AN ANALYSIS OF THE EFFECTS OF PHOTOSYNTHETICALLY ACTIVE

RADIATION AND RECREATION INDUCED TURBIDITY

IN THE SAN MARCOS RIVER ON THE VEGETATIVE

GROWTH OF TEXAS WILD RICE

(*ZIZANIA TEXANA* HITCHC.)

by

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A dissertation submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
with a Major in Aquatic Resources
and Integrative Biology
December 2018

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ACKNOWLEDGEMENTS

I wish to thank the following committee members for their guidance and expertise in the completion of this work: Dr. Tina M. Cade for your assistance and insight in gathering together data and putting it to paper, Dr. David E. Lemke and Dr. Weston H. Nowlin for the shared passion of plant taxonomy and early direction for my study. Dr. Robert D. Doyle for your long-time dedication to studying plants and the world they live in. A special debt of gratitude is reserved for Dr. Paula S. Williamson. Thank you for your time, brilliance and patience in helping me to complete this document. Throughout the countless hours I spent with you, I gained such an immense respect and admiration for you. I will be forever grateful for your commitment to see me through this. You and Dr. Tina M. Cade are an inspiration to all women in science.

I would like to thank Kristy Kollaus, Tom Heard, and the staff at the Meadows Center for Water and the Environment for their knowledge and professionalism in assisting me in all aspects of my field studies. A special gratitude is extended to Jacob Bilbo for his ingenuity and insight into mechanical aspects needed throughout the study periods.

I would like to thank my husband Lance and children, Tennessen and Tate, for their unconditional support as we all weathered changes in our lives together. Your love and encouragement are what carried me through.

I would also like to reserve my most gracious thank you to my mentor and friend Dr. Thom B. Hardy. For your willingness to take me onboard and see me through the challenges. For your wisdom and kindness throughout this entire journey. You are the sole reason this was all possible. I am forever indebted to you.

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ABSTRACT

Texas wild rice (*Zizania texana*; Poaceae) is an endangered perennial macrophyte known to occur only in the spring-fed San Marcos River, Hays County, Texas. Historically, Texas wild rice (TWR) was reported to reside in the upper 3 kilometers of the river, into the irrigation ditches, and approximately 300 meters behind Spring Lake dam. The current distribution of TWR is within the upper 5 kilometers with 97% of the population occurring within the upper 2.2 kilometers. Studies on various aquatic macrophytes have demonstrated that the availability of photosynthetically active radiation (PAR) is an important abiotic factor affecting a plant's biomass. The San Marcos River is impacted on a seasonal, weekly and diel basis by contact water recreation. Recreational activities can cause increases in suspended sediment induced turbidity resulting in a decrease in water clarity and reduction in ambient PAR. Two different studies were conducted to test the effect of a reduction in PAR on the vegetative growth of TWR. In the first study, the results of three *ex situ* experiments involving a reduction in PAR through shade frames (0%, 10%, 20%, 40%, and 80% PAR reductions) found that with only 20% ambient PAR (80% PAR reduction), above ground biomass, below ground biomass, above/below ground biomass, total biomass, shoot number, root number, and total leaf surface area of TWR plants were significantly reduced in two of the three experimental periods. The second study focused on periods of low and high contact recreation use, the suspended sediment induced turbidity response, and the impact on TWR biomass production. Results showed that differences in TWR biomass production existed along a longitudinal gradient in the river, when the Eastern Spillway (ES), which had very limited upstream recreational activity, was compared to the

downstream treatment sites located at Sewell Park (SP), Bicentennial Park (BP), and Ramon Lucio (RL), which all had substantial upstream recreational activity. Greater growth was found in TWR plants at the upstream ES site. Differences in biomass production are likely the result of lower levels of suspended sediment in the water column at the ES site, allowing for higher levels of photosynthetically active radiation (PAR). No difference in the amount of periphyton on the leaf surface of plants at the different study sites under either low or high contact recreation use was found. Therefore, periphyton does not account for observed differences in TWR growth. Additional factors related to habitat suitability requirements for TWR may also have contributed to differences in biomass production found along the longitudinal gradient in the river. Findings from this study suggest that locations in the river receiving more than 20% ambient PAR provide optimum habitat for the reintroduction of TWR. The results of these studies provide useful information for future conservation and management measures in the effort to restore TWR to once historical habitat, as well as continue to increase areal coverage in the San Marcos River.

I. INTRODUCTION

Texas wild rice (*Zizania texana*; Poaceae) is a monoecious perennial grass differing distinctively from other North American species of wild rice in its growth characteristics (Hitchcock 1933; Silveus 1933). The taxon is often found in water up to a meter or more in depth, rooting at the base and geniculate at the nodes (Silveus 1933). Multiple authors (Hitchcock 1933; Silveus 1933; Terrell et al. 1978) describe Texas wild rice (TWR) as possessing long linear leaves, up to two meters in length, which may float on or at a distance below the surface of the water or become aerial. Slender culms, up to five meters in total length, bend upward near the surface of the water and ascending portions may reach a length of up to one meter. Narrow erect panicles, 20 to 30 centimeters in length, bear lower staminate and upper pistillate branches. Staminate branches are ascending or somewhat spreading and reach five to ten centimeters in length. Upper pistillate branches are appressed or ascending and are up to seven centimeters in length. The terminal awn is somewhat flexible, one to two centimeters in length, with scattered prickle hairs that are slightly denser and longer at the base (Hitchcock 1933; Silveus 1933; Terrell et al. 1978).

First documented by G. C. Nealley in August 1892 (U.S. National Herbarium sheet 979361), the collection was erroneously labeled as *Zizania aquatica* (Terrell et al. 1978). The next collection was made by Ena A. Allen on July 10, 1921 (U.S. Herbarium sheet 1611456), and was correctly labeled by A. S. Hitchcock, presumably at a later date. W. A. Silveus, in 1932, first identified *Zizania texana* as a distinct species in a letter (preserved with the holotype in the U.S. National Herbarium) sent on April 4, 1932 to

Agnes Chase of the U.S. National Herbarium. A. S. Hitchcock then designated *Zizania texana* as a distinct species in 1933 (Hitchcock 1933; Terrell et al. 1978).

The genus *Zizania* L. is included in the rice tribe Oryzeae Dumort., a member of Poaceae. Oryzeae include approximately 10 to 12 genera and 70 to 100 species that are native to temperate, subtropical and tropical regions (Barkworth et al. 2007). In North America, four native genera (*Leersia* Sw., *Luziola* Juss., *Zizania* L., *Zizaniopsis* Döll & Asch.) and two introduced genera (*Hygroryza* Nees, *Oryza* L.) of the Oryzeae occur (Barkworth et al. 2007). The genus *Zizania*, includes four species. *Zizania latifolia* (Griseb.) Turcz. ex Stapf occurs in Asia while the other three species, *Zizania palustris* L., *Zizania aquatica* L., and *Zizania texana* Hitchc., are native to North America (Barkworth et al. 2007; Xu et al. 2010). *Zizania palustris* (Northern wild rice) and *Zizania aquatica* (Southern wild rice) are both annual species distributed throughout various states within the continental United States and Canada with some overlap occurring (Xu et al. 2010). *Zizania texana* is known only to occur in Texas and is endemic to the San Marcos River, Hays County (Poole et al. 2007) (Fig. 1.1). *Zizania texana* is geographically isolated by at least 640 km from known populations of the other *Zizania* species (e.g. *Z. aquatica*) located in Louisiana and Nebraska (Terrell et al. 1978; Xu et al. 2010).

Zizania texana is considered to be a relic of a larger population that inhabited the numerous streams of the Balcones Fault zone approximately 10,000 years ago (Horne and Kahn 1997; Xu et al. 2015). Because of the overlap in distributional ranges and morphological intermediates within other *Zizania* taxa, studies have been conducted to determine the phylogenetic relationships among the members of the North American clade. Brown (1950) and Dore (1969) established the base chromosome number to be $n = 15$ for North American *Zizania* taxa. Studies conducted by Duvall (1987) and Duvall and Biesboer (1989) suggested that a relationship exists between *Z. texana* and *Z. palustris* due to successful hybridization under artificial conditions, and between *Z. texana* and *Z. aquatica* based on isoelectric focusing profiles of seed protein. In a study conducted by Horne and Kahn (1997), data from isoenzyme and DNA sequencing compared the three North American *Zizania* species. Genetic distance based on isoenzyme variation indicated that *Z. texana* shared a closer relationship with *Z. palustris* than with Z. *aquatica*. In a later study, Xu et al. (2015) compared chloroplast DNA fragments and microsatellite loci and a close relationship was again found to exist between *Z. texana* and *Z. palustris.* These findings further support the hypothesis that *Z. texana* was isolated from ancestral populations of *Z. palustris* during northward postglacial expansion following the last glacial maximum in North America (Xu et al. 2015).

Figure 1.1 Distribution of Texas wild rice in the San Marcos River from the upper limits located in Spring Lake to above IH-35, Hays County, Texas.

Historically, TWR was reported residing in the upper reaches of the San Marcos River, its associated irrigation canals, and the headwaters of the river (Spring Lake) (Silveus 1933). The current distribution of TWR is limited to the upper 4.6 km of the river (Hutchinson and Ostrand 2015; Wilson et al. 2017).

TWR was listed as a federally endangered species by the United States Fish and Wildlife Service (USFWS 1978) due to the observed rate of decline in coverage and loss of sexual reproductive activity (Emery 1967). Early accounts of the decline in population size of this species and the extirpation from irrigation channels and beyond the first kilometer below Spring Lake Dam were noted by Emery (1967). Emery (1967) cited several factors thought to have probable effect on the rapid decline of TWR in the upper portion of the San Marcos River. Factors cited were river bottom dredging, floating vegetative debris, and sewage pollution. In a later assessment, Emery (1977) found that abatement of river bottom dredging had occurred along with regular collection of vegetative debris and upgrades in city sewage treatment facilities. However, the rate of decline had only slowed and had not resulted in the restoration of sexual reproduction or any appreciable increase in coverage via clones. These observed declines and identified factors underpinned the decision to designate the taxon as an endangered species (USFWS 1978).

Vaughan (1986) identified several additional factors suspected of affecting the distribution and abundance of TWR, including point and non-point source pollution, competition from introduced and native aquatic plant species, recreational use causing “knock-down” of inflorescences, and construction of dams causing a rise in the water level thus affecting growth and seed production. Altered sedimentation patterns, water depth and alteration in flow patterns due to the presence of dams, changes in sediment composition as a result of road and building construction, depletion of the seed bank by river bottom dredging and herbivory, and diminished spring flow related to ground water pumping have also been suggested as factors influencing the distribution of TWR (Power 1996).

The areal coverage of TWR has varied over time between 1976 and 2017 with the largest increases associated with TWR plantings initiated in 2013 (Figure 1.2; Emery 1977; Vaughan 1986; Hutchinson, *pers. comm.* 2014; Hathcock, *pers. comm.* 2018). Declines in coverage were observed through the 1980s. Cessation of river bottom dredging and restoration efforts starting in the late 1980s resulted in measurable increases in coverage (Poole 2002). Recent restoration activities as set forth in the Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan (EAHCP) (Edwards Aquifer Recovery Implementation Program 2012), including establishment of protected areas, removal of invasive and non-native aquatic plants, replanting TWR in the river, and increasing public awareness, have been instrumental in producing observed increases in coverage.

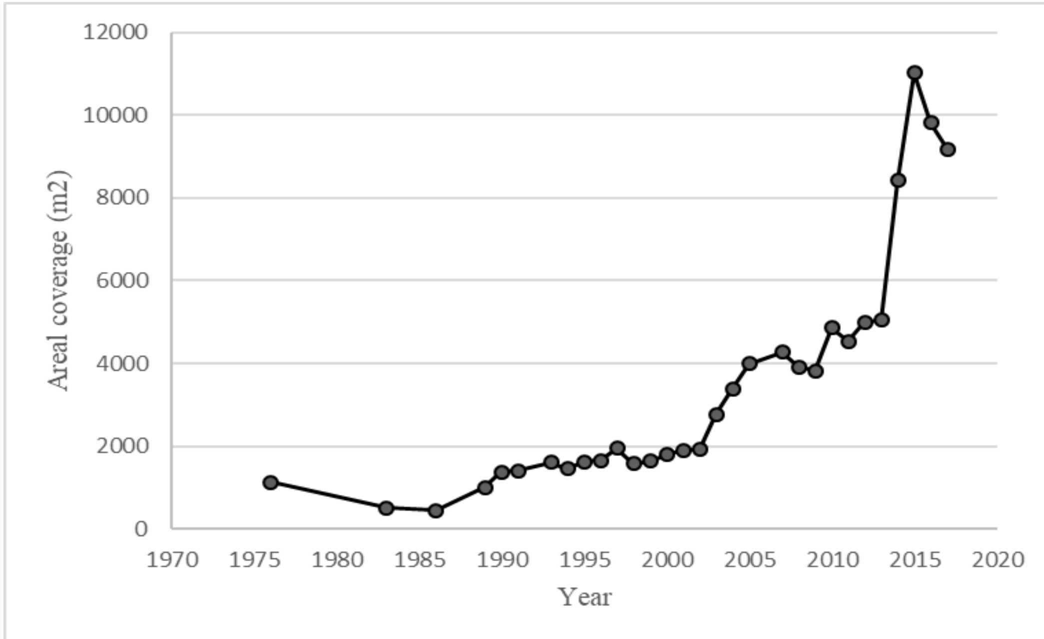

Figure 1.2 Changes in areal coverage (m^2) of Texas wild rice in the San Marcos River 1976-2017 (Emery 1977; Vaughan 1986; Hutchinson, *pers. comm.* 2014; Ostrand, *pers. comm.* 2017; Hathcock *pers. comm.* 2018).

Edwards Aquifer Recovery Implementation Program Habitat Conservation Plan

The San Marcos River arises from artesian springs along the Balcones Fault Line and is fed by emergent water from the Edwards Aquifer. The aquifer and San Marcos River serve as habitat for seven federally endangered and one threatened species: Fountain darter (*Etheostoma fonticola*), San Marcos gambusia (*Gambusia georgei*; presumed extinct), Texas blind salamander (*Eurycea rathbuni*), Comal Springs riffle beetle (*Heterelmis comalensis*), Comal Springs dryopid beetle (*Stygoparnus comalensis*), Peck's cave amphipod (*Styobromus pecki*), Texas wild rice (*Zizania texana*), and San Marcos salamander (*Eurycea nana*) (Edwards Aquifer Recovery Implementation Program 2012). These eight species depend directly on water in or discharged from the Aquifer. The EAHCP is a two-phase, fifteen-year plan formed by the Edwards Aquifer

Recovery Implementation Program (EARIP) in an effort to protect endangered and threatened species associated with the Edwards Aquifer, the San Marcos River, and the Comal River.

The EAHCP has been adopted by the Edwards Aquifer Authority, the City of San Marcos, Texas State University, the City of New Braunfels, the San Antonio Water System, Guadalupe-Blanco River Authority and Texas Parks and Wildlife Department. An Incidental Take Permit was issued by the United Stated Fish and Wildlife Service in 2013 which allows for the "incidental take" of endangered species as a result of otherwise lawful activities, such as recreational use of the San Marcos River and extraction of water from the Edwards Aquifer. The EAHCP includes minimization and mitigation measures designed to ensure covered activities, including recreational use of the river, do not impact the survival and recovery of protected species including TWR. Saunders et al. (2001) point out that recreational use of the river, resulting in increases in suspended sediment and turbidity, uprooting and physical damage to stands, and submergence of reproductive culms from tubing, canoeing, kayaking and swimming, presents challenges for the reintroduction, recovery, and management of TWR. Other authors have also noted that recreational use of the river is a factor that is potentially problematic for the growth and survival of TWR (Vaughan 1986; Bradsby 1994; Breslin 1997). Additional research would be important in quantifying the relationship between contact recreational use of the San Marcos River and the resulting increase in suspended sediment induced turbidity as it relates to the biomass production of TWR. Understanding the impact of recreational activities and their influence on TWR growth and survival is important to achieve the conservation goal of maintaining a TWR coverage of 8,000-15,450 m^2 in the river between Spring Lake Dam downstream through the IH-35 reach (Edwards Aquifer Recovery Implementation Program 2012).

Suspended Sediment Induced Turbidity and PAR Attenuation

Suspended sediment induced turbidity in an aquatic system can be problematic for aquatic plant life due to a decrease in water clarity and reduction in availability of photosynthetically active radiation (PAR) (Madsen et al. 2001). Barko et al. (1986) suggest that in most aquatic environments turbidity is a significant factor in limiting light availability, subsequently affecting aquatic macrophyte growth. Robel (1961) demonstrated that increased turbidity in aquatic systems resulted in decreased macrophyte biomass. In addition, increases in suspended sediment can lead to the accretion of inorganic material on periphyton resulting in shading to the leaf surface and leading to an increased depth of the boundary layer on the adaxial and abaxial surfaces of macrophyte leaves. These mechanisms related to suspended sediment induced turbidity were shown to be most pronounced in the top 30-40cm of water depth, seasonally important from spring to mid-June and negatively affected the vegetative growth and overall biomass production of macrophytes by inhibiting photosynthetic activity (~ 90% reduction in depth specific radiation reaching the macrophyte) and increasing the distance between gas exchange across the surface of leaves (Tóth 2013; Pedersen et al. 2013).

It has been suggested by Asaeda et al. (2004) that the continuous accumulation of periphyton, consisting mostly of epiphytes, on aquatic macrophytes may negatively affect biomass production. The resultant boundary layer created by the presence of periphyton concomitant with slow CO_2 diffusion rates in water has been shown to interfere with inorganic carbon transport in submersed macrophytes during photosynthesis (Smith and Walker 1980). The chlorophyll content and thickness of the epiphytic layer further leads to competition for light due to shading of the macrophyte by epiphyton (Jones and Sayer 2003; Tóth 2013).

Transparency within the water column and the efficacy of a photon to deliver energy to a plant may be affected by factors such as dissolved organic matter content, the concentration of suspended inorganic solids, and the density of suspended microorganisms (Bornette and Puijalon 2011). These factors contribute to increased turbidity levels and can affect the vertical attenuation of light in water often associated with a decrease in aquatic macrophyte productivity (Kirk 1994). Most aquatic macrophytes are found occurring at depths between zero and seven meters and is highly variable due to optical characteristics and light transmission/absorption properties of the overlying water column (Sculthorpe 1967; Pedersen et al. 2013). In general, macrophytes receive only a small fraction of full incident solar energy reaching the water surface due to deflection at the water surface, as well as absorption and scattering by suspended sediment, concentration of suspended microorganisms, and dissolved maerial (Kirk 1994). Sculthorpe (1967) considered the depth limit for most macrophytes to be when the water transparency allowed for less than one to four percent of ambient light to reach a plant. It has been suggested that TWR prefers shallow water depths less than one meter (Poole and Bowles 1999; Saunders et al.). However, recently TWR has been successfully planted and established in water depths in excess of 2 meters.

Dissertation Research Objectives

Previous research has focused on the effects of temperature, sediment preferences, and water velocity on the biomass production of TWR (Power 1996; Poole and Bowles 1999; Tolley-Jordan and Power 2007). Other studies focused on the influence of recreation and water quality as it relates to overall health and abundance of TWR (Vaughan 1986; Bradsby 1994; Breslin 1997). Tolman (2013) and Tolman et al. (2014) found that the spatial distribution of TWR in the upper San Marcos River was strongly associated with areas having high sunlight exposure. Results of these studies have been useful in identifying suitable habitat for TWR. However, an understanding of the effects of available PAR and suspended sediment induced turbidity on the vegetative growth of TWR is lacking.

In this dissertation, I will examine the effects of a reduction in PAR availability and the role of increased suspended sediment induced turbidity associated with recreational use in the upper San Marcos River on the vegetative growth of TWR. The results of this study will provide useful information for future conservation and management measures in the effort to restore TWR to historical habitat as well as to increase areal coverage in the San Marcos River.

The dissertation has three overall research objectives. The first objective is to experimentally examine the impact of a reduction in available PAR on the vegetative growth of TWR *ex situ*. The second objective is to determine the impact of suspended sediment-induced turbidity and associated accumulation of inorganic sedimentation within leaf surface periphyton on the vegetative growth of TWR *in situ* in the San Marcos River along a longitudinal gradient. Three independent studies representing one low recreational use and two high recreational use periods examined the impact of recreation induced turbidity and the impact on TWR biomass. The third objective is to examine the link between recreation induced turbidity and PAR attenuation and summarize its implication for TWR survivorship and restoration.

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II. AN ANALYSIS OF THE EFFECTS OF PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR) ON THE VEGETATIVE GROWTH OF TEXAS WILD RICE (*ZIZANIA TEXANA* HITCHC.) *EX SITU*

Introduction

A variety of abiotic factors including nutrients, velocity, substrate type, and water temperature are known to influence macrophyte growth and production (Bornette and Puijalon 2011). Availability and intensity of light is considered an important abiotic factor affecting aquatic plant biomass (Madsen 1993; Kirk 1994; Case and Madsen 2004). Scheffer (1999) found that light availability is a limiting factor in macrophyte colonization of benthic surfaces. Photosynthetically active radiation (PAR) availability is considered to be the factor most strongly correlated with coverage of aquatic plants (Hilton et al. 1970; Kirk 1994; Davies-Colley and Nagels 2008) and a decrease in available PAR can suppress the overall biomass production of macrophytes (Asaeda et al. 2004; Tóth 2013). PAR is influenced by the extent of riparian shading, especially in relatively narrow rivers where light attenuation by the water column is minimal (Julian et al. 2008). The San Marcos River in Hays County, central Texas, USA is relatively narrow, 5-15 meters wide, and ranges from 1-4 meters in depth at average flow rates (Terrell et al. 1978). Arising from artesian springs fed by the Edwards Aquifer, the mean annual flow of these headwater springs is 4.8 m^3sec^{-1}. The ecosystem serves as habitat for a variety of native and non-native aquatic plants including the federally endangered Texas wild rice (*Zizania texana* Hitchc.) (Edwards Aquifer Recovery Implementation

Program 2012). Texas wild rice (TWR) classified in the rice tribe (Oryzeae) of the Poaceae, is one of only four species in the genus *Zizania*, which is distributed throughout Asia and North America, and one of three species in North America (Xu et al. 2010). The current distribution of TWR is within the upper 5 kilometers of the San Marcos River with 97% of the population occurring in the upper 2.2 kilometers (Wilson et al. 2017). The enactment of the Edwards Aquifer Habitat Conservation Plan (EAHCP) in 2012 placed priority on the recovery and sustainability of TWR and established a goal of maintaining a minimum of 3,550 m^2 areal coverage of TWR in Spring Lake and the upper reaches of the river (Edwards Aquifer Recovery Implementation Program 2012).

In studies conducted by Poole and Bowles (1999) and Tolman et al. (2014), it was suggested that TWR biomass production is in part related to the high river water clarity and spatial distribution in high incident light locations. Tolman (2013) examined the influence of velocity, depth and light availability on the spatial distribution of TWR along three reaches in the upper portion of the San Marcos River. Tolman (2013) found the lowest areal coverage of TWR occurred in the narrowest segment of the river with the greatest extent of riparian canopy cover. These areas were characterized as having reduced incident light reaching the water surface (i.e., greater than 80 percent shading) based on seasonal ray casting and canopy cover estimated from leaf on and leaf off densitometer readings at the water surface. The results of Tolman's study suggest that light may play a role in the distribution of TWR within the river, but it remains unclear how differential light availability may impact TWR production, or what levels of available PAR would be associated with decreased growth and fitness. Understanding the influence of PAR on growth and areal coverage of TWR may help in restoration efforts and in determining suitable locations for reintroductions.

Since light availability may be an important determining factor governing the expansion of TWR coverage in the river system, the objective of this study is to examine the impact of a reduction in available PAR on the vegetative growth of TWR *ex situ*.

Methods and Materials

To test the effect of reduction in PAR on the vegetative growth of TWR, an *ex situ* study was conducted in a raceway located at the Freeman Aquatic Biology Building (FAB) on the campus of Texas State University, San Marcos, Texas, USA. I conducted sequential experiments between September 2015 and April 2016 involving the same range of PAR reduction. Because light availability changed seasonally, each experiment was treated independently. The initial study had a relatively longer baseline period (47 days) as well as growth period (47 days) when compared to the other two studies (Table 2.1) as a result of access issues from major flooding. The baseline period represents an initial grow out of plants from standardized cuttings of tillers after which a random sample was collected to determine starting conditions of above and below ground biomass. The growth period represents the actual treatment period.

Table 2.1 Study dates for baseline and treatment periods for the *ex situ* PAR study.

Study	**Baseline dates**	**Study treatment dates**
PAR I	*Aug. 5-Sept. 20, 2015	*Sept. 20- Nov. 5, 2015
PAR II	Dec. 16-Jan. 16, 2016	Jan. 16-Feb. 14, 2016
PAR III	Feb.19-March 18, 2016	March 18-April 16, 2016

* Protracted dates due to flood event (May 23-24; October 30)

For each of the three replicate study periods, I collected 120 tillers from approximately 75 TWR plants from the San Marcos River on a single day (Texas Parks and Wildlife permit #INT 17 02-21b). I standardized tiller size by removing all but two stems from the plant and trimming the remaining two stems to 20 cm in length. I also removed all but five roots and trimmed those to 5 mm in length. I then placed the tillers at 3 cm depth into individual pots containing soil. The soil used consisted of a commercially purchased blend (50 percent concrete sand and 50 percent loam, with 0.64cm pea size gravel spread on top of the mixture) utilized by the U.S. Fish and Wildlife Service San Marcos Aquatic Resource Center for TWR propagation. The tillers grew for four weeks, after which I randomly selected from surviving propagules 75 plants to be placed into PAR reduction treatment units.

I randomly assigned 15 TWR plants for each of three study periods to the control treatment (100 percent of ambient PAR conditions) or one of four experimental treatment units consisting of plants exposed to a reduction in ambient light. The four treatments consisted of PAR reduced by 10% (90% ambient light), 20% (80% ambient light), 40% (60% ambient light), and 80% (20% ambient light). I placed plants into treatment units 0.9m x 0.6m in size at a water depth of less than one meter and a velocity of 0.2-0.4$msec^{-1}$, which is within reported TWR suitability ranges (Poole and Bowles 1999; Saunders et al. 2001; Tolman 2013; Tolman et al. 2014). Three plastic plant trays (0.6m x 0.3m) containing five plant pots were placed in each treatment unit. Each treatment unit consisted of two corner placed stacked cinder blocks (40cm x 20cm x 15cm) secured with nylon cable ties and affixed with a constructed PVC (0.79cm diameter) shade frame (1.21m x 0.9m) fitted with high density polypropylene shade material positioned above the surface of the water (Fig. 2.1). PAR reductions were achieved by using combinations of suspended shade cloth. Targeted reductions in ambient PAR were determined beneath the suspended shade cloth and above the water level using dual a channel Li-Cor LI 1935A meter fitted with a 4π sensor. In addition, a submersible Tsunami pump (.04m^3s^{-1}) was positioned at the upstream end of each treatment unit to provide a consistent velocity at each treatment unit.

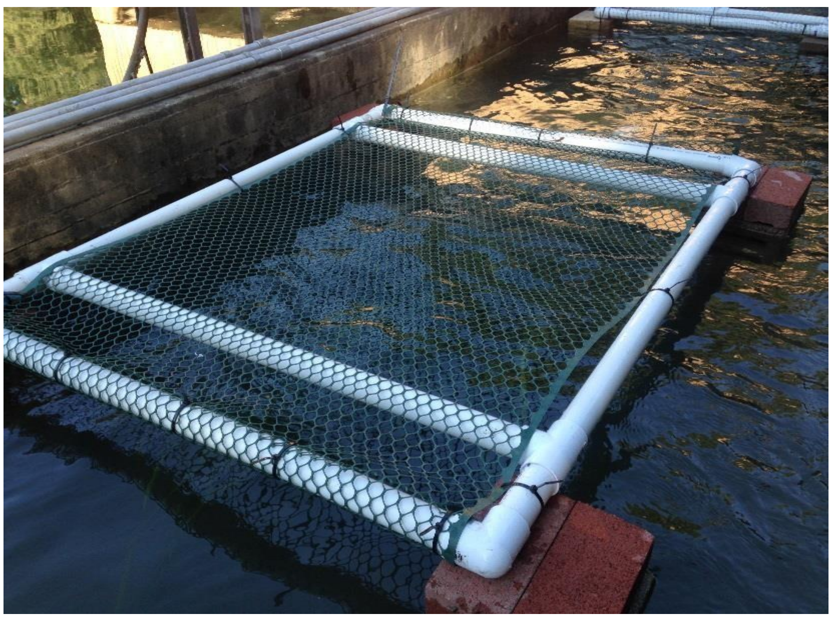

Figure 2.1 PAR experimental plot showing shade frame constructed from PVC and covered with polypropylene shade cloth.

At the onset, mid-point and end of each study period PAR was measured at the water surface, immediately below the water surface, at 10cm below the water surface and at the plant level using a dual channel Li-Cor LI 1935A meter fitted with a 4π sensor. PAR data were collected at midday under cloud free conditions when sunlight was at its maximum to minimize the variation between ambient light conditions due to time of day. I recorded velocity at the upstream point of each treatment unit using a Marsh-McBirney 2000 flow meter and top set wading rod. I recorded water temperature and pH using YSI 85 and Oakton Con +6 meters, respectively. I obtained daily meteorological data (ambient temperature, percent cloud cover, and precipitation) from the San Marcos Airport weather station located approximately 5km from the study site, from Weather Underground, Weather Spark, and Community Collaborative Rain, Hail, and Snow Network (Weather Underground 2016; Weather Spark 2016; Community Collaborative Rain, Hail, and Snow Network 2017). I obtained duration of daylight from the United States Naval Observatory website (United States Naval Observatory 2016).

At the end of the initial baseline growth and treatment periods (Table 2.1), I removed remaining plants and placed them into an individual plastic bag for each treatment for transport to the laboratory at the Freeman Aquatic Biology Building. In the lab, I separated shoots (leaves) from the root mass at the juncture. I recorded the number of individual roots (excluding root hairs) and leaves. I placed roots in a paper bag and dried them for 48 hours at 60˚C in a drying oven. I used a Li-Cor LI-3000C Portable Area meter to measure the leaf surface area (LSA) of all leaves and placed shoots in a paper bag and dried them for 48 hours in a 60˚C drying oven. After drying, I weighed the shoots and roots to determine dry biomass (g).

Statistical Analysis

Data collected from the three PAR experiments were checked for assumptions of normality and homogeneity of variance. When necessary, data were $\log_{10}$ transformed to attain normality. TWR growth data including dry weight biomass, the ration of above:below ground biomass, total biomass, shoot number, root number, and total leaf surface area were analyzed through a one-way ANOVA. If the result was found to be significant, the ANOVA was followed by a pairwise comparison of means using Tukey's HSD test at a minimum level of $p < 0.05$. All ANOVA and Tukey HSD tests were performed using R 3.4.0 studio statistical analysis.

Results

Figure 2.2 shows the day length during each *ex situ* PAR study period. Cumulative hours of daylight for PAR treatments I, II, and III were 1,134 (10.7 kW/m^2), 642 (5.8 kW/m^2), and 701 (8.7 kW/m^2), respectively. The difference in total daylight hours associated with the extended baseline grow out period and subsequent extension of the treatment period in the first experiment (PAR I) is almost twice that of the subsequent two treatment periods. Figures 2.3 and 2.4 show the daily percent cloud cover and daily total precipitation totals during each of the *ex situ* PAR study periods (Weather Underground 2016; Weather Spark 2016; Community Collaborative Rain, Hail, and Snow Network 2017). Precipitation data represent rainfall values collected at station number Tx-HYS 124 located approximately 3.7 km west northwest of San Marcos (Community Collaborative Rain, Hail, and Snow Network 2017). While indicative of rainfall in the

San Marcos area, it does not represent the total amount of rainfall in the watershed area contributing to the flood event of October 2015. Rainfall totals at station Tx-HYS 124 for October 30, 2015 and October 31, 2015 were 15.24mm and 292.00mm, respectively, and inundation of the raceway area located at the Freeman Aquatic Biology Building was a factor in the extended treatment period during PAR I experiment.

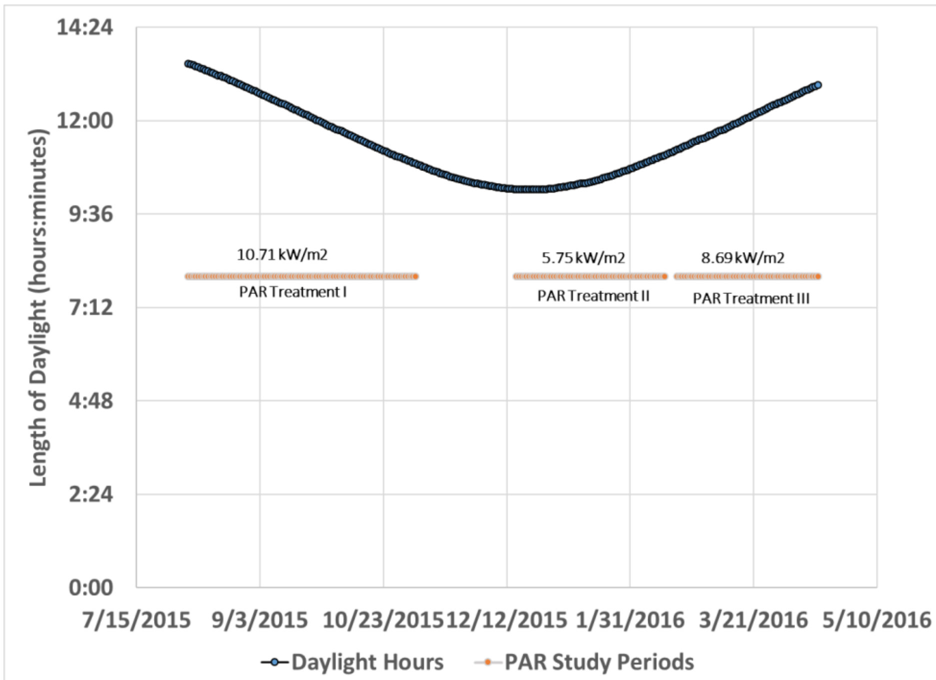

Figure 2.2 Length of daylight and total kilowatts per square meter during each of the three-ex *situ* PAR study periods. Length of day data are from United States Naval Observatory.

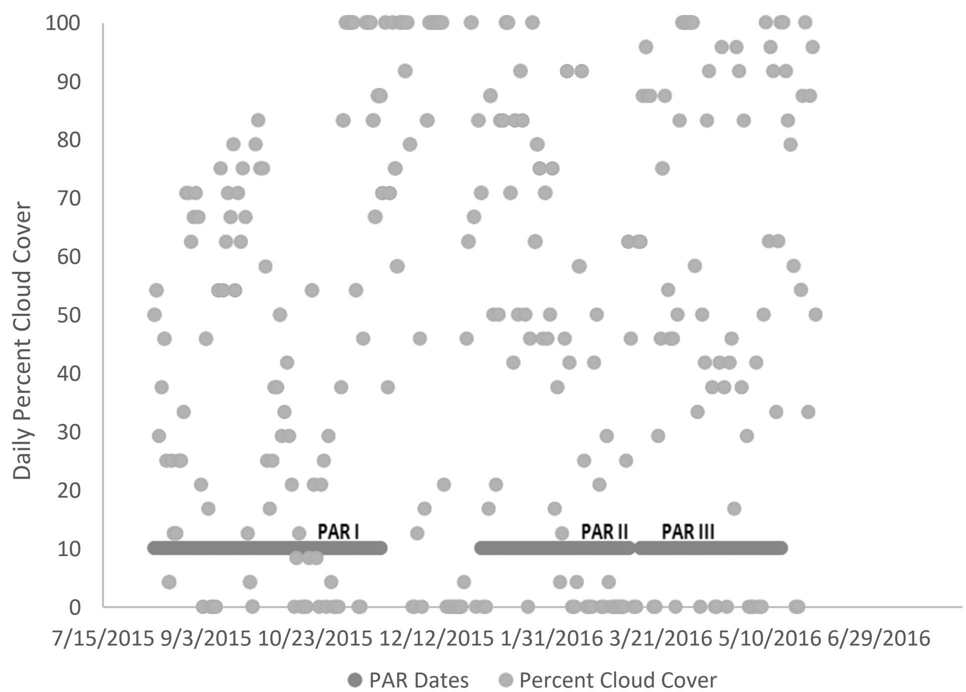

Figure 2.3 Percent cloud cover during the three PAR *ex situ* study periods conducted at the Freeman Aquatic Biology Building at Texas State University. Percent cloud cover data are from the Weather Spark and Weather Underground.

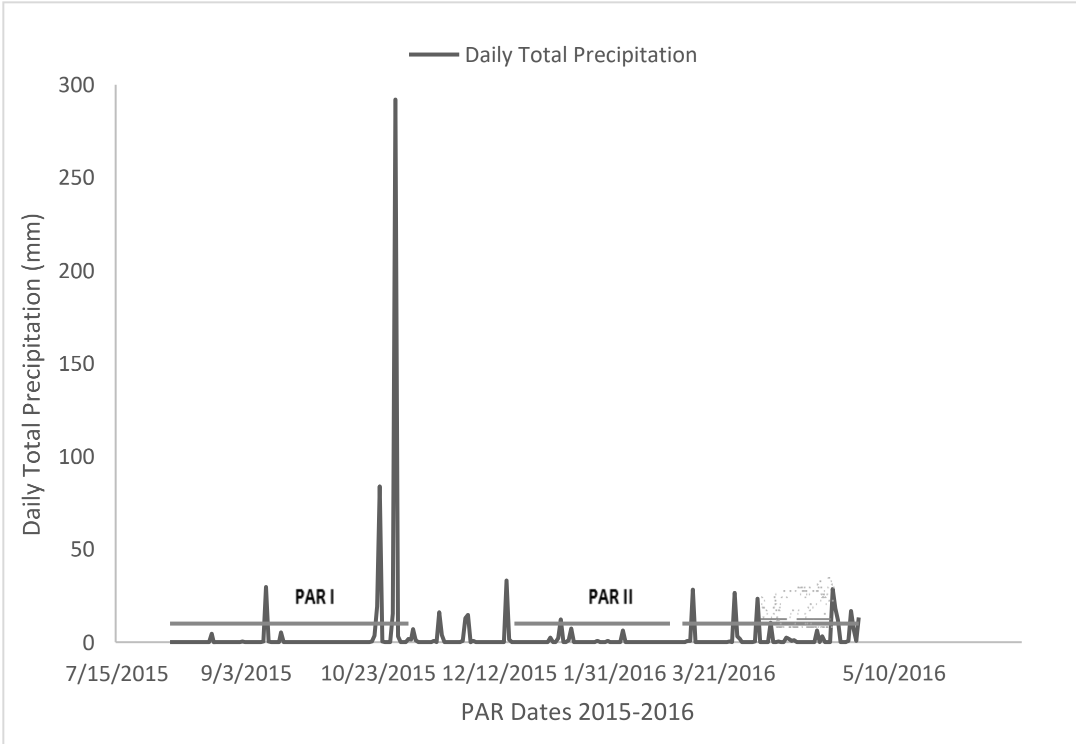

Figure 2.4 Daily total precipitation during the three PAR *ex situ* study periods conducted at the Freeman Aquatic Biology Building at Texas State University. Daily total precipitation (mm) data are from Community Collaborative Rain, Hail, and Snow Network.

There were some differences, although not significant ($F = 2.88$; $df = 2{,}209$; $\alpha = 0.06$), in the average daily percent cloud cover for the three consecutive PAR periods (41, 40, and 53 percent). Some of the growth data from the PAR I experimental period were omitted due to the October 31, 2015 flood, which resulted in inundation of the experimental raceway for several days. Plants were harvested approximately six days after the flood event when access was possible. Severe shoot entanglement was evident and precluded collection of specific individual values such as shoot numbers, number of broken shoots, and leaf area metrics for most plants. For the PAR I experimental period, all shoot and roots were still segregated, and the corresponding shoot and root dry weights were analyzed as in the other two PAR replicates.

An artesian well at the Freeman Aquatic Biology Building provided constant water quality properties over the entire *ex situ* treatment period where pH varied less than 0.2, temperature differences ranged less than 1.1˚C, while DO varies between 6.5 and 7.7 (see Table 2.2). Note that DO remained constant within each treatment period. Data collected for the three PAR experimental periods included, shoot dry weight, root dry weight, shoot number, root number, and total leaf surface area and are summarized in Tables 2.3 through 2.6.

Table 2.2 Mean physical and chemical properties measured during each of the PAR experimental periods and for each treatment (onset, mid-point, and end; n=3) in the experimental flume. Control is represented by 100 ambient light.

Study Period	**Percent ambient light**	**Depth (cm)**	**Velocity (m/s)**	**Temp (°C)**	**pH**	**DO (mg/L)**	**$PAR_{surface}$ ($\mu molm^{-2}s^{-1}$)**	**PAR_{10cm} ($\mu molm^{-2}s^{-1}$)**
	100	63.5 (63-64)	0.32 (0.22-0.42)	22.8 (22.5-22.9)	7.4 (7.3-7.4)	7.7 (7.7)	2250	1837
	90	66.5 (66-67)	0.32 (0.21-0.43)	22.8 (22.5-22.9)	7.4 (7.3-7.4)	7.7 (7.7)	2250	1756
PARI	80	68.8 (68.5-69)	0.32 (0.19-0.44)	22.8 (22.5-22.9)	7.4 (7.3-7.4)	7.7 (7.7)	2250	1403
	60	70.5 (70-71)	0.33 (0.21-0.44)	22.8 (22.5-22.9)	7.4 (7.3-7.4)	7.7 (7.7)	2032	874
	20	72.5 (72-73)	0.31 (0.21-0.41)	22.8 (22.5-22.9)	7.4 (7.3-7.4)	7.7 (7.7)	2032	360
	100	63.5 (63-64)	0.34 (0.22-0.45)	22.4 (21.7-22.1)	7.3 (7.2-7.4)	6.9 (6.9)	1982	1401
	90	66.5 (66-67)	0.36 (0.24-0.48)	22.4 (21.7-22.1)	7.3 (7.2-7.4)	6.9 (6.9)	1890	929
PARII	80	68.8 (68.5-69)	0.44 (0.21-0.48)	22.4 (21.7-22.1)	7.3 (7.2-7.4)	6.9 (6.9)	1654	1024
	60	70.5 (70-71)	0.32 (0.21-0.43)	22.4 (21.7-22.1)	7.3 (7.2-7.4)	6.9 (6.9)	1935	524
	20	72.5 (72-73)	0.31 (0.20-0.41)	22.4 (21.7-22.1)	7.3 (7.2-7.4)	6.9 (6.9)	1941	302
	100	63.5 (63-64)	0.39 (0.28-0.49)	22.3 (21.7-22.8)	7.4 (7.4)	6.5 (6.0-6.9)	2090	1530
	90	66.5 (66-67)	0.32 (0.24-0.40)	22.3 (21.7-22.8)	7.4 (7.4)	6.5 (6.0-6.9)	2090	1029
PARIII	80	68.8 (68.5-69)	0.35 (0.22-0.47)	22.3 (21.7-22.8)	7.4 (7.4)	6.5 (6.0-6.9)	1946	1132
	60	70.5 (70-71)	0.34 (0.28-0.40)	22.3 (21.7-22.8)	7.4 (7.4)	6.5 (6.0-6.9)	2065	629
	20	72.5 (72-73)	0.35 (0.25-0.44)	22.3 (21.7-22.8)	7.4 (7.4)	6.5 (6.0-6.9)	2046	409

(min-max values)

Table 2.3 Mean baseline and treatment values for shoot dry weight, root dry weight, above/below biomass, total biomass, shoot number, root number, and total leaf surface area for the *ex situ* PAR I study. Control is represented by 100% ambient light.

	Above ground (g) (Shoot)	**Below ground (g) (Root)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm^2)**
Baseline (15)	1.7 (±0.26)	0.55 (±0.08)	3.09	2.25 (± 0.19)	20 (±4)	30 (±3)	NA
100% ambient light (15)	11.48 (±2.39)	3.9 (±1.23)	2.94	15.39 (±3.49)	33 (±14)	86 (±9)	NA
90% ambient light (14)	10.17 (±1.22)	4.00 (±0.82)	2.54	14.18 (±1.78)	49 (±19)	100 (±12)	NA
80% ambient light (15)	9.87 (±2.03)	5.38 (±1.70)	1.83	15.25 (±3.52)	38 (±10)	87 (±12)	NA
60% ambient light (15)	8.88 (±2.69)	3.34 (±1.10)	2.66	12.22 (±3.66)	24 (±8)	71 (±13)	NA
20% ambient light (14)	4.33 (±0.62)	1.24 (±0.17)	3.49	5.57 (±0.73)	17 (±8)	57 (±6)	NA

Standard error in parentheses; n= number of plants remaining after baseline and treatment.

NA=Data not available due to leaf entanglement associated with October 2015 flood event.

Table 2.4 Mean baseline (n=14) and treatment values for shoot dry weight, root dry weight, above/below biomass, total biomass, shoot number, root number, and total leaf surface area for the *ex situ* PAR II study. Control is represented by 100% ambient light.

	Above ground (g) (Shoot)	**Below ground (g) (Root)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm^2)**
Baseline (15)	0.68 (±0.11)	0.22 (±0.03)	3.09	0.90 (±0.08)	10 (±1)	13 (±2)	197.93 (±31.50)
100% ambient light (15)	4.66 (±1.21) [c]	1.30 (±0.32) [c]	3.58	5.96 (±0.42)	31 (±9) [c]	52 (±10) [c]	1176.51 (±322.07) [c]
90% ambient light (14)	4.32 (±0.64) [c]	1.53 (±0.23) [c]	2.82	5.84 (±0.68)	49 (±8) [c]	61 (±8) [c]	1599.79 (±319.26) [c]
80% ambient light (15)	5.40 (±0.88) [c]	1.69 (±0.25) [c]	3.20	7.09 (±0.40) [c]	38 (±6) [c]	68 (±7) [c]	1626.85 (±228.66) [c]
60% ambient light (15)	3.41 (±0.59) [c]	0.82 (±0.15)	4.16	4.23(±0.26)	27 (±5)	41 (±7)	1381.86 (±205.68) [c]
20% ambient light (15)	2.88 (±0.49)	0.61 (±0.11) [ab]	4.72	3.49 (±0.14) [a]	21 (±3)	37 (±6) [a]	1058.18 (±182.82)

Standard error in parentheses; n= number of plants remaining after baseline and treatment.

[a] Values significantly different from 80% ambient light within a treatment period (α=0.05) using Tukey's test for post ANOVA.

[b] Values significantly different from 90% ambient light within a treatment period (α=0.05) using Tukey's test post ANOVA.

[c] Values significantly different from baseline within a treatment period (α=0.05) using Tukey's test post ANOVA.

Table 2.5 Mean baseline (n=11) and treatment values for shoot dry weight, root dry weight, above/below biomass, total biomass, shoot number, root number, and total leaf surface area, for the *ex situ* PAR III study. Control is represented by 100% ambient light.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm^2)**
Baseline (12)	0.31 (±0.04)	0.12 (±0.01)	2.58	0.43 (±0.01)	4 (±1)	4 (±1)	67.85 (±13.17)
100% ambient light (12)	1.88 (±0.35) [c]	0.43 (±0.06)	4.37	2.31 (±1.51) [c]	13 (±2)	22 (±4) [c]	446.75 (±84.66) [c]
90% ambient light (12)	1.52 (±0.51)	0.48 (±0.17) [c]	3.17	2.00 (±0.80) [c]	16 (±5) [c]	24 (±6) [c]	468.04 (±150.28) [c]
80% ambient light (11)	2.34 (±0.34) [c]	0.51 (±0.07) [c]	4.59 [bc]	2.85 (±1.05) [c]	17 (±1) [c]	25 (±4) [c]	643.14 (±73.46) [c]
60% ambient light (12)	1.62 (±0.21) [c]	0.38 (±0.06)	4.26 [bc]	2.00 (±0.71)	13 (±2)	22 (±3) [c]	417.77 (±57.62) [c]
20% ambient light (12)	0.85 (±0.13) [a]	0.19 (±0.02) [a]	4.47 [bc]	1.04 (±0.60) [a]	6 (±1) [ab]	11 (±1) [a]	243.77 (±33.27) [a]

Standard error in parentheses; n= number of plants remaining after baseline and treatment.

[a] Values significantly different from 80% ambient light within a treatment period (α=0.05) using Tukey's test for ANOVA.

[b] Values significantly different from 90% ambient light within a treatment period (α=0.05) using Tukey's test post ANOVA.

[c] Values significantly different from baseline within a treatment period (α=0.05) using Tukey's test post ANOVA.

Table 2.6 Results of ANOVA baseline and treatment values for shoot dry weight, root dry weight, above/below biomass, total biomass, shoot number, root number, and total leaf surface area, for the *ex situ* PAR II and III study periods.

PAR II	*F-value*	df	*p*	PAR III	*F-value*	df	*p*
SDW				**SDW**			
Baseline	5.05	5,84	<0.01	**Baseline**	5.32	5,64	<0.01
Treatment	1.14	4,66	0.32	**Treatment**	2.58	4,54	0.05
RDW				**RDW**			
Baseline	7.57	5,84	<0.00	**Baseline**	3.60	5,64	0.01
Treatment	4.14	4,70	0.01	**Treatment**	3.99	4,54	0.01
A/B				**A/B**			
Baseline	1.28	5,84	0.28	**Baseline**	5.91	5,64	<0.01
Treatment	1.09	4,70	0.37	**Treatment**	3.51	4,54	0.01
TB				**TB**			
Baseline	3.95	5,68	<0.01	**Baseline**	6.49	5,79	<0.00
Treatment	2.40	4,54	0.05	**Treatment**	2.55	4,60	0.05
SN				**SN**			
Baseline	4.93	5,84	<0.01	**Baseline**	5.22	5,80	<0.01
Treatment	2.13	4,68	0.09	**Treatment**	2.89	4,54	0.03
RN				**RN**			
Baseline	7.25	5,84	<0.00	**Baseline**	5.56	5,67	<0.01
Treatment	2.73	4,70	0.04	**Treatment**	2.26	4,57	0.05
TLSA				**TLSA**			
Baseline	4.93	5,84	<0.01	**Baseline**	5.54	5,64	<0.01
Treatment	2.62	4,70	0.04	**Treatment**	2.46	4,54	0.05

Significant differences in shoot biomass, root biomass, ration of above:below ground biomass, total biomass and shoot number existed in the PAR II and III experiments only. Significant differences in growth data (Table 2.3) in the PAR I experiment did not exist ($p_{shoot\ biomass} = 0.30$; $p_{root\ biomass} = 0.10$; $p_{above/below\ biomass} = 0.85$; $p_{total\ biomass} = 0.15$; $p_{shoot\ number} = 0.73$; $p_{root\ number} = 0.31$). Growth data from the PAR I experiment are provided, however given the extended initial growth period due to the October 31, 2015 flood inundation these results were excluded from further analysis (Table 2.1). In PAR II, plants exposed to 20% ambient light exhibited significantly lower root biomass ($p = 0.01$), total biomass ($p = 0.03$), and root number ($p = 0.05$) when compared to plants exposed to 80% ambient light (Tables 2.4, 2.5). In PAR III plants exposed to 20% ambient light exhibited significantly lower shoot biomass ($p = 0.02$), root biomass ($p < 0.01$), total biomass ($p = 0.03$), shoot number ($p = 0.03$), and root number ($p = 0.05$) when compared to plants exposed to 80% ambient light (Tables 2.4, 2.5). Additionally, plants exposed to 20% ambient light exhibited significantly lower root biomass ($p = 0.04$) in PAR II and the ration of above:below ground biomass ($p = 0.05$) and shoot number ($p = 0.02$ in PAR III when compared to 90% ambient light (Tables 2.4, 2.5).

Total leaf surface area of all plants in a given treatment unit was only calculated in the PAR II and PAR III experiments. It was not possible to collect leaf surface area in the PAR I experiment because of severe entanglement and breakage due to flooding. Total leaf surface area (Tables 2.4, 2.5) was significantly greater in treatment units exposed to 80% ambient light compared to plants exposed to 20% ambient light in PAR III ($p = 0.02$).

When compared to initial baseline significant differences existed for growth data in the PAR II and PAR III experiments. In PAR II, significantly greater values were obtained for shoot biomass ($p_{100\%} < 0.01$; $p_{90\%} = 0.01$; $p_{80\%} < 0.01$; $p_{60\%} < 0.05$), root biomass ($p_{100\%} = 0.01$; $p_{90\%} < 0.01$; $p_{80\%} < 0.01$), total biomass ($p_{80\%} = 0.01$), shoot number ($p_{100\%} < 0.05$; $p_{90\%} < 0.01$; $p_{80\%} = 0.01$), root number ($p_{90\%} < 0.01$; $p_{80\%} < 0.01$), and total leaf surface area ($p_{90\%} < 0.01$; $p_{80\%} < 0.01$; $p_{60\%} = 0.01$; $p_{20\%} < 0.01$). In PAR III, significantly greater values were obtained for shoot biomass ($p_{100\%} = 0.01$; $p_{80\%} < 0.01$; $p_{60\%} = 0.04$), root biomass ($p_{90\%} = 0.03$; $p_{80\%} = 0.02$), above/below biomass ($p_{100\%} = 0.01$; $p_{80\%} < 0.01$; $p_{60\%} = 0.04$), total biomass ($p_{100\%} = 0.01$; $p_{90\%} < 0.01$; $p_{80\%} < 0.01$; $p_{60\%} = 0.03$), shoot number ($p_{100\%} = 0.02$; $p_{90\%} < 0.01$; $p_{80\%} < 0.01$), root number ($p_{90\%} < 0.01$; $p_{80\%} < 0.01$), and total leaf surface area ($p_{100\%} = 0.02$; $p_{90\%} = 0.02$; $p_{80\%} < 0.0$; $p_{60\%} = 0.04$). When compared to baseline, in both PAR II and PAR III experimental periods, a significant increase in growth was recorded at 80% ambient light, and for above/below in PAR II, a significant decrease in growth was noted at 20% ambient light ($p = 0.01$).

Discussion

Light availability is of major importance in determining aquatic plant production and plant predominance and distribution in the aquatic environment (Kirk 1994; Case and Madsen 2004). Plant growth requires light and the availability of light in the aquatic environment may be impacted by factors such as the amount of incident radiation and back-reflectance at the water surface, optical properties within the water column, depth, and overhead shading due to riparian cover (Sculthorpe 1967; Kirk 1994; Bornette and Puijalon 2011; Tolman 2013; Pedersen et al. 2013). In addition, riparian shading or shading from adjacent macrophyte species may limit available light resulting in reduced growth. Shading of Northern wild rice, *Zizania palustris*, by giant bur reed (*Sparganium eurycarpum*) was found to be the major cause of reduced wild rice yield (Clay and Oelke 2017).

Studies with aquatic macrophytes have shown that shoot biomass, root biomass, and shoot number decrease under conditions of reduced ambient light availability. Clay and Oelke (2017) found a reduction in shoot dry weight in *Zizania palustris*, Northern wild rice, when plants were grown in a 47% shade treatment as compared to 100% ambient light. *Elodea canadensis*, *Myriophyllum spicatum*, and *Littorella uniflora* showed a reduction in root biomass at lower ambient light levels (Søndergaard and Bonde 1988; Sand-Jensen and Madsen 1991; Abernethy et al. 1996). Kurtz et al. (2003) investigated the impact of light reduction in *Vallisneria americana* and found a significant reduction in root biomass, shoot biomass, and above/below biomass occurred when plants were subjected to 21% and 8% ambient light. I found a similar result in that with 20% ambient light, root biomass (PAR II and III), shoot biomass (PAR III), and above/below biomass (PAR III) of TWR were significantly reduced when compared to the 80% and 90% ambient light treatments. Barko and Smart (1981) found that *Hydrilla*, and to a lesser extent *Myriophyllum*, exhibited decreased shoot number with increasing shade.
Similarly, TWR showed a significant decrease in shoot number at lower ambient light levels in the PAR III experiment.

Some plant species exhibit an increased leaf surface area when exposed to low light availability. One example is provided by the study by Aleric and Kirkman (2005) in which they investigated the growth response of the federally endangered terrestrial shrub *Lindera melissifolia* and found total leaf surface area to be greatest in 19% ambient light when compared to 58% and 100% ambient light. An increased leaf surface area allows greater interception of the little light that is available for photosynthesis. The opposite growth response was found in TWR, which exhibited a significant decrease in total plant leaf surface area at 20% ambient light in the PAR III experiment. Longer experiments under a broader range of light conditions might reveal if TWR is capable of a compensatory response in leaf structure resulting from light reduction.

Although submersed aquatic macrophytes are considered to be shade tolerant (Carr et al. 1997; Best et al. 2001), clearly their growth is impacted by limited light availability. In a survey conducted in 17 Florida streams, shading resulting from riparian vegetation was suggested to be a dominant factor in controlling the location and abundance of aquatic macrophytes (Canfield and Hoyer 2011).

PAR availability is considered most strongly correlated with areal coverage of aquatic plants and is influenced by the extent of riparian shading (Hilton et al. 1970; Davies-Colley and Nagels 2008; Julian et al. 2008). Tolman (2013) found TWR to be spatially distributed in areas having greater sustained direct sunlight on an annual basis compared to shaded areas from the distribution and riparian canopy characteristics. High shade areas were found to contain reduced aerial coverage or complete lack of TWR. The PAR experimental results are consistent with these findings and indicate TWR during the short-term early establishment growth period respond to reductions in PAR at levels greater than 40 percent. Additional experiments should target PAR reductions between 40 and 80 percent to better define the response point.

Shade tolerance and light-related morphological changes may offer competitive advantages for aquatic macrophytes in reduced light conditions resulting from riparian canopy coverage. Indeed, research has shown that morphological adaptations related to a rapid growth rate have enabled *Hydrilla verticillata* to grow under lower light conditions than nearly any other macrophyte species (Langeland 1996; Glomski and Netherland 2012). At only one percent light availability, *H. verticillata* can establish and displace native *Potamogeton* sp. and *Vallisneria americana* Michaux (Langeland 1996). This growth characteristic of Hydrilla may in part account for its aerial dominance in some reaches of the upper San Marcos River compared to other aquatic macrophytes.

My study results found a significant decrease in both above and below ground biomass with an 80 percent reduction in available PAR. The experimental design relied on PAR reduction increments that doubled the reduction increments. It is likely that the actual threshold of TWR growth response to PAR reduction lies between the 40 and 80 percent values. It should be noted that the observed response of TWR to PAR reduction in these experiments were associated with water depths less than a meter. It is reasonable to assume that the effect of PAR reduction at the water surface would result in differential plant growth as a function of increasing water depth due to light attenuation. Additional research using long term study periods are necessary to ascertain if this level of biomass reduction observed during short term early growth would result in differential fitness for the plant. Results from Tolman (2013) show that TWR is most often found in areas of high incident light and may reflect a lack of competitive advantage of TWR in lower light available locations. Mapping results for TWR also indicate that dense stands can be found in shaded areas when water depths are less than a meter but are less likely to be found in deeper sections of the San Marcos River downstream when water depths are in excess of 2 meters. This may reflect in part, reduced availability of PAR at the stream bed due to increased attenuation from turbidity as noted in the next chapter.

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III. IMPLICATIONS OF RECREATION INDUCED TURBIDITY IN THE SAN MARCOS RIVER ON THE BIOMASS PRODUCTION OF TEXAS WILD RICE (*ZIZANIA TEXANA* HITCHC.) *IN SITU*

Introduction

Transparency within the water column and the efficacy of a photon to deliver energy to a plant is affected by a suite of external factors such as dissolved organic matter (DOM) concentration in the water, the concentration of suspended solids, water depth, and the concentration of suspended microorganisms both in the water column and the density or thickness of materials on the surface of submerged aquatic macrophytes (Kirk 1994; Asaeda et al. 2004; Köhler et al. 2009; Bornette and Puijalon 2011). Scully and Lean (1994) indicated from studies in lakes that turbidity in the form of DOM is a dominant agent in the scattering, absorption, and reduction of available light, thus potentially contributing to the spatial and temporal variability in photosynthetic active radiation (PAR) attenuation. Suspended-sediment induced turbidity in an aquatic system can result in a decrease in water clarity and a reduction in availability of PAR (Madsen et al. 2001). It has been shown by Barko et al. (1986) that in aquatic environments, that turbidity is a significant factor in limiting light availability.

The decline in PAR with water depth resulting from increased turbidity is often associated with anthropogenic influences (Best et al. 2001). Recreational activities such as swimming, boating, and fishing can cause increased turbidity (Saunders et al. 2001;

Hall and Härkönen 2006). High levels of suspended solids in the water column can block or scatter light, which may negatively impact growth of submerged aquatic macrophytes due to reduction in the amount of PAR reaching photosynthetic active sites on the plant surface. Reduction in PAR can influence the production of biomass and may at times affect the morphology of aquatic macrophytes (Hudon et al. 2000; Best et al. 2001).

Light availability at the photosynthetically active sites on a submerged aquatic macrophyte is also influenced by the accumulation of periphyton on the leaves. The accumulation of periphyton, (epiphytic algae, cyanobacteria, heterotrophic microbes and detritus) can lead to reduction in the amount of light reaching the surface of macrophyte leaves, thus partially or indirectly influencing plant productivity and biomass (Orth et al. 1982). Periphyton accumulation can result in up to a 90% reduction in PAR reaching the plant leaf surface as well as increasing the length (depth) of the diffusive boundary layer affecting the exchange of gases and nutrients (Asaeda et al. 2004; Tóth 2013). The periphyton layer may also result in a feed-back loop in which it physically traps or accumulates more inorganic suspended solids, further reducing the amount of available PAR at the leaf surface (Davies and Walmsley 1985; Kemp et al. 2000).

Texas wild rice (*Zizania texana* Hitchc.) (TWR) is an endangered macrophyte that occurs only in the upper few kilometers of the spring-fed San Marcos River, Hays County, Texas, USA. The San Marcos River aquatic macrophyte community is impacted on a seasonal basis by contact water recreation such as kayaking, canoeing, swimming, tubing, wading, and fishing. Some areas of the river bed become denuded of all aquatic macrophytes and physical disturbance of the stream bed is known to increase suspended sediments in the water column (Breslin 1997; Saunders et al. 2001). Monitoring data show a correlation exists between intensity of contact recreation use of the river and increased turbidity levels on a diel, weekly, and seasonal basis (Crawford-Reynolds et al. 2016). These data show that the longitudinal pattern of turbidity in the river (i.e., upstream to downstream) coincides with the intensity of contact recreation (number of people) and seasonal periods of high contact recreational activity in the San Marcos River (Crawford-Reynolds et al. 2016).

However, it is currently unknown if the existing longitudinal gradient of recreational induced turbidity results in a concomitant reduction in PAR sufficient to negatively impact the growth dynamics of TWR. The objective of this research was to examine the impact of recreation induced suspended sediment turbidity on PAR, the accumulation of inorganic sediment on the leaf surface of TWR, and the subsequent potential to impact the vegetative growth of TWR *in situ* in the San Marcos River.

Methods and Materials

To analyze the effects of recreation induced suspended sediment associated with low to high recreational periods on TWR vegetative growth, I conducted *in situ* experiments at four locations in the upper reaches of the San Marcos River, Hays County, Texas, USA (Figure 3.1). This experiment examines the relationship between increased suspended sediment longitudinally due to recreation and PAR reduction with depth.

The study design consisted of examining TWR growth at four locations along the upper San Marcos River across two recreational activity periods in the river. The study period representing relatively low recreational use (termed Pre-Rec) was conducted from April 10 to May 29, 2015 (39 days) and two periods representing higher recreational use (termed High-Rec) conducted from May 29 to July 23, 2014 (56 days) and again during higher river discharges and higher recreational use from May 28 through July 20, 2015 (52 days) (Figure 3.2). These low versus high recreational use level time periods were selected based on river use observations over a three-year period (Crawford-Reynolds et al. 2016).

Figure 3.1 Locations of Texas wild rice study areas, automated data sondes, recreational use cameras, and USGS gauge in the San Marcos River, Hays County, Texas.

Study Area

As shown in Figure 3.1, the four study sites were located below the Eastern Spillway at Spring Lake Dam and ending downstream at Ramon Lucio Park. The Eastern Spillway study site is located above Sewell Park (ESSP; abbreviated ES); the Sewell Park study site is located below the second footbridge within Sewell Park (FBSP; abbreviated SP); the Bicentennial Park study site is located below Hopkins Street (BPHS; abbreviated BP); and the Ramon Lucio Park study site is located below the wooden bridge (WBRL; abbreviated RL).

To analyze the impact of suspended sediment induced turbidity resulting from contact recreational usage on attenuation of available PAR in the water column, I evaluated three sites longitudinally (upstream to downstream) along the San Marcos River. The sites (Fig. 3.1) analyzed were Sewell Park (SP; 29° 53.253"N, 97° 56.069"W), City Park (CP; 29° 53.160" N, 97° 56.143" W), and Bicentennial Park (BP; 29° 52.941" N, 97° 56.092" W). Turbidity values, using river water grab samples, and PAR availability data were collected at the three sites on three days in 2016 (Tuesday, August 9, 11:00-13:00; Saturday, September 3, 15:45-17:00; and Friday, October 7, 12:00-15:00).

Water Quality Data

Water quality data were obtained from the Edwards Aquifer Authority (EAA) Hydrologic Data report (Edwards Aquifer Authority (EAA) Hydrologic Data report 2015) and unpublished data (W. Nowlin, B. Schwartz, and T. Hardy) (Meadows Center for Water and the Environment 2016) real time water quality monitoring sondes (Manta 35) located above Sewell Park in the outfall from Spring Lake and downstream above the I-H35 Interstate bridge (Figure 3.1).

Daily river discharge (Figure 3.2) was obtained from the USGS gauge station (0817000) located in the San Marcos River below Spring Lake Dam (United States Geological Survey 2016). Precipitation data were obtained from the Community Collaborative Rain, Hail, and Snow Network (Community Collaborative Rain, Hail, and Snow Network 2015).

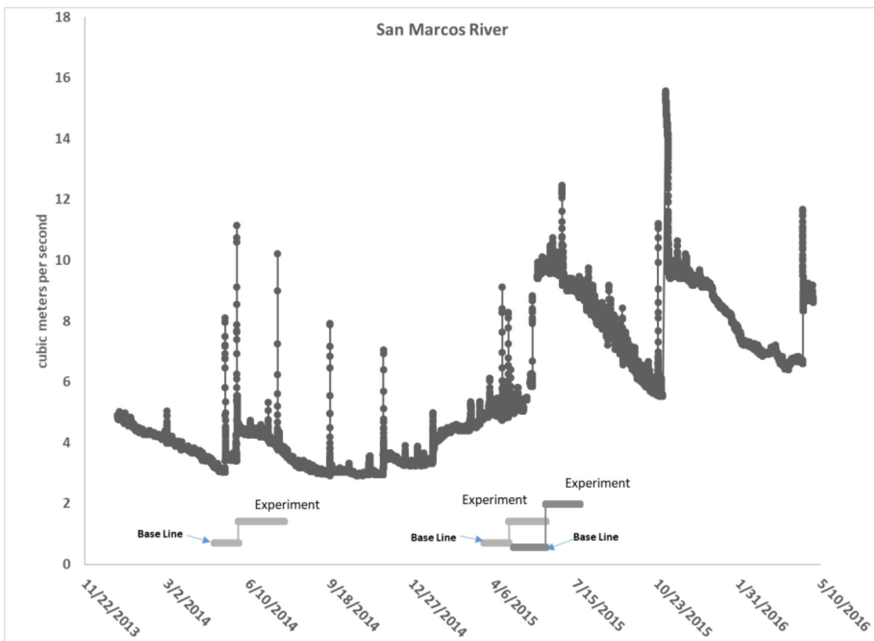


Figure 3.2 Daily and monthly average discharge (m^3s^{-1}) and *in situ* experimental dates in the San Marcos River. The change in experimental period in the graph (horizontal bars) is for clarification between the two consecutive experiments where baseline (initial growth period) overlapped with the end of the previous treatment period. Data from the United States Geological Survey.

Seasonal Recreational River Use

Previous research documented that seasonally, recreational use of the river is highest in the summer, moderate in the fall, and lowest in winter and spring (K. Huffaker, River Watchers, *pers. comm* 2015; Crawford-Reynolds et al. 2016). Stationary game cameras were positioned to quantify recreational use in the upper reaches of the river associated with more popular river access locations. The cameras recorded images documenting recreational use (number of boaters, people tubing, swimmers, anglers, and dogs) on an hourly basis at two locations in Sewell Park, one location at City Park and one location at Rio Vista Park (Figure 3.1). With the exception of the Eastern Spillway study site (minimal contact recreation), these locations represent data upstream of the *in situ* treatment locations. The study sites were also selected to provide maximum exposure to sunlight with a depth no greater than one meter based on data, the maximum suitable depth for TWR suggested by TPWD (Saunders et al. 2001).

Texas Wild Rice Propagation and Experimental Growth Parameters

For each of the three experimental time periods (two high-recreational use periods and one pre-recreational use period), 200 TWR plants were propagated from seed (Texas Parks and Wildlife permit #INT 17 02-21b) using a single 5L germination container in an outdoor flume at the USFWS San Marcos Aquatic Research Center (SMARC), San Marcos, Texas. Commercially purchased soil used for propagation and growth consisted of 50 percent concrete sand, 50 percent loam, and 0.64cm pea size gravel as recommended by SMARC staff for TWR propagation. Seeds were allowed to grow for two weeks after which 120 plants were randomly selected and transferred to 1.56L plastic plant containers. Plants were then allowed to grow for an additional two weeks. After the four-week total growth period, 75 plants were randomly selected for use in the *in situ* study. From these, 15 plants were randomly selected to measure initial plant size and biomass to use as a baseline at initiation of each study period. I randomly assigned the remaining 60 plants to one of four study sites in the river. The study sites were located in San Marcos River State Scientific Areas (SSA) established by Texas Parks and Wildlife Department to protect TWR and to minimize direct disturbance from recreational activities (EARIP 2012). Plant trays were positioned in locations that had approximately the same depth, velocity range and light exposure at the start of each experiment. Once the initial locations of plant trays were selected, they were not moved in response to apparent changes in depth and velocity due to changes in discharge. I placed three trays, each containing five randomly selected TWR plants, at each study site. Plants remained in the treatment study sites for a period of six to eight weeks. At the end of each treatment period (July 2014, May 2015, July 2015) I collected all remaining TWR plants in study trays for analysis. Upon retrieval of plants at the end of the experimental period, shoots were separated from the root mass at the juncture. The number of individual shoots and roots (excluding root hairs) was recorded. The longest intact shoot from each plant was selected for analysis. For this study, a shoot was defined as the length from the root-shoot juncture to the terminus of the leaf. Each selected shoot was measured for total leaf surface area and the entire shoot area was manually scraped with a gloved index finger five times on the adaxial and abaxial surfaces to remove material for total and non-volatile (inorganic) solids and chlorophyll-*a* analysis. Plant material was placed in a pre-cleaned container and filled to 1L with distilled water. Non-volatile solids (NVS) are a measurement of the amount of inorganic material trapped within periphyton on the leaf surface. Chlorophyll-*a* measurements reflect the amount of periphyton on the leaf surface. Care was taken not to scrape shoots in such a manner as to contaminate the samples with shoot material. A 20ml aliquot from each sample was filtered through pre-weighed Gelman A/E pre-ashed filters to assess total and non-volatile solids. Total solids were determined after drying at 60°C for 48h and then reweighing. Filters were subsequently ashed at 500°C for 4h and reweighed to determine non-volatile solids. A 20ml aliquot from the initial material collected from shoot surfaces was used to determine chlorophyll-*a* (Chl-*a*). Each 20 ml sample was filtered onto a weighed Gelman GFF pre-ashed filter. Filters were extracted with 90% acetone after 24h and analyzed using a Trilogy Laboratory fluorometer. All shoots and roots for each plant were separated and dried for 48 hours at 60˚C in a drying oven and weighed to determine shoot and root dry biomass.

PAR values were measured at the time of the water quality grab sample using a dual channel Li-Cor LI 1935A meter fitted with a 4π sensor at the water surface, immediately below the water surface, and at 10cm, 20cm, 30cm, and 40cm below the water surface. PAR and water quality grab samples for Sewell Park, City Park, and Bicentennial Park were collected downstream of any observed contact recreational activities at the site and closest to the thalweg of the river. The Sewell Park and Bicentennial Park collections occurred within the State Scientific Area (SSA) on river right. The collection site for City Park also occurred river right, but not within a SSA.

Two separate grab samples from the river water column were collected manually in a one-liter Nalgene bottle. The two grab samples were collected at each study site immediately following the collection of PAR values. After collection, samples were transported to the lab for total solids (TS) and non-volatile solid (NVS) analysis. Each 1L sample was filtered separately through pre-weighed Gelman A/E pre-ashed filters. Total solids were determined after drying at 60˚C for 48h and then reweighing. Filters were subsequently ashed at 288˚C for 4h and reweighed to determine non-volatile solids.

Statistical Analysis

Data were checked for assumptions of normality and homogeneity of variance. When necessary data were $\log_{10}$ transformed to attain normality. Differences within and among treatment periods for total solids, non-volatile solids, and chlorophyll-*a* data as well as TWR growth data including dry weight biomass, above/below ground biomass, total biomass, shoot number, root number, were analyzed through a one-way ANOVA. If a result was found to be significant, the ANOVA was followed by a pairwise comparison of means using Tukey's HSD test at a minimum level of $p < 0.05$. All ANOVA and Tukey HSD tests were performed using R 3.4.0 studio. Details of each analysis are included in the Results section, or in the captions to tables.

Results

Texas Wild Rice Growth Parameters

The numbers of individual plants at the end of the baseline period and the *in situ* study treatment periods and the average value for above ground, below ground, above/below ground biomass, total biomass, shoot number, and root number could be collected are provided in Tables 3.1 through 3.4. For the 2015 treatments, Tables 3.2 and 3.3 also provide the average value for TS, NVS and Chl-*a*. Note that TS, NVS and Chl-*a* were collected from TWR leaves during 2015 to examine potential differences associated with periphyton dynamics. Table 3.1 shows the remaining number of plants at each study site for each of the three *in situ* experiments. As can be seen from Table 3.1, substantial loss of plants occurred at both the Bicentennial Park and Ramon Lucio locations during the 2014 study period and at Ramon Lucio during the high recreation period in 2015. Loss of plants at Bicentennial Park during the 2014 *in situ* experiment was due to plants being smothered with fine sediment deposition. Plant loss at Ramon Lucio during 2014 was associated with vandalism.

Significant differences in shoot biomass, root biomass, above/below biomass, total biomass, shoot number, and root number of TWR existed among longitudinal study site locations during the three-time periods for the *in situ* San Marcos River study (Tables 3.1 through 3.3). TWR plants at the ES site exhibited significantly greater growth than did plants at the first downstream treatment site (SP site) during the high recreation periods. Significantly greater TWR shoot biomass (High- Rec 2014: $p < 0.01$; High-Rec 2015: $p = 0.01$), root biomass (High-Rec 2015: $p = 0.06$), above/below biomass (High-Rec 2014: $p < 0.01$), total biomass (High-Rec 2014: $p < 0.01$; High-Rec 2015: $p = 0.01$), shoot number (High-Rec 2015: $p < 0.01$), and root number (High-Rec 2015: $p < 0.01$) were found at the ES site compared to the SP site. A significantly greater shoot number was also found at the ES site compared to the first downstream treatment site at SP in the low recreation period in 2015 ($p = 0.01$).

Plants at the ES site exhibited significantly greater biomass compared to plants at the second downstream site (BP site) in 2014 during high recreation conditions ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} = 0.02$; $p_{above/below\ biomass} < 0.01$; $p_{total\ biomass} < 0.01$) and produced fewer roots ($p_{root\ number} = 0.02$). A significantly greater shoot biomass was also found at the ES site compared to the plants at the BP site in the high recreation period in 2015 ($p = 0.04$).

TWR plants at the ES site exhibited significantly greater growth during the three study periods when compared to plants at the farthest downstream site (RL site). Plants at the ES site exhibited significantly greater shoot biomass (High-Rec 2014: p = 0.02; Pre-Rec 2015: p = 0.04; High-Rec 2015: p < 0.01), above/below biomass (High-Rec 2014: p < 0.01), total biomass (High-Rec 2014: p = 0.01; Pre-Rec 2015: p = 0.04; High-Rec 2015: p = 0.01), shoot number (Pre-Rec 2015: p = 0.01; High-Rec 2015: p < 0.01), and root number (High-Rec 2015: p < 0.01).

Additional significant differences in TWR biomass existed in plants growing at the SP and RL treatment sites when compared to plants at the BP site in the 2015 study periods. When compared to the BP site, plants at the SP site exhibited significantly less growth in the Pre-Rec 2015 period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} = 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$). In the High-Rec 2015 study period only root number (p = 0.03) was found to be significantly lower in SP site plants. Plants growing at the RL treatment site exhibited significantly less growth compared to plants at the BP site in Pre-Rec 2015 ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} = 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$). In High-Rec 2015 only shoot biomass (p = 0.05) and root number (p = 0.01) were found to be significantly lower in the RL site plants.

Compared to initial baseline growth data significant differences were found in shoot biomass, root biomass, above/below biomass, total biomass, shoot number, and root number of TWR plants grown in the *in situ* San Marcos River study (Tables 3.1 through 3.3). TWR plants grown at the ES site exhibited increased growth in the High-Rec 2014 study period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} < 0.01$; $p_{above/below\ biomass} < 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$) and the High-Rec 2015 study period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} < 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} <$

0.01). TWR plants grown at the ES site also exhibited increased growth in the Pre-Rec 2015 study period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} = 0.02$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$).

When compared to baseline growth data in the High-Rec 2014 study period, plants grown at the SP site exhibited greater root biomass ($p = 0.01$) and increased shoot ($p < 0.01$) and root ($p < 0.01$) number, and in the High-Rec 2015 period increased root number ($p = 0.05$) Plants grown at the BP site exhibited significantly greater growth in the Pre-Rec 2015 study period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} < 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$). Plants grown at the BP site also exhibited increased growth in the High-Rec 2015 study period ($p_{shoot\ biomass} < 0.01$; $p_{root\ biomass} = 0.01$; $p_{total\ biomass} < 0.01$; $p_{shoot\ number} < 0.01$; $p_{root\ number} < 0.01$). However, plants grown at the RL site did not exhibit significant increased growth when compared to baseline values, except for greater above/below biomass ($p < 0.01$).

No significant differences in chlorophyll-*a* measurements were found, indicating no difference in the amount of periphyton on the leaf surface of plants at the different study sites under either High-Rec or Pre-Rec conditions. Differences in TS and NVS were also evident when comparing the Pre versus High-Rec 2015 results. However, significant differences in TS and NVS were found between sites, but only in the Pre-Rec 2015 study period. When compared to the BP site, lower amounts of TS and NVS were present on leaves of plants at the other study sites. When compared to plants at the BP site, significantly less TS ($p < 0.01$) and NVS ($p < 0.01$) were found on leaves of plants at the ES site. Leaves of plants located at the first downstream site (SP) were also found to be significantly lower in TS ($p < 0.01$) and NVS ($p < 0.01$) when compared to the BP site. Additionally, when the farthest downstream site (RL) was compared to the BP site significantly less TS ($p < 0.01$) and NVS ($p = 0.01$) were also found (Tables 3.2- 3.5). The number of individual plants at the end of the baseline and the study periods (High-Rec 2014, Pre-Rec 2015, High-Rec 2015) for which the data could be collected included, shoot dry weight, root dry weight, shoot number, root number, total solids, non-volatile solids, and chlorophyll-*a* are summarized in Tables 3.1-3.4.

Table 3.1 Mean baseline and treatment values for shoot dry weight, root dry weight, shoot/root, total biomass, shoot number, and root number for the *in situ* High-Rec 2014 study.

	Above ground (g) (Shoot)	**Below ground (g) (Root)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**
Baseline (15)	0.15 (±0.02)	0.03(±0.00)	5.00	0.17 (±0.03)	7 (±1)	8 (±1)
Eastern Spillway (10)	60.82(±13.51) [c]	1.99(±0.34) [c]	30.56 [c]	62.81(±3.70) [c]	49(±11) [c]	65(±7) [c]
Sewell Park (10)	4.83(±0.79) [a]	1.49(±0.33) [c]	3.24 [a]	6.32(±1.04) [a]	50(±6) [c]	61(±7) [c]
Bicentennial Park (5)	0.47(±0.19) [a]	0.33(±0.11) [a]	1.42 [a]	0.70(±0.42) [a]	24(±10) [c]	23(±6) [a]
Ramon Lucio (3)	1.24(±1.18) [a]	0.45(±0.35)	2.76 [a]	1.27(±1.07) [a]	38(±13)	28(±15)

Standard error in parentheses; n= number of plants remaining after baseline and treatment

[a] Values significantly different from the Eastern Spillway within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[b] Values significantly different from Bicentennial Park within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[c] Values significantly different from Baseline within a treatment period (α=0.05) using Tukey's test following an ANOVA.

Table 3.2 Mean baseline and treatment values for shoot dry weight, root dry weight, shoot/root, total biomass, shoot biomass, root number, total solids, non-volatile solids, and chlorophyll-*a* for the *in situ* Pre-Rec 2015 study.

	Above ground (g) (Shoot)	**Below ground (g) (Root)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total solids (mg/cm^2)**	**Non-volatile solids (mg/cm^2)**	**Chlorophyll-*α* (mg/cm^2)**
Baseline (15)	0.04 (±0.00)	0.01 (±0.00)	4.00	0.05 (±0.00)	5 (±0)	7 (±0)	NC	NC	NC
Eastern Spillway (15)	1.86(±0.39) [c]	0.80(±0.35) [c]	2.33	2.57(±0.78) [c]	35(±6) [c]	38(±6) [c]	344.63(±80.74) [b]	219.37(±64.29) [b]	1.96(±0.57)
Sewell Park (14)	0.50(±0.12) [b]	0.09(±0.02) [b]	5.56	0.59(±0.38) [b]	11(±2) [a b]	23(±3) [b]	269.56(±82.23) [b]	142.08(±55.41) [b]	3.52(±1.09)
Bicentennial Park (12)	3.42(±0.74) [a c]	0.73(±0.23) [c]	4.68	4.45(±0.99) [c]	51(±9) [c]	61(±11) [c]	1696.38(±383.20)	1220.25(±295.45)	3.83(±1.39)
Ramon Lucio (12)	0.29(±0.09) [a b]	0.07(±0.02) [b]	4.14 [c]	0.36(±0.31) [a b]	11(±2) [a b]	18(±2) [b]	62.17(±17.39) [b]	14.74(±10.25) [b]	4.16(±1.02)

Standard error in parentheses; n= number of plants remaining after baseline and treatment; NC data not collected

[a] Values significantly different from the Eastern Spillway within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[b] Values significantly different from Bicentennial Park within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[c] Values significantly different from Baseline within a treatment period (α=0.05) using Tukey's test following an ANOVA.

Table 3.3 Mean baseline and treatment values for shoot dry weight, root dry weight, shoot/root, total biomass, shoot number, root number, total solids, non-volatile solids, and chlorophyll-*a* for the *in situ* High-Rec 2015 study.

	Above ground (g) (Shoot)	**Below ground (g) (Root)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total solids (mg/cm^2)**	**Non-volatile solids (mg/cm^2)**	**Chlorophyll-*α* (mg/cm^2)**
Baseline (15)	0.15 (±0.02)	0.02 (±0.00)	7.50	0.16 (±0.02)	6 (±0)	10 (±1)	NC	NC	NC
Eastern Spillway (14)	8.58(±1.24) [c]	4.32(±1.22) [c]	1.99	14.71(±2.90) [c]	128(±14) [c]	105(±11) [c]	113.59(±31.23)	28.84(±13.10)	1.38(±0.26)
Sewell Park (15)	3.62(±0.73) [a]	0.60(±0.13) [a]	6.03	4.18(±1.01) [a]	39(±9) [a]	45(±6) [a b c]	132.68(±28.26	44.83(±14.38)	2.22(±0.30)
Bicentennial Park (14)	6.37(±1.44) [c]	4.25(±1.38) [c]	1.50	12.31(±2.38) [c]	111(±21) [a c]	91(±18) [c]	107.96(±30.61)	22.83(±15.55)	2.26(±0.56)
Ramone Lucio (4)	0.06(±0.01) [a b]	0.05(±0.00)	1.20	0.11(±0.01) [a]	9(±1) [a]	10(±0) [a b]	8.90(±3.51)	NC	2.18(±0.83)

Standard error in parentheses; n= number of plants remaining after baseline and treatment.

NC data not collected

[a] Values significantly different from the control within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[b] Values significantly different from Bicentennial Park within a treatment period (α=0.05) using Tukey's test following an ANOVA.

[c] Values significantly different from baseline within a treatment period (α=0.05) using Tukey's test following an ANOVA.

Table 3.4 Results of ANOVA baseline and treatment for shoot dry weight, root dry weight, above/below biomass, total biomass, shoot number, root number, and total solids, non-volatile solids, and chlorophyll-*a* for the *in situ* High-Rec (HR) 2014, Pre-Rec (PR) 2015, and High-Rec (HR) 2015 studies.

HR 2014	***F*-value**	**df**	***p***	**PR 2015**	***F*-value**	**df**	***p***	**HR 2015**	***F*-value**	**df**	***p***
SDW				**SDW**				**SDW**			
Baseline	16.54	4,40	<0.01	**Baseline**	15.13	4,63	<0.01	**Baseline**	13.38	4,55	<0.01
Treatment	11.82	3,26	<0.01	**Treatment**	11.89	3,49	<0.01	**Treatment**	6.46	3,41	<0.01
RDW				**RDW**				**RDW**			
Baseline	10.15	4,42	<0.01	**Baseline**	7.45	4,62	<0.01	**Baseline**	5.70	4,57	<0.01
Treatment	4.17	3,28	0.02	**Treatment**	5.86	3,49	0.00	**Treatment**	5.84	3,48	0.05
A/B				**A/B**				**A/B**			
Baseline	21.46	4,42	<0.01	**Baseline**	4.04	4,62	0.01	**Baseline**	1.29	4,55	0.29
Treatment	15.51	3,26	<0.01	**Treatment**	1.61	3,48	0.20	**Treatment**	1.06	3,41	0.38
TB				**TB**				**TB**			
Baseline	17.96	4,42	<0.01	**Baseline**	13.46	4,62	<0.01	**Baseline**	11.66	4,57	<0.01
Treatment	13.35	3,26	<0.01	**Treatment**	10.61	3,49	<0.01	**Treatment**	6.34	3,43	<0.01
SN				**SN**				**SN**			
Baseline	6.39	4,46	<0.01	**Baseline**	16.64	4,63	<0.01	**Baseline**	29.27	4,57	<0.01
Treatment	0.72	4,31	0.58	**Treatment**	12.34	3,49	<0.01	**Treatment**	18.83	3,43	<0.01
RN				**RN**				**RN**			
Baseline	13.96	4,42	<0.01	**Baseline**	13.62	4,63	<0.01	**Baseline**	19.33	4,57	<0.01
Treatment	3.96	3,28	0.02	**Treatment**	8.45	3,49	<0.01	**Treatment**	9.83	3,43	<0.01
				TS				**TS**			
				Treatment	15.09	3,49	<0.01	**Treatment**	1.48	3,10	0.24
				NVS				**NVS**			
				Treatment	7.65	3,34	<0.01	**Treatment**	0.08	2,18	0.92
				Chl-*α*				**Chl-*α***			
				Treatment	1.25	3,34	0.30	**Treatment**	1.1	3,40	0.36

Recreation Induced River Turbidity

Data from water quality sondes in the San Marcos River indicate that daily mean and median turbidity in the river varies temporally, with this variation likely related to the timing of recreational activities (Table 3.5). In general, turbidity in the river is higher during the period of April – October when recreational activities are higher. In addition, sonde data for turbidity (reported as Nephelometric Turbidity Units - NTU) are examined during the periods defined by this study in 2014 and 2015, turbidity in the river was generally higher during both High-Rec periods in 2014 and 2015 (Tables 3.6 and 3.7). It is also apparent from these data that the turbidity generally increases around two orders of magnitude from the upper portion at the Eastern Spillway (Aquarena Drive and Hopkins Street) to the more downstream sonde sites (Rio Vista and IH-35). In addition, the magnitude of diel variation in turbidity appears to be associated with the timing of recreational activities in the river (Figure 3.3). Indeed, examination of data during the July 4th weekend in 2015 indicates, that turbidity increased to >50 NTU for several hours during the daytime when large crowds of people were recreating in the river. In contrast, during a period of low recreational activity in late November 2015, the time series of NTUs showed very little diel variation and the magnitude never exceeded 7.5 NTU based on observed 15-minute readings.

Table 3.5 Long-term daily mean and median turbidity NTUs in the San Marcos River for 2015 at Rio Vista Park.

NTU	Mean	Median
Jan	2.8	0
Feb	13.3	1.4
Mar	3.1	1.9
Apr	53.4	2.3
May	15.6	1.8
June	17.2	0.9
July	18.3	1.2
Aug	101.8	16.5
Sept	66.4	1.1
Oct	16.8	0.3
Nov	17.1	2.1
Dec	2.9	1.8

Table 3.6 Long-term mean daily turbidity (NTUs) from Edwards Aquifer Authority Hydrologic Data reports and unpublished data (W. Nowlin, B. Schwartz, and T. Hardy) from real time water quality sondes for Aquarena through Hopkins in the San Marcos River for the experimental time periods in 2014 and 2015.

	pH	◦C	NTU	µS/cm
HI REC 2014 AQUARENA				
Min	7.10	21.46	0.00	571.20
Mean	7.17	22.82	2.08	598.14
Max	7.27	24.32	960.10	603.70
PRE REC 2015 AQUARENA				
Min	6.72	21.03	0.00	186.30
Mean	7.19	22.30	28.63	583.72
Max	7.35	23.74	3615.00	620.00
HI REC 2015 AQUARENA				
Min	6.90	22.04	0.00	364.70
Mean	7.25	22.54	141.23	618.61
Max	7.39	23.39	4795.00	633.20
HI REC 2014 HOPKINS				
Min	7.37	19.62	0.50	156.76
Mean	7.47	22.44	16.42	551.82
Max	7.63	24.00	1391.10	595.62
PRE REC 2015 HOPKINS				
Min	6.94	21.71	0.00	356.82
Mean	7.09	21.86	1.23	592.05
Max	7.25	23.18	127.50	624.15
HI REC 2015 HOPKINS				
Min	6.99	21.62	0.00	289.56
Mean	7.06	22.04	3.10	613.63
Max	7.25	23.16	129.40	621.30

Edwards Aquifer Authority sonde data (Aquarena)
Meadows Center for Water and the Environment sonde data (Hopkins)

Table 3.7 Long-term mean daily turbidity (NTUs) from Edwards Aquifer Authority Hydrologic Data reports and unpublished data (W. Nowlin, B. Schwartz, and T. Hardy) from real time water quality sondes for Rio Vista through IH-35 in the San Marcos River for the experimental time periods in 2014 and 2015.

	pH	◦C	NTU	µS/cm
HI REC 2014 RIO VISTA				
Min	7.44	21.76	1.40	238.90
Mean	7.52	22.91	7.68	597.43
Max	7.67	24.81	827.80	605.10
PRE REC 2015 RIO VISTA				
Min	6.81	20.81	0.00	181.70
Mean	7.31	22.28	20.22	576.36
Max	7.62	23.78	4757.00	602.10
HI REC 2015 RIO VISTA				
Min	7.23	22.00	0.00	138.50
Mean	7.35	22.55	3.47	612.50
Max	7.87	24.76	24.76	623.40
HI REC 2014 IH-35				
Min	7.00	19.07	0.00	101.34
Mean	8.16	22.49	5.57	511.73
Max	10.14	24.79	628.80	644.77
PRE REC 2015 IH-35				
Min	7.33	17.50	6.00	187.45
Mean	7.58	19.35	191.17	602.94
Max	8.02	31.29	3183.80	638.53
HI REC 2015 IH-35				
Min	6.66	17.95	29.10	171.09
Mean	7.57	18.86	232.03	598.59
Max	8.28	21.56	3278.10	636.08

Edwards Aquifer Authority sonde data (IH-35) and unpublished data from W. Nowlin, B. Schwartz, and T. Hardy sonde data (Rio Vista)

Figure 3.3 shows turbidity levels at Aquarena Bridge (lower boundary of the Eastern Spillway study site) and at Rio Vista during the July 4th, 2014 week associated with high recreation and late November 2014 during low recreation. Figure 3.4 shows an example of the turbidity response over time to rainfall events in the San Marcos River at Rio Vista.

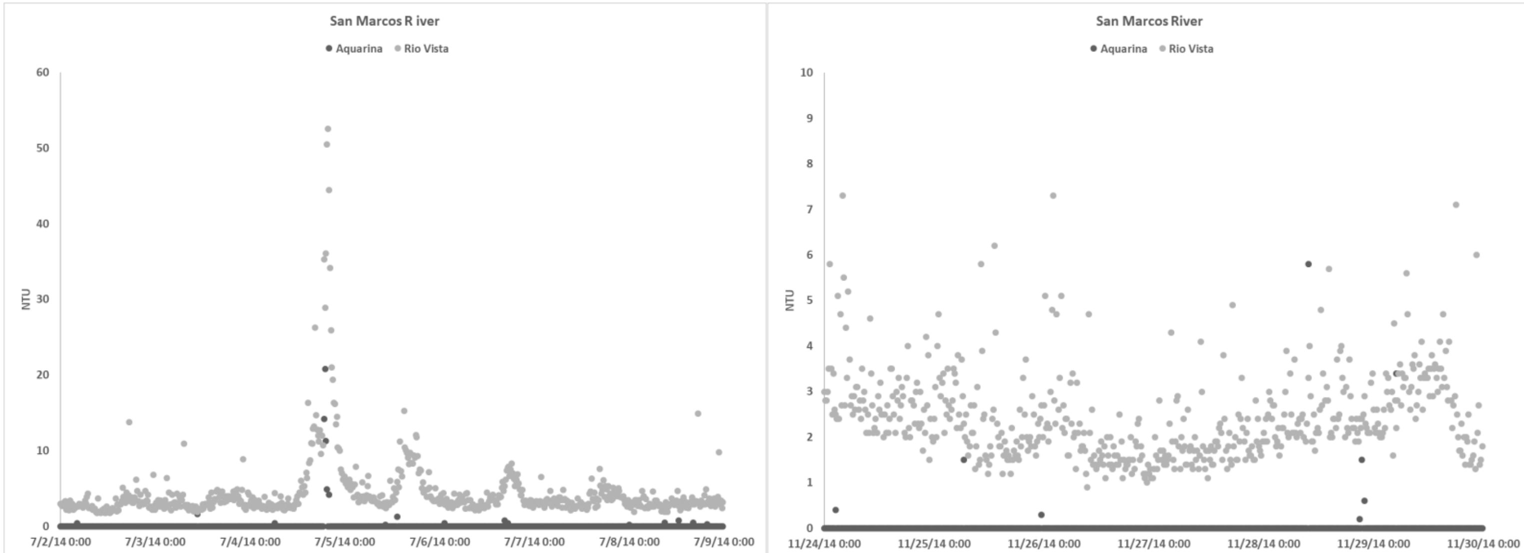


Figure 3.3 Diel, weekly and seasonal patterns in observed turbidity (NTUs) in the San Marcos River. Data from the Edwards Aquifer Authority Hydrologic Data and unpublished data (W. Nowlin, B. Schwartz, and T. Hardy) from real time water quality monitoring sondes.

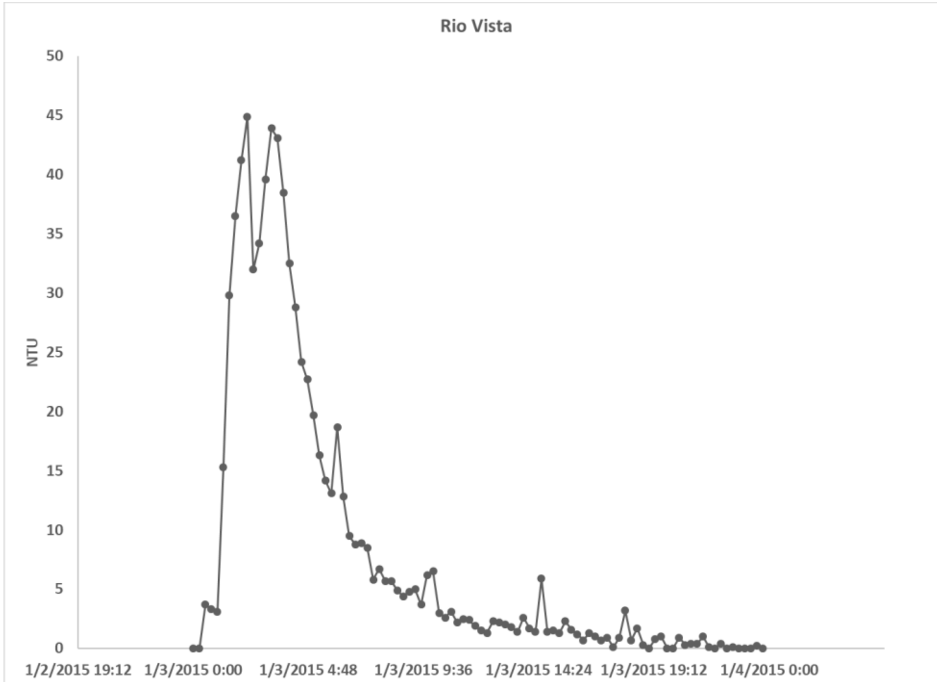


Figure 3.4. Turbidity (NTUs) response over time to rainfall events in the San Marcos River at Rio Vista. Data from the Edwards Aquifer Authority Hydrologic Data report and unpublished data (W. Nowlin, B. Schwartz, and T. Hardy) from real time water quality monitoring sondes.

Mean daily turbidity data demonstrate a significant increase (Aug 9, 2016: $F_{1,22}$ = 2893; p = 0.00; Sept 3, 2016: $F_{1,24}$ = 224; p = 0.00) from the upstream Aquarena site to the downstream Rio Vista site (Table 3.8) on both August 9 and September 3, demonstrating a longitudinal turbidity gradient along the river attributed to contact recreation between these two sites.

Table 3.8 Turbidity data (NTUs) for Edwards Aquifer Authority Hydrologic Data report for real time water quality sondes for Aquarena through Rio Vista in the San Marcos River for PAR collection dates in 2016.

	Min	Ave	Max
AUG 9, 2016 AQUARENA	0.00	0.00	0.00
AUG 9, 2016 RIO VISTA	2.48	2.69*	3.10
SEPT 3, 2016 AQUARENA	0.00	0.00	0.00
SEPT 3, 2016 RIO VISTA	3.58	4.84*	8.60

* Values significantly different from Aquarena (α=0.05) using Tukey's test post ANOVA

On August 9, recreational use of the river was high at SP (~ 400-500 individuals during the collection period), but minimal at the other two sites. On September 3 and October 7, recreational use in the river was higher at CP (~ 1000-2000 individuals) and BP (~1000-2000 individuals) than what was observed on August 9. I observed recreational use in the river at SP primarily to be swimming. CP recreation included swimming, as well as wading and tubing. CP is the location of the Lions Club tube rental facility and is the major entry point for tubing in the river. BP recreation was limited to primarily tubing.

Turbidity Effects on PAR

Table 3.9 shows the vertical light extinction coefficient at the water surface to a water depth of 40cm on the three collection dates at each study site. The data show greater light attenuation with depth at the most downstream site compared to upstream sites and is attributed to recreational induced turbidity.

Table 3.9 Slope data (*k*; μmolm^{-2}s^{-1}) for collection sites Sewell Park, City Park, and Bicentennial Park on collection dates.

Collection site	**AUG 9, 2016** **Depth *k***	**SEPT 3, 2016** **Depth *k***	**OCT 7, 2016** **Depth *k***
Sewell Park	y=-0.0025x+4.6066 R^2=0.883310	y=-0.0050x+4.5694 R^2=0.879510	y=-0.0036x+4.5956 R^2=0.883310
City Park	y=-0.0026x+4.6092 R^2=0.9621	y=-0.0093x+4.6118 R^2=0.9739	y=-0.0039x+4.6101 R^2=0.9904
Bicentennial Park	y=-0.0046x+4.5904 R^2=0.9501	y=-0.0197x+4.6821 R^2=0.8896	y=-0.0160x+4.6189 R^2=0.9761

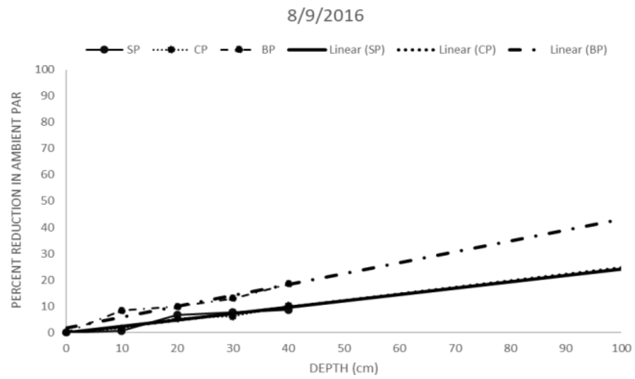
Table 3.10 represents the river water grab sample data for non-volatile solids on collection dates at SP, CP, and BP. Grab sample data on both August 9 and September 3 demonstrate a significant longitudinal increase in non-volatile solids from the upstream SP site to the more downstream CP ($F_{2,60}$ = 18.64; p < 0.00) and BP sites ($F_{2,60}$ = 33.24; p < 0.00). Increased non-volatile solids demonstrate a longitudinal gradient resulting from increasing suspended sediment from the upstream site to the more downstream site and may be attributed to upstream contact recreation.

Table 3.10 River water grab sample data for non-volatile solids (mg/mL) for collection sites located at Sewell Park, City Park, and Bicentennial Park.

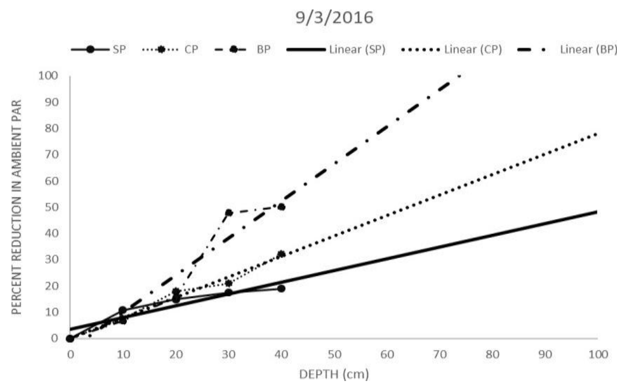
		Min	Ave	Max
AUG 9, 2016	**SP**	117.05	117.83	118.60
	CP	120.56	120.89*	121.21
	BP	117.70	119.55*	121.39
SEPT 3, 2016	**SP**	116.57	117.23	117.90
	CP	117.86	119.15*	120.43
	BP	118.04	119.58*	121.11
OCT 7, 2016	**SP**	117.50	118.77	120.03
	CP	117.63	118.63	119.63
	BP	118.90	119.23	119.56

* Values significantly different from Sewell Park (α=0.05) using Tukey's test post ANOVA

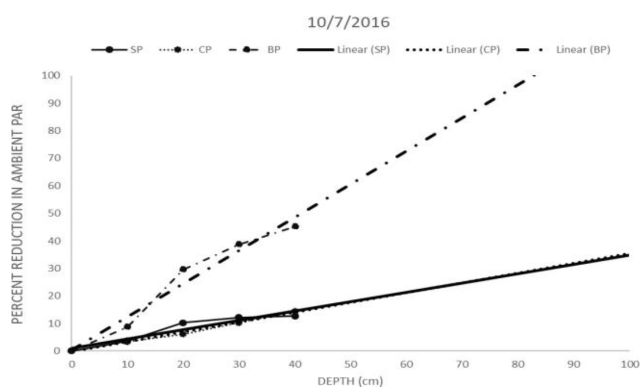
Figure 3.5 shows estimated reduction in ambient PAR up to depths of 100cm on the three collection dates. My research has shown that there is a significant reduction in TWR biomass at 20% ambient PAR or lower (see Chapter 2). On August 9, 40% ambient PAR was predicted at a depth of 100cm at BP. A level of 20% ambient PAR was predicted at depths of 60-70cm at BP on September 3 and October 7.



(a)



(b)



(c)

Figure 3.5 Percent reduction in ambient PAR (solid line) measured from water surface (0) to 40cm below surface and predicted model trend (dotted line) in percent reduction of ambient PAR from water surface to 100cm below surface at collection sites in the San Marcos River. The collection sites are Sewell Park (SP), City Park (CP), and Bicentennial Park (BP). Data were collected on (a) Tuesday, August 9, 2016; (b) Saturday, September 3, 2016; (c) Friday, October 7, 2016.

Discussion

The availability of light (e.g., photosynthetically active radiation, PAR) is an important abiotic factor affecting aquatic plant biomass (Madsen 1993; Blanch et al. 1998; Kurtz et al. 2003; Case et al. 2004; Stanton et al. 2010). Periphyton on the surfaces of macrophyte leaves has been shown to suppress overall biomass production due to reduced levels of photosynthetically active radiation (Asaeda et al. 2004; Tóth 2013). In studies conducted by Asaeda et al. (2004), Kiørbe (1980), and Yu-Zhi Song et al. (2017), decreases in growth and production for *Potamogeton pectinatus*, *Ruppia cirrhosa*, and *Myriophyllum spicatum* resulted from the shading effects associated with leaf area accumulation of epiphytic algae, a component of periphyton. However, no difference in the amount of periphyton on the leaf surface of plants at the different study sites under either High-Rec or Pre-Rec conditions was found in this study. Therefore, inhibition by periphyton does not account for observed differences in TWR growth for this study. It is hypothesized that the preference for TWR for areas of moderate to high velocity (Tolman et al. 2014) with long linear leaf structure that 'waves' in the velocity field may inhibit accumulation of fine sediments associated with leaf area periphyton. This dynamic with velocity is supported by study results during High-Rec 2014, where reduced flows and consequently velocity at the BP study site plant location resulted in higher TWR mortality due to increased siltation on the plant leaves. Initial propagation of TWR in ponds were unsuccessful as part of the EAHCP restoration efforts where germinated seedlings died after a few weeks. Placement of pumps within the ponds to generate a velocity field resulted in successful germination and growth sufficient to permit successful reintroduction to the San Marcos River

(Thom Hardy, *pers. comm.*, 2018).

Turbidity is known to be a significant factor limiting light availability and can result in reductions in vertical light attenuation in the water column (Robel 1961; Barko et al. 1986; Kirk 1994; Moore et al. 1997; Blanch et al. 1998; Doyle and Smart 2001). The availability of light in the aquatic environment has been demonstrated to be of major importance in plant productivity, predominance, and distribution (Kirk 1994; Case et al. 2004). Results from light reduction studies on *Potamogeton pectinatus*, *Hydrilla*, *Myriophyllum*, *Vallisneria americana*, *Zizania aquatica*, and *Z. palustris* have shown that when freshwater macrophytes were exposed to low light environments reductions in biomass did occur (Robel 1961; Barko and Smart 1981; Blanch et al. 1998; Doyle and Smart 2001; Kurtz et al 2003; Pillsbury and Bergey 2009; Clay and Oelke 2017). Results from the Chapter 2 study on light reduction in the absence of turbidity, parallel these findings and demonstrate that TWR exhibits a decrease in biomass when exposed to low PAR availability.

Increased turbidity from urbanization and recreational activity has been shown to impact plant growth in aquatic systems. Increased turbidity levels can reduce vertical light attenuation in the water column (Kirk 1994), resulting in a reduction in photosynthetically active radiation reaching plants at a given water depth. Pillsbury and McGuire (2009) studied the impacts of urbanization on the growth of Southern wild rice, *Zizania aquatica*, in the Great Lakes. They found decreases in rice and other aquatic macrophytes to be related to urbanization within the watershed leading to among other factors, increased turbidity. Hall and Härkönen (2006) found that increased wave action from recreational motor boats resulted in increased turbidity in the water column, which resulted in reduced growth and increased mortality of *Zizania aquatica*. Increases in recreation induced suspended sediment and associated turbidity have been suggested to be problematic for *Zizania texana* through the reduction in available light in the water column (Bradsby 1994). Sonde data show turbidity generally increases around two orders of magnitude from the upper portion of the San Marcos River at the Eastern Spillway (ES) to the farthest downstream treatment site at Ramon Lucio (RL). The ES site has very limited upstream contact recreational activity compared to the downstream treatment sites, which all have substantial upstream recreational activities. PAR reduction with depth due to turbidity suggests that, at times, recreation induced turbidity has the potential to impact TWR biomass production. This reduction is most likely to occur with increasing water depths. Significant differences in shoot biomass, root biomass, above/below biomass, total biomass, shoot number, and root number existed among plants growing at longitudinal study site locations. In general, plants at the furthest upstream ES site grew significantly better than plants growing further downstream. These results indicate that the increased longitudinal turbidity gradient due to contact recreation has the potential to impact TWR growth dynamics.

In addition to the potential effects of a longitudinal turbidity gradient affecting depth dependent magnitude of PAR, water quality data indicate a decrease in CO_2 concentration moving from upstream to downstream in the San Marcos River (Saunders et al. 2001). TWR is a CO_2 obligate (Power and Doyle 2004) and therefore the CO_2 gradient may in part account for some of the observed differences in growth rates.

In previous work by Tolman (2013) and Tolman et al. (2014), light availability was suggested to be a factor that should be considered in determining locations to reintroduce TWR in the San Marcos River. Results indicated that stream areas with open sky or low canopy cover (i.e., low riparian shading) had higher areal coverage of TWR. Köhler et al. (2009) found a significant reduction in biomass of submerged macrophytes (*Sagittaria sagittifolia*, *Riccia fluitans*, *Potamogeton pectinatus*, *Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Sparganium emersum*) was detected at high shade levels, associated with riparian coverage and depth, when compounded by leaf area epiphytes. Planting in deeper river sections with high canopy cover in combination with high levels of turbidity may reduce the effectiveness of restoration efforts due to reduced PAR availability at the river bottom.

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IV. IMPLICATIONS FOR MANAGEMENT AND RESTORATION FOR TEXAS WILD RICE (*ZIZANIA TEXANA* HITCHC.)

The Edwards Aquifer is a highly utilized resource providing the primary source of drinking water to over two million people within south-central Texas and relied on extensively to support municipal, industrial, and agricultural water needs throughout this region. The aquifer is also the source of the two largest spring systems in Texas that directly create the headwaters of the Comal and the San Marcos rivers. In particular, the San Marcos Springs have been characterized as having the most constant discharge regime of any spring system in the southwestern United States, and in contrast, to the Comal Springs which ceased flowing during the drought of record in the 1950s, have never ceased flowing within recorded history (Brune 1981). These spring systems are also characterized by high water quality, constant temperatures, pH, and dissolved ion concentrations (Saunders et al. 2001; Edwards Aquifer Recovery Implementation Program 2012). The aquifer, springs, and associated headwaters of the Comal and San Marcos rivers provide habitat for eight endemic species that are federally-listed as threatened or endangered. These includes the endangered Texas wild rice (TWR; *Zizania texana*) which is confined to the upper few kilometers of the San Marcos River above the confluence with the Blanco River. The species was listed due to its single spatially restricted population and threats including reduced or cessation of flow from aquifer pumping, changes in runoff hydrograph characteristics due to urbanization in the watershed, deterioration of water quality from point and non-point sources of pollution, habitat modifications, introduced non-native plant and animal species and impacts from water-based recreation (USFWS 1996; Edwards Aquifer Recovery Implementation Program 2012).

Growing concerns over the increased utilization of the Edwards Aquifer, population growth and impacts from these combined threats resulted in a lawsuit by the Sierra Club in 1991 to force the United States Fish and Wildlife Service (USFWS) to designate critical habitat and undertake protective measures for these ecosystems. This lawsuit caused the Texas Legislature to create the Edwards Aquifer Authority (EAA), which was tasked with regulating pumping from the aquifer, implement critical period management restrictions on pumping, and to develop a program "to ensure that the continuous minimum spring flows of the Comal Springs and the San Marcos Springs are maintained to protect endangered and threatened species to the extent required by federal law . .." (Edwards Aquifer Recovery Implementation Program 2012). Beginning in 2006, the USFWS undertook a collaborative stakeholder process to develop a plan to guide protection and recovery of the federally listed species dependent on the Edwards Aquifer and associated spring systems. In 2007, the Texas Legislature passed legislation directing the EAA and other state resource agencies to develop a USFWS-approved plan by 2012. This process created the Edwards Aquifer Recovery and Implementation Plan (EARIP) process which led to the approval and adoption of the Edwards Aquifer Habitat Conservation Plan (EAHCP) in 2012 (Edwards Aquifer Recovery Implementation Program 2012).

The EAHCP identified specific biological targets for each listed species necessary to ensure recovery from a repeat of the drought of record for both the Comal and San Marcos Spring systems. Within the San Marcos River, this included specific areal targets for TWR by river sections between the spring headwaters downstream and the confluence with the Blanco River. These areal targets were based on an assessment of historical mapping of TWR and other aquatic macrophyte species by river reach conducted since the mid-1980s. Beginning in 2013, applied restoration actions identified within the EACHP began with removal of non-native aquatic macrophytes and planting of TWR and other native aquatic macrophytes. It was recognized that the success of the reintroduction and or establishment of TWR within target river reaches was predicated on an understanding of TWR habitat requirements and river reach specific characteristics. The EACHP included an Adaptive Management element which would necessarily need to be responsive to several factors including propagation strategies for TWR, reach and spatially specific success of planting methods and subsequent monitoring of the dynamics of competitive interactions between TWR and other aquatic macrophytes. It was also recognized that habitat alterations in some river sections have resulted in deeper and slower areas upstream of impoundments that may impede restoration success, especially given known longitudinal increases in recreation induced turbidity.

Previous studies have investigated a variety of habitat requirements of TWR that include consideration of physical, chemical and biological properties important to inform and guide restoration strategies. This includes evaluations of germination, growth responses and resource partitioning due to differences in substrate, oxygen concentrations, CO_2, depth, velocity and aquatic macrophyte associations (Power 1996; Poole and Bowles 1999; Power and Doyle 2004; Saunders et al. 2001; Tolman 2013;

United States Department of the Interior 2013; Tolman et al. 2014). It is noted that in some instances, refinements in the understanding of specific requirements have changed over time given subsequent research results and field-based monitoring of the EAHCP applied restoration efforts.

Power and Fonteyn (1995) examined the effects of dissolved oxygen concentration, depth of seeds in the substrate and substrate characteristics on seed germination success and leaf area of TWR. They concluded that higher seed germination rates were associated with low dissolved oxygen concentrations and clay versus sand substrates. TWR also had greater leaf area in clay versus sand substrates. It should be noted that in these experiments, the clay substrates were rich in organic material collected from a pond while the sand was derived from a hardware store with little or no organic content. Power (1996) extended this work by examination of above ground biomass and stem density using naturally occurring sandy clay versus gravel substrates obtained from the San Marcos River in areas adjacent to established TWR stands. Higher above ground biomass and stem densities were obtained in the sandy clay soil treatments compared to gravel and may have reflected the higher organic matter, nitrogen and phosphorus within the sandy clay compared to the gravel utilized. Results also suggested that these growth characteristics responded positively to an increasing velocity gradient between 0.05-0.12 m s^{-1} and 0.40-0.49 m s^{-1}.

This early work is in contrast to assessments of river substrate characteristics associated with established TWR stands (Poole and Bowles 1999). They found TWR in coarse, sandy substrates with relatively low organic matter content and in areas with moderate current velocities and depths less than 1 meter. The apparent differences in reported suitable substrate characteristics is attributed to the experimental methodology differences between these studies. EAHCP based restoration efforts have conclusively demonstrated high success rates for TWR plantings in coarser substrates such as sand and gravel and low success rates in finer substrates such as clay (T. Hardy, *pers. comm.* 2018). Poole and Bowles (1999) also reported that sampled areas where TWR was absent had depths that ranged between 1.76-1.85 meters. Most TWR was found in depths ranging between 0.72-0.83 meters. Current velocities for TWR stands ranged between 0.46-1.01 m s^{-1}. They also noted that TWR stands were most often associated with native aquatic macrophytes versus non-natives. Sampled transect locations where TWR was absent were characterized with current velocities between 0.09-0.22 m s^{-1}. Notably, they characterized areas lacking TWR as typically deeper, with slower current velocities and substrates consisting primarily of fine, clay-based soils. These conditions are descriptive of river conditions upstream in backwater areas from impoundments. Power and Bowles (1999) specifically noted that the longitudinal distribution of TWR in the San Marcos River apparently reflects the effects of such impoundments and that TWR does not grow in those areas immediately behind dams (Rio Vista and Cape's Dam) where lentic conditions are approached. They further noted that factors such as increased sedimentation, turbidity and water depth, with decreases in velocity produce unsuitable habitat conditions for TWR. This has implications on restoration strategies by target reaches longitudinally since these impoundments and river characteristics are spatially located in the lower extant of the San Marcos River.

Saunders et al. (2001) developed a habitat model for TWR based on depth, velocity and substrate. Field sampling at a limited number of TWR stands were used to develop habitat suitability criteria that generally reflected the ranges of depth, velocity and substrate reported in Poole and Bowles (1999). Preferred depths were reported to be between 0.23-1.0 meter while preferred velocities ranged between 0.06-0.61 m s^{-1}. Substrate preferences were characterized as sand and fine to small gravels. Field measurements of turbidity during the study showed increasing variability and higher mean values in a downstream longitudinal direction that was attributed to increases in suspended sediment. Their turbidity data also clearly reflected the seasonal influence of water recreation with the highest reported turbidities associated with the summer period and successively higher turbidity values at each successive downstream study station and reflected trends noted by Groeger et al. (1997). This study underscored the longitudinal gradient in seasonal turbidity related to suspended sediment from recreation and attributed the lack of aquatic macrophytes in river sections below Cape's Dam and upstream of Cummings Dam to the increased depth and lack of light penetration to the stream bottom. However, no implications to TWR in upstream sections were noted.

Power (2002) found a positive response in TWR total biomass where plants grown at velocities between 0.25-0.37 m s^{-1} had 28.42 grams dry weight (gdw) compared to only 4.24 gdw for plants grown in 0.0-0.01 m s^{-1}. In addition, resource partitioning differed between velocity gradients and two phenotypes were evident. TWR grown in relatively higher current velocities exhibited higher net productivity, a well-developed root system, and proportionally allocated more biomass to non-reproductive organs (49.2% gdw root biomass in faster velocities vs 24.8% gdw root biomass at slower velocities). TWR grown in relatively slower flowing water, had lower net productivity and proportionally allocated more biomass to reproductive organs (22.1% gdw reproductive culm in faster velocities site vs. 65.0% gdw reproductive culm in slower velocities). These results suggest that restoration efforts in deeper slower habitat conditions in the backwater reaches in the lower sections of the San Marcos River may be problematic to meet targeted areal restoration goals.

EAHCP annual mapping has clearly shown dense TWR stands in the upper half of the San Marcos River at water depths in excess of two and three meters where water clarity remains very high independent of recreational activities (EAHCP 2017). However, in the lower one-half of the river in the deeper backwater sections above Rio Vista and Cape’s Dam, TWR is typically only found in water less than 1 meter at the stream margins. This is attributed to PAR reductions with depth due to suspended sediment induced turbidity and supported by monitoring of EAHCP restoration efforts. TWR stands planted in a 2.5-meter-deep section of the San Marcos River less than 100 meters upstream of I-35 with high turbidity died within 3 months while plantings in adjacent river locations in less than 1 meter have persisted (EAHCP 2017).

The implications of increased turbidity in conjunction with deeper river sections and associated PAR reductions affecting TWR distributions is further supported by results reported by Hardy and Raphelt (2015). Figure 4.1 provides a comparison of PAR attenuation as a function of depth compared between Spring Lake (no recreation) and the backwater at Cape’s Dam on the same day (Saturday) during moderate recreation activity on the river.

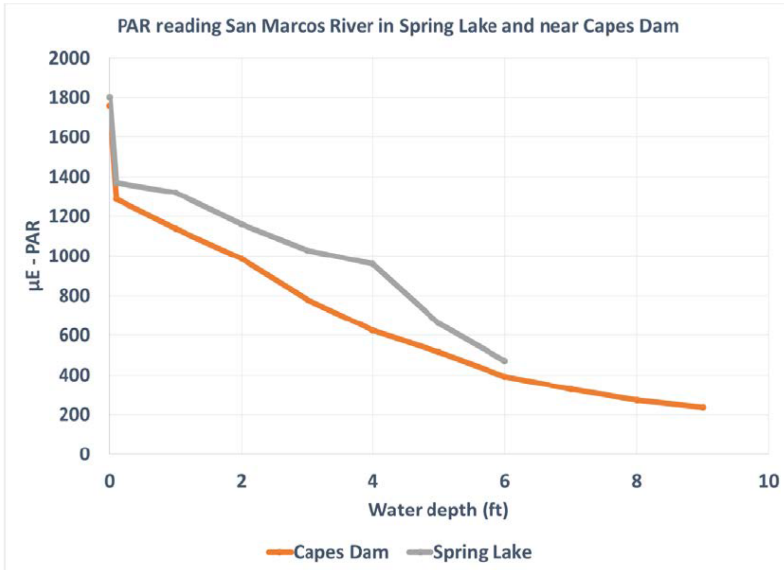

Figure 4.1. Comparison between PAR in Spring Lake versus the backwater at Cape's Dam (Hardy and Raphelt 2015).

Figure 4.2 shows the associated distribution of TWR stands in the vicinity of Cape's Dam during the same period. This section of the San Marcos River was mapped for TWR stands where 126 TWR (total area of 237 m^2) were found within the 0.5 kilometer stretch of the San Marcos River upstream of Cape's Dam. Notably, only 58 TWR stands were found in water depths between 1 and 1.5 meters, while the remaining TWR stands were found in water depths less than 0.6 meters. Approximately 80 percent of TWR stands were located more than 250 meters upstream of Cape's Dam while only 16 TWR stands were located within the 100-meter section just upstream of Cape's Dam. The section of the San Marcos River immediately upstream of Cape's Dam is almost devoid of aquatic macrophytes along the thalweg (depths greater than 3 meters) of the channel which is attributed to light attenuation at the stream bed (Hardy and Raphelt 2015). These data show that both the longitudinal distribution of TWR immediately above Cape's Dam is confined to relatively shallow water locations, which likely reflects the reduction in PAR due to recreational induced turbidity. The 100-meter section of the

SMR upstream of Cape's Dam is oriented such that very little riparian shading occurs on a seasonal basis and that this is therefore not a controlling factor for TWR at this location.

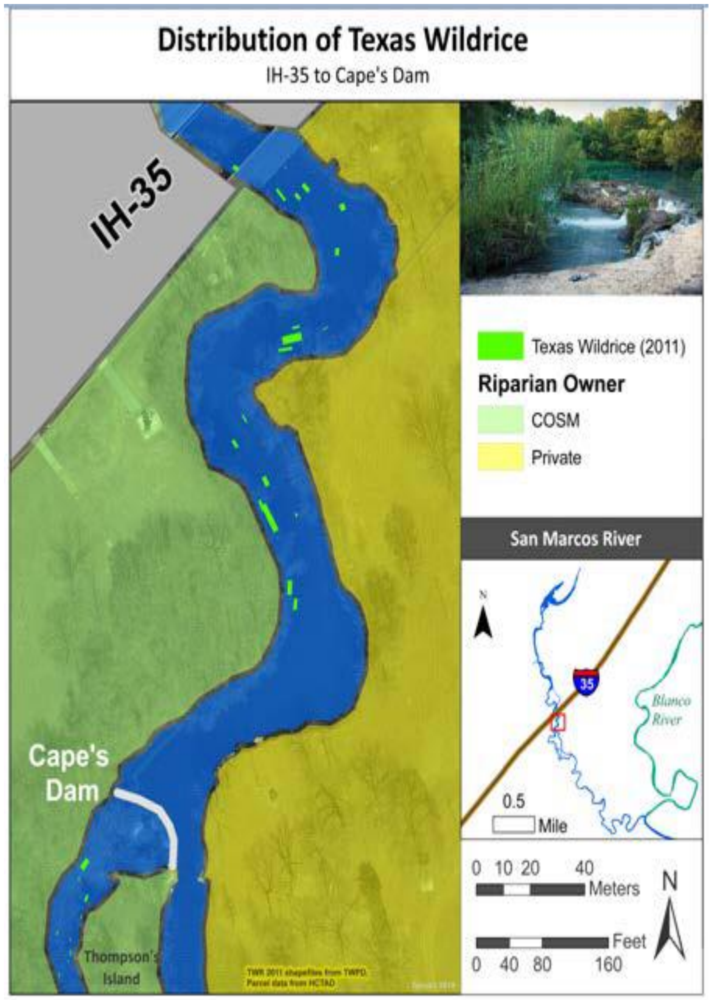

Figure 4.2. Historical distribution of TWR stands mapped in the SMR. (Hardy and Raphelt 2015).

Power and Doyle (2004) demonstrated that TWR is a CO_2 obligate plant and therefore potentially impacted by longitudinal changes in CO_2 concentrations. The San Marcos Springs are derived from karst, limestone strata and have relatively high CO_2 concentrations at the spring orifices. High turbulence at the outflow at Spring Lake Dam results in rapid equilibrium with atmospheric concentrations. Bio-West (2004) collected pH and alkalinity and computed CO_2 concentrations between August 2000 and August 2002 at ten locations from the spring headwaters downstream to below Cape's Dam. Carbon dioxide concentrations in the headwaters ranged between 7 and 25 mg/L and dropped to 5-15 mg/L at the outflow below Spring Lake Dam and 2-6 mg/L at the most downstream sampling location. Experimental data on TWR above, below and total biomass exposed to a gradient of CO_2 concentrations between low (6 mg/L), moderate (12 mg/L) and high (21.5 mg/L) over a six-week period showed sustained growth at all three concentrations. Only a somewhat lower and statistically different below ground biomass was observed for the moderate concentration treatment, while no statistical differences were found between the lowest and highest CO_2 concentrations. These results suggest that the CO_2 gradient within the San Marcos River is not likely a concern for applied restoration efforts targeting TWR.

Tolman (2013) utilized three-dimensional topography derived from LIDAR to compute the riparian canopy structure in the San Marcos River between the headwaters and the confluence with the Blanco River. Densiometer readings at over 300 locations associated with each riparian species during leaf on and leaf off periods were used to estimate the light characteristics at the water surface using ray casting techniques on a seasonal basis. The amount of 'shading' was compared to the distribution of all mapped TWR stands and results suggested that TWR stands were located in areas of the San Marcos River were incident light was greater than ~75 percent (or less than ~ 25 riparian shading). These results were used in conjunction with a spatially explicit hydrodynamic/habitat suitability model for TWR at 0.25 meter resolution (Hardy et al., 2011) to identify specific spatial locations with high light regimes where TWR habitat suitability based on depth, velocity, and substrate was between 0.75 and 1.0 but occupied by non-native aquatic macrophytes. These areas were targeted at the onset of the EAHCP for non-native aquatic macrophyte removal and planting of TWR. Monitoring of non-native aquatic plant removal in these areas adjacent to established TWR stands since 2013 has demonstrated that TWR successfully expands laterally and in a downstream direction without supplemental TWR planting. In these areas in which more extensive non-native aquatic plant removal has been undertaken, planting of TWR derived from tillers has been over 98 percent effective at establishment of TWR.

My dissertation results focused on early growth characteristics of TWR based on the short-term response of tillers to a gradient of PAR reductions and the *in situ* growth responses to the seasonal and longitudinal gradient of recreational induced turbidity. Study results showed no difference in non-volatile suspended solids or periphyton chlorophyll-*a* concentrations on TWR leaves with *in situ* conditions that reflected the longitudinal gradient and seasonal pattern of recreational induced turbidity. This is attributed to the hydrodynamics associated with the preference of TWR for moderate current velocities where the root ball of the plant effectively behaves as a cylinder and generates a Von Karmen Street effect downstream where the velocity vortices shed in an alternative pattern downstream (left then right) that induces the side to side waving of the TWR leaves. This is believed to result in a self-cleaning mechanism that reduces the accumulation of sediment on the periphyton or leaf surface. This speculation is supported by my *in situ* study results where reduced flow rates resulted in a reduced velocity field at Bicentennial Park and the TWR plants were lost from smothering by fine silt. No other plants in the experimental period that remained in moderate velocities were affected.

My study results also suggest that PAR reductions somewhere between 40 and 80 percent begin to exhibit reductions in TWR growth. This is supported by the empirical observations of reduced distributions of TWR in the backwater sections of the San Marcos River and the negative response of the TWR restoration efforts in deeper sections at IH-35 as noted above. During the summer of 2018, TWR plantings have been undertaken in the deeper backwater section upstream of Rio Vista but insufficient time has elapsed to assess the effectiveness of these efforts.

Overall, the cumulative research, study results and results of the applied restoration efforts of EAHCP strongly suggest that successful TWR restoration is demonstrably effective in water depths up to 3 meters in the upper sections of the San Marcos River where current velocities and turbidity are at a minimum. Successful TWR planting efforts in the lower San Marcos River are likely to be restricted to stream margins in water depths less than 1 to 1.5 meters. Selection of specific locations are to some extent mediated since target planting areas, currently associated with discharges near the median flow (~5 m^3s^{-1}), must focus on river bed sections that are expected to remain wet during a repeat of the drought of record in which the SMR is expected to drop to ~2.3 m^3s^{-1} (EARIP 2012).

Overall success of TWR restoration as part of the EAHCP is evident by the increased total areal coverage over time (Figure 1.2) as well as the high success rate of TWR plantings by restoration reach, which include some areas downstream in deeper river sections below City Park where turbidity remains somewhat lower compared to reaches farther downstream. The EAHCP (2017) report that during the 2016 restoration period, a total of 1115 TWR plants were planted in the Cypress Island reach (below City

Park) and that 1375 TWR plants were planted in the IH-35 reach. These efforts increased the areal coverage for TWR in the Cypress Island reach by 115 m^2 and by 194 m^2 in the IH-35 reach. It is notable that almost all the TWR areal coverage expansion and planting efforts within both these reaches targeted stream areas in higher light regimes and with river depths less than 1 meter under lower than median flow conditions (~5 m^3s^{-1}). For both these reaches, utilizing the combined strategies from Tolman (2013) (high light regimes), Hardy et al. (2011) (high light regime and suitable depth and velocity), Crawford-Reynolds et al. (2016) and this study (PAR and turbidity responses by TWR), these TWR restoration efforts were over 98 percent successful (EAHCP 2017). Study results on the response of TWR to availability of PAR or PAR reductions due to the seasonal and longitudinal patterns of recreation induced turbidity are consistent with the observed success of TWR restoration within the SMR that have empirically incorporated these results over the past two years.

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