SUSPENDED SEDIMENT IMPACTS ON TEXAS WILD-RICE AND OTHER AQUATIC PLANT GROWTH CHARACTERISTICS AND AQUATIC MACROINVERTEBRATES

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Executive Summary

Empirical data from the San Marcos River has suggested a relationship between seasonal and longitudinal intensity of recreation and higher turbidity levels that may influence aquatic macrophyte growth and macroinvertebrate community characteristics. This report examined the influence of turbidity related to suspended sediments on a longitudinal and seasonal basis on Texas wild-rice (Zizania texana) growth dynamics as well as the growth response of Texas wild-rice to a gradient of photosynthetically active radiation (PAR). Seasonal and longitudinal responses in the distribution and composition of macrophytes and associated characteristics in the benthic and drift invertebrate communities were concurrently examined.

Ex situ experiments on reduction in PAR on the vegetative growth of Texas wild-rice were conducted three times over the course of the study. In each experiment, Texas wild-rice plants were subjected to 100%, 90%, 80%, 60%, and 20% available PAR for approximately 6 weeks. The initial experimental period was impacted by severe flooding that inundated experimental plants for several days which prevented collection of individual leaf specific values due to entanglement and breakage. Results of the three PAR studies demonstrated that Texas wild-rice growth is negatively impacted by low light availability. In general, shoot, root, and total biomass, as well as total leaf surface area were significantly lower at 20% PAR availability. No significant reductions in plant growth metrics were observed with 80% or higher PAR availability. *Ex situ* experimental results were consistent with empirical assessments of light availability and persistent Texas wild-rice stands based on seasonal ray-casting modeling in the San Marcos River (Tolman 2013). Overall, these results suggest that relatively large PAR reductions (e.g., < 20% availability), whether from riparian shading or turbidity due to suspended sediment can result in reduced growth characteristics.

In situ experiments were conducted within the San Marcos River at four locations between the outfall of Spring Lake (headwaters and low recreation intensity) and approximately 2 kilometers downstream. Study sites generally reflected an increased intensity of recreational use within the river during relatively high recreational periods (i.e., April through September). Two of the in situ experiments were conducted during high recreation use periods while the third *in situ* experiment was conducted during a low recreation period (i.e., spring). A significant loss of experimental plants were observed in the 2014 high recreation study period at the two most downstream study sites due to smothering in fine sediments associated with backwater affects from major flooding in the Blanco River, vandalism, and herbivory. In general, we found significant differences in root, shoot and total biomass of Texas wild-rice over the longitudinal gradient in the San Marcos River associated with recreational intensity. We also found that total solids and non-volatile solids deposited on Texas wild-rice leaves tended to increase in a downstream direction, but were lower at the most downstream site (Ramon Lucio). The lower amount of material on leaves at the Ramon Lucio site was attributed to settling of fine sediments above the Rio Vista dam just upstream of the site. The in situ study results also suggest that Texas wild-rice is likely 'self-cleaning' due to the interplay of leaf movement in its preferred velocity regime where fine sediments remain suspended in the water column. Study results also indicate that turbidity levels are indicative of the intensity of recreational use whether within a day, between days of the week, or seasonal. Turbidity levels were approximately two orders of magnitude higher between the upstream study site compared to downstream study sites during high recreation periods. Conversely, there was very little longitudinal variation in turbidity levels during low recreation periods (e.g., winter).

Longitudinal and seasonal characteristics of the aquatic macroinvertebrate community and macrophytes were evaluated at three study sites within the San Marcos River. The upstream study site represented a relatively low recreation intensity area near the outflow of Spring Lake, the middle study site (City Park) and lowest study site (Ramon Lucio) represented locations downstream of intense recreational use during late spring to early fall. At each study site for each seasonal sampling period, the availability of aquatic macrophytes and substrate were mapped and utilized to guide stratified random sampling based on proportional sampling for benthic macroinvertebrates. In addition, invertebrate samples representing the 24-h periodicity in macroinvertebrate drift were collected at each site. A total of 134,488 invertebrates representing 73 taxa were collected among 480 drift samples. In general, drift densities were highest at the upstream study site and decreased in a downstream direction. Drift densities at all study sites were lower in the winter compared to spring, summer and fall sampling periods. A total of 40,288 invertebrates representing 60 taxa were collected from benthic samples. Drift taxa richness was highest at the upstream study site and decreased in a downstream direction. Conversely, benthic diversity increased in a downstream direction primarily due to the high number of *Hyallella sp*. in the upstream samples. We did not detect an increase in macroinvertebrate drift densities during daylight hours associated with high recreation use. We attribute this to the fact that actual bed disturbance occurs in localized areas that are denuded of aquatic macrophytes very early in the recreation season. It is also possible that dislodged drift may have settled out above the sampling locations which were downstream of the high recreation use areas (i.e., below City Park and below Rio Vista). Finally, we suspect that the increased turbidity associated with small grain (silt) size in the suspended sediment remains entrained in the water column with even small or moderate velocity fields in the channel. Habitat associations of the macroinvertebrates were similar to what has been previously reported (Diaz et al., 2015; Fries and Bowles, 2002) as well as the composition in terms of functional feeding groups. Composition of functional feeding groups in the drift reflected the invertebrate species pool which was present in the benthos over all study periods and study sites. These study results indicate that the macroinvertebrate benthos and drift at Site 1 are minimally impacted by recreation or turbidity as would be expected given its location immediately below Spring Lake Dam and the protected State Scientific Area which limits direct contact recreation. Site 2 exhibited little impact associated with recreation induced turbidity on a seasonal basis and maintains a robust aquatic vegetation community that supports the aquatic macroinvertebrates. However, at Site 3 our results indicate that substrate size and water column turbidity influence macroinvertebrate community structure. This area lies downstream of the Rio Vista Dam that was constructed in 2006. Vegetation and substrate monitoring during the period from 2000 to the present (Bio-West 2016) in this reach of the San Marcos River has documented both channel changes (depth decreases) and the aquatic vegetation in this reach has dramatically declined from pre-dam construction periods.

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1.0 Introduction

Excessive suspended sediments in aquatic systems can lead to negative impacts on plants, macroinvertebrates and fish. Suspended sediment loads decrease water clarity, increase the attenuation of light in the water column, and lead to less light available for macrophyte and algal photosynthesis. In addition, suspended sediments can also settle onto benthic surfaces, essentially smothering plant and animal life. The San Marcos River is a clear-water system with dense macrophyte stands and high invertebrate diversity (Groeger et al. 1997; Diaz et al. 2010). Preliminary data collected with automated water quality logging sondes at 15 minute intervals have indicated that there are strong longitudinal patterns of increasing turbidity in the river as well as strong diel, weekly, and seasonal patterns of turbidity in the river (unpublished data). Much of this temporal periodicity coincides with periods of high recreational activity in the upper river and there is a concern that recreationally-mediated turbidity may affect the organisms in the river. For example, the attenuation of photosynthetically active radiation (PAR) by increased turbidity may affect the fitness and performance of endangered Texas wild-rice (*Zizania texana*) or other native vegetation in the river. However, it remains unknown if extended diel, weekly, and seasonal impacts of reduced PAR associated with suspended sediments have an effect on the productivity or biomass of Texas wild-rice (TWR) or other aquatic plants within the San Marcos River and whether these impacts cascade to the macroinvertebrate community in the river.

The research focuses on assessing the potential implication of suspended sediment within the San Marcos River. This report provides the results from three study components as indicated below:

1. Texas wild-rice
    a. *Ex situ* photosynthetically active radiation
    b. *In situ* suspended sediment due to low versus high river recreation
2. Aquatic macroinvertebrate community
    a. Seasonal differences due to low versus high river recreation

The implication of reduced PAR on TWR growth dynamics in an *ex situ* experimental design are presented first, followed by results obtained from *in situ* experiments conducted within the San Marcos River associated with a longitudinal gradient approximated by increased intensity of water based recreation. This is then followed by the macroinvertebrate study that examined the benthic and drift community dynamics on a seasonal basis associated at three study sites along the longitudinal gradient of recreation use within the river.

1.1 Background

1.1.1 Texas wild-rice (*Zizania texana*)

Texas wild-rice is a monoecious perennial grass differing distinctively from other North American species of wild-rice in its habit of growth (Silveus 1933). According to Silveus (1933), TWR is a floating perennial, rooting and geniculating at the nodes and growing up to three meters underwater. The linear leaves are one to two meters long. Culms reach a length of up to four to five meters. Emergent portions of the culm are up to a meter or more in length and bear inflorescences. 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). In 1921, Ena A. Allen collected a sample that was correctly labeled by A. S.

Hitchcock, presumably at a later date. Recognition as a distinct species, *Zizania texana*, was made by A. S. Hitchcock in 1933. The taxon is classified as one of four species included in the rice tribe (Oryzeae). Members of the tribe are disjunctly distributed throughout Asia and North America, with three occurring in North America (Xu et al. 2010). In a study of comparative phylogenetics of wild-rice, Xu et al. (2015) found that *Zizania texana* had the lowest genetic diversity among the three North American species. Texas wild-rice is known to occur only in the upper 2.4 kilometers of the spring-fed San Marcos River, Hays County, Texas (Figure 1.1). 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).

Figure 1.1 Geographic distribution of TWR in the San Marcos River, Hays County, Texas and location of TWR *in situ* study sites.

Texas wild-rice was listed as a federally endangered species (USFWS 1978) due to the observed rate of decline in coverage and loss of sexual reproductive activity (Emery 1977). Early accounts of the decline in population of the species were first described by Emery (1967). At this time, extirpation of the species from the irrigation channels and beyond the first kilometer of Spring Lake Dam was noted by Emery. He attributed the decline of TWR to anthropogenic activities, such as submergence of inflorescence by floating vegetative debris, overharvesting of the species, dredging of the river channel, and the release of raw sewage from the San Marcos Wastewater Treatment Plant into the San Marcos River. In a later assessment, Emery (1977) found that although previously cited activities had abated, restoration of sexual reproduction had not resulted, nor had any appreciable increase in coverage occurred via clones, subsequently leading to the designation as an endangered species with the USFWS in 1978 (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 species (flora and fauna), recreational use causing "knock-down" of inflorescences, and construction of dams causing a rise in the water level thus effecting growth and seed production. Altered sedimentation patterns, changes in sediment composition as a result of road and building construction, depletion of soil seed bank by river plowing and herbivory, and diminished spring flow related to ground water pumping have also been suggested as factors influencing distribution of TWR (Power 1996).

Texas wild-rice coverage has fluctuated over time. Figure 1.2 shows the areal coverage of TWR in square meters from 1976 to 2014 (EAHCP 2012, EAHCP 2014). Declines in coverage were noted throughout the early 1980s. Cessation of aquatic dredging and restoration efforts starting in the late 1980s resulted in measurable increases in coverage (Poole, unpublished report 2001). Recent restoration activities as set forth in the Edwards Aquifer Habitat Conservation Plan (2012) including planting TWR in the river, establishment of protected areas, removal of non-native aquatic vegetation, and increased public awareness have all contributed to continued increases in coverage.

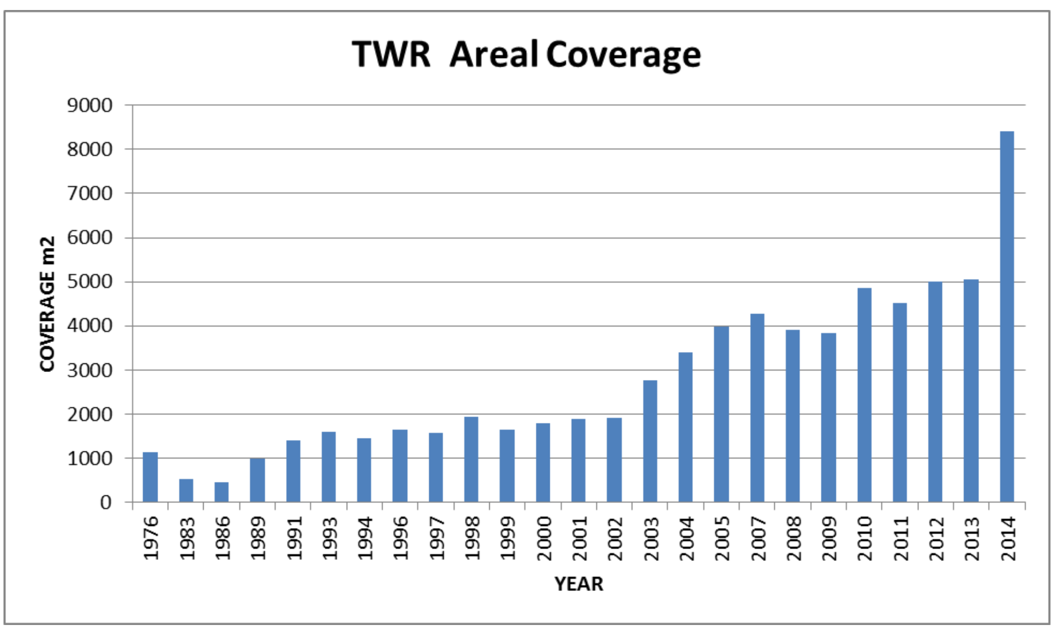

Figure 1.2 Changes in areal square meter coverage of TWR in the San Marcos River 1976-2014 (EAHCP 2011, EAHCP 2014).

1.1.2 Overview of Suspended Sediment Induced Responses in Aquatic Communities

A variety of factors including nutrients, velocity, substrate type, and water temperature are known to influence macrophyte growth (Hynes, 1970). However, availability of light is the single most important abiotic factor affecting a plant's biomass (Madsen 1993; Case and Madsen 2004). Scheffer (1998) pointed out that light availability is also a limiting factor in macrophyte colonization. Plants depend on light interception and absorption for photosynthesis and enough light must be absorbed for photosynthesis to result in a net increase in biomass in order for the plant to grow and reproduce. As the amount of light decreases, the plant reaches a point where the products of photosynthesis are equal to the products consumed by respiration or the Light Compensation point, and no net growth occurs and CO_2 uptake is equal to O_2 release. As light levels further decrease, the plant begins to consume more photosynthate for respiration than can be produced by photosynthesis and plants begin to lose biomass and can ultimately die. Photosynthetically active radiation is considered most strongly correlated with areal coverage of aquatic plants (Dodds and Welch 2000; Hilton et al. 2006; Davies et al. 2008). A decrease in available PAR has been demonstrated to suppress the overall biomass production of macrophytes (Asaeda 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).

Suspended sediment induced turbidity in an aquatic system can be problematic for plant life due to a decrease in water clarity and reduction in availability of 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 induced turbidity concomitant with accretion of periphyton material have been associated with subsequent formation of a boundary layer on the adaxial and abaxial surface of macrophyte leaves. This has been suggested to negatively affect the vegetative growth and overall biomass production of macrophytes by inhibiting photosynthetic activity and increasing the distance between gas exchange across the surface of leaves (Tóth 2013). Sediment may also damage plants by abrasion, scouring, and burial, while sediment deposition may encourage species shifts because of a change of substrate.

Concomitant with other environmental factors, i.e. changes in velocity, light limitation and nutrient levels, the concentration of periphyton affects the growth and morphology of aquatic macrophytes. It has been suggested by Asaeda et al. (2004) that the continuous accumulation of periphyton, consisting mostly of epiphytic algae, on the leaves of aquatic macrophytes may negatively affect biomass productivity. 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 the inorganic carbon transport in submersed macrophytes during photosynthesis (Smith and Walker 1980). The chlorophyll content and thickness of the epiphytic layer further leads to a competitive interaction for light between periphyton and its macrophyte host (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 solids (inorganic), and 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 2011). Most aquatic macrophytes are found occurring at depths between zero and seven meters (Sculthorpe 1967; Pedersen et al. 2015). In general, macrophytes receive only a small fraction of full incident solar energy due to deflection at the water surface, as well as absorption and scattering by suspended sediment (Kirk 2011). Sculthorpe (1967) considered the depth limit for most to be when the water transparency allowed for less than one to four percent of light to reach a plant.

1.1.2.1 Texas wild-rice

Poole and Bowles (1999) suggested that TWR has evolved to rely on the transparency of the clear San Marcos Spring water to deliver adequate light for photosynthesis. It has been suggested through previous observations that TWR prefers shallow water depths less than one meter (Poole and Bowles 1999; Saunders et al. 2001), but this limit may be an artifact from the *in situ* observations that were impacted by the longitudinal increases in suspended sediment within the San Marcos River.

Previous research has focused on the effects of temperature, sediment preferences, and velocity on the biomass productivity 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) assessed and characterized variables influencing spatial distribution of TWR such as depth, velocity, substrate and shading. The results of these studies have aided 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.

1.1.2.2 Macroinvertebrates

Sediment-induced changes in a water body may result in changes to the composition of an aquatic community (Wilber, 1983). Large volumes of suspended sediment will reduce light penetration that can suppress photosynthetic activity of phytoplankton, algae, and macrophytes. These primary productivity changes then cascade into secondary and tertiary trophic levels due to fewer photosynthetic organisms available to serve as food sources for many invertebrates, fish, and other herbivores (e.g., turtles). This cascading effect can therefore result in overall invertebrate numbers that may also decline, which in turn can lead to decreased fish populations.

Increased levels of sediment may also interfere with essential functions of organisms. This includes the potential for the numbers of filter-feeding invertebrates to decline if their filter mechanisms are choked by suspended particles (James et al., 1979). Settling of suspended solids can also negatively impact benthic aquatic communities. Sediment deposition may obscure sources of food, quantity and quality of habitat, hiding or refuge places, and nesting sites (Wilber, 1983). In some instances, some aquatic insects will drift with the current in an attempt to move out of the affected area. Sediment deposition may also shift benthic invertebrate community structure. For example, species that prefer low-silt substrates, such as mayflies, stoneflies, and caddisflies, may be replaced by silt tolerant communities of oligochaetae, pulmonate snails, and chironomid larvae (James et al., 1979).

2.0 *Ex situ* Experiments: Reduction in Photosynthetically Active Radiation (PAR) and Vegetative Growth of Texas wild-rice (*Zizania texana*)

2.1 Introduction

The San Marcos River, is relatively narrow (5-15 meters wide) and ranges from 1 to 4 meters (m) in depth at average flow rates (Terrell et al. 1978). Arising from artesian springs fed by the Edwards Aquifer, the ecosystem serves as habitat for native and non-native aquatic plants including one federally endangered species, TWR (EAHCP 2012). The enactment of the Edwards Aquifer Habitat Conservation Plan (EAHCP) in 2013, placed priority on the recovery and sustainability of TWR and established a goal of maintaining no less than 3,550 m^2 areal coverage of TWR in Spring Lake and the upper reaches of the river (EAHCP 2012).

Tolman (2013) examined the influence of velocity, depth and light availability on spatial distribution of TWR at three points in the upper reaches of the San Marcos River. Tolman (2013) found the least amount of TWR areal coverage occurred in the narrowest segment of the river with the greatest extent of riparian canopy cover where light availability was only 28 percent. With interest in increasing areal coverage of TWR an understanding of the influence of PAR on growth may help in restoration efforts. Specifically, in determining the best locations to reintroduce plants. Since light availability may be an important determining factor governing the successful expansion of TWR in the river, the objective of this study is to test the impact of a reduction in available PAR on the vegetative growth of TWR *ex situ*.

2.2 Materials and Methods

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 on the campus of Texas State University, San Marcos, TX. Three independent studies were conducted from September 2015 to April 2016 involving the same range of PAR reductions during each study period. The initial PAR treatment had a somewhat protracted baseline period as well as growth period as shown in Table 2.1. 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 (e.g., above and below ground biomass). The growth period represents the actual treatment period. The experimental apparatus was targeted for Spring Lake but after a two week trial period, it was determined that excessive buildup of extraneous plant material was continuous; associated in part to aquatic plant harvesting activities within Spring Lake. This resulted in development of the experimental setup in the raceway at the Freeman Aquatic Building as noted (Table 2.1).

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); re-location of study from Spring Lake to Freeman Aquatic Building (PAR I)*

For each of the three replicate study periods, a total of 120 tillers of TWR were collected from the San Marcos River on a single day. Tiller size was standardized by removing all but two stems from the plant and trimming the remaining two stems to 20 cm in length. All but five roots were removed and the remaining 5 roots were trimmed to 5 mm in length. This approach is similar to the technique by Vaughan (1986) and utilized by Texas State for native plant propagation as part of the EAHCP. Tillers were then placed in individual pots containing soil at a standardized depth 3 cm. The soil used consisted of a local blend of mulch, humus, sand, and pea size gravel utilized by the U.S. Fish and Wildlife Service San Marcos Aquatic Resource Center for TWR propagation. The tillers were grown for four weeks, after which 90 plants were randomly selected for the experiments and baseline analysis as noted below.

A total of 15 plants were randomly selected from the available 90 plants to measure baseline growth parameters prior to starting the experimental treatment. The 75 remaining plants were randomly assigned to the control (100 percent ambient PAR) or to one of four experimental treatments consisting of a reduction in PAR. The four treatments consisted of PAR reduced by 10% (90% available PAR), 20% (80% available PAR), 40% (60% available PAR), and 80% (20% available PAR). Individual plants were placed in plots 0.9m x 0.6m in size at a depth of less than one meter and a velocity of 0.4-0.2m/sec, which is within the species' suitability preference (Hardy et al. 2010; Saunders et al. 2001; Poole and Bowles 1999). Three plastic plant trays (.6cm x .3cm) containing five plant pots were located in each plot. Each plot consisted of two corner placed stacked cinder blocks (40.64cm x 20.32cm x 15.24cm) secured with nylon cable ties (45cm) and affixed with a constructed PVC (7.63cm) shade frame (1.21m x 0.9m) fitted with high density polypropylene, lock-stitch shade material positioned above the surface of the water (Figure 2.1). PAR gradients were achieved by using combinations of suspended (polypropylene) shade cloth resulting in target light availability. In addition, a submersible Tsunami pump (.04 cms) was positioned at the upstream end of each treatment unit to provide a consistent velocity between sequential plots.

Figure 2.1 . PAR experimental plot showing constructed PVC shade material frame and sequential PAR reduction panels.

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 when sunlight was at its maximum to minimize the variation between ambient light conditions due to time of day. Velocity was recorded using a Marsh-McBirney 2000 flow meter and top set wading rod. Water temperature and pH were measured approximately every two weeks using a YSI 85 and Oakton Con +6 meter. Daily meteorological data (ambient temperature, cloud cover, precipitation) were obtained from the San Marcos Airport weather station located approximately 5 km from the study site. Duration of daylight was obtained from the United States Naval Observatory (website - http://aa.usno.navy.mil/data/docs/RS_OneYear.php).

At the end of the initial growth (baseline) and treatment periods (see Table 2.1 for dates and length), plants were removed and placed into plastic bags for transport to the laboratory at the Freeman Aquatic Building. In the lab, leaves and corresponding leaf sheaths were separated from the root mass at the juncture. The number of individual roots (excluding root hair) and leaves were counted for each plant. A Li-Cor LI-3000C Portable Area meter was used to measure leaf surface area (LSA) of leaves. Leaves and roots for each plant were then dried for 48 hours at 60˚C in a drying oven and then weighed to determine dry biomass. Significance of treatment differences for TWR growth was analyzed by ANOVA followed by Tukey's HSD ($p \leq 0.05$) to compare treatment means where differences were found. Statistical analysis was performed using Excel.

2.3 Results

Figure 2.2 shows the relationship between day length and each corresponding *ex situ* PAR study period. Cumulative hours of daylight for PAR treatments I, II, and III were 1134, 642, and 701, 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. Figure 2.3 shows the daily percent cloud cover and daily total precipitation totals during each of the *ex situ* PAR study periods.

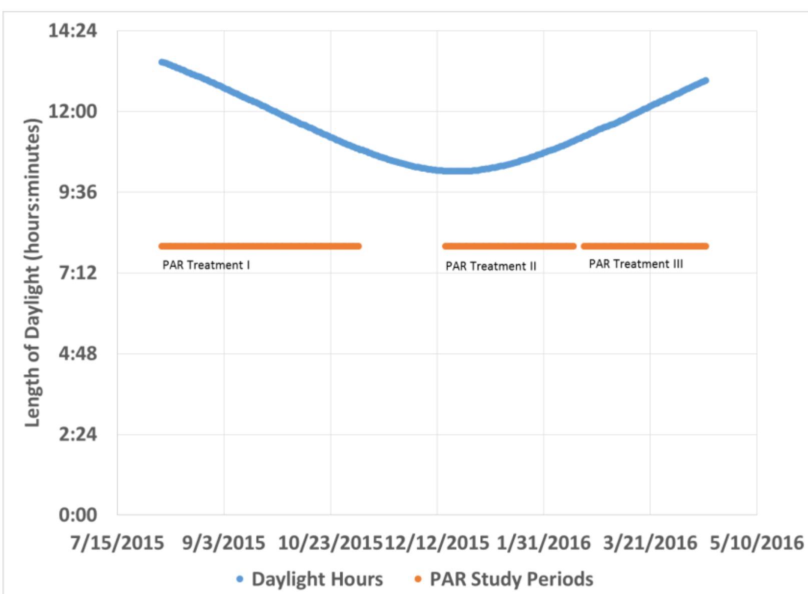

Figure 2.2 Day length during each of the three *ex situ* PAR study periods.

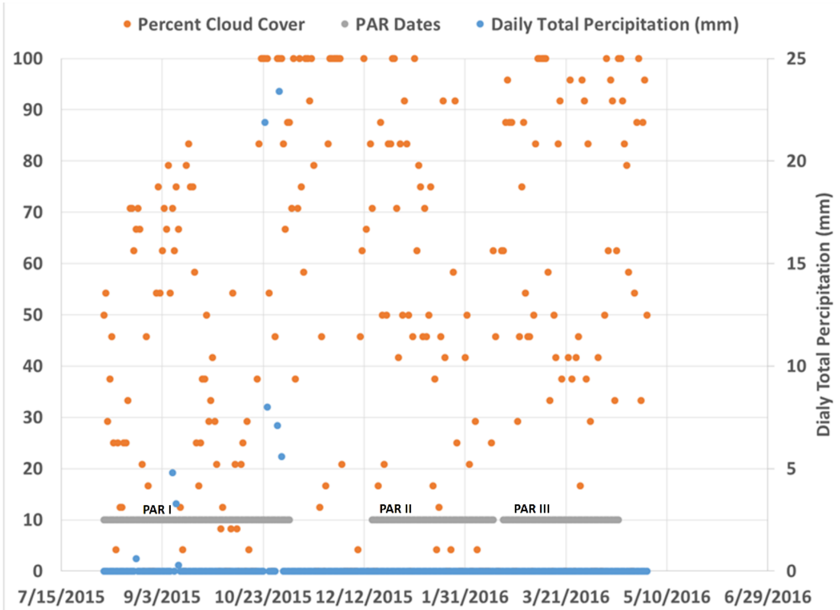

Figure 2.3 Percent cloud cover and daily total precipitation during the three PAR ex situ study periods conducted at the Freeman Aquatic Center at Texas State University. Meteorological data is from the San Marcos airport station.

There were some differences in the average daily percent cloud cover for the three consecutive PAR periods (41, 40, and 53 percent). However, the initial PAR study period was impacted by the October 31, 2015 flood as illustrated in Figure 2.4, which resulted in the experimental flume being underwater water 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 individual leaf specific values for most plants such as shoot numbers, number of broken shoots, and leaf area metrics. For this study 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.

The artesian well source water at the Freeman Aquatic Building provided constant water quality properties over the entire *ex situ* treatment period as shown in Table 2.2. Plant parameters at the end of baseline periods are provided in Table 2.3.

Figure 2.4 Flood inundation at the Freeman Aquatic Building during the October 31, 2015 flood event impacting the last six days of the initial PAR experiment.

Table 2.2 Physical and chemical properties of the PAR treatments in the experimental raceway

			PARI					PARII					PARIII		
Percent PAR reduction	CTL	10	20	40	80	CTL	10	20	40	80	CTL	10	20	40	80
Min Depth (cm)	63	66	68.5	70	72	63	66	68.5	70	72	63	66	68.5	70	72
Avg Depth (cm)	63.5	66.5	68.8	70.5	72.5	63.5	66.5	68.8	70.5	72.5	63.5	66.5	68.8	70.5	72.5
Max Depth (cm)	64	67	69	71	73	64	67	69	71	73	64	67	69	71	73
Min Velocity (m/s)	0.22	0.21	0.19	0.21	0.21	0.22	0.24	0.21	0.21	0.2	0.28	0.24	0.22	0.28	0.25
Avg Velocity (m/s)	0.32	0.32	0.32	0.33	0.31	0.34	0.36	0.44	0.32	0.31	0.39	0.32	0.35	0.34	0.35
Max Velocity (m/s)	0.42	0.43	0.44	0.44	0.41	0.45	0.48	0.48	0.43	0.41	0.49	0.4	0.47	0.4	0.44
Min Temp °C	22.5	22.5	22.5	22.5	22.5	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Avg Temp °C	22.8	22.8	22.8	22.8	22.8	22.4	22.4	22.4	22.4	22.4	22.3	22.3	22.3	22.3	22.3
Max Temp °C	22.9	22.9	22.9	22.9	22.9	22.1	22.1	22.1	22.1	22.1	22.8	22.8	22.8	22.8	22.8
Min pH	7.3	7.3	7.3	7.3	7.3	7.2	7.2	7.2	7.2	7.2	7.4	7.4	7.4	7.4	7.4
Avg pH	7.4	7.4	7.4	7.4	7.4	7.3	7.3	7.3	7.3	7.3	7.4	7.4	7.4	7.4	7.4
Max pH	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
Min DO (mg/L)	7.7	7.7	7.7	7.7	7.7	6.9	6.9	6.9	6.9	6.9	6.5	6.5	6.5	6.5	6.5
Avg DO (mg/L)	7.7	7.7	7.7	7.7	7.7	6.8	6.8	6.8	6.8	6.8	6.0	6.0	6.0	6.0	6.0
Max DO (mg/L)	7.7	7.7	7.7	7.7	7.7	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9
PAR Air Sun µmol^{2}s^{-1}	2250	2250	2250	2032	2032	1982	1890	1654	1935	1941	2090	2090	1946	2065	2046
PAR 10cm Sun µmol^{2}s^{-1}	1837	1756	1403	874	360	1401	929	1024	524	302	1530	1029	1132	629	409

Table 2.3 Average baseline TWR parameters for the three ex situ PAR I-III studies.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm^2)**	**Number broken leaves**
Study								
PAR I (14)	1.7 (0.26)	0.55 (0.08)	2.25	0.17 (0.03)	20 (4)	30 (3)	560.40 (97.18)	8 (1)
PAR II (14)	0.68 (0.11)	0.22 (0.03)	0.90	0.05 (0.00)	10 (1)	13 (2)	197.93 (31.50)	1 (0)
PAR III (11)	0.31 (0.04)	0.12 (0.01)	0.43	0.16 (0.02)	4 (1)	4 (1)	67.85 (13.17)	1 (0)

Standard Errors in parentheses.

The number of individual plants at the end of each PAR treatment for which data could be collected are provided in Table 2.4. Tables 2.5 through 2.7 summarize the average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface for baseline and each of the three PAR treatments. The relationships for each measured plant metric are shown in Figures 2.5 through 2.10.

Table 2.4 Number of plants remaining at the end of the treatment period for TWR ex situ PAR I-III study.

	Above ground (g) (Shoot)	Below ground (g) (Roots)	Shoot number	Root number	Total leaf surface area (cm^2)	Number broken leaves
PAR I						
Control (100%)	**15**	**15**	**15**	**15**	**15**	**15**
10% light reduction	**14**	**15**	**15**	**14**	**15**	**15**
20% light reduction	**15**	**15**	**15**	**15**	**15**	**15**
40% light reduction	**15**	**15**	**15**	**15**	**15**	**15**
80% light reduction	**14**	**15**	**15**	**14**	**15**	**15**
PAR II						
Control (100%)	**15**	**15**	**14**	**15**	**15**	**15**
10% light reduction	**15**	**15**	**14**	**15**	**15**	**15**
20% light reduction	**15**	**15**	**14**	**15**	**15**	**15**
40% light reduction	**15**	**15**	**14**	**15**	**15**	**15**
80% light reduction	**15**	**15**	**14**	**15**	**15**	**15**
PAR III						
Control (100%)	**12**	**12**	**12**	**12**	**12**	**12**
10% light reduction	**12**	**12**	**12**	**12**	**12**	**12**
20% light reduction	**11**	**11**	**11**	**11**	**11**	**11**
40% light reduction	**12**	**12**	**12**	**12**	**12**	**12**
80% light reduction	**12**	**12**	**12**	**12**	**12**	**12**

Table 2.5 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the ex situ PAR I study.

	Above ground (g) (Shoot)	Below ground (g) (Roots)	Above/ Below	Total biomass (g)	Shoot number	Root number	Total leaf surface area (cm^2)	Number broken leaves
Treatment								
Control (100%)	11.48 (2.39)	3.9 (1.23)	2.94	15.39	33 (14)	86 (9)	**	15 (4)
10% light reduction	10.17 (1.22)	4.00 (0.82)	2.54	14.18	49 (19)	100 (12)	**	27 (10)
20% light reduction	9.87 (2.03)	5.38 (1.70)	1.83	15.25	38 (10)	87 (12)	**	13 (4)
40% light reduction	8.88 (2.69)	3.34 (1.10)	2.66	12.22	24 (8)	71 (13)	**	14 (6)
80% light reduction	4.33 (0.62) $^{a\,b}$	1.24 (0.17) $^{a\,b}$	3.49	5.57 $^{a\,b}$	17 (8)	57 (6)	**	12 (6)

**Data not available due to leaf entanglement associated with October 2015 flood event

Standard error are in parentheses (n=14-15)

^{a} Values significantly different from the control within a treatment period (α=0.05) using Tukey's test post ANOVA

^{b} Values significantly different from 20% light reduction within a treatment period (α=0.05) using Tukey's test post ANOVA

Table 2.6 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the ex situ PAR II study.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm²)**	**Number broken leaves**
Treatment								
Control (100%)	4.66 (1.21)	1.30 (0.32)	3.58	5.96	31 (9)	52 (10)	1176.51 (322.07)	6 (2)
10% light reduction	4.32 (0.64)	1.53 (0.23)	2.82	5.84	49 (8)	61 (8)	1599.79 (319.26)	5 (2)
20% light reduction	5.40 (0.88)	1.69 (0.25)	3.20	7.09	38 (6)	68 (7)	1626.85 (228.66)	4 (1)
40% light reduction	3.41 (0.59)	0.82 (0.15)	4.16	4.23	27 (5)	41 (7)	1381.86 (205.68)	3 (1)
80% light reduction	2.88 (0.49)	0.61 (0.11) [b]	4.72	3.49 [b]	21 (3)	37 (6)	1058.18 (182.82) [b]	3 (1)

Standard error are in parentheses (n=14-15)

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

[b] Values significantly different from 20% light reduction within a treatment period (α=0.05) using Tukey's test post ANOVA

Table 2.7 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the ex situ PAR III study.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Total leaf surface area (cm²)**	**Number broken leaves**
Treatment								
Control (100%)	1.88 (0.35)	0.43 (0.06)	4.37	2.31	13 (2)	22 (4)	446.75 (84.66)	2 (1)
10% light reduction	1.52 (0.51)	0.48 (0.17)	3.17	2.00	16 (5)	24 (6)	468.04 (150.28)	3 (1)
20% light reduction	2.34 (0.34)	0.51 (0.07)	4.59	2.85	17 (1)	25 (4)	643.14 (73.46)	4 (1)
40% light reduction	1.62 (0.21)	0.38 (0.06)	4.26	2.00	13 (2)	22 (3)	417.77 (57.62)	3 (1)
80% light reduction	0.85 (0.13) [a b]	0.19 (0.02) [a b]	4.47	1.04 [a b]	6 (1)	11 (1)	243.77 (33.27) [a b]	2 (0)

Standard error are in parentheses (n=11-12)

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

[b] Values significantly different from 20% light reduction within a treatment period (α=0.05) using Tukey's test for ANOVA

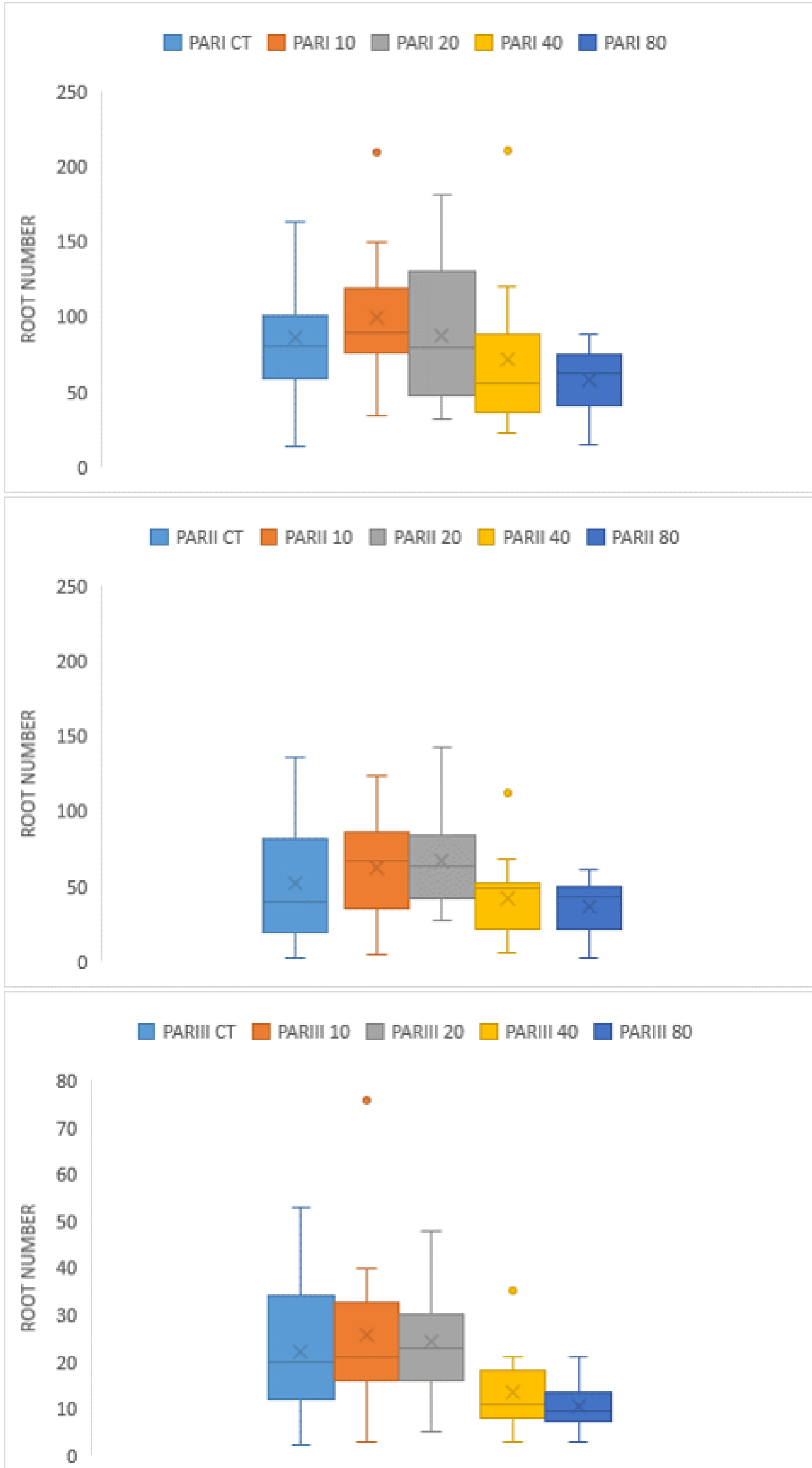

Figure 2.5 Number of roots observed under decreasing amounts of PAR for each ex situ treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

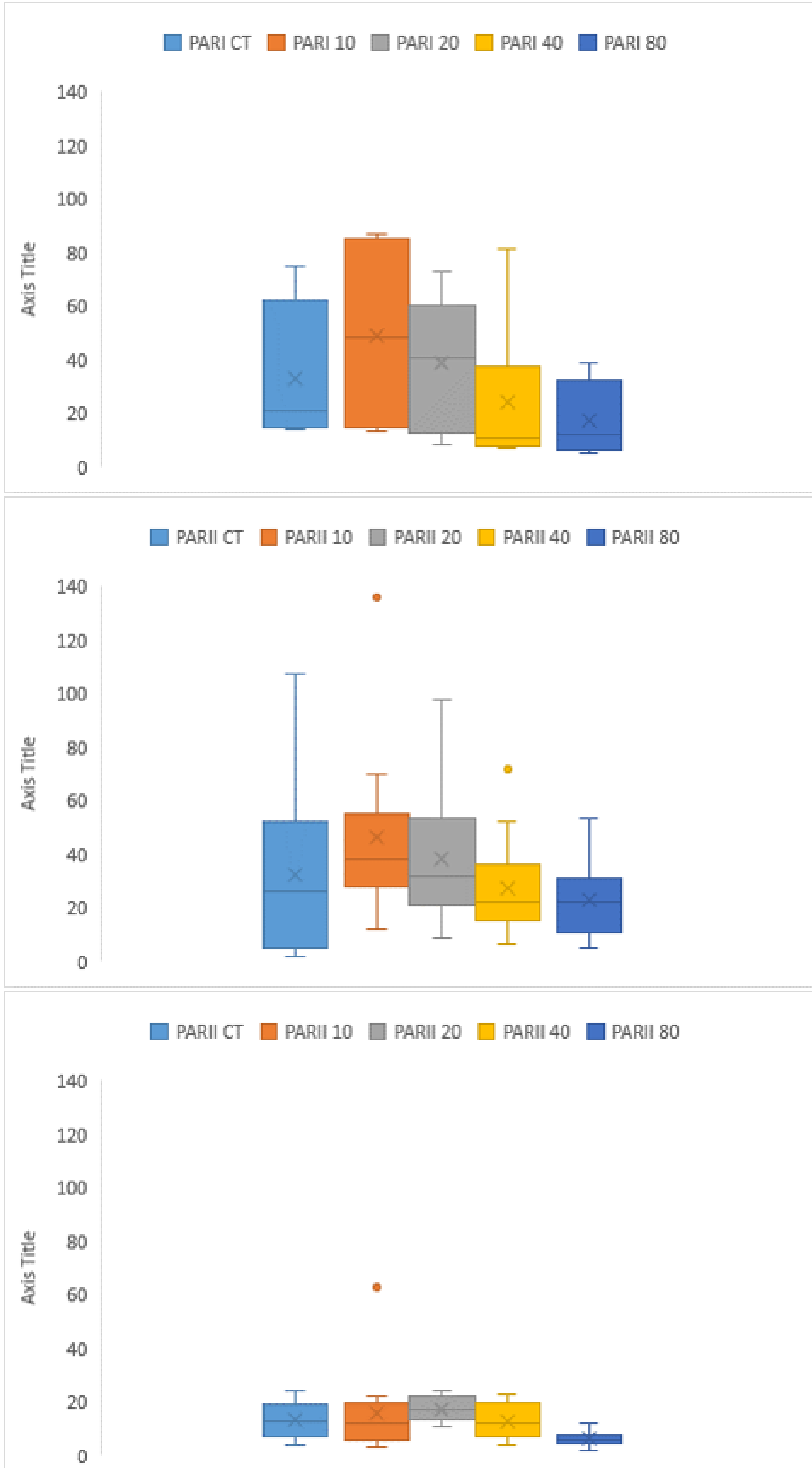

Figure 2.6 Number of shoots observed under decreasing amounts of PAR for each *ex situ* treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

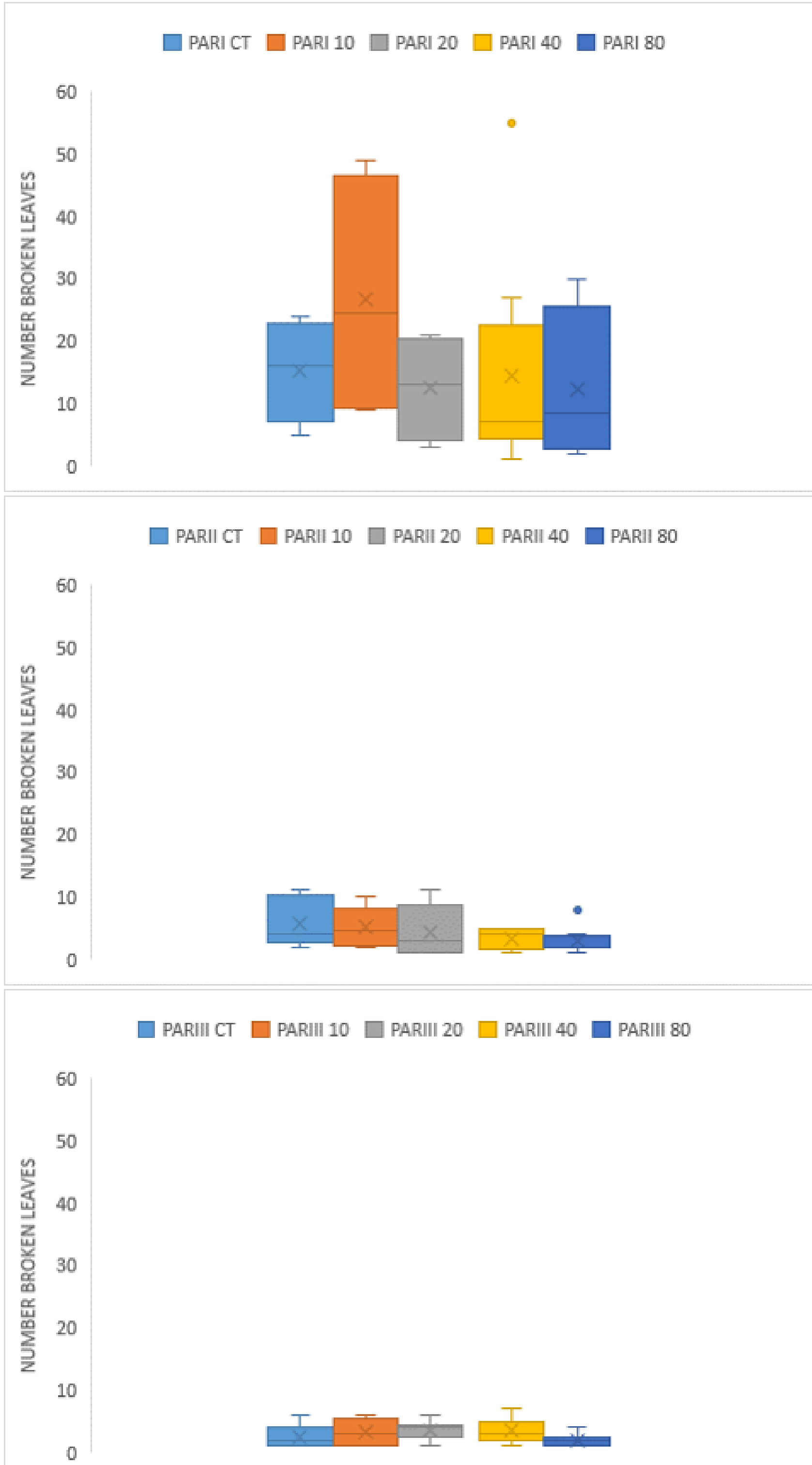

Figure 2.7 Number of broken leaves observed under decreasing amounts of PAR for each ex situ treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

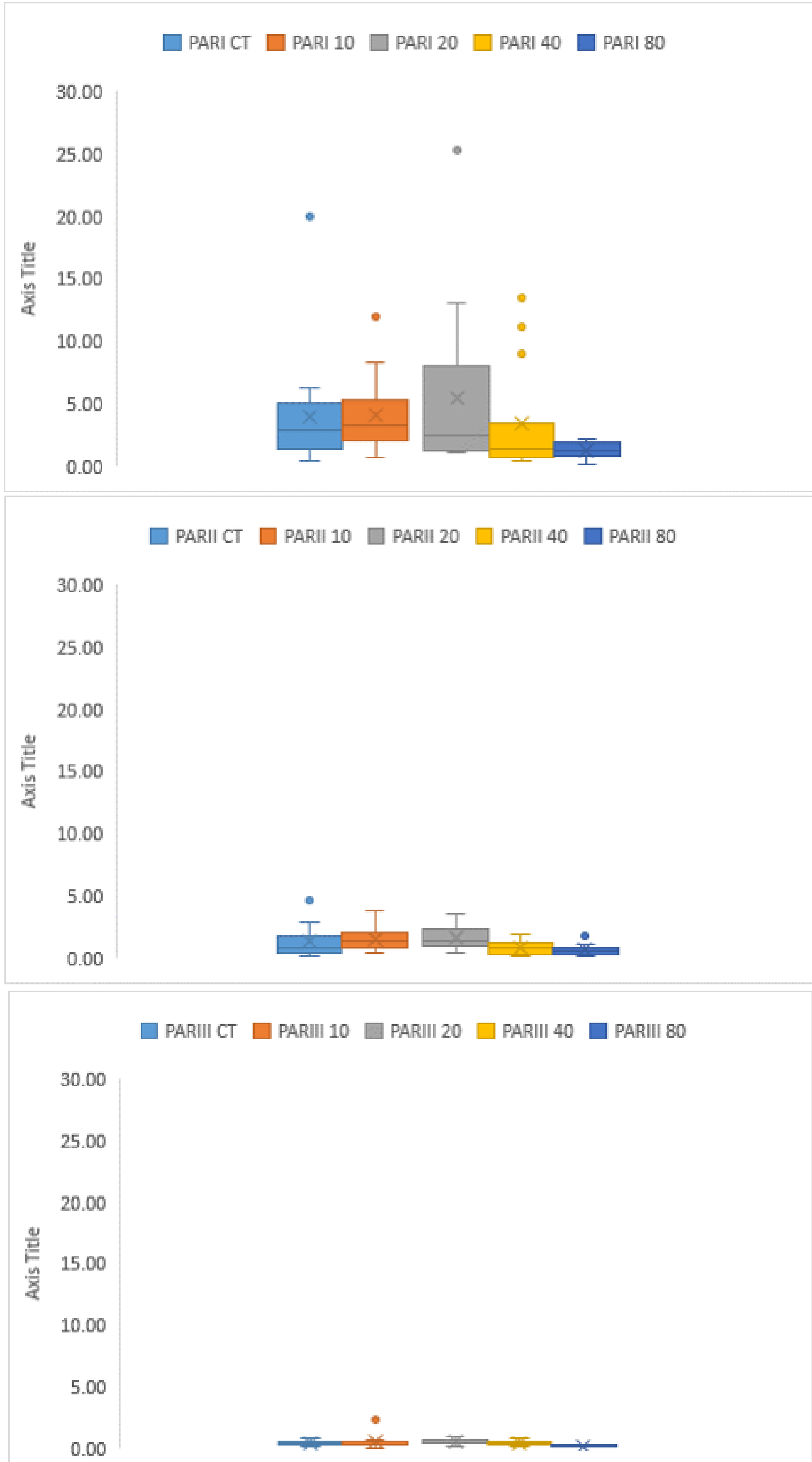

Figure 2.8 Root dry weight observed under decreasing amounts of PAR for each *ex situ* treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

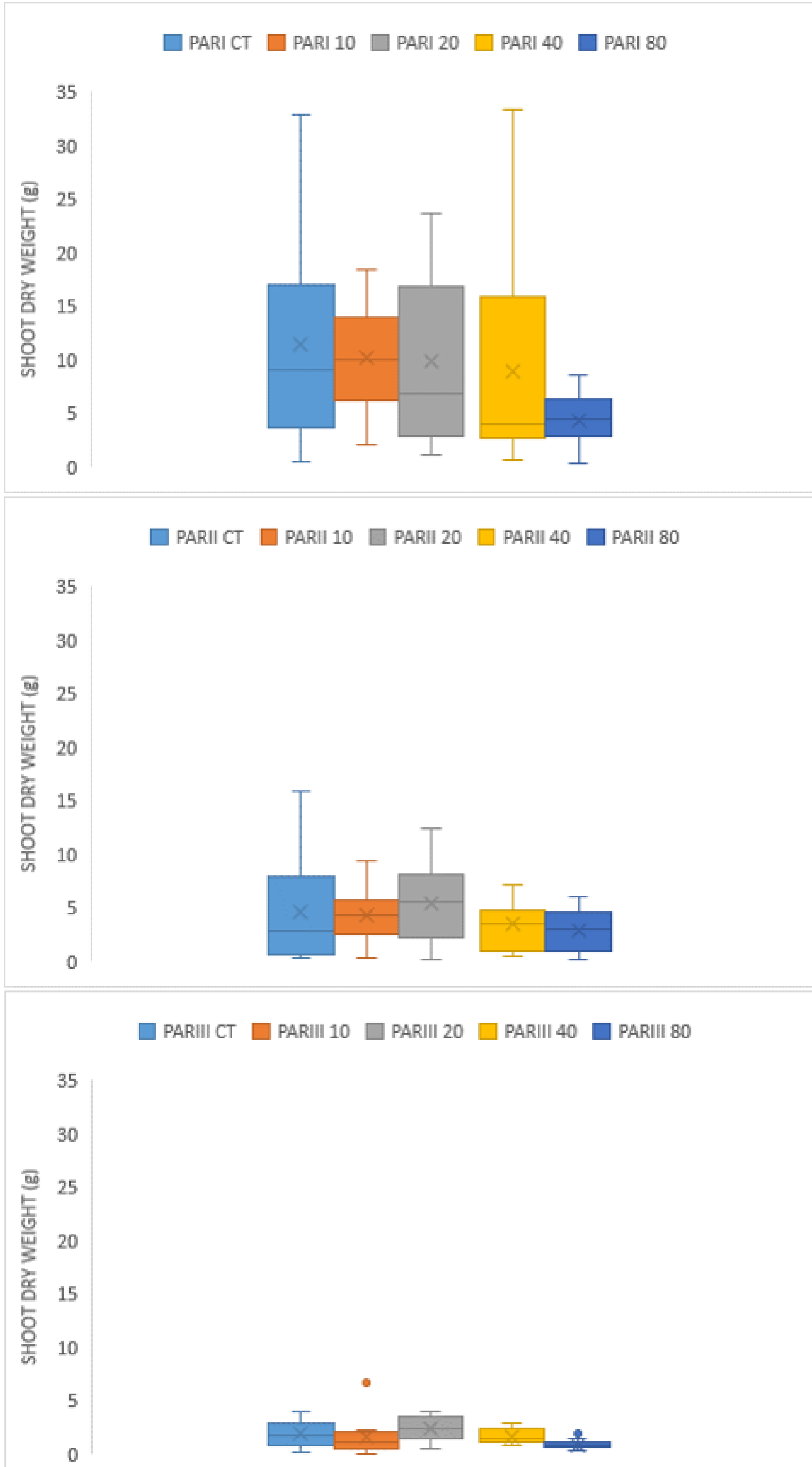

Figure 2.9 Shoot dry weight observed under decreasing amounts of PAR for each *ex situ* treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

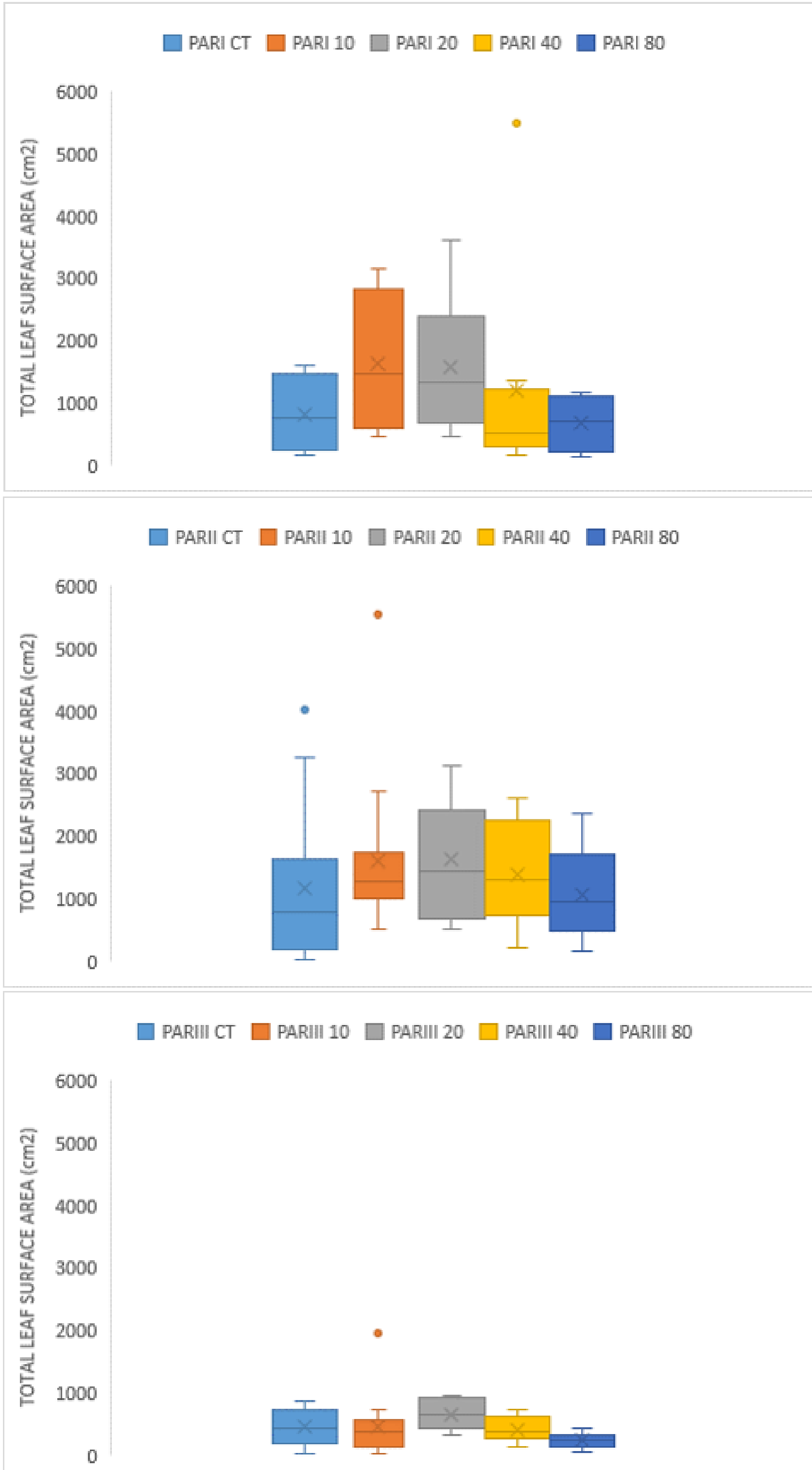

Figure 2.10 Total leaf surface area observed under decreasing amounts of PAR for each *ex situ* treatment and overall average of all treatments (CT = control). Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

Significant differences in shoot, root, and total biomass, as well as total leaf surface area existed in PAR I, II, and III experiments. In both the PAR I and PAR III studies, plants exposed to 20% PAR exhibited significantly less shoot biomass (PAR I: $p = 0.009$; PAR III: $p = 0.020$), root biomass (PAR I: $p = 0.048$ and PAR III: $p = 0.004$), and total biomass (PAR I: $p = 0.0147$; PAR III: $p = 0.000$) compared to plants exposed to 100% PAR. Additionally, a significant difference existed in PAR II for total biomass ($p = 0.013$). However, there was no significant difference between root and shoot biomass in the PAR II experiment ($p_{root} = 0.065$; $p_{shoot} = 0.184$). Plants exposed to 20% PAR exhibited significantly less root biomass (PAR I: $p = 0.027$; PAR II: $p = 0.001$; PAR III: $p < 0.001$), shoot biomass (PAR I: $p = 0.017$; PAR II: $p = 0.013$; PAR III: $p < 0.001$), and total biomass (PAR I: $p = 0.015$; PAR II: $p = 0.006$; PAR III: $p = 0.000$) compared to those exposed to 80% PAR in PAR I, II and III experiments. Plants exposed to 20% available PAR resulted in having a total biomass that was approximately less than half of 100% PAR and 80% PAR availability for all three experiments (Tables 2.5 through 2.7).

Total leaf surface area was only calculated in the PAR II and PAR III experiments. It was not possible to collect leaf surface area data in the PAR I experiment because the leaves became entangled in debris as a result of the October 2015 flood event (Fig. 2.4). Attempts to untangle them resulted in severe fragmentation. Total leaf surface area (Tables 2.6 and 2.7) was significantly greater in plants exposed to 80% PAR compared to plants exposed to 20% PAR in both PAR II ($p = 0.023$) and in PAR III ($p = <0.001$). No other significant differences in total leaf surface area were found.

2.4 Discussion

Results of the three PAR studies demonstrated that TWR growth is impacted by limited light availability. Relationships between light availability and corresponding depth limits for seagrass growth have been developed for many species (Duarte 1991; Dennison 1987; Nielsen et al. 2002). The minimum light requirements of seagrasses investigated by Duarte (1991) and Dennison et al., (1993) ranged from 4% to 30% incident light availability. Kurtz et al. (2003) further investigated the light requirements of *Vallisneria americana*, and found that a reduction in biomass was apparent when plants were exposed to 21% and 8% light availability due to shading. With only 20% light available, shoot, root, and total biomass, as well as total leaf surface area of TWR plants were significantly reduced in PAR I, PAR II, AND PAR III study periods. Both the above- and below-ground biomass values, as well as total biomass and total leaf surface area for the 20% light availability treatment were significantly lower than that of the control (100%) and 80% light availability treatments. PAR availability is considered most strongly correlated with areal coverage of aquatic plants and is influenced by the extent of riparian shading, especially in relatively narrow rivers where light attenuation by the water column is minimal (Davies et al. 2008; Dodds and Welch 2000; Hilton et al. 2006; Julian et al. 2008). The results of this PAR study parallel Tolman's (2013) study, which found the least amount of TWR areal coverage occurred in the narrowest segment of the San Marcos River with the greatest extent of riparian canopy cover and where light availability was only 28 percent. Thus, light availability is one important factor that needs consideration in determining locations to reintroduce TWR in the river.

It is apparent from these results that although we standardized the number and length of both root and shoots at the beginning of each PAR replicate treatment, there was a significant difference in all measured plant metrics between each successive PAR experiment for the baseline data. Somewhat higher values for plant metrics were expected given the longer baseline growth period associated with PAR I. However, the different length in daylight hours between PAR II and PAR III (642 versus 701) seems improbable as a causative factor for the observed differences, especially given the increasing trend for daylight hours between the PAR II and PAR III periods. Furthermore, the constant water quality characteristics over the entire study period (Table 2.2), as well as the similar amount of ambient cloud shading over the period of the experiments (i.e., an average of 41, 40, and 53 percent) cannot account for the observed differences between baseline for the PAR treatments, especially when comparing the results between PAR II and PAR III. We speculate that this observed pattern was related in some way to unknown differences in source tillers used in the propagation.

3.0 *In Situ* Experiments: Suspended Sediment Induced Turbidity Associated with Recreational River Use on the Vegetative Growth of Texas wild-rice (*Zizania texana*)

3.1 Introduction

The availability of light is the single most important abiotic factor affecting a plant's biomass (Madsen 1993). 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 inorganic suspended solids, and microorganisms (Bornette and Puijalon 2011). These factors contribute to increased turbidity levels that can reduce the vertical attenuation of light in water (Kirk 2011). Suspended sediment induced turbidity in an aquatic system can result in a decrease in water clarity and reduction in availability PAR (Madsen et al. 2001). It has been suggested by Barko et al. (1986) that in most aquatic environments turbidity is a significant factor in limiting light availability, subsequently negatively affecting aquatic macrophyte growth. For example, Robel (1961) demonstrated that increased turbidity in aquatic systems resulted in decreased biomass in *Potamogeton pectinatus*.

Light availability is also influenced by accumulation of periphyton, including epiphytic algae, on the leaves of aquatic macrophytes. The accumulation of a periphyton may result in a boundary layer diminishing the amount of light reaching the surface of macrophyte leaves, thus partially or indirectly influencing plant productivity and biomass (Orth and Van Montfrans 1984). The accumulation of periphyton on the surfaces of macrophyte leaves concomitant with a decrease in available PAR has been demonstrated to suppress the overall biomass production of macrophytes (Asaeda et al. 2004; Tóth 2013).

Recreational activity such as swimming, tubing, boating and fishing can cause increased turbidity in bodies of water (e.g., Hall and Härkönen 2006). High levels of suspended solids in the water column can reduce and/or limit the amount of light available, which may negatively impact growth of aquatic macrophytes.

Texas wild-rice (*Zizania texana*; Poaceae) is an endangered macrophyte known to occur only in the spring-fed San Marcos River, Hays County, Texas. The San Marcos River is impacted on a seasonal basis by contact water recreation such as kayaking, canoeing, swimming, tubing, wading, and fishing that results in physical disturbance and increased suspended sediments in the water column (Breslin 1997; Saunders et al. 2001). Preliminary data suggest a strong correlation exists between increasing turbidity levels (diel, weekly, and seasonal) and intensity of contact recreational use of the river (Thomas Hardy; Weston Nowlin, unpublished data). The data suggest that the strong longitudinal pattern of turbidity in the river (i.e., upstream to downstream) coincides with intensity of contact recreation (number of people) and seasonal periods of high contact recreational activity in the San Marcos River.

Poole and Bowles (1999) and Saunders et al. (2001) suggested that TWR has evolved to rely on the transparency of the clear river water to allow adequate light for photosynthesis. It is unknown if increased turbidity and the resulting decrease in PAR associated with suspended sediments affects the productivity and biomass of TWR. Therefore, the objective of this study component was to analyze the effects of increased turbidity and accumulation of periphyton associated with increased recreational activity on the vegetative growth of TWR based on an *in situ* experimental design.

3.2 Material and Methods

In situ experiments were conducted at four locations in the upper reaches of the San Marcos River, Hays County, Texas, to compare the effects of recreational related suspended sediment (low use to high use) on TWR vegetative growth (see Figure 1.1). The study period representing low recreational use was conducted from April 10 to May 29, 2015 (39 days) and the period representing high recreational use was conducted during May 29 to July 23, 2014 (56 days). The high recreational use period was repeated during higher river discharges from May 28 through July 20, 2015 (52 days) (Figure 3.1). These periods were selected based on river use observations collected by The Meadows Center for Water and the Environment (MCWE) over the past three years (T. Hardy, *unpublished data*).

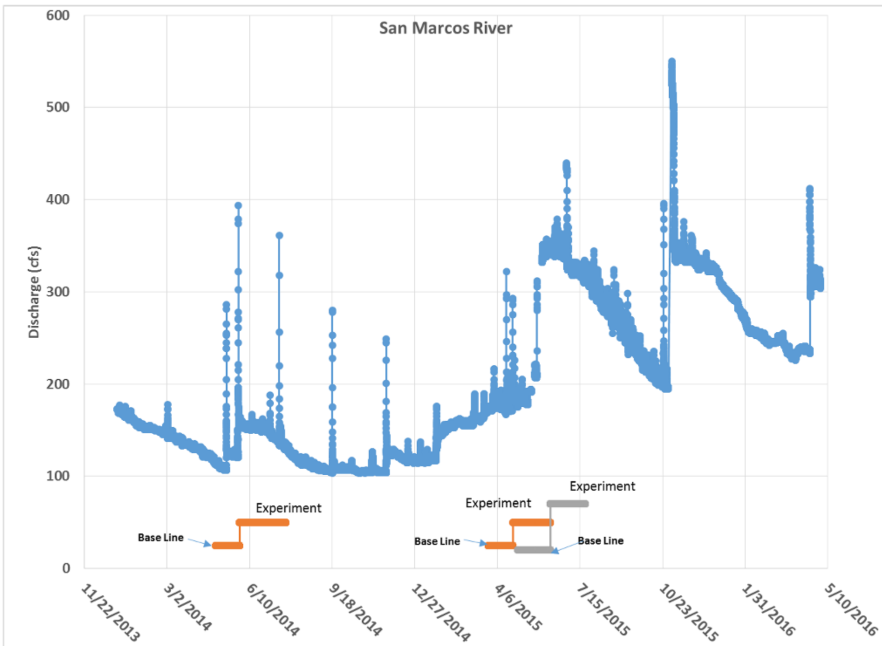

Figure 3.1 Daily average discharge (cfs) and *in situ* experimental dates in the San Marcos River. The change in experimental period color bars in the graph are for clarification between the two consecutive experiments where baseline overlapped with the end of the previous treatment period.

Figure 3.1 shows the daily discharge of the San Marcos River, mean daily discharges, corresponding baseline grow-out period (SMARC) and the dates over which the *in situ* river experiments were conducted. It is apparent that the discharge magnitude and variability was different over each *in situ* experimental period. 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 location of plant trays were selected, they were not moved in response to apparent changes in depth and velocity 'at the plant' due to changes in discharge.

Seasonally, recreational use of the river is highest in the summer, moderate in the fall, and low in winter and early spring (see Section 5 below; Kevin Huffaker, River Watchers, *pers. comm.*; MCWE, unpublished data). Stationary game cameras were used to quantify recreational use in the upper reaches of the river associated with river access locations. The cameras record recreational use (number of 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 (see Section 5). With the exception of the Eastern Spillway study site, these locations integrate data upstream of the *in situ* treatment locations. Water quality data were obtained from the EAHCP real time water quality monitoring stations at the Aquarena Drive Bridge and Rio Vista falls stations. The study sites were located in San Marcos River State Scientific Areas (SSA) established by Texas Parks and Wildlife Department to protect TWR (Edwards Aquifer Habitat Conservation Plan 2012) to minimize direct disturbance from recreational activities. The study sites were also selected to provide maximum exposure to sunlight with a depth no greater than one meter.

The *in situ* experiments at four study sites were located below the Eastern Spillway at Spring Lake Dam and ending downstream at Ramon Lucio Park (see Figure 1.1):

1. Eastern Spillway (ES)
2. Sewell Park (SP)
3. Bicentennial Park (BP)
4. Ramon Lucio Park (RL)

At the onset, mid-point and end of each study period PAR and velocity data was collected. PAR was measured at the water surface, immediately below the water surface, at 10cm, and at the plant level using a dual channel Li-Cor LI 1935A meter fitted with a 4π sensor. PAR data was collected at midday when sunlight is at its maximum to minimize the variation between ambient light conditions due to time of day. Velocity at the plants was recorded using a Marsh-McBirney 2000 flow meter and top set wading rod at the depth of plants contained in plant trays. Daily river discharge was obtained from the USGS gage station (0817000) located in the San Marcos River below Spring Lake Dam. Hourly meteorological data (e.g. temperature, cloud cover, precipitation, etc.,) was obtained from the San Marcos Airport weather station ~5 km from the study site.

For each of the three experimental treatments (two - high recreational use periods and one - low recreational use period), 200 TWR plants were propagated from seed using a single 5-L germination container in an outdoor raceway at the San Marcos Aquatic Research Center, San Marcos, Texas. Soil used for propagation and growth was a local proprietary blend of mulch, humus, sand, and pea size gravel utilized by the U.S. Fish and Wildlife Service San Marcos Aquatic Resource Center for TWR propagation. Seeds were allowed to grow for two weeks then 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 this four-week total growth period, 75 plants were randomly selected for use in the in situ study. From these 75 plants, 15 plants were randomly selected to measure initial plant size and biomass to use as a baseline at initiation of the *in situ* experiments.

The remaining 60 plants were randomly assigned to one of four study sites in the river. Three trays, each containing five randomly selected TWR plants, were placed 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) all remaining TWR in study trays were collected for analysis.

Leaves were separated and corresponding leaf sheaths from the root mass at the juncture. The number of individual leaves and roots (excluding root hair) were recorded. The longest intact leaf from each plant was selected for periphyton analysis as follows. Each selected leaf was measured for total leaf surface area and the entire leaf area was manually scraped five times on the adaxial and abaxial surface. Care was taken not to scrape leaves in such a manner to contaminate the periphyton samples with leaf material. Collected material was filtered and analyzed for total solids (TS), non-volatile solids (NVS) and

Chlorophyll-α. All leaves and roots for each plant were separated and dried for 48 hours at 60˚C in a drying oven and weighed to determine leaf and root dry biomass. The number of individual plants at the end of the baseline period for the *in situ* study treatment for which data could be collected are provided in Table 3.1. The number of individual plants at the end of each *in situ* treatment period for which data could be collected are provided in Table 3.2. The average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area at each study site are provided in Tables 3.3 through 3.5 for each study period. For the 2015 treatments, Table 3.4 and 3.5 also provide the average Total Solids (TS), Non-Volatile Solids (NVS) and Chlorophyll-α (Chl-α). Note that TS, NVS and Chl-α were not collected from TWR leaves during 2014. These results are shown in Figures 3.2 through 3.8. 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, significant 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 during the 2014 *in situ* experiment were due to plants being smothered with fine sediment deposition. Plant loss at Ramon Lucio during 2014 was associated with vandalism and suspected accidental displacement. Significance of differences within and among treatment periods for TWR growth was analyzed by ANOVA followed by Tukey's HSD ($p \leq 0.05$) to compare treatment means where differences were found. Statistical analysis was performed using Excel.

Table 3.1 Average number of individual Texas wild-rice plants remaining at the end of the baseline growth period.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**
Study Period (n)						
Hi-Rec 2014 (15)	0.15 (0.02)	0.03 (0.00)	5.00	0.17 (0.03)	7 (1)	8 (1)
Pre-Rec 2015 (15)	0.04 (0.00)	0.01 (0.00)	4.00	0.05 (0.00)	5 (0)	7 (0)
Hi-Rec 2015 (15)	0.15 (0.02)	0.02 (0.00)	7.50	0.16 (0.02)	6 (0)	10 (1)

Standard errors are in parentheses after the mean.

Table 3.2 Number of plants remaining at the end of the treatment period for TWR *in situ* Turbidity study.

	Above ground (g) (Shoot)	Below ground (g) (Roots)	Shoot number	Root number	Total solids (mg/cm^2)	Non-volatile solids (mg/cm^2)	Chlorophyll-α (mg/cm^2)
HR2014							
Eastern Spillway (ES; Control)	10	10	10	10	10	10	10
Sewell Park	10	10	10	10	10	10	10
Bicentennial Park	4	5	4	5	4	4	4
Ramon Lucio	2	3	2	3	2	2	2
PR2015							
Eastern Spillway (ES; Control)	15	15	15	15	15	15	15
Sewell Park	14	14	14	14	14	14	14
Bicentennial Park	12	12	12	12	12	12	12
Ramon Lucio	12	12	12	12	12	12	12
HR2015							
Eastern Spillway (ES; Control)	14	14	14	14	14	14	14
Sewell Park	15	15	15	15	15	15	15
Bicentennial Park	12	14	12	14	12	12	12
Ramo Lucio	4	4	4	4	4	4	4

3.3 Results

Significant differences in root, shoot, and total biomass of TWR existed between longitudinal study site locations during all three time periods for the *in situ* San Marcos River study (Tables 3.3, 3.4, and 3.5). Regardless of the experimental period, there was greater TWR shoot biomass (Hi-Rec 2014: $p < 0.001$; Pre-Rec 2015: $p = 0.002$ and Hi-Rec 2015: $p = 0.002$), root biomass (Hi-Rec 2014: $p = 0.045$; Pre-Rec 2015: $p = 0.021$ and Hi-Rec 2015: $p = 0.004$), and total biomass (Hi-Rec 2014: $p < 0.001$, Pre-Rec 2015: $p = 0.004$ and Hi-Rec 2015: $p = 0.001$) at the Eastern Spillway (ES) control site than the first downstream treatment site at Sewell Park (SP). The shoot, root and total biomass of TWR plants at the Bicentennial Park (BP) site was significantly lower in the Hi-Rec period in 2014 ($p_{shoot} = 0.017$; $p_{root} = 0.005$; $p_{total} = 0.007$). However, during the Pre-Rec and Hi-Rec study periods in 2015, the shoot biomass (Pre-Rec 2015: $p = 0.058$ and Hi-Rec 2015: $p= 0.254$), root biomass (Pre-Rec 2015: $p = 0.386$ and Hi-Rec 2015: $p = 0.971$) and total biomass (Pre-Rec 2015: $p = 0.315$ and Hi-Rec 2015: $p = 0.315$) of TWR plants at the BP treatment site did not differ from TWR plants at the ES site. Although total TWR biomass was not significantly different between the ER and the farthest downstream Ramon Lucio (RL) treatment site during the Hi-Rec period in 2014 ($p = 0.081$), there was significantly less TWR root, shoot and total biomass at Ramon Lucio (RL) for the Pre-Rec period in 2015 ($p_{shoot} = 0.001$, $p_{root} = 0.028$, $p_{total} = 0.003$), and only shoot and total biomass significantly differed in Hi-Rec ($p_{shoot} = 0.026$, $p_{total} = 0.010$) periods in 2015.

The mean number of broken leaves per TWR plant, in general, did not significantly differ between the ES control site and all of the downstream treatment sites across all of the study periods (Tables 3.3 – 3.5), with the only exceptions being that the number of broken leaves per plant was significantly lower at the

ES site when compared to the BP site during the Pre-Rec (p = 0.049) and Hi-Rec periods in 2015 (p < 0.001).

Significant differences in total solids (TS) and non-volatile solids (NVS) existed between longitudinal study site locations during Pre-Rec 2015. A significant difference for total solids and non-volatile solids was only found to exist in plants located in Bicentennial Park (p =0.029) and Ramon Lucio (p = 0.043) when compared to the Eastern Spillway. An increasing concentration in TWR leaf surface TS and NVS concentration existed in a longitudinal direction from the Eastern Spillway to the Bicentennial Park treatment site. However, TWR plants located in Ramon Lucio treatment site exhibited a lower concentration in TS and NVS for the Pre-Rec 2015 study period. During study periods Pre-Rec and Hi-Rec 2015, no significant differences existed for chlorophyll-α (Table 3.3-3.5).

Table 3.3 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the *in situ* Hi-Rec 2014 study.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Number broken leaves**
Treatment site							
Eastern Spillway (ES; Control)	60.82(13.51)	1.99(0.34)	30.56	62.81(3.70)	49(11)	65(7)	19 (4)
Sewell Park	4.83(0.79) [a]	1.49(0.33) [a]	3.24	4.59(1.04) [a]	50(6)	61(7)	26 (4)
Bicentennial Park	0.47(0.19)	0.33(0.11) [a]	1.42	0.70(0.42) [a]	24(10)	23(6)	10 (3)
Ramone Lucio	1.24(1.18) [a]	0.45(0.35) [a]	2.76	1.27(1.07)	38(13)	28(15)	19 (14)

Standard error are in parentheses

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

Table 3.4 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the *in situ* Pre-Rec 2015 study.

	Above ground (g) (Shoot)	**Below ground (g) (Roots)**	**Above/ Below**	**Total biomass (g)**	**Shoot number**	**Root number**	**Number broken leaves**	**Total suspended solids (mg/cm²)**	**Non-volatile suspended solids (mg/cm²)**	**Chlorophyll-α (mg/cm²)**
Treatment site										
Eastern Spillway (ES; Control)	1.86(0.39)	0.80(0.35)	2.33	2.57(0.78)	35(6)	38(6)	5 (1)	344.63(80.74)	219.37(64.29)	1.96(0.57)
Sewell Park	0.50(0.12) [a]	0.09(0.02) [a]	5.56	0.59(0.38) [a]	11(2)	23(3)	2 (0)	269.56(82.23)	142.08(55.41)	3.52(1.09)
Bicentennial Park	3.42(0.74) [a]	0.73(0.23)	4.68	4.45(0.99)	51(9)	61(11)	10 (2)	1696.38(383.20) [a]	1220.25(295.45) [a]	3.83(1.39)
Ramone Lucio	0.29(0.09) [a]	0.07(0.02) [a]	4.14	0.36(0.31) [a]	11(2)	18(2)	5 (1)	62.17(17.39) [a]	14.74(10.25) [a]	4.16(1.02)

Standard error are in parenthesis

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

Table 3.5 Average number of roots, shoots, broken leaves, root dry weight, shoot dry weight, total leaf surface area for the *in situ* Hi-Rec 2015 study.

	Above ground (g) (Shoot)	Below ground (g) (Roots)	Above/ Below	Total biomass (g)	Shoot number	Root number	Number broken leaves	Total suspended solids (mg/cm^2)	Non-volatile suspended solids (mg/cm^2)	Chlorophyll-α (mg/cm^2)
Treatment site										
Eastern Spillway (ES; Control)	8.58(1.24)	4.32(1.22)	1.99	14.71(2.90)	128(14)	105(11)	14 (6)	113.59(31.23)	28.84(13.10)	1.38(0.26)
Sewell Park	3.62(0.73) [a]	0.60(0.13) [a]	6.03	4.18(1.01) [a]	39(9)	45(6)	6 (1)	132.68(28.26)	44.83(14.38)	2.22(0.30)
Bicentennial Park	6.37(1.44)	4.25(1.38)	1.50	12.31(2.38)	111(21)	91(18)	56 (7)	107.96(30.61)	22.83(15.55)	2.26(0.56) [a]
Ramone Lucio	0.06(0.01) [a]	0.05(0.00)	1.20	0.11(0.01) [a]	9(1)	10(0)	1 (0)	8.90(3.51)	0.00(0)	2.18(0.83)

Standard error are in parenthesis

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

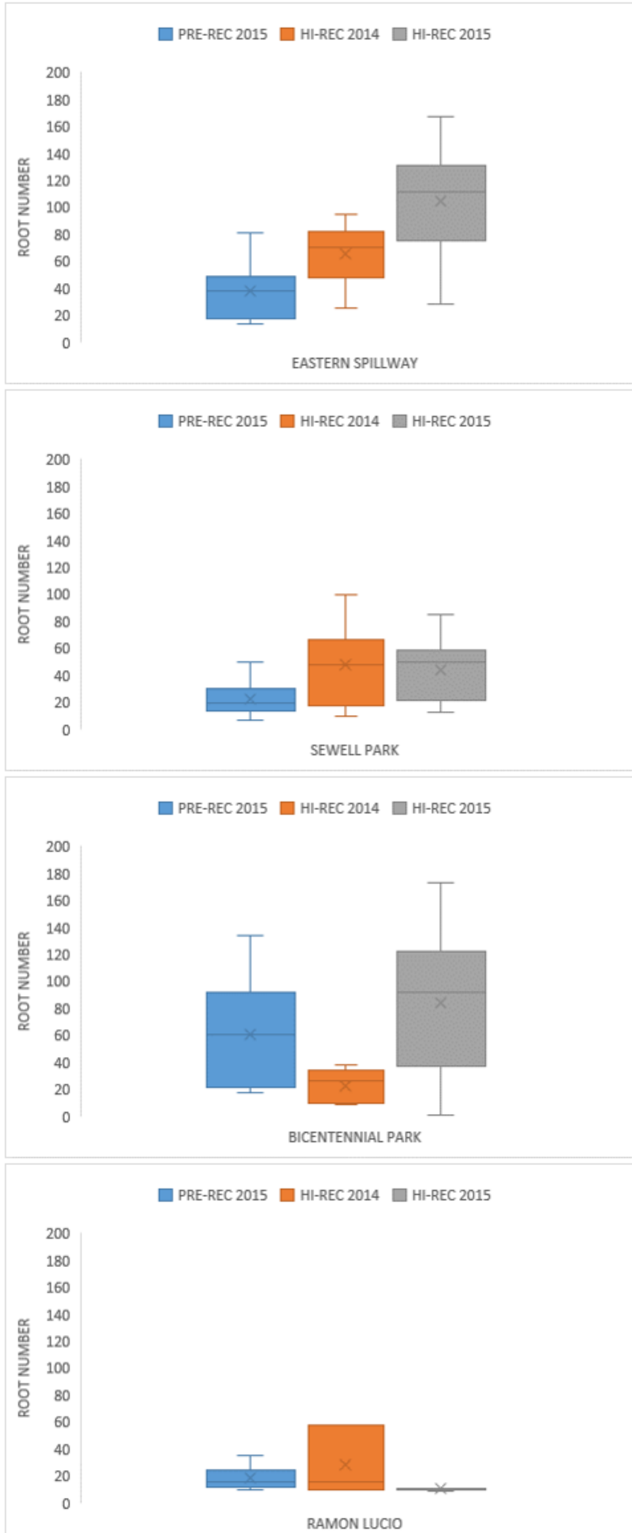

Figure 3.2 Number of roots for each *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

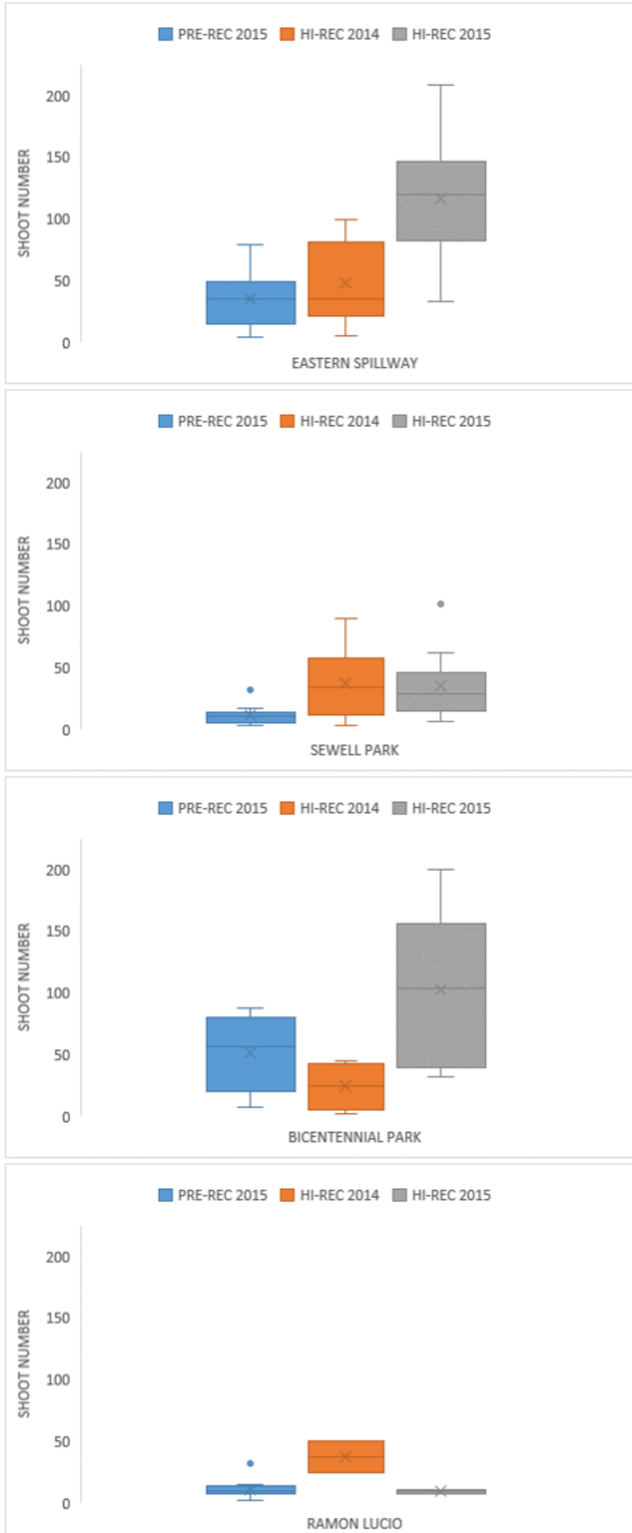

Figure 3.3 Number of shoots for each *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

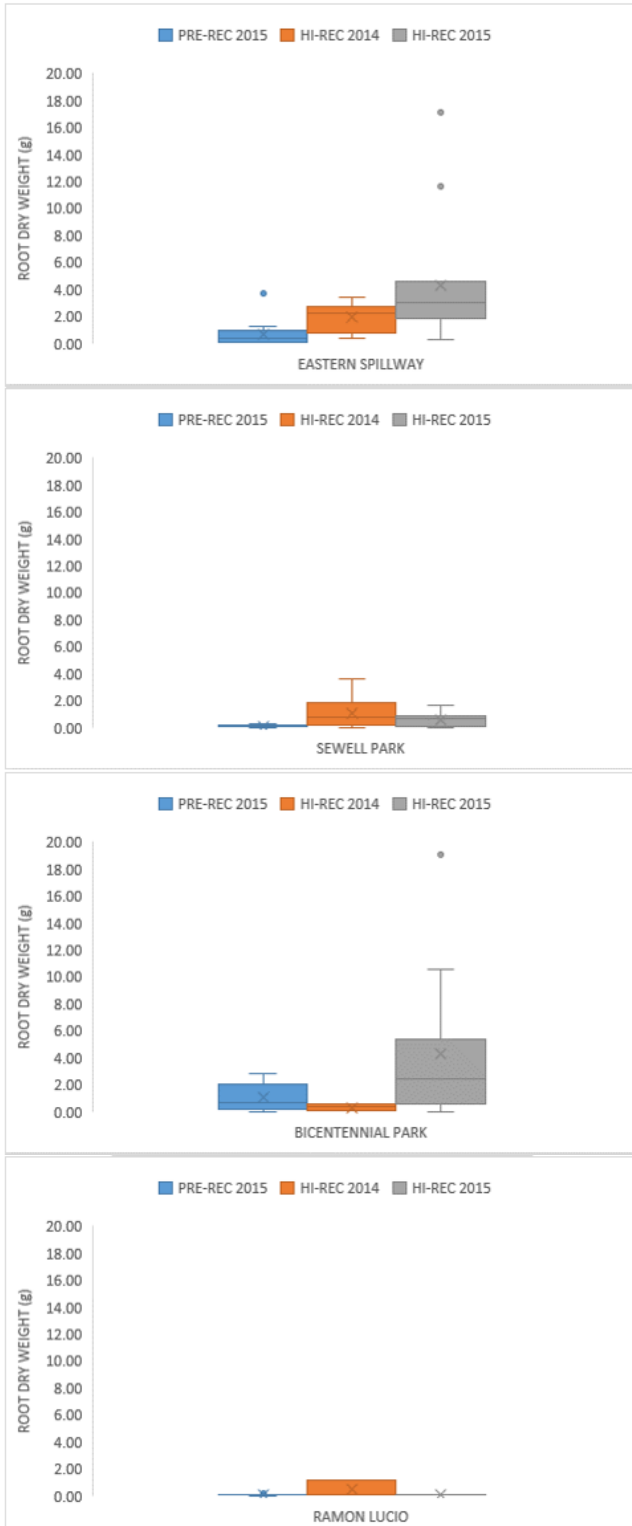

Figure 3.4 Root dry weight (g) for each *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

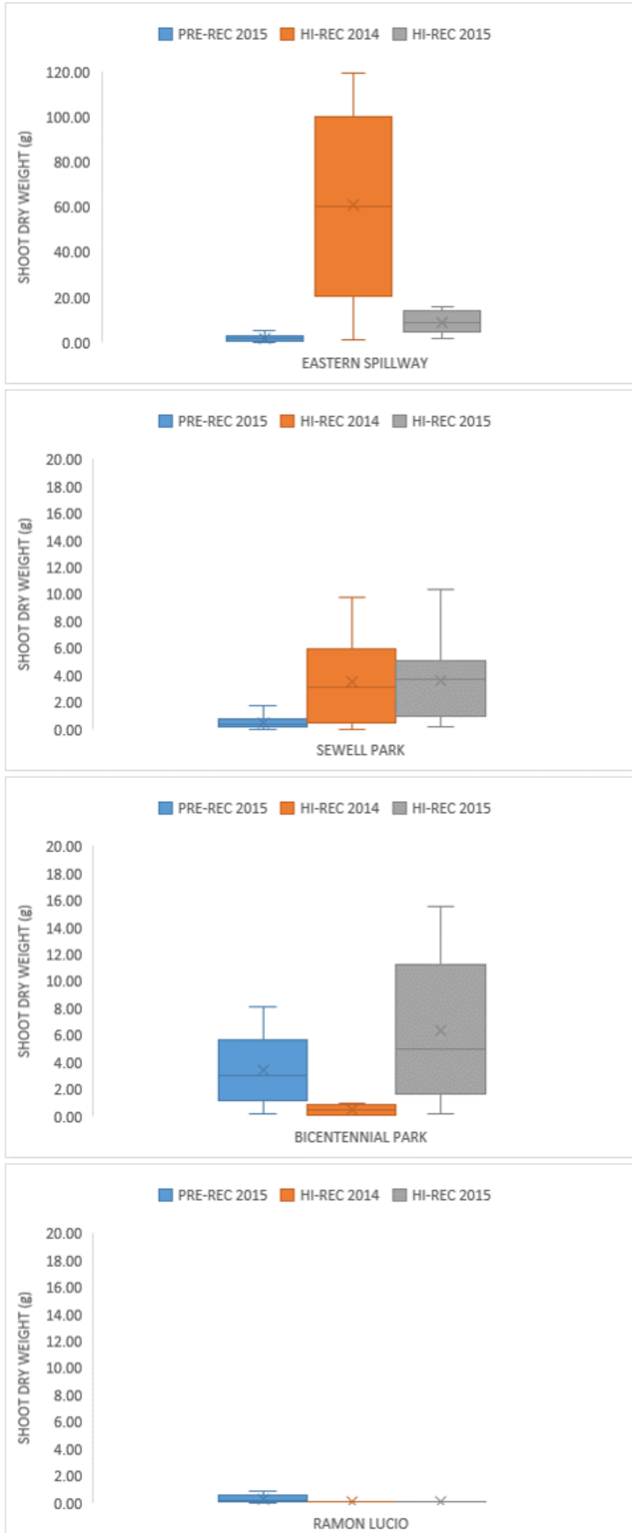

Figure 3.5 Shoot dry weight (g) for each *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values. NOTE change in scale for the Eastern Spillway plot.

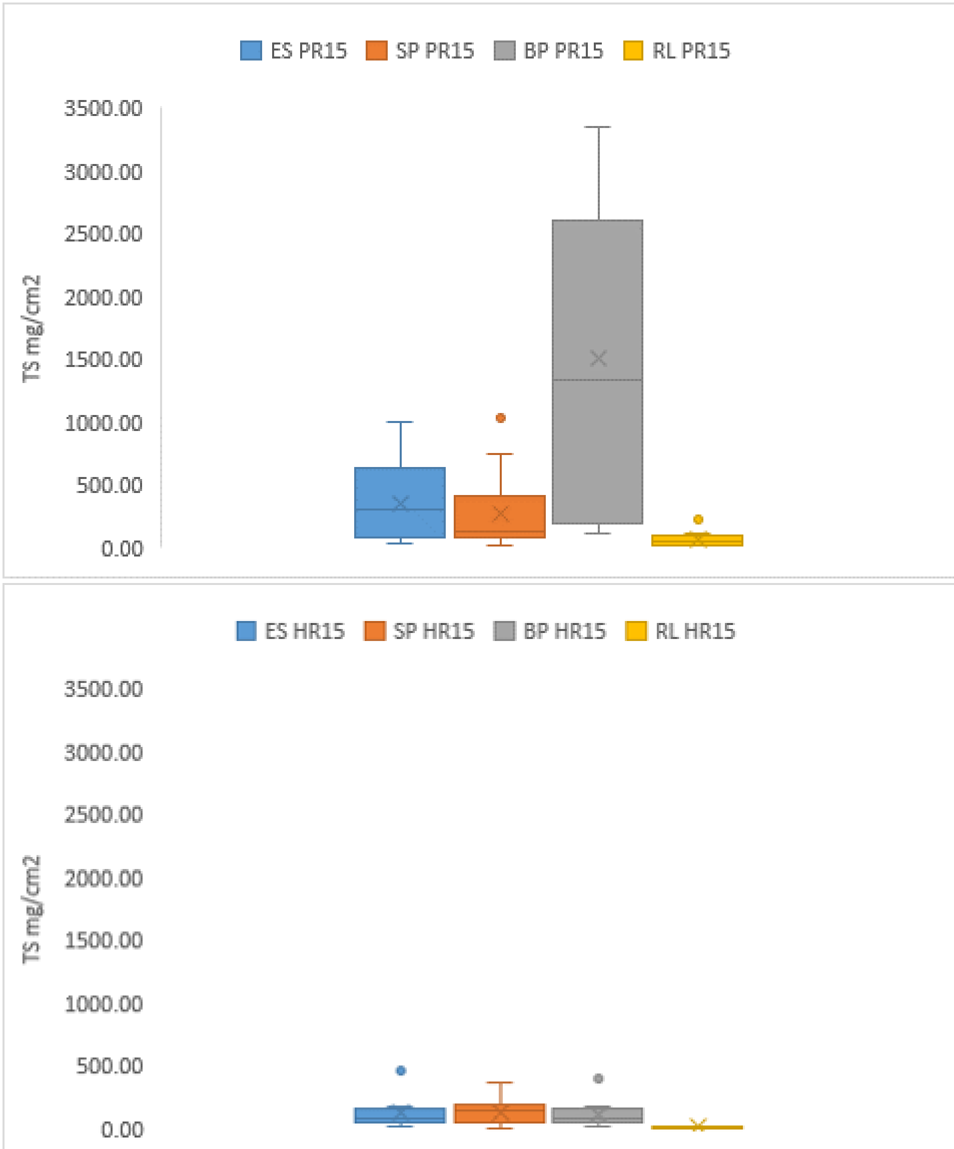

Figure 3.6 Total Solids (mg/cm^2) for each 2015 *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

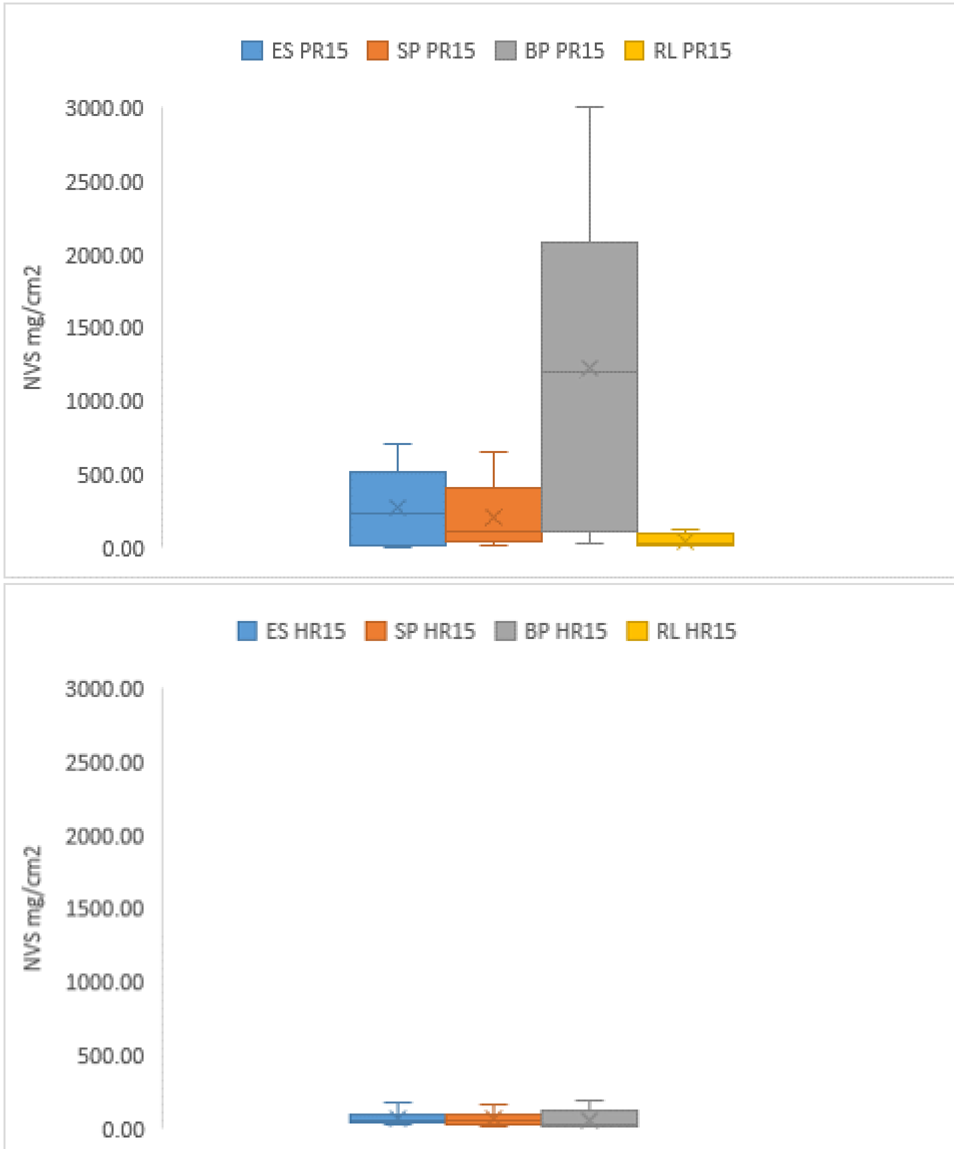

Figure 3.7 Non-Volatile Solids (mg/cm^2) for each 2015 *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

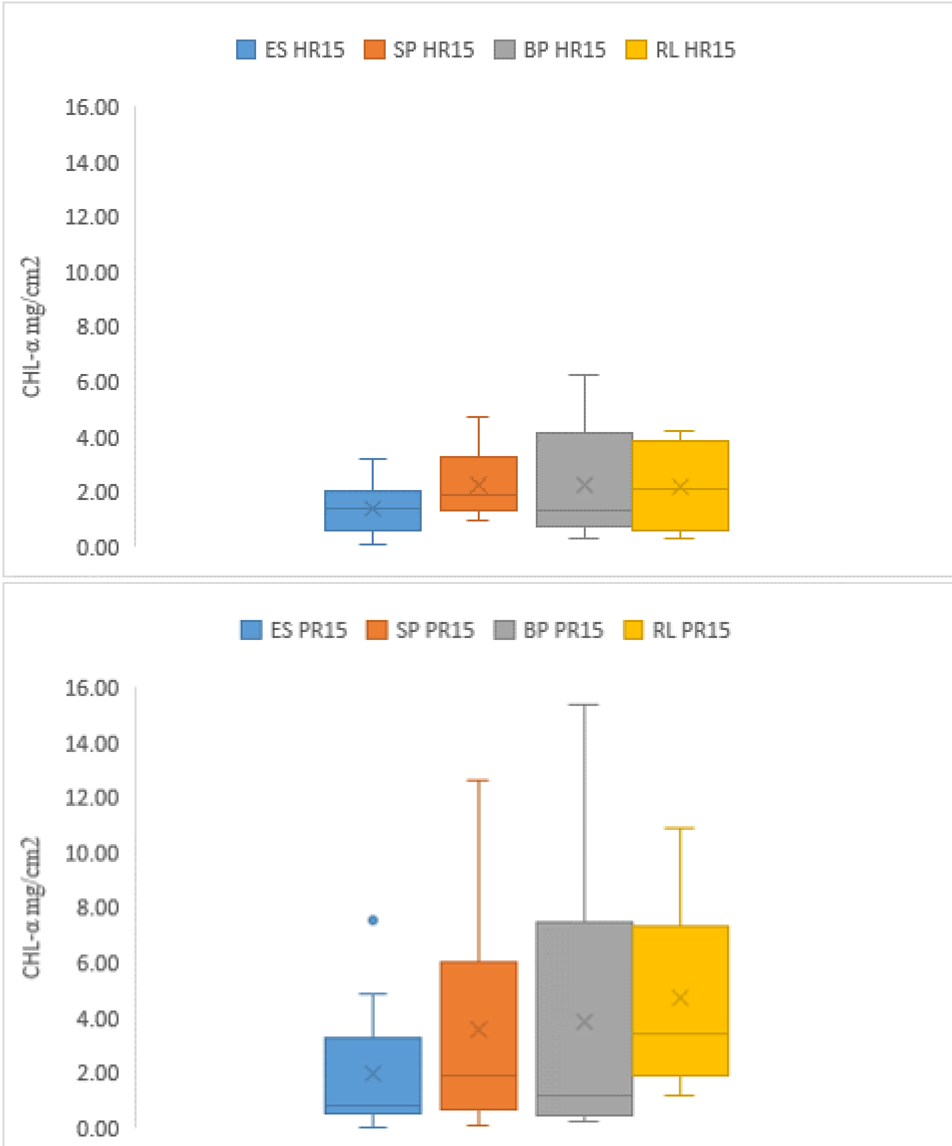

Figure 3.8 Chlorophyll-a (mg/cm^2) for each 2015 *in situ* treatment for each sampling station. Shaded boxes represent the central distribution of 50 percent of the values above and below the mean and whiskers show the minimum and maximum observed values.

Suspended sediments in an aquatic environment can attenuate light, thereby reducing the amount of light for primary producers. Data from water quality sondes in the San Marcos River indicate that daily mean and median turbidity in the river differs temporally, with this variation likely related to the temporal variation in recreational activities (data from 2015; Table 3.6); in general, turbidity in the river is higher during the period of April – October when recreational activities are higher. In addition, when sonde data are examined during the periods defined by this study in 2014 and 2015, turbidity across sites in the river was generally higher during both of the Hi-Rec periods in 2014 and 2015 (Table 3.7). It is also apparent from these data that the turbidity generally increases around two orders of magnitude from the upper portion of the river (Aquarena Drive and Rio Vista Park) to the more downstream sonde sites (Cheatham Street and IH-35 crossings). In addition, the magnitude of diel variation in turbidity appears to be associated with the timing of recreational activities in the river (Figure 3.9). Indeed, examination of data during the July 4th weekend 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 (late November 2015) showed very little diel variation and individual NTU values based on data collected every 15-min never exceeded 7.5 NTU.

Table 3.6 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
Sep	66.4	1.1
Oct	16.8	0.3
Nov	17.1	2.1
Dec	2.9	1.8

Table 3.7 Long-term mean daily turbidity (NTUs) for EAA and MCWE water quality sondes at various points in the San Marcos River for the experimental times periods in 2014 and 2015.

	pH	◦C	NTU	µS/cm
Hi-Rec 2014				
AQUARENA	7.25 (0)	22.54 (0.01)	141.2 (8.18)	618.61 (0.18)
RIO VISTA	7.52 (0)	22.91 (0.01)	7.9 (0.46)	597.43 (0.20)
Pre-Rec 2015				
AQUARENA	7.17 (0)	22.82 (0.01)	2.1 (0.23)	598.14 (0.17)
HOPKINS	7.09 (0)	21.86 (0.01)	1.2 (0.06)	592.05 (0.58)
RIO VISTA	7.31 (0)	22.28 (0.01)	20.2 (0.21)	576.36 (0.7)
CHEATHAM	7.55 (0)	19.35 (0.01)	191.2 (5.28)	602.94 (0.76)
I-35	7.55 (0)	19.39 (0.05)	191.2 (5.28)	602.94 (0.76)
Hi-Rec 2015				
AQUARENA	7.19 (0)	22.30 (0.01)	28.7 (1.79)	583.72 (0.91)
HOPKINS	7.06 (0)	22.04 (0.01)	3.1 (0.05)	613.63 (0.21)
RIO VISTA	7.35 (0)	22.55 (0.01)	3.5 (0.31)	612.56 (0.35)
CHEATHAM	7.57 (0)	18.86 (0.01)	232.0 (9.50)	598.59 (0.60)
I-35	7.51 (0)	19.25 (0.01)	129.2 (5.54)	611.88 (1.00)

Figure 3.9 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 3.10 shows an example of the turbidity response over time to rainfall events in the San Marcos River at Rio Vista.

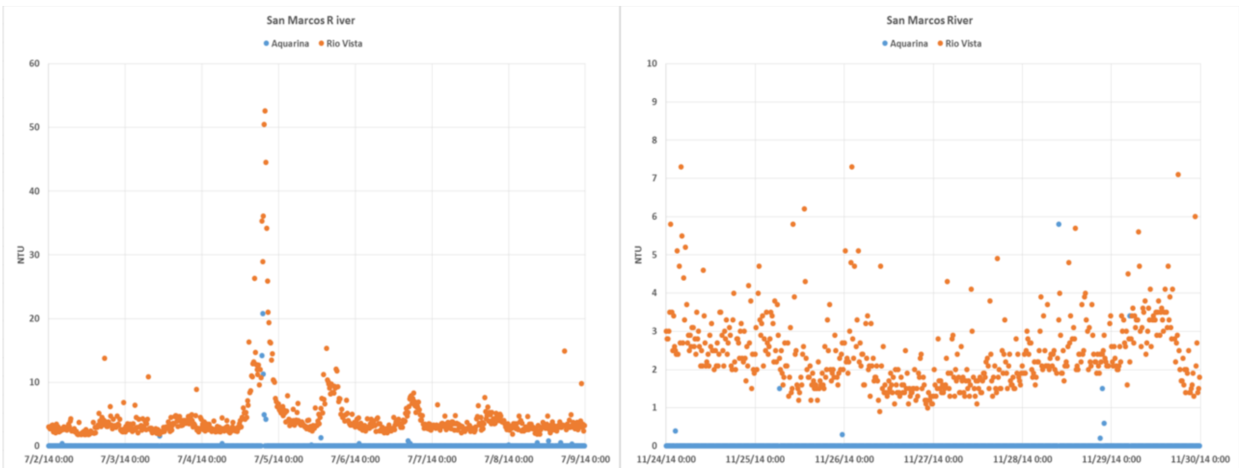

Figure 3.9 Diel, weekly and seasonal patterns in observed turbidity in the San Marcos River.

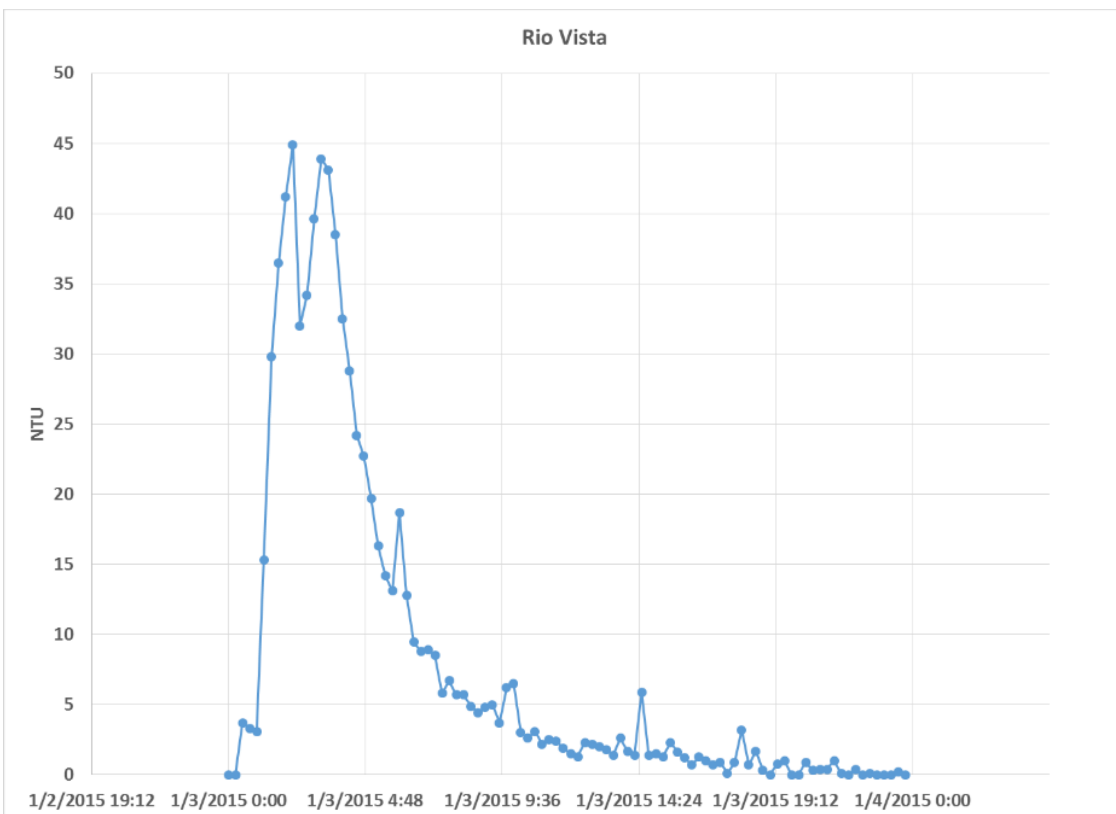

Figure 3.10 Turbidity response over time to rainfall events in the San Marcos River at Rio Vista.

3.4 Discussion

In the *in situ* study examining the spatial variation in the biomass production of TWR at sites in the San Marcos River with different intensities of recreational activities, we found that there were general differences in TWR biomass production when the control site (ES), which had very limited to no upstream recreational activity, and the downstream treatment sites, which all had substantial upstream recreational activities, and recreational activities in the vicinity of each of the experimental plots. It is noted that TWR is a CO_2 obligate plant (Doyle and Power 2004) and there is a known gradient of diminishing CO_2 concentration moving from upstream to downstream. Generally, the control site had greater shoot, root and total TWR biomass when compared to downstream treatment sites during all of the study periods, with the exception of the BP site in the Pre-Rec 2015 study period. These general differences in TWR biomass suggests that there is possibly relationship between human access and activity in the river and the biomass production of TWR *in situ*. A number of previous studies have highlighted a variety of human and anthropogenic factors which could affect TWR production. Increases in suspended sediment and associated turbidity has been suggested to be problematic for TWR through deposition of material on leaves and through the reduction in available light in the water column (Vaughan 1986; Bradsby 1994; Breslin 1997). Recreational activities, such as kayaking, canoeing, swimming, and tubing have been hypothesized to cause physical disturbance to TWR and cause breakage of shoots (Breslin 1997; Saunders et al. 2001). In addition, upstream recreational activities may damage and break plants and this fragmented material may coalesce into floating vegetation mats that move downstream with the current and may become entangled with TWR leaves. Changes in water velocity and river discharge may also affect TWR production by reducing the deposition of material on leaf surfaces and by creating depth-velocity gradient habitats which are higher production environments for TWR (Poole and Bowles 1999). Lastly, herbivory by macroinvertebrate consumers in the river (i.e., crayfish) may reduce TWR biomass estimates of net biomass production (Power 1992). In the present study, we can address the potential effects of several of these factors (e.g., recreational access, turbidity, plant breakage, and inter-annual differences in river discharge, but we cannot directly evaluate the influence of rafting vegetation fragments and herbivory on the production of TWR in the San Marcos River.

It appears that the increased turbidity in the river varies both temporally and spatially, and that most of the baseline (i.e., not storm-event) turbidity was associated with recreational activities. It, however, remains to be seen whether this variation in turbidity has the potential to affect the amount of light in the water column that is available for TWR growth. Based on the light attenuation study (Section 2.0), a reduction of light ~80% has the potential to significantly reduce TWR growth, but a further analysis is required to determine if the turbidity levels within the San Marcos River are indeed high enough to reduce light by this magnitude. As noted in Figure 3.10, turbidity reverts to background levels in under a day for short duration pulse flow events and likely not a factor on TWR growth dynamics.

We hypothesized that increased turbidity associated with recreational activities would lead to increased deposition of sediment and other materials on TWR plant leaves and thereby reduce the net growth of TWR plants in the San Marcos River. In this study, we were able to collect data on the areal concentration ($\mu g/cm^2$) of total sediments and non-volatile suspended (inorganic) sediments during the Pre-Rec and Hi-Rec periods during the higher flow year of 2015. We found that one site downstream from the control site had higher sediment concentrations on leaves (BP) and that one downstream site (RL) had lower sediment concentrations than the control site during the Pre-Rec period. Further, we found that there were no significant differences among the control site and the downstream treatment sites during the Hi-Rec period. These data suggest that larger-scale patterns in recreational activities in the river likely did not have a strong effect on the amount of materials deposited on leaf surfaces. These data also suggest that there are among-site differences in sediment deposition and accumulation on leaf surfaces that are associated with site-specific conditions, such as depth-velocity gradients present within a site and the amount of local or immediate upstream recreational disturbance. In addition, the lack of differences between the control site and the downstream sites during the Hi-Rec period in 2015 may also be associated with the higher discharge in the river during that time period. Deposition was likely minimized with increasing discharge associated with leaf movement within swifter flowing water (Figure 3.1). Daily discharge in the river during this time period was >200 cfs and flows of this magnitude may be high enough to keep TWR leaves relatively clear of accumulated sediment. Although we did not estimate sediment accumulation on TWR leaves in the lower flow 2014 experiment, we found that TWR plants at the BP site were completely covered with deposited sediments and only 4 out of the 15 plants (26%) survived to the end of the experiment. This is in contrast to survival patterns during the Hi-Rec period in 2015, where TWR plants at the BP site exhibited an 80% survival rate (12/15 plants). This finding clearly indicates a need for further studies which examine the role of inter-annual variation in flows on sediment accumulation on TWR growth and fitness.

In the present study, we found little evidence that recreational activities upstream and near our experimental plots had an effect on the breakage and loss of leaves of TWR plants. The proportion of total shoots (leaves) which were broken did not greatly vary between the control and the downstream treatment sites (Tables 3.3 – 3.5). Across all sites during the Hi-Rec 2014 study period, an average of 46% (39-52%) of TWR leaves were broken. In the higher-flow 2015 year, the TWR plants in the Pre-Rec study period exhibited an average of 25% broken shoots (14-46%) and the Hi-Rec study period plants had an average of 27% (11-51%) broken shoots. One reason why we did not observe differences between the control and downstream treatment sites in the amount of total and proportional breakage in TWR plants was that the downstream plants were largely contained within designated State Scientific Areas (SSAs) which limit the amount of recreational access; SSAs have fencing and signage designed to keep swimmers, tubers, and boaters out of the TWR stands and this may prevent substantial breakage. Power (1996) examined the impact of rafting vegetation mats on TWR and suggested that entrapment of free-floating vegetation on TWR may lead to breakage and damage to plants. Although, we did not directly evaluate the amount of rafted material on our TWR plants, the lack of differences among the control site and the downstream treatment sites in terms of the total and proportional breakage of shoots indicates that there is unlikely a systematic difference among sites with regard to the impacts of rafted materials.

4.0 Synthesis of Texas wild-rice Experiments

Cumulatively, the data collected for this study indicate that there are likely several important factors that influence growth of TWR in the upper San Marcos River. In the PAR study (Section 2.0), reduction of light levels below ambient light had an impact on the biomass production of TWR, but reduction of growth was not substantial until an 80% reduction in available light had occurred. However, we were not able to directly relate the observed patterns of turbidity in the river during the *in situ* experiments (Section 3.0) to the PAR reductions. At present additional data is being collected on the relationship between PAR and in situ turbidity. As noted previously, we strongly suggest a future study evaluate the relationship between ambient turbidity and ambient light in the river and its impact on the growth of TWR. In addition, this study clearly demonstrates that periods of high recreation are associated with increased turbidity in the upper San Marcos River, but the magnitude of those effects are dependent upon the spatial location in the river (i.e., more upstream versus more downstream). Results of the *in situ* study indicate, that TWR generally exhibited lower net growth rates (lower biomass at the end of the experimental period) at the downstream treatment sites when compared to the upstream control site. However, it is critical to note that downstream sites were established in the SSAs which are designed to minimize the localized impacts of recreational activities on TWR; our data indicate that the TWR plants within these SSAs still had lower net growth than the most upstream site with very limited upstream recreational activity. It remains to be determined whether or not these observed spatial differences in TWR growth are related to recreational activity or to the spatial variation with factors not examined by this study, such as differences in microhabitat quality or herbivory.

5.0 Seasonal and Longitudinal Dynamics of the Aquatic Macroinvertebrate Community and Associated Habitat Structure within the San Marcos River

5.1 Introduction

The purpose of this study was to examine several aspects of the macroinvertebrate community within the San Marcos River. Currently, no study has quantified both patterns in macroinvertebrate drift and benthic community structure simultaneously in the San Marcos River. Information on drift patterns and benthic macroinvertebrate habitat relationships is necessary to understand mechanisms for species persistence within the river and can aid in management strategies. Additionally, given the continued urbanization and increasing population in San Marcos metropolitan area, understanding the relationships among instream habitat and biotic responses are necessary to mitigate against anthropogenic alterations. The upper San Marcos River is a major tourist attraction and reportedly draws close to 500,000 visitors annually for recreational activities (Earl et al 2002). Heavy recreational activities, land use, sewage and septic tank discharge, storm water run-off, non-source point pollution, bank erosion, and invasive species are the major concerns facing the river (HCP 2012). Specific objectives of this study were to (1) describe seasonal and longitudinal patterns of habitat associations among the benthic macroinvertebrate community within the San Marcos River and (2) quantify seasonal and longitudinal drift rates of the macroinvertebrate community in the San Marcos River, given patterns of seasonal recreation use of the river corridor.

5.2 Materials and Methods

5.2.1 Study Area

The San Marcos River originates from multiple springs sources in the headwaters located in Spring Lake and is the second largest spring system in Texas (Brune 1981). The water quality of the upper 8 km of the San Marcos is considered very high but becomes more turbid as it flows downstream (Groeger et al 1997). Water temperature stays relatively consistent at around 22°C (Hannan and Dorris, 1970). The upper San Marcos River contains the critical habitat for several species of concern including three federally listed endangered species: Fountain Darter, *Etheostoma fonticola* (USFWS 1970), the San Marcos salamander, *Eurycea nana* (USFWS 1980), and Texas wild-rice, *Zizania texana*) (USFWS 1978).

5.2.2 Study Sites

This study was conducted at three sampling locations on the upper 3 km of the San Marcos River: Site 1- Upper Sewell Park, Site 2- City Park and Site 3- Above IH 35 (Figure 5.1). Study sites were selected to represent a longitudinal gradient in water quality changes, habitat, and recreation use within the San Marcos River. Recreational activities like tubing, swimming, snorkeling, diving and kayaking are common to all sites.

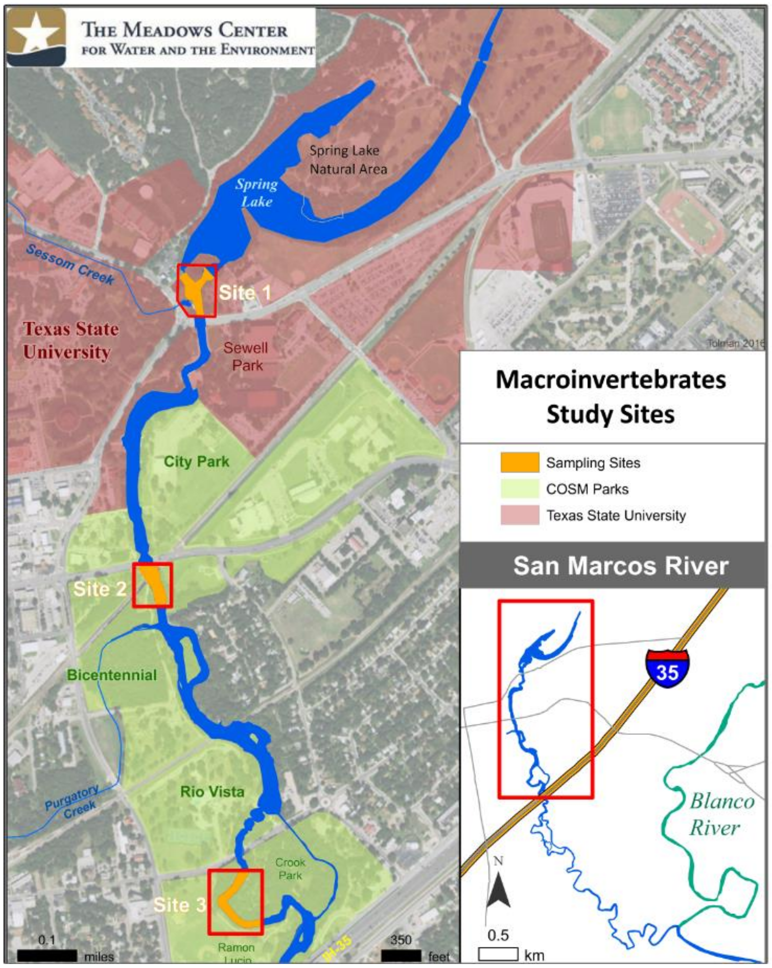

Figure 5.1 Macroinvertebrate site map for the San Marcos River.

5.2.3 Recreation Counts

PlotWatcher Pro game cameras were placed at three locations on the upper San Marcos River identified as high recreational areas. Areas were preliminarily identified as recreation hotspots based on river accessibility by bank as well as known entry/pull out points for tubers and kayakers. Cameras were placed facing the river allowing for the widest view possible. One camera was placed in Sewell Park on Texas State University Property, one at City Park, and one at Rio Vista Park. Each camera was programmed to capture images once an hour for nine hours a day (dusk to dawn) and images were downloaded once a month. Each picture was reviewed and only individuals in physical contact with the river were counted as a person recreating in the river; individual humans and dogs along the bank were not included in counts. Recreation was divided into categories: tubing, vessel (kayaking, canoeing, etc.), swimming, anglers, and dogs. Seasons defined for assessing recreation were: Spring (March – May): Summer (June – August), Fall (September – November), and Winter (December – February). We assessed recreation use for one month within each season.

5.2.4 Drift Sampling

Drift samples were conducted seasonally at each site over a 24h period April 2015 – December 2015. Table 5-1 denotes dates drift collections were completed at each site. Drift nets (0.45 by 0.25 m, 500um mesh) were located at constricted stream sections which funneled stream discharge and drift nets were distributed across a horizontal transect to capture the range of current velocities at each stream section. Drift nets were supported by two metal fence posts and placed at least 5 cm above the substratum to prevent crawling insects from entering the nets (Brewin et al. 1994). Drift nets were serviced every 2 hours (Neale et al. 2008) or when debris buildup affected sampling efficiency. The inlet velocity at the net opening, along with water depth were measured using a Marsh McBirney Flo-Mate 2000. Water temperature, dissolved oxygen, and pH were measured with a YSI 85 model and turbidity was recorded using a Hanna hand-held turbidity meter at the beginning of each sampling interval. After each sampling period, all contents collected in the drift nets were preserved in 95% ethanol and transported to the lab for identification and counting.

Table 5.1 Sampling dates for drift and benthic macroinvertebrate collections.

Macroinvertebrate Sampling Periods								
	Spring		Summer		Fall		Winter	
	Drift	Benthic	Drift	Benthic	Drift	Benthic	Drift	Benthic
Site 1	April 20, 2015	April 21, 2015	July 13, 2015	July 15, 2015	September 28, 2015	September 22, 2015	December 15, 2015	December 8, 2015
Site 2	April 15, 2015	April 16, 2015	July 8, 2015	July 8, 2015	September 22, 2015	September 24, 2015	December 9, 2015	December 10, 2015
Site 3	April 14, 2015	April 14, 2015	July 6, 2015	July 6, 2015	September 21, 2015	September 21, 2015	December 7, 2015	December 7, 2015

5.2.5 Benthic Sampling

Benthic samples were collected seasonally at each study site within the San Marcos River. Table 5.1 denotes the dates benthic collections were completed at each site. Twenty samples were collected from each study site and a proportional sampling method was used to determine the number of samples per habitat type based on the three most abundant vegetation species and open substrate present during each sampling event. Vegetation and substrate maps (Figure 5.2) were produced before each sampling event to serve as a basis for selecting the dominant vegetation and substrate types within each study sites. Table 5.2 indicates the number of benthic samples per habitat type (vegetation or substrate) collected at each site per season based on available habitat.

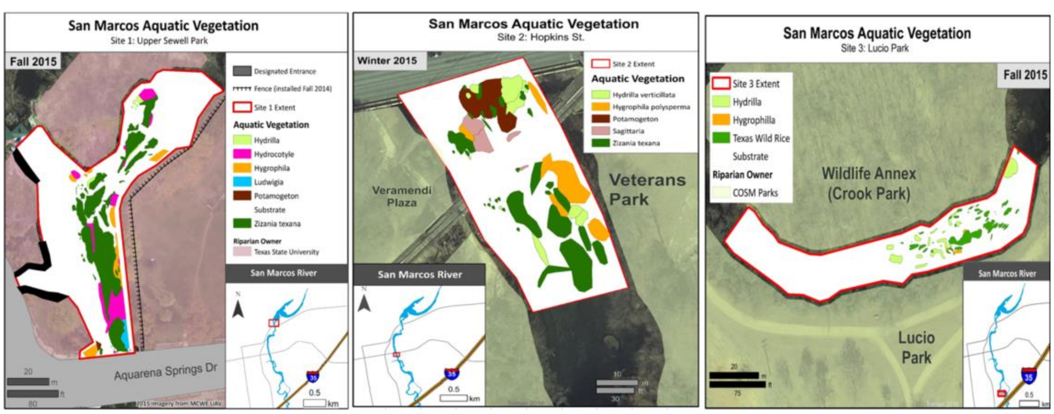

Figure 5.2 Example of substrate and vegetation maps at each study site utilized to select random locations for benthic samples.

Table 5.2 Number of samples collected per cover type (vegetation or substrate) based on site maps produced prior to each sampling event.

Benthic Proportional Sampling													
Cover type		Spring			Summer			Fall			Winter		
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Substrate	Cobble	5	2	-	5	3	-	5	3	5	5	5	5
	Gravel	5	3	5	5	3	5	3	3	2	5	5	5
	Sand	-	1	5	-	2	5	2	2	-	2	3	5
	Silt	1	3	1	1	1	1	1	1	3	1	-	-
Vegetation	*Hydrilla verticillata*	3	4	3	3	3	3	3	4	3	1	2	1
	Hydrocotyle	3	-	-	3	-	-	3	-	-	-	-	-
	Hygrophila polysperma	-	3	3	-	3	3	-	-	3	1	2	1
	Sagittaria	-	-	-	-	-	-	-	3	-	-	-	-
	Zizania texana	3	4	3	3	5	3	3	4	4	5	3	3

Sampling points within either substrate or vegetation habitats were randomly selected. If possible, a minimum of three replicate samples were collected from each selected vegetation type for each study site. Benthic sampling was conducted by laying a 0.25m^2 quadrat in each selected sampling point and a 0.25 m x 0.25 m drift net (500-µm mesh) was placed at the downstream side of the quadrat for collection of macroinvertebrates (Diaz et al 2012). Substrate or vegetation in each quadrat was agitated for approximately 30 seconds to enable the dislodging and collection of invertebrates. Materials collected from disturbing the substrate or vegetation were transferred into a Whirl-Pak bag and preserved with 95% ethanol. For each sampled quadrat, water velocity and water depth were measured with a Marsh McBirney Flo-Mate 2000 after sampling. Visual estimates of vegetation type and cover and substrate type and cover were recorded. Substrate types in each quadrat were defined following the Wentworth grain size classification scale. At each site, water temperature, dissolved oxygen, and pH were measured with a YSI 85 model and turbidity was recorded using a Hanna hand-held turbidity meter.

5.2.6 Laboratory analysis

Invertebrates were separated from debris and vegetation and sorted into vials containing 95% ethanol. Identification was made to the lowest practical taxonomic level, mainly down to family, based on taxonomic keys (Cummins et al. 1985; Merritt and Cummins 2008; Pete Diaz 2012; TCEQ (Surface Water Quality Monitoring Procedures, Volume 2. 2014). All macroinvertebrates were placed in functional feeding groups (FFG): scrapers, collector-gatherers, filtering collectors, predators, and shredders based on Merritt and Cummins et al (2008) and TCEQ (2014).

5.2.7 Statistical Analysis

Taxonomic richness (*S*), and Shannon-Weiner (*H'*) diversity, and the relative abundance of taxa and FFGs were calculated for benthic and drift macroinvertebrate samples among sites and seasons. Renkonen similarity index was calculated to assess similarities in the benthic macroinvertebrate community among the three sites (Renkonen 1938). Principal component analysis (PCA; Canoco 4.5, Microcomputer Power 2002) was used to examine variation in habitat characteristics within and among sites. Environmental data were *z*-score transformed for PCA and qualitative data (i.e., site and season) were denoted as dummy variables in CCA. Canonical correspondence analysis (CCA) was used to assess the relationship between environmental gradients and the benthic macroinvertebrates community. Two separate CCA analyses were performed for vegetation and substrate samples since we wanted to identify the unique contributions of vegetation separate from the substrate. Care was taken to minimize substrate disturbance when sampling vegetation in an effort to reduce 'mixed' sampling in vegetation versus substrate. Drift density was calculated with the following equation (Hauer and Lamberti 2011):

Drift density = [(N)(100)]/[(t)(W)(H)(V)(3600s/h)]

Where N = number of invertebrates per sample; t = time; W = width of net; H = net height; and V, water velocity at net opening (m/s). Spearman rank correlation was calculated to assess the relationship of FFG's observed in the drift related to FFG's found in the benthic community (Spearman 1904).

5.3 Results

5.3.1 Recreation counts

Table 5.3 denotes each month recreation counts were completed for each site (i.e., Sewell Park, City Park, and Rio Vista). Months from 2013 and 2014 were used to assess recreation use because they were the most complete period of record. Images were collected through 2015 but due to several unforeseen factors, (e.g., freezing temperatures, flooding, sun glare, camera and battery malfunction, insect nest building on camera lens) images were not captured sufficiently to document recreation activities.

Table 5.3 Dates of recreation count sampling periods.

Recreation Count Sampling Periods				
	Spring	Summer	Fall	Winter
Sewell Park	March 2014	June 2014	September 2013	December 2013
City Park	April 2014	June 2014	September 2013	December 2013
Rio Vista	April 2014	July 2014	October 2013	December 2013

Figure 5.3 illustrates the total number of individuals per recreation type among sites across all seasons. Tubing and swimming accounted for most of the recreation activity (>90%). Recreation activity was highest during the summer in City Park and Rio Vista Dam sites, but was higher in Fall (September) at the Sewell Park site. Among sites, the Rio Vista Dam location had the highest amount of recreational activity with most of the recreation activity consisting of tubers and swimmers. Figure 5.4 illustrates the mean number of recreationists per weekday among seasons. Among all weekdays, recreation numbers were highest in summer among all sites with spikes in the number of people observed during Saturday and Sunday. Weekday recreation numbers were similar for fall and spring and very little recreation activity was observed during winter.

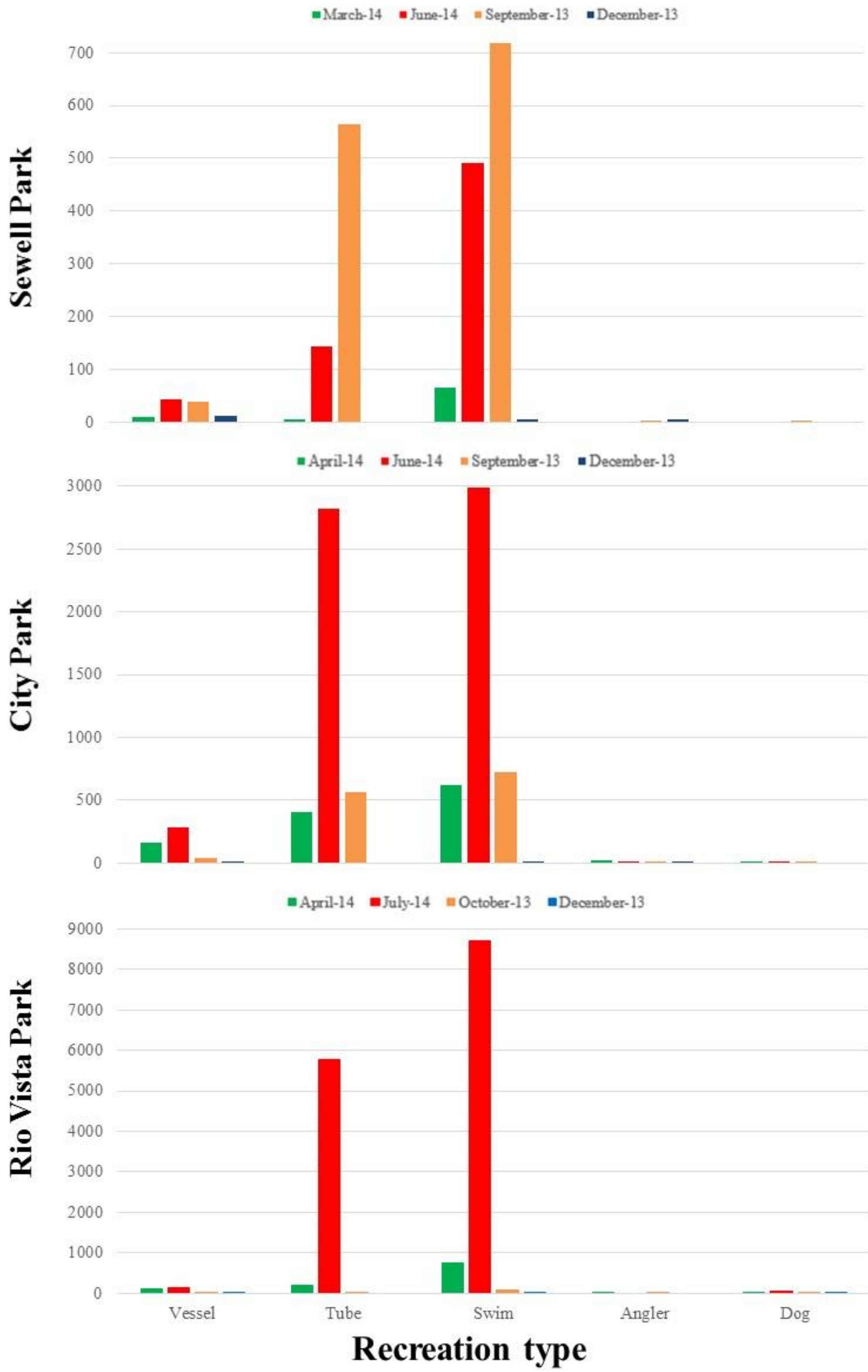

Figure 5.3 Total number of individuals per recreation type for each season captured by Sewell Park, City Park, and Rio Vista Park game cameras.

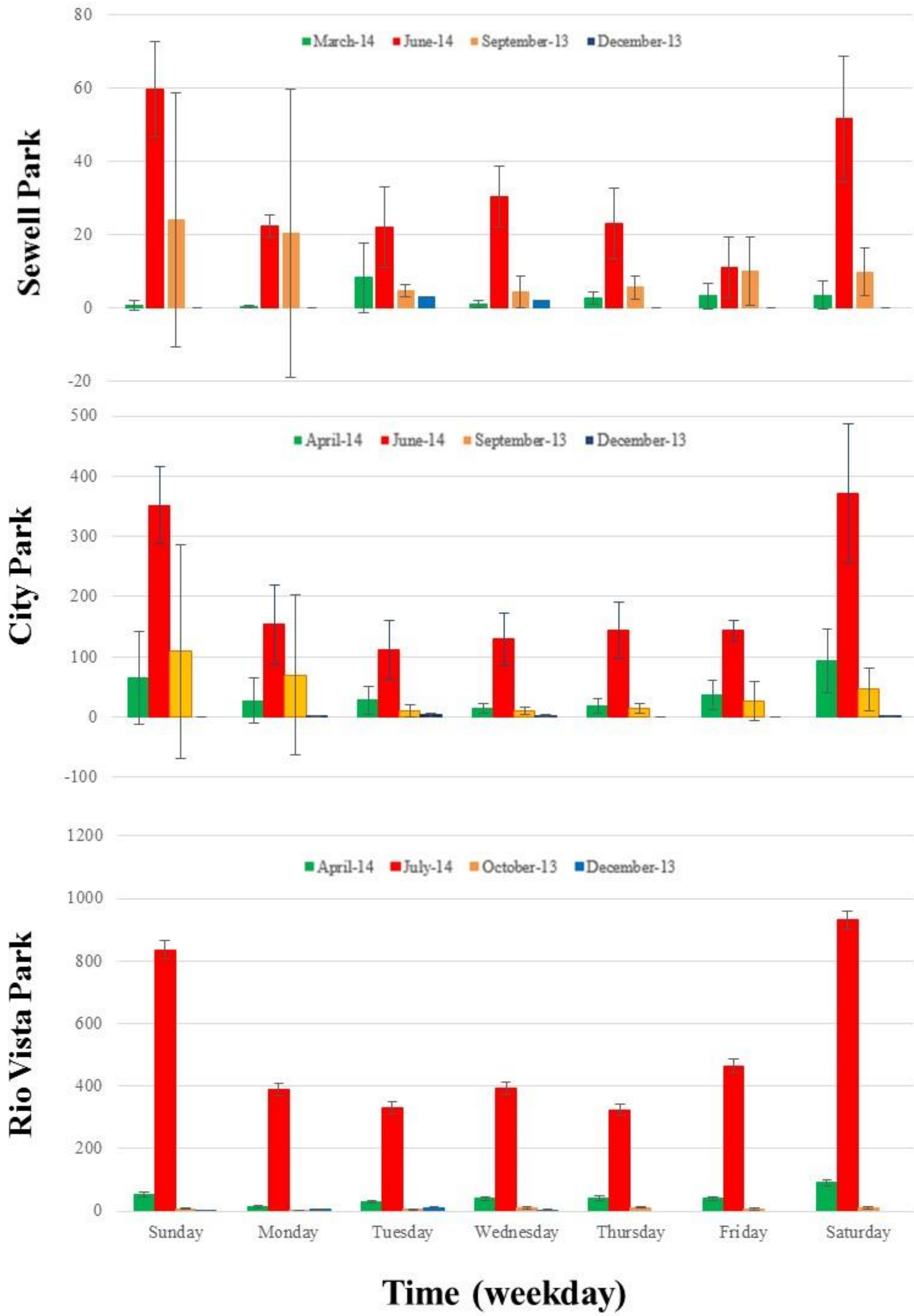

Figure 5.4 Mean and SD of recreation per weekday for all recreation types for each season captured by Sewell Park, City Park, and Rio Vista Park game cameras.

5.3.2 General habitat characteristics

The mean and range of measured physical parameters for each site are noted in Table 5.4. Water quality parameters were consistent among sites, except turbidity, which was slightly higher (0.71 FTU) than Site 2 (0.29) or Site 1 (0.11). Available depth and current velocities were similar among all sites with slightly higher current velocities at Site 1 and Site 3.

Table 5.4 Mean (range) of physical parameters observed at each site on the San Marcos River April 2015 – December 2015.

Physical Parameters*			
	Site 1	Site 2	Site 3
Conductivity (µS)	606 (594-614)	605.5 (596-616)	605.3 (594-616)
Depth (m)	2.2 (1.2-3.7)	2.7 (1.3-3.7)	2.3 (0.8-4.5)
D.O. (mg/l)	8.09 (7.37-8.45)	8.18 (7.37-8.32)	8.25 (7.98-8.75)
pH	7.71 (7.43-8.16)	7.66 (7.52-7.88)	7.77 (7.62-8.04)
Vegetation Cover (%)	40.3 (0-99)	49.45 (0-100)	40.06 (0-100)
Velocity (m/s^2)	0.41 (0.01-0.97)	0.38 (0.01-0.77)	0.37 (0.01-0.95)
Temperature (°C)	21.97 (21.7-22.37)	22.25 (21.7-23.2)	21.74 (21.2-22.24)
Turbidity (FTU)**	0.11 (0.05-0.24)	0.29 (0.12-0.48)	0.71 (0.39-0.9)
*Parameters displayed as Mean (Min. - Max.); ** 1 FTU = 1 NTU			

Principal component results are shown in Figure 5.5. Principal components axis 1 and 2 explained 22.9% of the variation in habitat measurements taken among 240 benthic samples. Principal Component Axis 1 explained 12.3% of the variation and described a water quality gradient with water temperature (0.81), conductivity (0.76), turbidity (-0.56), pH (0.46) and water depth (0.40) having strong PC 1 loadings. Principal Component Axis 2 explained 10.6% of the variation and described a substrate, vegetation, and current velocity gradient with silt (0.73), current velocity (-0.68), gravel (0.57), *Hygrophila* (0.49), and percent vegetative cover (0.33) having the strongest loadings along the axis. Site 1 and 2 [mean sample scores and (±SE)] were positively associated with PC axis 1 and Site 3 was negatively associated with PC axis 1.

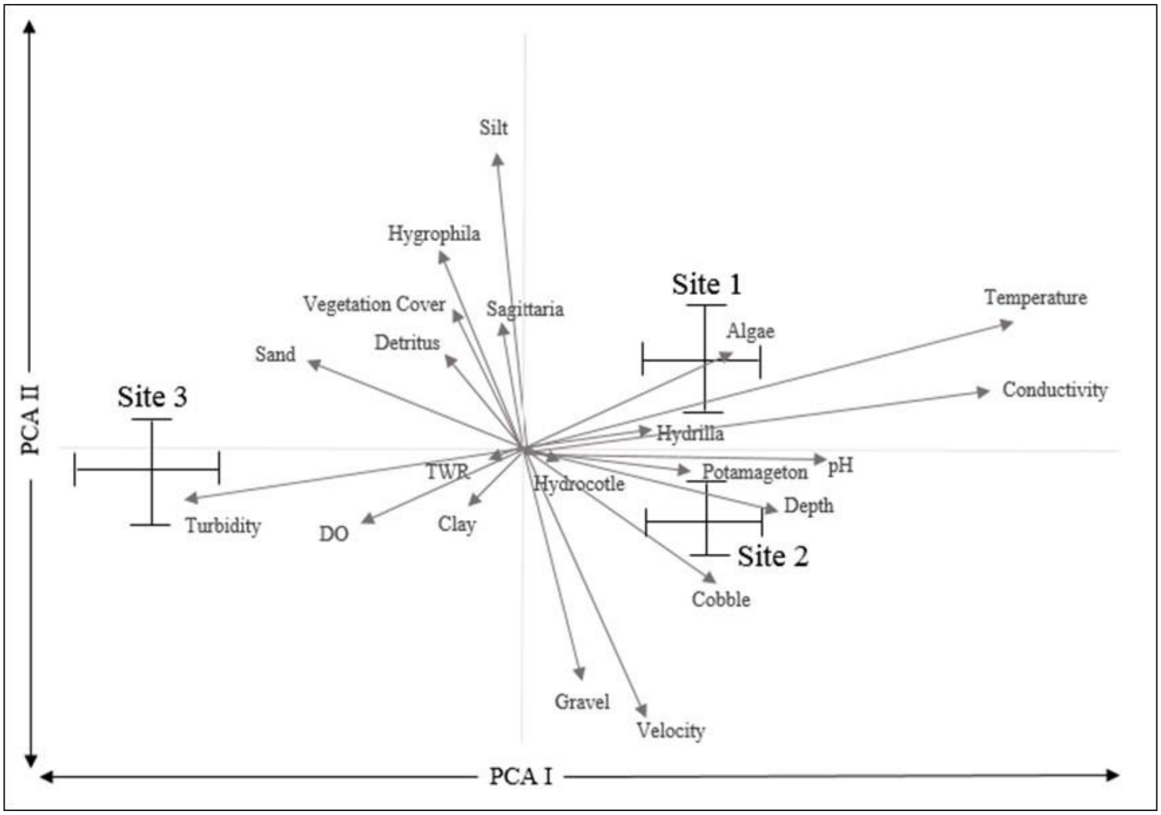

Figure 5.5 Principal component analysis bi-plot for measured environmental parameters and general habitat characteristics by site for habitat sampled on the San Macros River during April 2015 – December 2015.

5.3.3 Macroinvertebrate drift community

A total of 134,488 invertebrates representing 73 taxa were collected among 480 drift samples (Table 5.3). Site 1 had the highest total number of drifting invertebrates (77,792), followed by Site 2 (31,723), and Site 3 (24,973). A total of 32,275 invertebrates were collected during our spring sampling period, 40,701 in summer, 55,679 in fall and 5,843 in winter. Among sites and season, Hyalellidae had the greatest relative abundance at 52% followed by Leptohyphidae (28%), Beatidae (10%), Chironomidae (4%), and Petrophila (1%).

Hyalellidae was most abundant taxa at Site 1 with a relative abundance of 69% but decreased in abundance downstream with a relative abundance of 41% at Site 2 and 13% at Site 3. Leptohyphidae increased in relative abundance downstream with an abundance of 17% at Site 1, 31% at Site 2 and 59% at Site 3. The greatest relative abundance (16%) of Baetidae was at Site 2 with abundances of 6% and Site 1 and 12% at Site 3.

Hyalellidae was most abundant during the summer with a relative abundance of 64% with relative abundances of 49%, 48% and 25% for spring, fall and winter respectively. Leptohyphidae relative abundance was highest in the spring at 34% with relative abundances of 18%, 31% and 32% for summer, fall and winter, respectively. Baetidae had the highest relative abundance in winter at 16% with relative abundances of 7%, 8% and 11% for spring, summer and fall, respectively. Chrironomidae showed an increase in relative abundance in winter (12%) with the lowest in spring at 2%.

Across all seasons, Site 1 had the highest drift densities compared to Site 2 and Site 3 (Figure 5.6). Highest drift densities occurred at Site 1 during the summer and fall between 20:00 and 23:00 hours. Drift densities at Sites 2 and 3 were relatively low compared to Site 1 (magnitude of 10 less) . Drift rates were higher during hours of darkness and lowest during mid-day hours. Winter drift densities was lowest among seasons at all sites.

Table 5.5 Relative abundance by taxa (%), total *N*, taxa richness, and Shannon-Wiener Diversity for drift macroinvertebrates collected from the San Marcos River (April 2015 – December 2015).

Taxa	Site 1	Site 2	Site 3	Total	Taxa	Site 1	Site 2	Site 3	Total
Baetidae	6.088	15.995	12.209	9.561	*Trichopteran*	0.003	0.038	0.032	0.016
Baetodes	0.166	0.258	0.404	0.232	*Lepidoptera*	-	0.003	-	0.001
Caenidae	0.058	0.044	0.104	0.063	*Crambidae*	0.118	0.350	0.312	0.209
Ephemeroptera	0.012	0.006	0.040	0.016	*Paraponyx*	0.318	0.662	0.809	0.490
Ephemeridae	0.010	0.120	0.032	0.040	*Petrophila*	1.265	1.485	1.213	1.307
Heptageniidae	0.013	0.057	0.048	0.030	*Dytiscidae*	0.014	0.009	0.004	0.011
Leptohyphidae	16.752	31.104	58.511	27.892	*Elmidae*	0.154	0.054	0.164	0.132
Leptophlebiidae	0.012	0.022	0.296	0.067	*Gyrinidae*	-	-	0.004	0.001
Anisoptera	0.359	0.129	0.044	0.246	*Haliplidae*	-	-	0.004	0.001
Coenagrionidae	0.423	0.725	0.741	0.553	*Hydrophilidae*	0.010	0.003	0.012	0.009
Corduliidae	0.033	0.003	0.004	0.021	*Phanocerus*	0.072	0.151	0.460	0.163
Gomphidae	0.001	0.016	0.008	0.006	*Psephenidae*	0.005	0.006	-	0.004
Libelluloidea	0.001	-	-	0.001	*Scritidae*	-	-	0.012	0.002
Odonata	-	0.006	-	0.001	*Ceratopogonidae*	0.012	0.006	0.020	0.012
Zygoptera	0.022	0.022	0.188	0.053	*Chironomidae*	2.293	4.211	7.696	3.749
Calopterygidae	-	-	0.016	0.003	*Culicidae*	0.030	0.025	0.012	0.025
Lestidae	0.001	-	-	0.001	*Empididae*	0.003	0.069	0.020	0.022
Hemiptera	0.004	-	0.004	0.003	*Ephrydidae*	0.015	0.006	0.004	0.011
Ambrysus	0.015	0.016	-	0.013	*Hemerodromia*	0.039	0.202	0.220	0.111
Belostomatidae	0.012	0.019	0.052	0.021	*Simulidae*	0.122	0.095	0.248	0.139
Corixidae	0.012	0.054	0.064	0.031	*Stratiomydae*	0.072	0.050	0.092	0.071
Cryphocricos	0.006	-	0.032	0.010	*Tipulidae*	0.003	-	-	0.001
Gerridae	0.018	0.009	0.012	0.015	*Annelid*	0.015	0.013	0.020	0.016
Limnocoris	0.017	0.038	0.072	0.032	*Cladocera*	0.937	0.350	0.112	0.645
Pleidae	0.003	0.003	-	0.002	*Copepoda*	0.021	0.047	0.040	0.030
Hebridae	-	-	0.004	0.001	*Decapoda*	-	0.050	0.032	0.018
Veliidae	0.009	0.003	0.052	0.016	*Hirudinea*	0.031	0.016	0.036	0.028
Corydalidae	0.003	-	0.044	0.010	*Hyalellidae*	68.936	41.431	13.062	52.073
Glossosomatidae	0.118	0.293	0.613	0.251	*Hydrachnidae*	0.067	0.331	0.276	0.168
Hydroptilidae	0.022	0.082	0.092	0.049	*Oligochaete*	0.006	0.003	0.012	0.007
Leptoceridae	0.006	0.016	0.012	0.010	*Ostracod*	0.032	0.025	0.088	0.041
Nectopsyche	0.018	0.274	0.108	0.095	*Platyhelmenthes*	0.018	0.003	0.020	0.015
Oecitis	0.014	0.003	0.008	0.010	*Nematoda*	-	-	0.008	0.001
Oxyethira	0.827	0.599	0.344	0.683	*Hydropsychidae*	0.080	0.243	0.452	0.187
Trianodes	0.005	0.038	0.008	0.013					
Helicopsychidae	0.014	0.003	0.120	0.031	**Total N**	77,792	31,723	24,973	134,488
Neureclipsis	0.005	-	0.036	0.010	**Taxa richness**	64	60	65	73
Philopotamidae	0.228	0.088	0.168	0.184	**Taxa Diversity**	0.91	1.74	1.64	
Polycentropodidae	0.004	0.016	0.008	0.007					

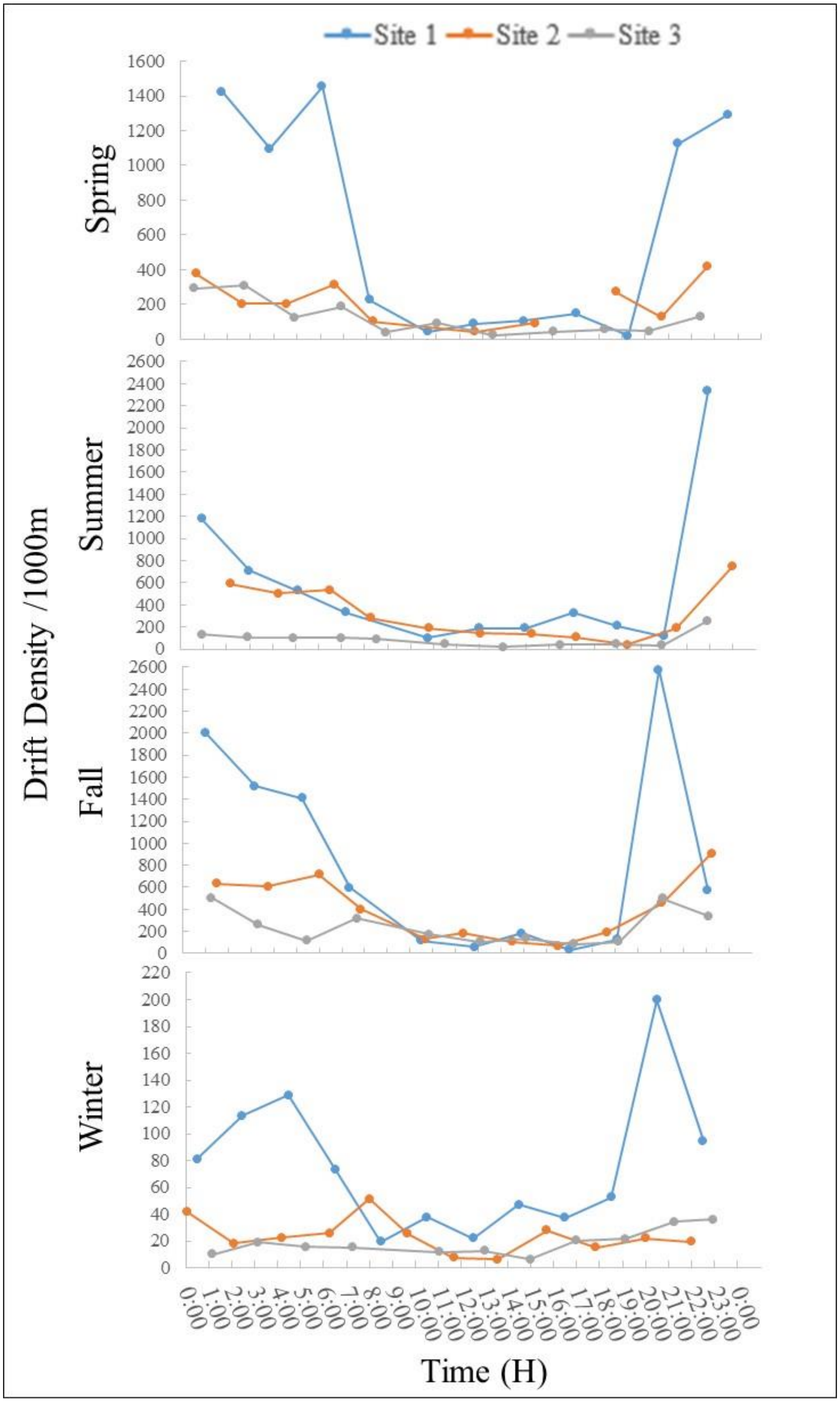

Figure 5.6 Drift densities/1000m for Sites 1-3 on the San Marcos River among seasons (April 2015 – Dec 2015).

5.3.4 Benthic macroinvertebrate community

A total of 40,288 invertebrates representing 60 taxa were collected (Table 5.4). Overall, Hyalella was the most abundant group (46%) followed by Baetid mayflies (20%), Leptohyphid mayflies (11%), and Chironomidae (7%). Taxa richness was highest at Site 1 (55) followed by Site 2 (50), and Site 3 (49). Shannon-Wiener Diversity was highest at Site 3 (2.38) and lowest at Site 1 (1.50). Renkonen similarity index for the three sites ranged between 0.53 – 0.78 with the greatest similarity between Site 1 and Site 2 (0.78) whereas the similarity index was lowest between Site 1 and Site 3 (0.53).

Table 5.6 Relative abundance by taxa (%), total N, taxa richness, and Shannon Diversity (*H'*) for benthic macroinvertebrates collected from the San Marcos River (April 2015 – December 2015).

Species	Site 1	Site 2	Site 3	Total	Species	Site 1	Site 2	Site 3	Total
Baetidae	13.45	27.00	21.23	19.99	*Paraponyx*	0.13	0.10	0.30	0.16
Baetodes	0.01	0.04	0.06	0.03	*Petrophila*	0.08	0.04	0.09	0.07
Caenidae	0.01	0.01	-	<0.01	*Berosus*	0.01	-	-	<0.01
Ephemeridae	-	0.01	0.04	0.01	*Elmidae Gen.*	0.70	0.22	0.72	0.54
Heptageniidae	0.01	-	0.02	0.01	*Phanocerus*	0.11	0.05	0.18	0.11
Hexagenia	-	0.01	-	<0.01	*Psephenidae*	0.02	-	-	0.01
Leptohyphidae	7.03	9.74	18.75	10.75	*Ceratopogonidae*	0.01	-	-	<0.01
Leptophlebiidae	0.08	0.04	1.16	0.32	*Chironomidae*	3.73	5.95	14.41	7.03
Zygoptera	0.49	0.33	0.99	0.56	*Ephrydidae*	0.01	-	-	<0.01
Anisoptera	0.07	0.04	0.13	0.07	*Hemerodromia*	0.14	0.35	0.19	0.22
Ambrysus	0.14	0.21	0.15	0.16	*Simulidae*	1.05	0.55	2.31	1.18
Belostomatidae	-	0.01	-	<0.01	*Stratiomyidae*	0.02	0.01	0.01	0.02
Cryphocricos	0.02	0.05	0.06	0.04	*Annelid*	1.01	1.03	1.09	1.03
Gerridae	0.01	-	0.02	0.01	*Bivalvia*	-	0.06	0.07	0.04
Limnocoris	0.28	1.26	2.13	1.06	*Cladocera*	0.04	0.01	0.05	0.03
Veliidae	0.01	-	0.01	<0.01	*Copepoda*	0.01	0.01	0.04	0.01
Corydalidae	-	-	0.01	<0.01	*Decapoda*	0.01	0.04	0.34	0.10
Sialidae	0.01	0.01	-	<0.01	*Hirudinea*	0.05	0.05	2.04	0.52
Glossosomatidae	2.76	3.91	5.74	3.86	*Hyalellidae*	62.82	44.92	18.24	46.04
Hydroptilia	0.04	0.23	0.16	0.13	*Hydrachnidae*	0.13	0.40	1.39	0.52
Leptoceridae	0.01	0.01	0.03	0.02	*Oligochaete*	0.36	0.32	0.19	0.31
Nectopsyche	0.40	0.53	0.69	0.51	*Ostracod*	1.89	1.01	1.03	1.39
Oecitis	0.02	0.02	0.02	0.02	*Platyhelmenthes*	0.53	0.20	0.45	0.40
Oxyethira	0.52	0.32	0.17	0.36	*Mesogastropoda*	0.88	0.34	0.59	0.62
Trianodes	0.01	0.00	0.02	0.01	*Corbiculidae*	0.01	0.01	0.11	0.03
Helicopsychidae	0.13	0.04	2.10	0.57	*Hydropsychidae*	0.29	0.24	0.36	0.29
Hydrobiosidae	0.02	-	-	0.01	*Limnophila*	0.11	0.01	0.03	0.05
Neureclipsis	0.01	-	0.01	<0.01	*Thiaridae*	0.09	0.11	1.77	0.49
Philopotamidae	0.16	0.03	0.13	0.11	*SHRIMP*	0.04	0.11	0.10	0.08
Polycentuopodidae	0.02	-	-	0.01	**N=**	**16,782**	**13,939**	**9,567**	**40,288**
Crambidae	0.05	0.04	0.05	0.05	**Taxa richness**	**55**	**49**	**50**	**60**
					Taxa diversity	**1.50**	**1.70**	**2.38**	

5.3.5 Invertebrate – Vegetation Associations

Canonical Correspondence Analyses (Figure 5.7) axes 1 and 2 explained 15.3% of the variation in the San Marcos River benthic macroinvertebrate community among vegetation habitats. Physical parameters and season strongly associated with CCA axis 1 were Fall (0.81), TWR (-0.50), gravel (-0.49), *Hydrocotyle* sp. (0.47), and current velocity (-0.43). Physical parameters strongly associated with CCA axis 2 were gravel (0.57), current velocity (0.51) *Hydrocotyle sp.* (0.44), sand (-0.39), and *Hygrophila sp.* (-0.34).

Among macroinvertebrate species associated with CCA axes 1 and 2, Ostracods, Nectopsyche, and Petrophila were more abundant in fall among several vegetation types (*Hydrocotyle*, *Potamogeton*, and *Sagittaria*) over cobble substrate. Hirudinea, Decapoda, Crambidae, Hydroptila, Shrimp, Hydrachnidae, and Zygoptera were most abundant at Site 2 in *Hygrophila* over fine substrates. Helicopsychidae, Thiaridae, Simulidae, and Limnocoris were found most often in vegetation over gravel substrates in higher current velocities. Hyalellidae, Chironomidae, Leptohyphidae, and Baetidae were common among all available habitats. Other species such as Baetodes, Glossosomatidae, Hemerodromia, Hydropsychidae were more abundant in spring and summer within TWR over gravel substrates. Species more common at Site 1 in areas of greater depth within Potamogeton were Philopotamidae, Platyhelmenthes, Mesogastropoda, Elmidae, Anisoptera, and Cladocera.

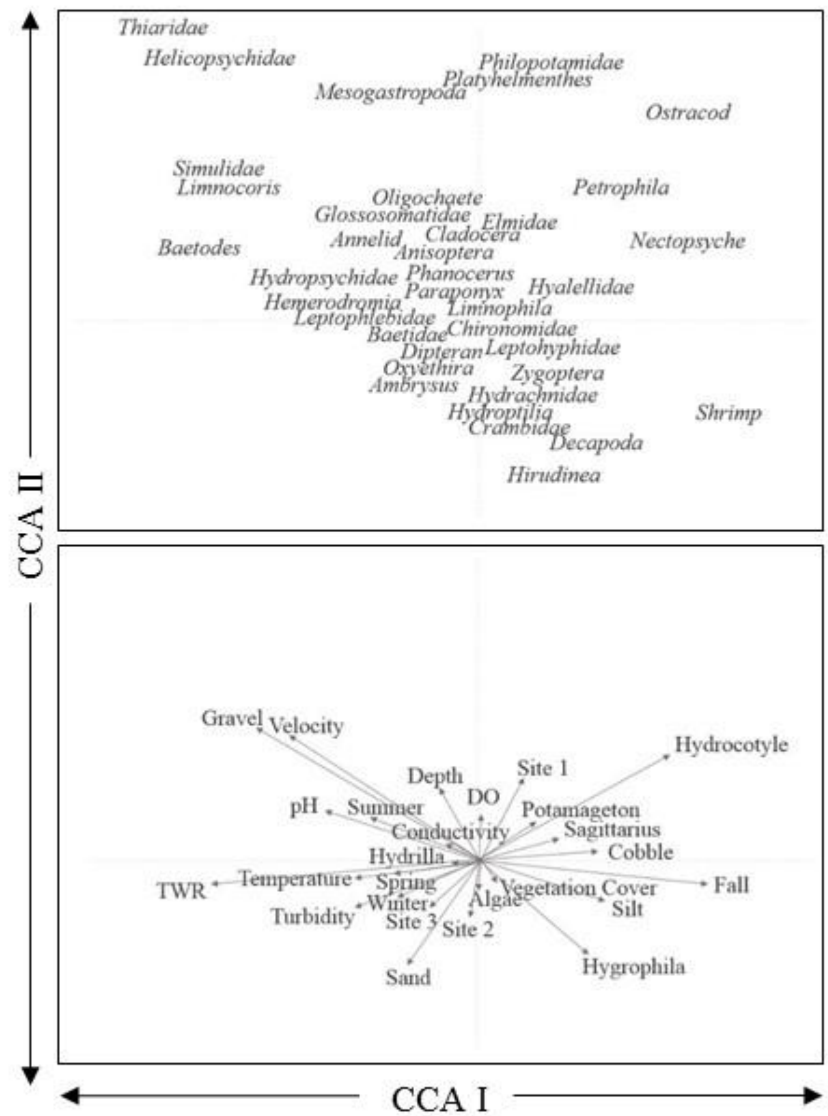

Figure 5.7 Canonical correspondence analysis bi-plots for macroinvertebrate species among vegetation samples (upper) and environmental parameters, site, and season from San Marcos River (April 2015 – December 2015).

5.3.6 Invertebrate – Benthic Substrate Associations

Canonical Correspondence Analyses (CCA) (Figure 5.8) axes 1 and 2 explained 23.9% of the variability in the San Marcos River benthic macroinvertebrate community among open substrate habitats. Physical parameters, water quality, and site strongly associated with CCA axis 1 were Site 3 (0.71), turbidity (0.62), Site 1 (-0.52), current velocity (0.34), and silt (-0.29). Physical parameters and season strong associated with CCA axis 2 were Spring (0.72), current velocity (-0.51), cobble (-0.44), Fall (-0.42), and sand (0.28). Among macroinvertebrate species associated with CCA 1 and 2, Limnocoris, Ambrysus, Leptophlebiidae, Hydropsychidae, Philopotamidae, Nectopsyche, Glossosomatidae, and Elmidae were more abundant during fall and winter in areas of higher current velocities over cobble substrates. Helicopsychidae and Hirudinea were more abundant in spring over gravel substrates. Petrophila, Zygoptera, Anisoptera, Baetidae, Oxyethira, and Hemerodromia were more common at Site 2 during fall over cobble substrates. Corbicula, Thiaridae, and Mesogastropoda, Simulidae, and Bivalvia were most abundant during spring. Chironomidae, Annelids, Hyalellidae, Platyhelmenthes, and Leptohyphidae were common among all available habitats and seasons.

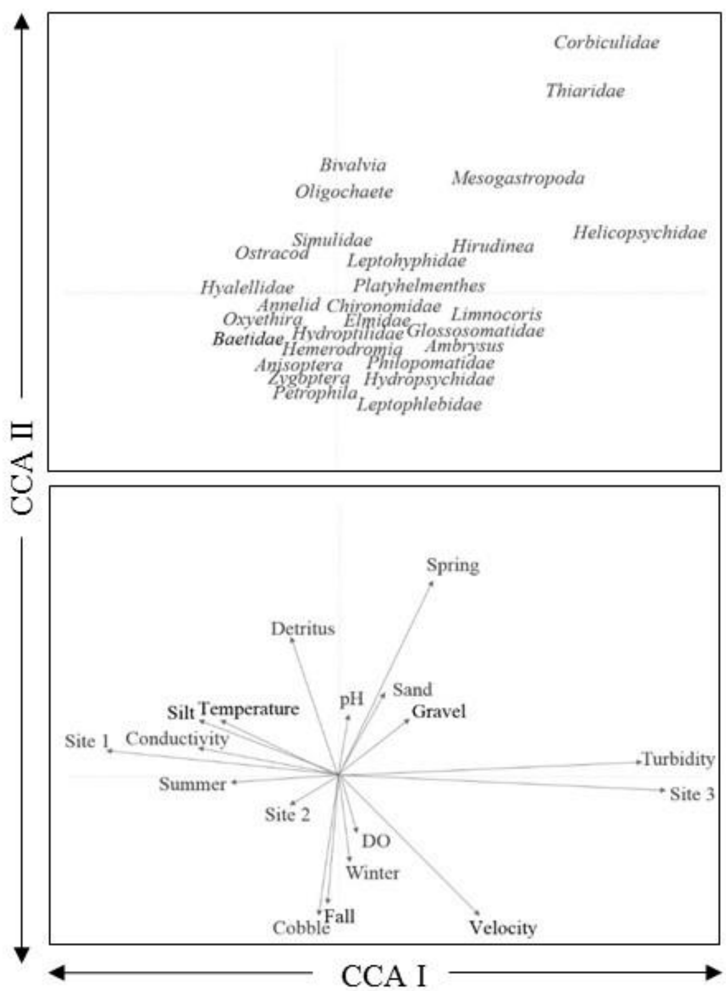

Figure 5.8 Canonical correspondence analysis bi-plots for macroinvertebrate species among substrate samples (upper) and environmental parameters, site, and season from San Marcos River (April 2015 – December 2015).

5.3.7 Functional Feeding Groups (FFG)

Collector gatherers accounted for most of the composition of the FFG for all sites and seasons except for Site 3 in the spring, which was dominated by scrapers (Figure 5.9). Filtering collectors comprised the lowest relative abundance of FFG among all sites and seasons. Relative abundance of collector gatherers in drift samples increased from upstream to downstream, except for the winter, which decreased from 64% at Site 1 to 55% at Site 3. Scrapers among benthic samples increased from upstream to downstream in spring and summer. For example: during spring sampling, scrapers observed in the benthos increased from 27% at Site one to 47% at Site three. Overall, scrapers had a higher relative abundance in benthic samples (20%) compared to drift samples (10%), filter collectors had a higher relative abundance in benthic samples (3%) than in drift samples (1%), and shredders also had a slightly higher relative abundance in benthic (21%) than in drift samples (20%). Collector gatherers was the only FFG that had a higher relative abundance in drift samples than in benthic samples (63% and 49% respectively). Spearman Rank correlation (Table 5.7) showed a very strong correlation between the FFG of benthic and drift macroinvertebrates. The correlation results ranged from 0.8 to 1.0 among sites and for all seasons. Site 3 (0.8 – 0.9) showed the lowest correlation among sites and for all seasons.

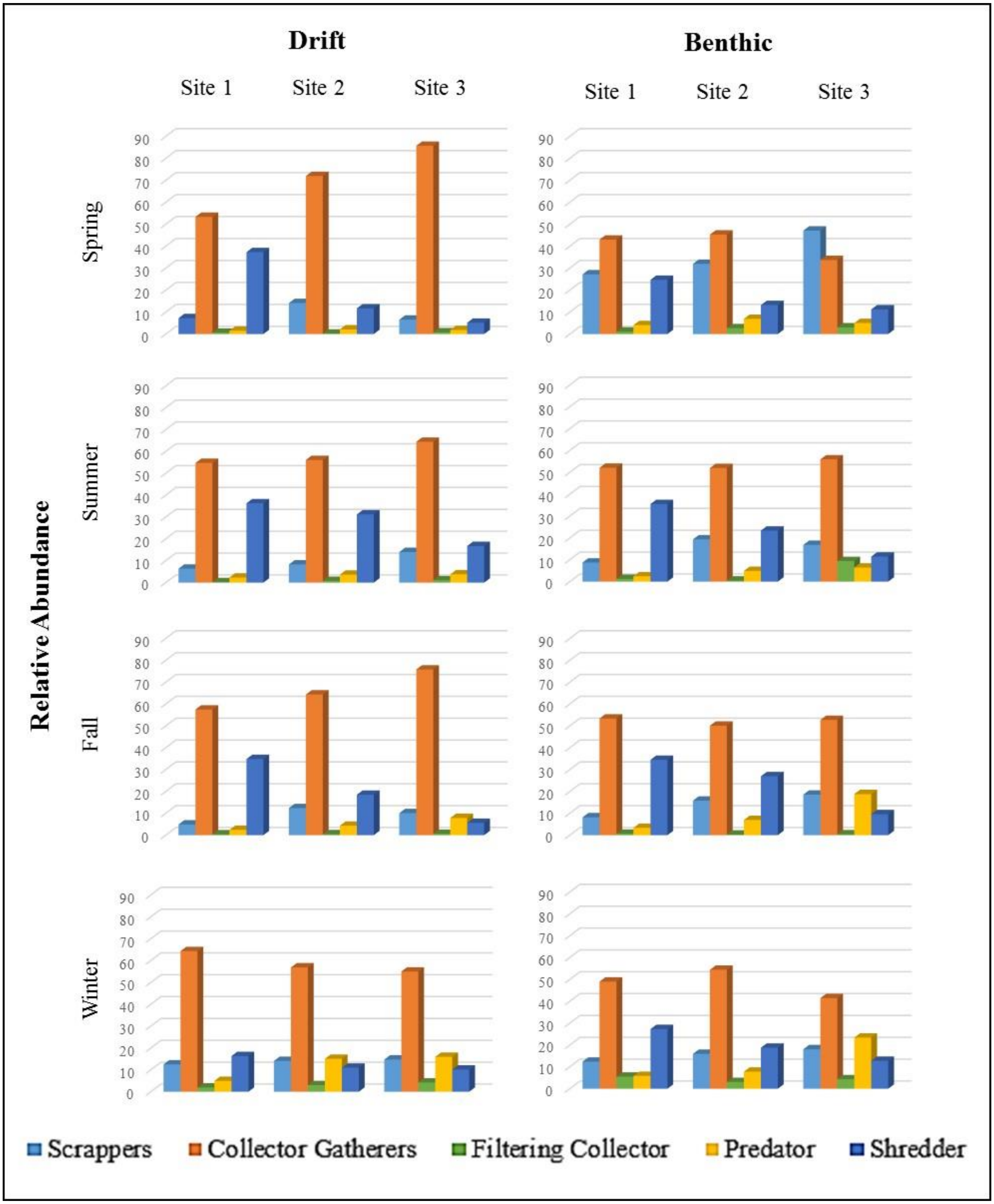

Figure 5.9 Relative abundance for FFG in the drift and benthic samples for Sites 1-3 on the San Marcos River among seasons (April 2015 – Dec 2015).

Table 5.7 Spearman's correlation coefficient for FFG in the drift and benthic samples for all sites and seasons in the San Marcos River (April 2015 – Dec 2015).

Spring	**Site 1**		**Site 2**		**Site 3**	
FFG	Benthic	Drift	Benthic	Drift	Benthic	Drift
			Spring			
Scrapers	724	1387	579	792	1109	502
Collector Gatherers	1145	10188	821	4037	793	6556
Filtering Collector	29	116	48	11	71	58
Predator	108	289	125	118	118	141
Shredder	658	7122	239	655	264	395
Spearman Coefficient	**0.90**		**1.00**		**0.90**	
			Summer			
Scrapers	505	1593	1153	936	286	611
Collector Gatherers	3018	13678	3103	6327	950	2809
Filtering Collector	72	54	28	87	159	47
Predator	142	581	293	409	109	165
Shredder	2057	9058	1394	3518	195	730
Spearman Coefficient	**1.00**		**1.00**		**0.80**	
			Fall			
Scrapers	497	1478	792	1937	942	1251
Collector Gatherers	3240	17334	2513	10081	2681	9421
Filtering Collector	40	109	13	69	19	76
Predator	203	730	348	678	955	981
Shredder	2093	10503	1353	2891	488	700
Spearman Coefficient	**1.00**		**1.00**		**0.90**	
			Winter			
Scrapers	497	1478	792	1937	942	1251
Collector Gatherers	3240	17334	2513	10081	2681	9421
Filtering Collector	40	109	13	69	19	76
Predator	203	730	348	678	955	981
Shredder	2093	10503	1353	2891	488	700
Spearman Coefficient	**1.00**		**1.00**		**0.90**	

5.4 Discussion

Macroinvertebrate drift densities followed the typical circadian pattern observed in other river systems and did not show an increase in macroinvertebrate drift during the day when recreation is occurring. This is attributed to several factors. Bed disturbance is localized primarily at specific spatial locations such as at a tube rental vendor location and City of San Marcos-managed river access points. These areas are quickly denuded of aquatic vegetation early in the recreation season and remain so until aquatic vegetation recovery occurs during the fall and winter when river access dramatically drops (EAHCP biological monitoring data between 2000 and 2015). This may also reflect that drift had settled out upstream of our study sites. Other forms of contact recreation, such as kayaking and tubing do not typically disturb the river bed outside of direct access areas. Measured turbidity levels appear to be low enough as to not precipitate a drifting response and may in part be related to the small grain size (silt like) suspended sediment that remains entrained in the water column at relatively low velocities. Depositional areas are typically associated with lateral stream margins or in backwater areas upstream of low head dams in the system.

Site 1 (immediately below Spring Lake) had the highest drift densities but lowest diversity and was due to the very high number of *Hyalella* in the samples. Spring Lake maintains a very high density of Hyalellidae that likely serves as a continual source, especially given the aquatic vegetation maintenance every few days as part of Spring Lake glass bottom boat operations (Diaz et al. 2015). Aquatic vegetation maintenance results in large number of vegetation fragments washing downstream for several days after each cutting period and we suspect that some macroinvertebrates remain trapped in the vegetation and transported downstream below Spring Lake dam.

Drift densities in winter were lowest among all sites and is attributed to either seasonal emergence patterns and/or influenced by a large flood/scouring event that occurred only six weeks prior to sampling event. Habitat associations of macroinvertebrates were similar to what was previously observed by Diaz et al. (2015) and Fries and Bowles (2002). In addition, functional feeding groups in the drift reflect invertebrates available in the benthos over all sampling periods and study sites. Overall community composition in terms of functional feeding groups was similar to results reported by Fries and Bowles (2002) for the San Marcos River ~ 2 km below the lowest sampling station at Ramon Lucio.

Study results documented a diverse and dense macroinvertebrate community in both the benthos for a variety of aquatic vegetation types and previously undocumented characteristics within Texas wild-rice stands. This is important given that the EAHCP is targeting non-native vegetation removal and planting of native aquatic vegetation specifically to meet target densities of the endangered fountain darter (*Etheostoma fonticola*) derived from species specific vegetation darter densities. The aquatic vegetation restoration effort is also targeting increased areas of Texas wild-rice. We had previously documented Texas wild-rice use by the endangered fountain darter and the results of this study documents the presence of key macroinvertebrate species (e.g., Hyalellidae) that are important components of the fountain darter diet.

Study results indicate that the macroinvertebrate benthos and drift at Site 1 are not impacted by recreation or turbidity as would be expected given its location immediately below Spring Lake Dam and associated with the protected designation as a State Scientific Area that limits direct contact recreation. Site 2 appears to show very little impact associated with recreation induced turbidities on a seasonal basis and maintains a robust aquatic vegetation community that supports the aquatic macroinvertebrates both in the benthos and drift. However, Site 3, based on the CCA results indicate that substrate and turbidity are factors influencing the macroinvertebrate community. This area lies downstream of the Rio Vista Dam (kayak park) that was constructed in 2006. Vegetation and substrate monitoring during the period from 2000 to the present (Bio-West 2016) in this reach of the San Marcos River has documented both channel changes (depth decreases) and the aquatic vegetation in this reach has dramatically declined from pre-dam construction periods. Loss of aquatic vegetation obviously has a direct impact to the macroinvertebrates.

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