of the river (segments A, B, and C) where large populations of *Z texana* currently exist. Twenty-six sites were chosen in lower segments (E to M) where populations are currently much lower. This split design will allow us to compare plantings in areas known to be suitable habitat (because of current abundance of the species) with areas believed to be favorable habitat but currently lacking extensive colonies of the species. Plantings in each section (Figure 4.5) were made as follows:

Segment A – 8 sites
Segment B – 8 sites
Segment C – 8 sites
Segment D – none (backwater from Rio Vista Dam)
Segment E – 5 sites
Segment F – 5 sites
Segment G – none (backwater from Cape's Dam)
Segment H – none
Segment I – none
Segment J – 5 sites
Segment K – 5 sites
Segment L – 4 sites
Segment M – 2 sites

We planted each of the four planting types (coarse mesh pack, fine mesh pack, whole plant, and fresh tiller) at each location on a square approximately 0.25 meters by 0.25 meters at each of fifty sites (Figure 4.6). We selected areas with minimal differences in depth and velocity within the square as possible. Each of the four planting types was placed at a random corner of a square. I generated the random order for each site with Microsoft Excel’s (2007) random number generator. Position 1 was at the upper left corner of the square (facing upstream), position 2 the upper right, position 3 the lower left, and position 4 the lower right.

At planting, we recorded the position of each site with a Trimble GeoXH handheld GPS unit (post-processing resolution of 30 cm) and the maximum leaf length and basal width of the whole plant and tiller. We also measured river depth, velocity, tree canopy cover, type of neighboring plants (within or immediately next to the planting square) and their percent cover, and substrate. We measured velocity with a Marsh-McBirney Flo-Mate, setting the top-setting-wading rod to measure flow at 60% of river

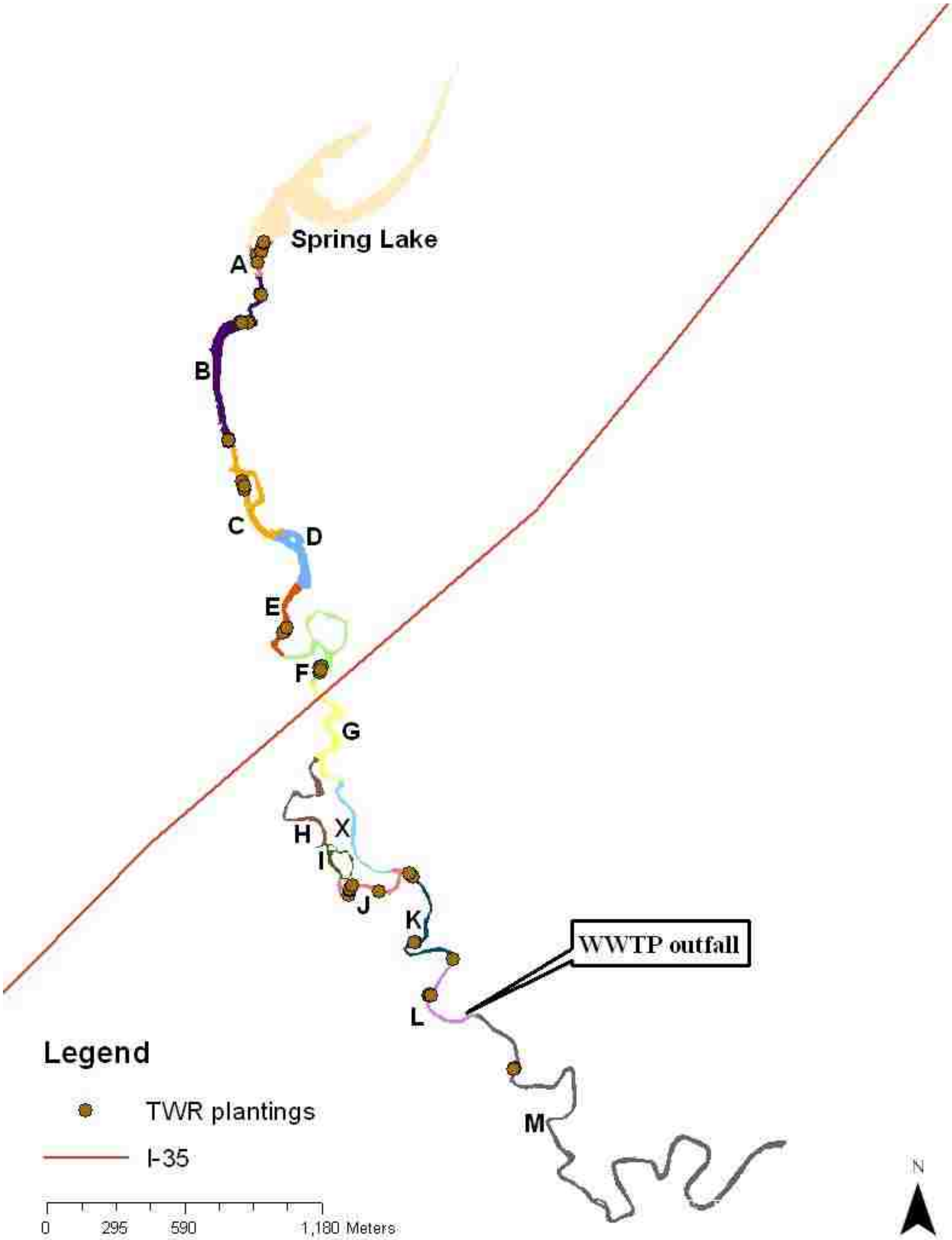

Figure 4.5 Map of *Z. texana* planting locations in this study

Figure 4.6 *Z. texana* planting site (whole plant top left, tiller bottom left, coarse seed pack bottom right, fine seed pack top right (tag not visible))

depth. We measured percent canopy cover as densiometer readings with a Forestry Suppliers, Inc. convex spherical densiometer, taking readings in the directions north, south, east, and west and averaging these readings.

Plants were surveyed for survival on April 6, 2011 and June 21, 2011, one and three months post-planting, respectively. In June we also measured the length and width of the whole plant, tiller, and any emergent seedlings.

At the April survey, we found six out of eight tillers in segment A had not survived. These tillers were the ones taken from plants grown in the raceways. We had noted at the time of planting that these tillers were smaller on average than the ones taken from plants in Segment B (2.26 cm^2 basal area for tillers from the raceway plants versus 24.74 cm^2 for tillers from Segment B). We replaced these six tillers in segment A and five others (one from segment C, two each from segments J and K) with tillers from Segment B. These tillers planted in April were anchored the same as the others but not tagged. Because they were planted later than the general plantings, these April tillers are not included in the results or in any of the statistical analyses, but will be considered separately in the discussion.

Results

The number and percentage of *Z. texana* transplants surviving or seedling emergence after one month (April 2011) and three months (June 2011) are shown in Table 4.1. Tillers obtained from Segment B (that is, all tillers except the eight planted in Segment A) are noted separately. Transplants of whole plants had the highest survival rate, and whole plants and tillers had a much higher survival rate than either of the seed packs. Plants emerging from seed packs had a steeper loss between one and three months (76% and 62%, respectively, for fine and coarse packs) than did tillers and whole plants (46% and 24%, respectively).

Survival for the planting methods was significantly different at both survey periods (April, $\chi^2 = 64.55$, $p < 0.01$; June, $\chi^2 = 59.33$, $p < 0.01$). We can reject the null hypothesis that the survival percentage is equal among methods of plantings for both time periods.

Table 4.2 shows how survival differed in river segments A, B, and C versus segments E, F, J, K, L, and M.

Survival was independent of segment grouping for both the April 2011 ($\chi^2 = 0.05$, $p = 0.82$) and the June 2011 ($\chi^2 = 0.94$, $p = 0.33$) surveys. The same held true if seeds were excluded from the test (that is, tillers and whole plants only) for the April 2011 ($\chi^2 = 2.46$, $p = 0.12$) and the June 2011 ($\chi^2 = 2.45$, $p = 0.12$) surveys.

Table 4.1 *Z. texana* planted and survival at subsequent surveys

	March 2011	April 2011		June 2011	
Planting method	Planted	# Alive	Percent	# Alive	Percent
Fine seed pack	50	13	26.00%	5	10.00%
Coarse seed pack	50	17	34.00%	4	8.00%
All tillers	50	39	78.00%	21	42.00%
Seg. B tillers only	42	37	88.10%	20	47.62%
Whole plant	50	46	92.00%	35	70.00%
Totals (not including replaced tillers)	200	115	57.50%	65	32.50%

Table 4.2 Survival of *Z. texana* in segments A, B, C vs. segments E, F, J, K, L, M

	March 2011	April 2011		June 2011	
Segments	Planted	# Alive	Percent (%)	# Alive	Percent (%)
A, B, C	96	56	58.33%	28	29.17%
E, F, J, K, L, M	104	59	56.73%	37	35.58%
Totals	200	115	57.50%	65	32.50%

Table 4.3 shows the physical characteristics of each of the fifty sites and the size (basal area) of plants, all measured at time of planting. For the purpose of performing chi-square analyses, I divided the characteristics into two or three categories, using natural breaks and distribution of the data for continuous data. These categories are also shown in Table 4.3.

Table 4.3 Summary of data collected at each of 50 planting sites at time of planting. Categories are divisions used for chi-square analyses.

Factor	Mean ± standard deviation (range)	Category 1 (*n*)	Category 2 (*n*)
Depth (m)	0.75 ± 0.22 (0.31 to 1.22)	0.31 to 0.74 (104)	0.74 to 1.22 (96)
Velocity (m/s)	0.44 ± 0.28 (0.04 to 1.02)	0.04 to 0.42 (104)	0.42 to 1.02 (96)
Canopy cover (%)	47 ± 31 (0 to 99)	0 to 50 (108)	51 to 99 (92)
Presence of neighbor	— —	Yes (44)	No (156)
Substrate[a]	— —	Fine (72)	Coarse (128)
Basal area when planted (cm^2)			
Tiller	21.14 ± 23.92 (0.79 to 78.54)	0.79 to 19.99 (35)	20.00 to 78.54 (15)
Whole plant	121.03 ± 133.59 (0.79 to 706.86)	0.79 to 79.99 (28)	80.00 to 706.86 (22)

[a] Fine = silt or sand, Coarse = gravel or cobble

As mentioned previously, planting sites were chosen generally following Poole and Bowles' (1999) *Z. texana* habitat guidelines. However, some planting sites were outside this zone for one or more parameters. Survival percentage of each planting type did not differ notably from plantings within Poole and Bowles' guidelines. Of the fifty sites, six had depths greater than the recommended one meter. Of these twenty-four plantings, seven survived as of the June survey (zero of twelve seed packs, two of six tillers (33.33%), and five of six whole plants (83.33%)). Eight sites had velocities less than the recommended 0.46 m/s or greater. Of these thirty-two plantings, ten survived as of the June survey (one of eight fine seed packs (12.5%), one of eight coarse seed packs (12.5%), three of eight tillers (37.5%), and five of eight whole plants (62.5%)).

Using the categories in Table 4.3, I performed chi-square analyses to test if survival was independent of those factors (Table 4.4). The only significant factors for the April 2011 survey were depth (negative impact on whole plants of deeper depth) and the basal area of the plant at time of planting (initial size) for tillers alone and tillers and whole plants combined (higher than randomly expected survival for larger plants). Significant factors for the June 2011 survey included depth (negative impact on whole plants of deeper depth), the presence of neighboring plants (negative impact on whole plants) and the basal area of the plant at time of planting (initial size) for tillers alone, whole plants alone, and tillers and whole plants combined (all with higher than randomly expected survival for larger plants).

We measured the basal area of surviving plants at the June 2011 survey and calculated the growth since time of planting for tillers and whole plants (Table 4.5). I divided the surviving plants based on growth data into three categories based on natural breaks and distribution of the data for the purpose of a chi-square analysis. Dead plants were excluded from this analysis.

The relative rate of growth was independent of planting methods as of the June survey ($\chi^2 = 0.20$, $p = 0.90$). We can accept the null hypothesis that there is no difference in growth between tillers or whole plants.

Table 4.4 Chi-square (χ^2) tests for independence of survival vs. other factors for each survey date

Factor	χ^2	
(degrees of freedom)	Apr 2011	Jun 2011
Depth (1)		
All plantings	1.18	0.30
Seed packs	1.29	0.23 [b]
Tillers	0.24	1.42
Whole plants	4.01 [a,b]	3.91 [a]
Tillers & whole plants	0.45	0.20
Velocity (1)		
All plantings	1.18	0.30
Seed packs	1.29	0.23 [b]
Tillers	0.04	0.00
Whole plants	0.92 [b]	0.55
Tillers & whole plants	0.45	0.20
Presence of neighbor (1)		
All plantings	0.06	0.70
Seed packs	1.60	0.00 [b]
Tillers	0.29 [b]	0.07 [b]
Whole plants	1.99 [b]	4.05 [a,b]
Tillers & whole plants	1.32 [b]	1.27
Substrate (1)		
All plantings	1.03	0.25
Seed packs	1.62	0.03 [b]
Tillers	0.00 [b]	0.74
Whole plants	0.37 [b]	0.07
Tillers & whole plants	0.12	0.60
Canopy cover (1)		
All plantings	0.79	0.11
Seed packs	0.93	0.01 [b]
Tillers	0.00	0.14
Whole plants	0.77 [b]	1.38
Tillers & whole plants	0.26	0.25
Initial size (basal area) (1)		
Tillers	5.02 [a]	2.80 [a]
Whole plants	1.94 [b]	2.85 [a]
Tillers & whole plants	7.71 [a]	6.51 [a]

[a] $p < 0.10$

[b] Contains some expected contingency cell values of < 5

Table 4.5 Size (basal area) and percentage growth of *Z. texana*. Categories are divisions used for chi-square analyses. Dead plants not included in analysis (29 tillers, 15 whole plants).

Planting method	Plants surviving as of Jun 2011 mean initial area (cm^2)	Plants surviving as of Jun 2011 mean initial area (cm^2)	Average area growth (%)	Category 1 (*n*)	Category 2 (*n*)	Category 3 (*n*)
Tiller	24.46	241.06	886%	Smaller or same size (7)	< 10x growth (6)	>10x growth (8)
Whole plant	146.02	1146.25	685%	Smaller or same size (11)	< 10x growth (12)	>10x growth (12)

Discussion

My *Z. texana* plantings had three month survival rates of 9% for seed packs, 42% for tillers (48% if only Segment B tillers are considered), and 70% for whole plants. A higher survival rate for transplants of whole plants rather than germinated seeds in the wild is not unexpected. For example, in terrestrial environments, Maschinski and Wright (2006) had 2% of seeds survive versus 59-97% of whole plants for time periods of less than one year to four years; Jusaitis and others (2004) had 15% of seeds survive versus 93% of whole plants after four to nine weeks; Guerrant and Kaye (2007) found seed survival rates of 0-48% and whole plant survival rates of 0-90% after 1-9 years, with the whole plant survival rate being higher in all cases except one. My seeds' survival rate at one month of 30% was in line with these studies, although lower than the approximately 75% to near 100% Rose and Power (2002) reported for germination rates of *Z. texana* seeds aged 3-5 months. However, their study was done in static water in laboratory conditions and measured weekly; it is certainly possible that in my study more seeds germinated but were lost due to flow conditions, human disturbance, or herbivory before the one month survey. This possibility is supported by the high germinated seedling loss rate (70%) between one month and three months. We had hoped the seed packs would improve germinated seedling survival rate by providing an anchor for the new seedlings, but the anchored packs did not appear to improve seedling retention rate post germination.

Given the higher survival rate, one might think transplanting whole plants should be the recommended method for future *Z. texana* restoration plantings. However, one should take into account the cost-benefit balance for each method. Seeds provide genetic variability but require extensive labor to collect and store, especially since *Z. texana* does not produce abundant viable seed. Assembling seed packs adds about an extra thirty minutes per pack to the time for collection and storage. Whole plants also require considerable space and time, including propagation and maintaining of plants over extended periods, maintenance of running-water raceways, and extra labor to transport the plants along the river (multiple trips needed to transport large plants versus easy to carry seeds or tillers). Conversely, tillers generally require little time to gather and can be transplanted immediately or within a couple of days if stored in water. In my study, one person gathered the great majority of our tillers in Segment B (Sewell Park) within about 20-30 minutes. A study using similar asexual propagule plantings (Orth, Harwell, Fishman 1999) found similar results – a 73% one month survival rate and time for collection, sorting, and planting of about 21 seconds each, lending support to the idea that using would be fast, have a reasonable survival rate, and have the lowest cost/benefit ratio of the various transplant methods. As further support, my results indicate that tillers were growing during my study proportionately to whole plants, with no significant difference between the two.

Using asexual propagules does have the disadvantage of potentially not providing genetic diversity. There are two possible solutions to this issue in the case of *Z. texana*. Tillers could be gathered randomly from large stands where Richards and others (2007) found high genetic diversity or tillers could be gathered from one or more segments to be planted in different segments. This should provide a reasonable possibility of maintaining genetic diversity at a low cost. Seeds could also be used to breed plants offsite to guarantee sexual reproductive diversity as long as an offsite population is necessary. Germinated seedlings could be more closely monitored and protected in this situation.

As we attempted to select planting sites that roughly fit within the previously defined habitat guidelines, it is not surprising that most environmental factors did not significantly affect short-term survival. Deeper depths (defined as >0.75 m in this study) and presence of neighboring plants (of which 82% were the invasives *H. verticillata* and *H. polysperma* in my study) negatively affected the whole plants after three months. These findings are consistent with Poole and Bowles (1999), who found *Z. texana* was found primarily in shallower depths and in the presence of other native rather than invasive macrophytes. These two items should be monitored when transplanting whole plants. The only other factor significantly affecting short-term survival was tiller and whole plant size at time of planting, in which case bigger was better. This is likely because larger plants have a larger base to deflect the initial stress from water flow upon transplanting. Other factors that might affect short-term survival, such as herbivory or human disturbance, may have a higher effect on larger plants due to increased chance of contact. Since larger plants survived at a higher rate, herbivory or human disturbance factors were likely not considerable.

As mentioned previously, all tiller transplants other than those done in Segment A were collected from large colonies in Segment B. These Segment B tillers were larger and appeared healthier than the ones we gathered from whole plants raised in raceways offsite and transplanted in Segment A. The survival rate for the Segment B tillers was 88% and 48% after one and three months, respectively, compared to 25% and 12.5% for the smaller raceway tillers. In addition, we planted new Segment B tillers at 11 sites where tillers were gone after one month (6 in Segment A, 1 in Segment C, 2 in Segment J, and 2 in Segment K). Of these 11 tillers, 6 (55%) survived as of the June survey two months later. These results further support that large tillers should provide a reasonable survival rate compared to higher cost whole plants.

Despite the obviously higher density of *Z. texana* in the upper portion of the river, I found no significant difference in either the one month or three month survival rate among all plantings between the uppermost segments (A, B, and C) and the downstream segments. This indicates that although *Z. texana* populations are currently much lower in the downstream segments, I have shown that appropriately chosen planting sites can support at least the short-term survival of seedlings or transplants throughout the river. In addition, in 2004 Paula Powers of the USFWS planted several *Z. texana* plants in Segments K and L just above and below the power lines and many of those plants have were still present in 2011 (Doyle 2012, Baylor University, Waco, TX, personal communication). My results and the previous successes should encourage additional plantings or seedings in downstream segments to further spread the population throughout its critical habitat.

BIBLIOGRAPHY

[SRCC] Southern Regional Climate Center. 1997. Rainfall frequency/magnitude atlas for the south-central United States. Baton Rouge (LA): Louisiana State University. SRCC Technical Report nr 97-1. 39 p.

[USDA] United States Department of Agriculture Aerial Photography Field Office. National Agriculture Imagery Program (NAIP) Digital Ortho Photo Image (dated 5/3/2010) [Internet]. Salt Lake City, UT: USDA; c2010 [cited 2011 August 28]. Available from: http:// data.tnris.org/datadownload.

[USGS] United States Geological Survey. 1/3-Arc Second National Elevation Dataset [Internet]. Sioux Falls, SD: U.S. Geological Survey; c2009 [cited 2011 September 18]. Available from: http://www.tnris.org/.

Alexander ML, Doyle RD, Power P. 2008. Suction dredge removal of an invasive macrophyte from a spring-fed river in central Texas, USA. Journal of Aquatic Plant Management 46:184-5.

Alexander ML. 2008. Seed germination response of *Zizania texana* (Poaceae: Oryzae) to soil inundation. Journal of the Botanical Research Institute of Texas 2(1):673-676.

Angerstein MB and Lemke DE. 1994. First records of the aquatic weed *Hygrophila polysperma* (Acanthaceae) from Texas. Sida Contrib Bot 16(2):365-71.

Baattrup-Pedersen A and Riis T. 1999. Macrophyte diversity and composition in relation to substratum characteristics in regulated and unregulated Danish streams. Freshwater Biology 42(2):375-385.

Beaty HE. 1976. Transplanting *Zizania texana* (Texas Wildrice) in Bell County, Texas. Temple, Texas: Self-published. 13 p. Available from: Smithsonian Institution Libraries.

Bernhardt ES, Palmer MA, Allan JD, Alexander G, Barnas K, Brooks S, Carr J, Clayton S, Dahm C, Follstad-Shah J, and others. 2005. Ecology: Synthesizing U.S. river restoration efforts. Science 308(5722):636-7.

Bowles DE and Arsuffi TL. 1993. Karst aquatic ecosystems of the Edwards Plateau region of central Texas, USA: A consideration of their importance, threats to their existence, and efforts for their conservation. Aquatic Conservation: Marine Freshwater Ecosystems 3(4):317-29.

Bradsby DD. 1994. A recreational use survey of the San Marcos River [MS thesis]. San Marcos (TX): Southwest Texas State University. 82 p. Available from: Texas State University Albert B. Alkek Library.

Breslin SL. 1997. The impact of recreation on Texas Wild-rice [MS thesis]. San Marcos (TX): Southwest Texas State University. 69 p. Available from: Texas State University Albert B. Alkek Library.

Callaway RM. 1995. Positive interactions among plants. Botanical Review 61(4):306-349.

Campbell GS. 1977. New York, New York: Springer-Verlag. 159 p.

Devall LL. 1940. A comparative study of plant dominance in a spring-fed lake [MA thesis]. San Marcos (TX): Southwest Texas State College. 49 p. Available from: Texas State University Albert B. Alkek Library.

Doyle RD. 2002. Development of techniques for establishment of native aquatic vegetation in the Comal and San Marcos Rivers, Texas. Unpublished report: U.S. Fish and Wildlife Service. Final Report nr IAG No. 1448-20181-97-N795. 109 p.

Doyle RD. 2001. Expansion of the exotic aquatic plant *Cryptocoryne beckettii* (Araceae) in the San Marcos River, Texas. Sida Contrib Bot 19(4):1027-38.

Doyle RD, Power P, Kennedy K. 2000. Reintroduction potential of Texas wild rice (*Zizania texana*) into the San Marcos River, Texas. Proceedings, Wild Rice Research and Management Conference; 2000; Carlton, Minnesota. p. 179-84.

Doyle RD, Mullins M, Bormann RL. 2011. Final summary of research activities. Unpublished report: U.S. Fish and Wildlife Service, Baylor University. Cooperative Agreement No. 201815J828. 25 p.

Doyle RD, Francis MD, Smart RM. 2003. Interference competition between *Ludwigia repens* and *Hygrophila polysperma*: Two morphologically similar aquatic plant species. Aquat Bot 77(3):223-34.

Emery HP. 1977. Current status of Texas wild rice (*Zizania texana* Hitchc.). The Southwestern Naturalist 22(3):393-4.

Emery WHP and Guy MN. 1979. Reproduction and embryo development in Texas Wildrice (*Zizania texana* Hitchc.). Bulletin of the Torrey Botanical Club 106(1):29-31.

Emery WHP. 1967. The decline and threatened extinction of Texas Wild Rice (*Zizania texana* Hitchc.). The Southwestern Naturalist 12(2):203-4.

ESRI Inc. 2010. ArcMap [computer program]. Version 10.0. Redlands, CA: ESRI Inc.

Flores J and Jurado E. 2003. Are nurse-protégé interactions more common among plants from arid environments? Journal of Vegetation Science 14(6):911-916.

Giller PS. 2005. River restoration: Seeking ecological standards. Editor's introduction. J Appl Ecol 42(2):201-7.

Gómez-Aparicio L. 2009. The role of plant interactions in the restoration of degraded ecosystems: A meta-analysis across life-forms and ecosystems. J Ecol 97(6):1202-14.

Gould FW. 1975. The grasses of Texas. College Station, Texas: Texas A&M University Press. 653 p.

Gregg WW and Rose FL. 1982. The effects of aquatic macrophytes on the stream microenvironment. Aquat Bot 14:309-24.

Greipsson S. 2011. Restoration ecology. Sudbury, MA: Jones & Bartlett Learning. 408 p.

Groeger AW, Brown PF, Tietjen TE, Kelsey TC. 1997. Water quality of the San Marcos River. The Texas Journal of Science 49(4):279-94.

Guerrant Jr EO and Kaye TN. 2007. Reintroduction of rare and endangered plants: Common factors, questions and approaches. Aust J Bot 55(3):362-70.

Guyton, W. F and Associates. 1979. Geohydrology of Comal, San Marcos, and Hueco Springs. Report 234. Austin, Texas: Texas Department of Water Resources. 85 p.

Hardy TB. 2009. Technical assessments in support of the Edwards Aquifer science committee "J charge" flow regime evaluation for the Comal and San Marcos River systems. Edwards Aquifer Recovery Implementation Program Available from: http://www.earip.org/Science/12-29-09%20Final_Hardy_Report.pdf

Hitchcock AS. 1933. New species and new names of grasses from Texas. Journal of the Washington Academy of Sciences. 23:449-56.

Hungerford RD, Nemani RR, Running SW, Coughlan JC. 1989. MT-CLIM: A mountain microclimate simulation model. Ogden, UT: U.S. Agricultural Department, U.S. Forest Service (US). Research Paper INT-414. 54 p. Available from: http://digitalcommons.usu.edu/govdocs_forest/54.

Jusaitis M, Polomka L, Sorensen B. 2004. Habitat specificity, seed germination and experimental translocation of the endangered herb Brachycome muelleri (Asteraceae). Biol Conserv 116(2):251-66.

Kolar CS and Lodge DM. 2001. Progress in invasion biology: predicting invaders. Trends in Ecology and Evolution 16(4):199-204.

Langeland KA. 1996. *Hydrilla verticillata* (L.f.) Royle (Hydrocharitaceae), "The perfect aquatic weed". Castanea 61(3):pp. 293-304.

Larned ST, Suren AM, Flanagan M, Biggs BJF, Riis T. 2006. Macrophytes in urban stream rehabilitation: establishment, ecological effects, and public perception. Restor Ecol 14(3):429-40.

Lemke DE. 1989. Aquatic macrophytes of the Upper San Marcos River, Hays Co., Texas. The Southwestern Naturalist 34(2):289-91.

Lind OT. 1985. Handbook of common methods in limnology. 2d ed. Dubuque, IA: Kendall/Hunt Publishing Company.

Maass J, Vose JM, Swank WT, Martínez-Yrízar A. 1995. Seasonal changes of leaf area index (LAI) in a tropical deciduous forest in west Mexico. For Ecol Manage 74(1–3):171-80.

Malmqvist B and Rundle S. 2002. Threats to the running water ecosystems of the world. Environ Conserv 29(2):134-53.

Maschinski J and Wright SJ. 2006. Using ecological theory to plan restorations of the endangered Beach jacquemontia (Convolvulaceae) in fragmented habitats. Journal for Nature Conservation 14(3–4):180-9.

NCDC Climate-Radar Data Inventories [Internet]. Asheville, North Carolina: National Climatic Data Center; c2012 [cited 2011 September 17]. Available from: http://www4.ncdc.noaa.gov/cgiwin/wwcgi.dll?wwDI~StnSrch~StnID~20025996#DAF.

Ogden AE, Spinelli AJ, Horton J. 1985. Hydrologic and hydrochemical data for the Edwards Aquifer in Hays and Comal Counties. San Marcos, Texas: Edwards Aquifer Research and Data Center, Southwest Texas State University. Report nr EARDC R2-85.

Orth RJ, Harwell MC, Fishman JR. 1999. A rapid and simple method for transplanting eelgrass using single, unanchored shoots. Aquat Bot 64(1):77-85.

Owens CS, Madsen JD, Smart RM, Stewart RM. 2001. Dispersal of native and nonnative aquatic plant species in the San Marcos River, Texas. Journal of Aquatic Plant Management 39:75-9.

Owens CS, Shad A, Tripe J, Dodd L, Glomski L. 2010. San Marcos River survey - 2009 feasibility. Lewisville, Texas: US Army Corps of Engineers (US) [USACE] - Lewisville Aquatic Ecosystem Research Facility. 41 p.

Oxley FM, Echlin A, Power P, Tolley-Jordan L, Alexander ML. 2008. Travel of pollen in experimental raceways in the endangered Texas Wild rice (*Zizania texana*). The Southwestern Naturalist 53(2):169-74.

Poole JM. 2002. Historical distribution of Texas wild-rice (*Zizania texana*) from 1989 to 2001. Section 6 final report. Albuquerque, New Mexico: United States Fish and Wildlife Service. 22 p.

Poole J and Bowles DE. 1999. Habitat characterization of Texas wild-rice (*Zizania texana* Hitchcock), an endangered aquatic macrophyte from the San Marcos River, TX, USA. Aquatic Conservation: Marine and Freshwater Ecosystems 9(3):291-302.

Power P. J. 1995. Reintroduction of Texas Wildrice (*Zizania texana*) in Spring Lake: some important environmental and biotic considerations. Southwestern rare and endangered plants: Proceedings of the second conference; September 11-14, 1995; Flagstaff, AZ. 179 p.

Power P. 2002. Resource allocation patterns and phenotypic variation in the endangered Texas Wildrice (*Zizania texana*, Poaceae). Sida Contrib Bot 20(2):571-82.

Power P. 1997. Moisture, seeds, and reproductive failure in Texas Wildrice (Z*izania texana*). The Southwestern Naturalist 42(4):435-40.

Power P. 1996a. Effects of current velocity and substrate composition on growth of Texas Wildrice (*Zizania texana*). Aquat Bot 55(3):199-204.

Power P. 1996b. Growth of Texas Wildrice (*Zizania texana*) in three sediments from the San Marcos River. Journal of Aquatic Plant Management 34:21-23.

Power P and Doyle RD. 2004. Carbon use by the endangered Texas Wild rice (*Zizania texana*, Poaceae). Sida Contrib Bot 21(1):389-98.

Power P and Fonteyn PJ. 1995. Effects of oxygen concentration and substrate on seed germination and seedling growth of Texas Wildrice (*Zizania texana*). The Southwestern Naturalist 40(1):1-4.

Puente C. 1976. Statistical analysis of water-level, springflow, and streamflow for the Edwards Aquifer in south-central Texas. U.S. Geological Survey Open-File Report nr 76-393. 59 p.

R Development Core Team. 2011. R: A Language and Environment for Statistical Computing [computer program]. Version 2.13.1. Vienna, Austria: R Foundation for Statistical Computing. Available from: http://www.R-project.org. System requirements: PC with Unix-like, Windows, and Mac families of operating systems.

RECON Environmental I, Hicks & Company, Zara Environmental LLC, BIO-WEST. 2011. Edwards Aquifer recovery implementation program habitat conservation plan. 425 p. Available from: http://earip.org/. Accessed February 18, 2012.

Richards CM, Antolin MF, Reilley A, Poole J, Walters C. 2007. Capturing genetic diversity of wild populations for *ex situ* conservation: Texas wild rice (Z*izania texana*) as a model. Genetic Resources and Crop Evolution 54(4):837-48.

Riis T. 2008. Dispersal and colonisation of plants in lowland streams: success rates and bottlenecks. Hydrobiologia 596(1):341-51.

Riis T and Sand-Jensen K. 2006. Dispersal of plant fragments in small streams. Freshwat Biol 51(2):274-86.

Riis T, Schultz R, Olsen H, Katborg C. 2009. Transplanting macrophytes to rehabilitate streams: experience and recommendations. Aquat Ecol 43(4):935-42.

Rose F and Power P. 2002. Maintenance of conservation population of Texas Wildrice (*Zizania texana*). Austin, TX: U.S. Fish and Wildlife Department. Federal Agreement No. 1448-00002-96-0856. 21 p.

Rosen DJ. 2000. *Cryptocoryne beckettii* (Araceae), a new aquatic plant in Texas. Sida Contrib Bot 19:399-401.

Running SW, Nemani RR, Hungerford RD. 2003. MT-CLIM for Excel [computer program]. Version 4.3. Missoula, Montana: University of Montana. Available from: http://www.mmnt.net/db/0/0/firecenter.umt.edu/pub/models/mtclim-xl. Accompanied by: 1 manual. System requirements: PC with Unix-like or Windows families of operating systems. Microsoft Excel spreadsheet software.

Running SW, Nemani RR, Hungerford RD. 1987. Extrapolation of synoptic meteorological data in mountainous terrain and its use for simulating forest evapotranspiration and photosynthesis. Can J for Res 17(6):472-83.

Saunders KS, Mayes KB, Jurgensen TA, Trungale JF, Kleinsasser LJ, Aziz K, Fields JR, Moss RE. 2001. An evaluation of spring flows to support the upper San Marcos river spring ecosystem, Hays County, Texas. Austin (TX): Texas Parks and Wildlife Department. Rivers Studies Report No 16. 33 p.

Silveus WA. 1933. Texas grasses. San Antonio, Texas: The Clegg Co. 782 p.

Slattery RN and Fahlquist L. 1997. Water-quality of the San Marcos Springs riverine system, San Marcos, Texas, July-August 1994. Austin, Texas: U.S. Geological Survey. 4 p.

Smart RM, Dick GO, Doyle RD. 1998. Techniques for establishing native aquatic plants. Journal of Aquatic Plant Management 36:44-9.

Stachowicz JJ. 2001. Mutualism, facilitation, and the structure of ecological communities. Bioscience 51(3):pp. 235-246.

Staton LL. 1992. Assessment of changes in the aquatic macrophyte community in the upper San Marcos River [MS thesis]. San Marcos (TX): Southwest Texas State University. 50 p. Available from: Texas State University Albert B. Alkek Library.

Sutton DL. 1995. Hygrophila is replacing Hydrilla in south Florida. Aquatics 17:4-10.

Terrell EE, Emery WHP, Beaty HE. 1978. Observations on *Zizania texana* (Texas Wildrice), an endangered species. Bulletin of the Torrey Botanical Club 105(1):50-7.

Thornton PE and Running SW. 1999. An improved algorithm for estimating incident daily solar radiation from measurements of temperature, humidity, and precipitation. Agric for Meteorol 93(4):211-28.

Thornton PE, Hasenauer H, White MA. 2000. Simultaneous estimation of daily solar radiation and humidity from observed temperature and precipitation: an application over complex terrain in Austria. Agric for Meteorol 104(4):255-71.

Tolley-Jordan LR and Power P. 2007. Effects of water temperature on growth of the federally endangered Texas Wild rice (*Zizania texana*). The Southwestern Naturalist 52(2):201-8.

U.S. Fish and Wildlife Service. 1996. San Marcos/Comal (revised) recovery plan. Albuquerque, New Mexico: U.S. Fish and Wildlife Service. 121 p.

U.S. Fish and Wildlife Service. 1978. Endangered and threatened wildlife and plants. Federal Register 43(81):17910-6.

Zar JH. 2010. 5th ed. Upper Saddle River, New Jersey: Prentice Hall, Inc. 944 p.