Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos Springs/River Aquatic Ecosystem

FINAL 2004 ANNUAL REPORT

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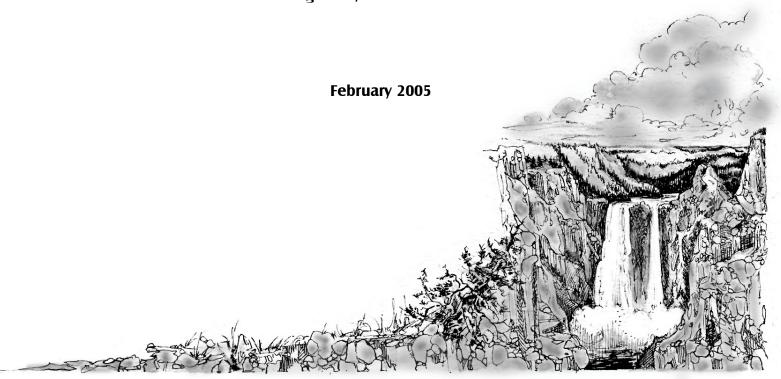


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EXECUTIVE SUMMARY

During this study, valuable information has been gathered on current population dynamics of each listed species found in the San Marcos Springs/River ecosystem (except those that are primarily subterranean) and their habitat conditions. These data were collected under a Comprehensive Monitoring effort that included regular, quarterly samples to provide baseline information and during Critical Period events that were triggered by infrequent, extremes in discharge that might affect each species. Because above-average flow conditions occurred during most of 2000-2004, there were no low-flow Critical Period events, but three high-flow Critical Period events in the San Marcos Springs/River ecosystem during that time.

There were multiple components used to make assessments of the population condition of each species and of habitat availability. Overall water quality was assessed quarterly throughout the entire San Marcos River and in Spring Lake during 2000-2002 using standard parameters (water temperature, dissolved oxygen, pH, and conductivity) as well as conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids). This resulted in a detailed assessment of baseline conditions, which were then augmented with standard parameters collected during each subsequent sample effort. This assessment suggested that all water quality parameters had relatively narrow ranges that were providing high quality conditions for aquatic life use. A continuous record of water temperature was also maintained in multiple locations within the San Marcos Springs/River ecosystem during the study using temperature loggers. These loggers revealed a high degree of thermal uniformity throughout the San Marcos River despite the wideranging conditions experienced during the study. Apart from acute disturbances, water temperatures fluctuated by less than 2°C daily and by approximately 1°C seasonally at the two sites located directly below Spring Lake in the eastern (chute) and western (dam) spillways. Further downstream in the City Park and IH-35 reaches temperatures varied by greater than 2°C daily and approximately 2.5°C seasonally. The maximum deviation was approximately 7°C from the average value in the late summer (observed in 2001) but these data are suspect since no other site had similar readings. Overall, water temperature data have not presented any cause for concern during the study, but it will continue to be important to evaluate low-flow conditions. Fixed station photographs also documented physical changes in habitat at multiple locations throughout the San Marcos Springs/River ecosystem. Additional low-flow data are vital to improve understanding of water quality conditions that may be expected at various discharges.

For the fountain darter, habitat use is largely influenced by aquatic vegetation and assessments of habitat availability were conducted by mapping this vegetation during each sampling event. Throughout the study, the amount of aquatic vegetation varied more in the IH-35 Reach than the City Park or Spring Lake Dam reaches. Flooding resulted in a temporary reduction in total aquatic vegetation coverage in all reaches, but affected the IH-35 Reach most dramatically and the City Park Reach the least. However, many plant types quickly responded to the scouring flows with rapid re-growth and expanded to a total coverage that exceeded the pre-flooding condition. The plant type that was most susceptible to scour during flooding in the San Marcos Springs/River ecosystem was *Cabomba*, which occurs in areas of deep silt. It was also the highest quality habitat type (of those sampled quantitatively) for fountain darters in the San Marcos Springs/River ecosystem. It remains unclear how various vegetation types will respond to low-flow conditions and whether each plant type will support the same densities of fountain darters under such conditions.

There was a substantial increase in total coverage of Texas wild-rice throughout the San Marcos River in 2000-2004. The scouring events in 2001, 2002 and 2004 have had a positive long-term impact on Texas wild-rice total coverage. Although there were short-term losses following each flood, the total coverage in the river has increased to the highest levels recorded during this study or by the Texas Parks and Wildlife (TPWD). These scouring events appear to stimulate growth of individual stands and also result in displacement of small Texas wild-rice plants, which settle into new areas and grow rapidly. Stands of Texas wild-rice that remain in place after flooding are generally smaller, but they experience rapid growth. Despite the increased total coverage in the river, the total coverage of the species in the lower portions of its range (below the IH-35 Bridge) remains very small. Unlike most areas above the IH-35 Bridge, no plants were displaced from upstream of these areas during flood events to compensate for lost plants. Also, plants that remained after flooding did not experience the rapid growth that occurred in upstream habitats. This trend downstream of IH-35 traces back to the massive flood of 1998 when the TPWD data revealed significant declines in areal coverage following that event; the Texas wild-rice population in those areas has never recovered from that event and continues to decrease with each significant flood event.

Direct sampling of the fountain darter occurred in the same reaches with aquatic vegetation used to stratify random sample locations. There are fewer abundant vegetation types in the San Marcos Springs/River ecosystem than in the Comal Springs/River ecosystem and the densities of fountain darters observed in the various vegetation types did not range as widely. The relatively narrow range of densities supported by the various vegetation types suggest that the type of vegetation is not as important in the San Marcos Springs/River ecosystem as the presence/absence of any type of vegetation. The average densities of fountain darters in all drop net sites in each vegetation type and maps of total vegetation coverage were used to estimate fountain darter abundance in each reach during 2000-2004. These rough estimates were subject to a wide variability in density estimates so results were normalized and presented as a proportion of the maximum. The results show a relatively consistent estimate of the fountain darter population during 2000-2004 with some decrease associated with each high-flow event. The drop net data were also used to develop linear regressions using the three variables of most importance to fountain darter densities (vegetation, reach, and discharge). These results revealed that discharge has had limited influence on fountain darter densities with the discharge that has occurred in 2000-2004. However, the limited data from low-flow conditions did not allow an extensive statistical assessment of fountain darter population dynamics.

Drop net data also provided information on the overall size-class distribution for the San Marcos Springs/River ecosystem, which continues to indicate a healthy fish assemblage. However, the distribution is shifted towards larger fish than those observed in the Comal Springs/River ecosystem and this may be a function of lower quality habitat in the two sampled reaches compared with the Comal River reaches. Also, currents tend to be stronger in the San Marcos River reaches and may contribute to finding larger, but fewer, fish. In general, the number of fountain darters per net in the San Marcos River was much lower than in the Comal River. This is likely related to the quality of habitat sampled in each ecosystem and the ability of the habitat to support various densities of fountain darters. In the Comal River, habitat tends to be much more favorable for fountain darters, and densities are much higher (although densities in Spring Lake on the San Marcos River are very high – based on visual [SCUBA] observations).

In addition to evaluating fountain darter population dynamics, the entire fish community was assessed by calculating species diversity in each sample reach during each sample effort in 2000-2004. There was no difference in diversity between the two reaches and no discernable patterns of change in

diversity at the reach level during 2000-2004. One additional area of concern to fountain darters, the density of giant ramshorn snails (*Marisa cornuarietis*) was monitored in 2000-2004. By all indications the densities of giant ramshorn snails observed in the San Marcos Springs/River ecosystem during the study period to pose no serious threat to the aquatic vegetative community (i.e., fountain darter habitat). However, because of the impact that this exotic species can have under heavier densities, close monitoring of this species should continue. The gill parasite that has been reported to infect the fountain darter in the Comal Springs/River ecosystem was not visually evident in fountain darters collected from the San Marcos Springs/River ecosystem during 2000-2004.

Observations of the San Marcos salamander have varied in number within and between locations during 2000-2004, but individuals have been observed in each sample location during each sample period. Filamentous algae was abundant in most sampling events conducted during 2000-2004 in the sample site adjacent to the former Aquarena Springs Hotel and the deep site in Spring Lake and required clearing the algae prior to sampling efforts. This may have impacted sampling efficiency. Regardless, the Hotel site consistently had the highest number of San Marcos salamanders. This site provided highly suitable habitat with consistent springflow, abundant cover, and an abundant food supply. The deep site in Spring Lake had lower San Marcos salamander densities, but it also provided high-quality habitat. Population estimates have varied greatly between seasons at each site, overshadowing any seasonal or discharge-related trend that may be present. A lack of substantial low-flow data precludes discussion of potential influences of lower flows on the population at this time.

As described above, the data in this report remain preliminary due to the lack of low-flow data that are necessary to make a complete analysis. More data from low-flow periods (particularly from an extended low-flow period) are essential to fully evaluate the biological risks associated with future critical periods (high or low flow). Although quarterly sampling events do not yield vital low-flow data, this sampling is extremely important to maintain a continuous understanding of current conditions in order to be prepared for a low-flow period and monitor changes that may occur. Sampling only during a low-flow event will not provide the necessary context to adequately assess such changes.

This study remains the most comprehensive biological evaluation that has ever been conducted on the San Marcos Springs/River ecosystem. Variable flow conditions encountered to date have provided an excellent confirmation that the study design is well suited to address the concerns of variable flow and water quality on the biological resources in the Comal and San Marcos Springs/River ecosystems. As noted in previous annual reports, this study meets three critical criteria to assure the greatest possible success in assessing impacts to biological communities of variable flow conditions: (1) the endangered species are evaluated directly (some studies make conclusions based on surrogate species and attempt to describe dynamics of the endangered species), (2) continuous sampling is used to evaluate current conditions to properly assess changes relative to flow variation (one-time sampling events or limited sampling during particular seasons will not yield accurate conclusions), and (3) multiple collection techniques are used to evaluate multiple components of the ecosystem (important observations may be missed using limited sampling means).

INTRODUCTION

This study, conducted from 2000-2004, was initiated in response to concerns about the impact of low recharge and correspondingly low spring discharge on the listed species found within the Comal and San Marcos Springs/River ecosystems. Management of the water resources within the Edwards Aquifer is dependent on accurate knowledge of the quantity and quality of the water required by each species as well as other habitat needs. Changes in springflow associated with fluctuating recharge conditions lead to changes in water quality and habitat availability. Some fluctuation in habitat conditions is beneficial, but severe changes in springflow conditions may reduce habitat suitability and the ability of that habitat to effectively support individual species' populations over long periods of time. However, it is difficult to discern a precise shift from "good conditions" to "poor conditions" for a given species resulting from the total discharge from each spring. This study was initiated to gather more information on population dynamics of each species under a range of possible discharge conditions in order to enhance predictive ability of a population-level response to a given discharge.

The design of this study focused on evaluating the critical questions of population responses to changes in water quality and other concerns associated with variable flow conditions occurring in the San Marcos and Comal Springs/River ecosystems. The monitoring and research plan was developed in coordination with the Authority and USFWS in May 2000 with additional input from a Technical Advisory Group (TAG) during the scoping process. The study included monitoring and research efforts directed toward each of the eight threatened and endangered species found in the Comal and San Marcos Springs/River ecosystems. These include two fish species, fountain darter (Etheostoma fonticola) and San Marcos gambusia (Gambusia georgei), two salamanders, Texas blind salamander (Eurycea rathbuni) and San Marcos salamander (Eurycea nana), one plant species, Texas wild-rice (Zizania texana) and three invertebrates, Comal Springs dryopid beetle (Stygoparnus comalensis), Comal Springs riffle beetle (Heterelmis comalensis), and Peck's cave amphipod (Stygobromus pecki). Of these, only the San Marcos salamander is listed as threatened by the U.S. Department of Interior, Fish and Wildlife Service (USFWS), the rest are all listed as endangered species. The San Marcos gambusia is likely extinct since no individuals have been collected since 1982, despite an intensive search effort in 1990 (USFWS 1996). One additional species that was monitored during this study was the undescribed Comal Springs salamander (Eurycea sp.)

Each of these species has a restricted distribution range limited to springs associated with the Edwards Aquifer, and many are found in either Comal Springs or San Marcos Springs, but not both. Prior to initiation of this study, only the fountain darter was believed to occupy both Spring ecosystems, but recent collections of the Comal Springs riffle beetle in Spring Lake at the headwaters of the San Marcos River (R. Gibson, U.S. Fish and Wildlife Service, pers. comm.) reveal that this species is also found in both ecosystems. Information gathered during this study (BIO-WEST 2002a) also indicates that the Comal Springs riffle beetle is more widely distributed in the Comal Springs/River ecosystem than previously believed. Among the other species, San Marcos gambusia, San Marcos salamander and Texas wild-rice occur only in the San Marcos River, while the Texas blind salamander is found in the aquifer below San Marcos and nearby springs. Two of the three invertebrates, Comal Springs dryopid beetle and Peck's cave amphipod, are found only in Comal and nearby springs (i.e., Hueco and Fern bank springs).

During this study, valuable information was gathered on water quality and habitat conditions for each of these species as well as monitoring data on current population dynamics of each. For those species that

are primarily subterranean, Comal Springs dryopid beetle and Peck's cave amphipod, information is less extensive due to sampling limitations. There were multiple components incorporated into this study because sampling efforts were unique to each species and for general water quality conditions.

The objectives of the water quality analysis were to delineate and track water chemistry throughout the ecosystem; monitor controlling variables (i.e., flow, temperature) with respect to the biology of each ecosystem; monitor any alterations in water chemistry that may be attributed to anthropogenic activities; and evaluate consistency with historical water quality information. This was conducted with quarterly water quality sampling throughout the entire San Marcos River for two years including standard parameters (water temperature, dissolved oxygen, pH, and conductivity) and conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids). In addition, nine sites were sampled in the headwaters of the San Marcos River, Spring Lake. A continuous record of water temperature was also maintained in multiple locations within the San Marcos Springs/River ecosystem during the study using temperature loggers. Fixed station photographs documented physical changes in habitat at multiple locations throughout the San Marcos Springs/River ecosystem.

The primary habitat sampling component to this study was directed at aquatic vegetation. Aquatic vegetation presence/absence and species composition have a substantial influence on the distribution and presumably the abundance of several of the threatened/endangered species, particularly the fountain darter. Because it is a primary variable in fountain darter population dynamics, very precise maps (<1m accuracy) of vegetation composition in each sample reach were created during each sample event to document the abundance of all aquatic vegetation and monitor fluctuations associated with season and discharge.

Of the sampling efforts directed at individual species, the fountain darter component was the most extensive. To establish a clear understanding of fountain darter habitat associations and evaluate population responses to changes in flow, sampling was conducted using two methods in multiple sample reaches. Drop netting, enclosing a 2-m² area and sampling exhaustively, provided valuable quantitative information on fountain darter densities in each of the dominant vegetation types and allowed for evaluation of potential seasonal and discharge-related responses of the population. Dip netting provided information on fountain darters using habitat along the river margins, which was not typically sampled with the drop net and also augmented information on smaller size-classes.

The San Marcos salamander was monitored in multiple locations throughout this study to evaluate relative distribution among known habitat locations and to examine population responses to changes in springflow. The sample areas included two locations in Spring Lake and one site just downstream of the Spring Lake dam in the eastern spillway. Timed surveys in each of these areas provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge. During these surveys, qualitative visual observations of fountain darters in the lake were also noted.

The final sample component in the San Marcos Springs/River ecosystem was to estimate the density of various exotic fish species and to evaluate the potential that predators may be consuming one or more of these threatened/endangered species in large numbers. This was conducted by using gill nets and evaluating stomach contents of captured fish for the presence of any threatened/endangered species (primarily fountain darters and salamanders). Because of limited sample sizes early in the study, rod-and-reel sampling was added to supplement gill net sampling for this study component. Rod-and-reel

sampling allowed researchers to target larger sunfish and small- to intermediate-sized bass, which are the most likely piscine predators on fountain darters and salamanders. As a result of using both rod-and-reel and gill net sampling, sufficient baseline data had been collected by the final 2002 sample to discontinue this component except during "Critical Period" events.

In addition to each of the study components described above, several individual research efforts that developed during the course of this study were conducted by BIO-WEST, Inc. (BIO-WEST). The details of each study is not covered in this document but can be found in the individual reports cited here. A laboratory study of fountain darter reproductive response to parasites and fluctuations in temperature (BIO-WEST 2002b) revealed that parasites did not affect reproductive capability, but reaffirmed earlier work by Brandt et al. (1993) and Bonner et al. (1998) that reproduction in the laboratory is reduced when water temperature reaches 26°C regardless of daily temperature fluctuations. In 2004, BIO-WEST conducted a laboratory study on the influence of fluctuations in discharge on water quality and determined that carbon dioxide was a potentially limiting factor affecting growth and structural support of several plants in the San Marcos Springs/River ecosystem including Texas wild-rice (BIO-WEST 2004b).

The data for the primary study components were gathered during regular, quarterly "Comprehensive" sample efforts to provide a baseline of information on population dynamics of each species under "normal" discharge conditions. However, the study design also incorporated sampling events that occurred following infrequent, extremes in discharge (Critical Period events) to evaluate the response of each species and its habitat to such events. Unfortunate to the primary goals of this study, the sampling events resulting from the infrequent, extremes in discharge have been primarily high discharge events, but the data have yielded valuable information nonetheless. The scouring effects of flooding had oftendramatic results on aquatic vegetation and population abundance of fountain darters. The only period of low discharge on the San Marcos River occurred in 2000, but discharge did not decrease enough to trigger a Critical Period sample and the low flows were relatively short-lived. Therefore, it is difficult to make direct evaluations of the water quality conditions that would be expected with a repeat of these discharge levels (or lower discharge conditions) or the response of each species' population and its habitat to such events.

As a result of the lack of low-flow conditions and need for establishment of long-term monitoring efforts, this study has been extended and will hopefully yield the desired information on the effects of low discharge on each listed species in the Comal and San Marcos Springs/River ecosystems. This valuable information will improve the ability to predict the response of each population to such conditions if and when they occur again in the future. The continuity of baseline information is also extremely valuable due to the dynamic nature of a population-level response to changing habitat conditions. A population that has experienced "good conditions" for an extended period and has expanded in abundance to capitalize fully on extensive availability of high quality habitat will not have the same response to a rapid decline in discharge that a population with moderate to low quality conditions immediately preceding such an event. The only way to maintain knowledge of current population conditions is through regular monitoring, which reveal trends in population abundance and prevent a lack of baseline data on conditions immediately prior to a low-recharge event, since low-flow conditions can occur rapidly due to the geophysical characteristics of the Edwards Aquifer.

METHODS

There are two major elements to the sampling design of this study. Comprehensive sampling is conducted during the spring, summer and fall (winter was sampled in 2001 and 2002) to develop a thorough understanding of baseline conditions and seasonal variation in the biological community. The other component is a Critical Period effort in which sampling events are triggered by uncommon discharge events that have the potential to alter dynamics of the biological community, whether short or long-term. These discharge events include both critical low-flow and flooding discharge events. Both types of sampling events involve similar sampling components.

In total, the following sampling components are incorporated into the study:

- ➤ Water quality evaluation
 - Standard parameters (DO, pH, spCond, temperature)
 - Conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids)
 - Thermistors for continuous record of water temperature
 - Fixed station photographs for qualitative evaluation of temporal variation
- > Detailed mapping of aquatic vegetation (fountain darter habitat) in study reaches
 - Texas wild-rice annual survey
- > Texas wild-rice physical observations
- > Fountain darter sampling
 - Drop nets
 - Dip nets (time constrained surveys)
- > San Marcos salamander observations
- > Exotic / Predation evaluation

A thorough description of the methodology for each sampling component is provided below.

In 2000-2002 all sample components were incorporated into each Comprehensive and Critical Period sampling event. In 2003 and 2004, slight modifications were made to the protocol for a sampling event on the San Marcos Springs/River ecosystem in which some components were removed from Comprehensive samples. Modifications to the monitoring program were discussed among BIO-WEST, the Edwards Aquifer Authority (Authority), and the U.S. Fish and Wildlife Service (USFWS) during a meeting in August 2002 and implemented beginning with the fall 2002 Comprehensive sampling event. The most notable change was to remove the water quality component from Comprehensive sampling events due to the comprehensive assessment that was completed in 2002 on the relatively constant water quality conditions that occur under the majority of flow conditions. One water quality component, regular monitoring of thermistors, did remain a part of each sample effort to maintain a continuous record of water temperature at all sites. Another component that was removed from Comprehensive sampling events was the exotic/predation study because there was little evidence to suggest predators were consuming threatened or endangered species under most flow conditions. Low-flow Critical Period sampling events would have included the water quality and exotic/predation sample components to evaluate potential effects at low-flow, but no low-flow Critical Period events occurred between 2001 and 2004.

There was one other modification between samples conducted in the San Marcos Springs/River ecosystem in 2000-2002 and those conducted in 2003-2004, which was the removal of the winter Comprehensive sample. Data collected during the first two years of the study provided sufficient information to adequately describe baseline conditions during this season. Low-flow conditions are most likely to occur during the late summer and early fall, so it was considered to be more critical to have continuous monitoring of conditions immediately preceding and following this timeframe each year.

Springflow

Total discharge data in the San Marcos River were acquired from U.S. Geological Survey (USGS) Water Resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2000). The discharge data for the San Marcos River ware taken from USGS gage 08170500 at the University Drive Bridge in the city of San Marcos. This site represents the cumulative discharge of the springs that form the San Marcos River system. In addition to the cumulative discharge measurements that were used to characterize this ecosystem during sampling, spot measurements of water velocity were taken during each sampling event using a Marsh McBirney model 2000 portable flowmeter.

Low-Flow Critical Period Sampling

This project was initiated during a period of limited recharge in the summer of 2000. Although these conditions led to low discharge in the Comal River and triggered low-flow critical period sampling in that ecosystem, it did not trigger a full sampling event in the San Marcos Springs/River ecosystem. The first low-flow trigger for a full sampling event is 100cfs and discharge did not decrease below 108cfs during 2000-2004. There were additional Texas wild-rice observations (scheduled for 120cfs and every 5 cfs decline) during August and September 2000, but no other low-flow data for the San Marcos Springs/River ecosystem. The rainfall event in September that raised discharge above trigger levels in the Comal Springs/River ecosystem also increased discharge in the San Marcos Springs/River ecosystem and there have been primarily higher-than-normal flows since that time. As a crucial component of the study, more biological data during low-flow periods is essential to adequately assess population-level impacts during these periods. A thorough analysis of all available data is presented in this report, but the continuation of this monitoring and research effort will allow collection of more critical low-flow data.

High-Flow Critical Period Sampling

Although there were no low-flow discharge events in the San Marcos Springs/River ecosystem in 2000-2004 to adequately evaluate that component of the study, three significant flood events provided valuable information to describe high-flow impacts (Figure 1). These events occurred in November 2001, July 2002, and June 2004 (the event in August 2001 in the Comal Springs/River ecosystem was not considered a high-flow event in the San Marcos Springs/River ecosystem). The 24-hour mean discharges for the four events were, respectively, 1,019, 668, and 439cfs. Although the most significant event in the Comal Springs/River ecosystem was in July 2002, the localized rainfall from that event did

not impact the San Marcos Springs/River ecosystem to the same extent. The largest event in the San Marcos Springs/River ecosystem occurred in November of 2001. A full sampling event occurred, to the extent possible, in response to each event after a sufficient time elapsed (approximately one week) to allow conditions to stabilize after the flushing flows subsided in order to evaluate post-flooding effects. In two instances these samples corresponded with the summer Comprehensive sampling event (July 2002 and June 2004), whereas the other November 2001 sample was an additional event. The exotic/predator sampling was not incorporated into the additional samples. In some instances, dip net and/or drop net sampling for fountain darters was not possible during these events due to high water. In addition to these three high-flow Critical Period sampling events, a high-flow trigger occurred between November 17-24 2004 (24-hr mean discharge during this time was not calculated because flows overtopped the gage). However, sufficient data had been collected on high-flow impacts and only a visual assessment (including photographs) was conducted along with mapping of vulnerable stands of Texas wild-rice plants.





Figure 1. Photographs of (a) the City Park (Lion's Club) and (b) University Drive Bridge two days after the high-flow event in November 2004 (peak discharge unknown). The water is 12-18 inches above normal in the photos.

Water Quality

Dr. Alan Groeger of Texas State University supervised all aspects of the water quality component in 2000-2002, and the chemical analyses for each Comprehensive sampling event was conducted in Dr. Groeger's laboratory at Texas State University (formerly Southwest Texas State University). Conventional water chemistry parameters were determined from "grab" samples (described below). Standard physico-chemical parameters were measured using a Hydrolab data sonde. That data resulted in a baseline water quality assessment that is described in detail in the 2002 annual report (BIO-WEST 2003) and summarized in the Results section of this document. In 2003-2004 the water quality component was discontinued during Comprehensive and high-flow Critical Period sampling events and no low-flow Critical Period sampling events occurred during that time. An additional water quality component, water temperature, was monitored with loggers (thermistors) that were placed in select water quality stations along the San Marcos River and downloaded at regular intervals to provide continuous record of water temperatures in these areas. This component of the water quality effort has been conducted throughout the study (2000-2004).

Water quality was evaluated in the headwaters of the San Marcos River in Spring Lake and in several sites downstream. A total of nine sites were used to characterize water quality conditions in Spring Lake and the same number of sites chosen to represent the water quality conditions in the Upper San Marcos River down to the City of San Marcos wastewater treatment outfall (Figure 2). In each sample site, a Hydrolab profile was conducted taken during Comprehensive and Critical Period sampling events in 2000-2002. A hydrolab profile measured water temperature, conductivity compensated to 25°C, pH, and dissolved oxygen. In addition, the water depth at sampling point and observations of local conditions were noted. These Hydrolab measurements were taken at the surface at all water quality stations. In addition, these same parameters were measured at each fountain darter sample (drop net) location at the surface, mid-depth, and near the bottom when there was stratification.

In addition to hydrolab profiles, water "grab" samples were taken in each of the nine sites in Spring Lake and all sites in the San Marcos River to evaluate conventional water chemistry parameters. These samples were taken in 1-liter polyethylene bottles with caps and analyzed in the laboratory. Prior to sample collection, the bottles were soaked in Contrad 70 overnight, rinsed repeatedly in DI water, and rinsed once in Milli-Q water before being dried for 24 hours. At the sampling site, each bottle was rinsed with river water prior to sample collection; all samples were collected from under the surface of the water to avoid surface-active particulates and floating debris. Samples were then stored in the dark, under ice, for the remainder of the collection period. Samples were transported to the laboratory within 4-6 hours and warmed to room temperature, at which point the samples were partitioned into fractions for the following analyses. Whole water samples were also frozen for a few weeks prior to some analyses; once frozen the samples are stable for many months. Table 1 summarizes the parameters, methodology, minimum analytical levels (MAL) and minimum detection limits (MDL) of the data gathered from this chemistry analysis.

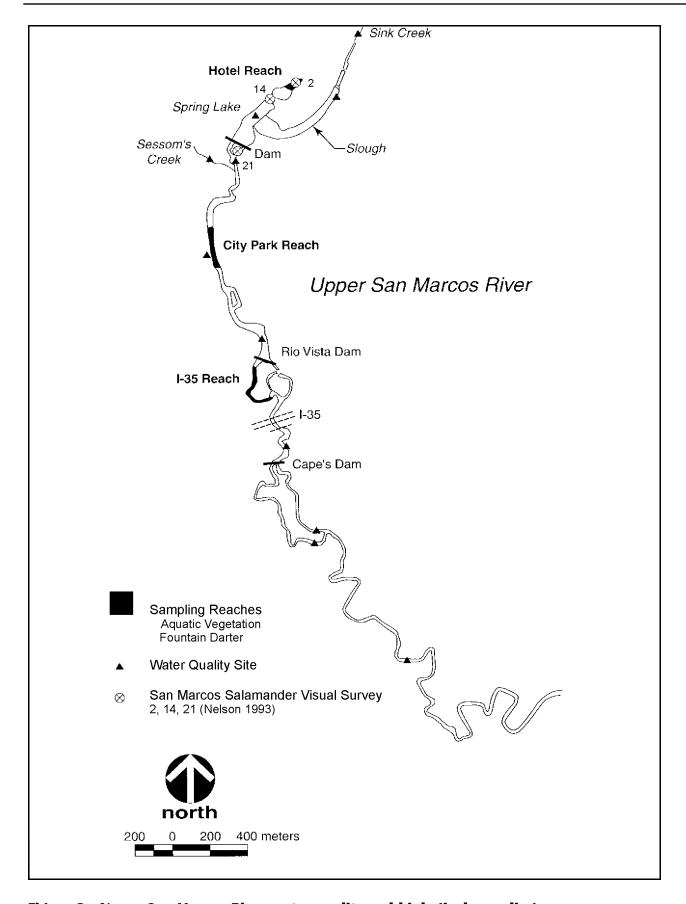


Figure 2. Upper San Marcos River water quality and biological sampling areas.

Table 1. Parameters, analytical methodology, minimum analytical levels, and minimum detection limits for water chemistry analyses conducted on water quality grab samples.

PARAMETER	METHOD	MINIMUM ANALYTICAL LEVELS (per liter)	MINIMUM DETECTION LIMITS (per liter)
Nitrate Nitrogen	UV Spectroscopy	≈10.0 µgª	≈3.0 µg
Total Nitrogen	UV Spectroscopy	10.0 μg	<5.0 μg
Ammonium	Fluorometric	7 μg	2 μg
Soluble Reactive Phosphorous	Spectroscopy	3 µg	0.5 μg
Total Phosphorous	Spectroscopy	5 μg	3 µg
Alkalinity	Potentiometric	Appropriate	
Total Suspended Solids	Gravimetric	Appropriate	

^a micrograms.

Alkalinity, Turbidity, and Total Suspended Solids (TSS): All samples were immediately titrated to determine alkalinity, then sampled for nephelometric turbidity units and filtered onto prewashed, preweighed filters for determination of total suspended solids (TSS). Determination of TSS followed the methodology outlined in Standard Methods for the Examination of Water and Wastewater (APHA 1992).

Soluble Reactive Phosphorus (SRP): Soluble reactive phosphorus (SRP) and nitrate were usually analyzed within 48 hours. SRP was measured following Strickland and Parsons (1972) in which the sample is filtered with a 0.45 micrometer (μm) pore size filter and allowed to react with a composite reagent containing molybdic acid (ammonium molybdate and sulfuric acid), ascorbic acid, and potassium antimonyl tartrate. The resulting complex heterpolic acid is reduced in situ to give a molybdenum blue solution, the extinction of which is measured at 885 nanometers (nm) and plotted on a standard curve.

Nitrate: Nitrate analysis was conducted via the method described by Crumpton et al. (1992), in which the nitrate concentration is determined by using ultraviolet (UV) spectroscopy to measure the absorbance at 224-228 nm and a second derivative is calculated for that value. This derivative is linear to the concentration of nitrate ion in natural waters, assuming that the samples are reasonably clear. The second derivative function is calculated using a software package designed by Dr. Groeger.

Total Nitrogen (TN) and Total Phosphorus (TP): Total nitrogen was analyzed using the same process as nitrate following a persulfate digestion and autoclave heating period of 30 minutes at 121° C and 15 pounds per square inch (PSI). The TP analysis was similar to the method for soluble reactive phosphorus (APHA 1992). The sample was first digested by the persulfate oxidation technique and then subjected to the ascorbic acid method for determination of the TP content.

Ammonium: The ammonium concentration was determined following the outline of Holmes et al. (1999). The method uses fluorescence of the sample minus background fluorescence and matrix effects against a standard curve. Protocol B was followed for systems with ammonium concentrations generally exceeding 0.5 micromole per liter (µmol/L). The method uses a fluorometer equipped with a Turner designs optical kit 10-AU, near UV mercury vapor lamp, a 350 nm interference excitation filter with a 25 nm bandpass, a 410-600 nm combination emission filter, and a 1:75 attenuator plate.

In addition to the water quality collection effort, habitat evaluations were conducted using fixed station photography. Fixed station photographs allowed for temporal habitat evaluations and included an upstream, a cross-stream, and a downstream picture taken at each water quality site depicted on Figure 2.

Aquatic Vegetation Mapping

Vegetation mapping was conducted in all three San Marcos Springs/River sample reaches (Spring Lake Dam, City Park, and IH-35) during Comprehensive and Critical Period sampling events. These maps were created using a Trimble Pro-XRS global positioning system (GPS) unit with real-time differential correction capable of sub-meter accuracy. The GPS unit was linked to a Fujitsu Stylistic 2300 laptop computer with Aspen software that displays field data as it is gathered and improves efficiency and accuracy. The GPS unit and computer were placed in a 3-meter (m) Perception Swifty kayak with the GPS unit antenna mounted on the bow. The aquatic vegetation was identified and mapped by gathering coordinates while maneuvering the kayak around the perimeter of each vegetation type at the water's surface. Vegetation stands less than 0.5 m in diameter were not mapped.

In addition to mapping all of the vegetation found within the three study reaches, all Texas wild-rice in the entire San Marcos River was mapped annually during the summer Comprehensive sampling effort. Each Texas wild-rice plant was mapped using the same mapping methodology except that plants smaller than 0.5 m in diameter were mapped as a single point.

Texas Wild-Rice Physical Observations

Surveys were conducted in the upper reach of the San Marcos River during each Comprehensive and Critical Period sampling effort to identify, map, and record any stands of Texas wild-rice that were considered to be in vulnerable areas at the beginning of the study. Texas wild-rice stands were considered to be in vulnerable areas if they possessed one or more of the following characteristics: (1) occurred in shallow water, (2) revealed extreme root exposure because of substrate scouring, or (3) generally appeared to be in poor condition. For this study a stand of Texas wild-rice is defined as a contiguous group of plants that are growing no closer than 45 centimeter (cm) from any other stand(s) of Texas wild-rice. These monitoring efforts were designed following discussions with Dr. Robert Doyle, currently with Baylor University, and Ms. Paula Power, formerly with the USFWS National Fish Hatchery and Technology Center, San Marcos.

After an evaluation of the general condition of all stands of Texas wild-rice along the San Marcos River from Spring Lake Dam to the confluence with the Blanco River in September 2000, 19 representative stands were selected for study. These included eight stands in the Sewell Park Reach, eight stands in the

reach from Rio Vista Dam to IH-35, and three stands between Cape's Dam and the City of San Marcos sewage treatment facility (one of the latter stands was lost in 2001).

The aerial coverage of most vulnerable Texas wild-rice stands were determined by GPS mapping (described above), but some smaller stands were measured using maximum length and maximum width. The length measurement was taken at the water surface parallel to streamflow and included the distance between the base of the roots to the tip of the longest leaf. The width was measured at the widest point perpendicular to the stream current (this usually did not include roots). The length and width measurements were used to calculate the area of each stand according to a method used by the TPWD (J. Poole, TPWD, pers. comm.) in which percent cover was estimated for the imaginary rectangle created from the maximum length and maximum width measurements.

Qualitative observations were also made on the condition of each Texas wild-rice stand. These qualitative measurements included the following categories: the percent of the stand that was emergent (and how much of that was in seed), the percent covered with vegetation mats or algae buildup, any evidence of foliage predation, and a categorical estimation of root exposure. Notes were also made regarding the observed (or presumed) impacts of recreational activities. Each category was assigned a number from 1 to 10 for each stand, with 10 representing the most significant impact.

Flow measurements were taken at the upstream edge of each vulnerable Texas wild-rice stand and depth was measured at the shallowest point in the stand. A cross-section of the river was taken along the shallowest depth in which flow, depth, and substrate composition were measured at 1-m intervals across the entire width of the river. To complement all of the measurements made during each survey, video images were taken during several Comprehensive and Critical Period samples using an underwater video camera.

Fountain Darter Sampling

Drop Net Sampling

A drop net is a sampling device previously used by the USFWS to sample fountain darter and other fish species in the Comal and San Marcos Springs/River ecosystems. The design of the net is such that it encloses a known area (2 square meters [m²]) and allows a thorough sample by preventing escape of fishes occupying that area. A large dip net (1 m²) is used within the drop net and is swept along the length of the river substrate 15 times to ensure complete enumeration of all fish trapped within the net. For sampling during this study, drop net locations were selected using aquatic vegetation maps (with a grid overlay) to randomly select sites that were stratified by dominant aquatic vegetation type. The dominant vegetation types used in each reach were defined at the beginning of the study, but modified with dramatic changes in vegetation composition within a site.

At each location the vegetation type, height, and areal coverage were recorded, along with substrate type, mean column velocity, velocity at 15 centimeters (cm) above the bottom, water temperature, conductivity, pH, and dissolved oxygen. In addition, vegetation type, height, and areal coverage, along with substrate type, were noted for all adjacent 3-m cell areas. Fountain darters were identified, enumerated, measured for standard length, and returned to the river at the point of collection. The same measurements were taken for all other fish species, except for abundant species where only the first 25 individuals were measured; a total count was recorded for a drop net sample beyond the first 25

individuals in such instances. Fish species not readily identifiable in the field were preserved for identification in the laboratory. All live giant ramshorn snails were counted, measured, and destroyed, while a categorical abundance was recorded (i.e., none, slight, moderate, or heavy) for the exotic Asian snails (*Melanoides tuberculata* and *Thiara granifera*) and the Asian clam (*Corbicula* sp.). A total count of crayfish (*Procambarus* sp.) and grass shrimp (*Palaemonetes* sp.) was also recorded for each dip net sweep.

Drop Net Data Analysis

The fisheries data collected with drop nets were analyzed in several ways. Calculations of fountain darter density in the various vegetation types during 2000-2004 provide valuable data on species/habitat relationships. These average density values were also used with aquatic vegetation mapping data on total coverage of each vegetation type by sampling effort to create estimates of the population abundance in each reach (fountain darter density within a vegetation type x total coverage of that vegetation type in the given reach). Because there were generally only two drop net samples in each vegetation type within each reach, density estimates between sampling efforts had great variation and population estimates based on those densities would be greatly influenced by this variation. Part of the variation would be due to changes in environmental conditions (discharge, temperature, etc.) that had occurred since the last sample, but part would be natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints) it is impossible to tell how much of the variation is attributed to each source within a given sampling effort. Using the average density of fountain darters across all samples for a given vegetation type does not account for changes in density across samples (differences associated with changes in environmental conditions), but the increased sample size substantially reduces the high natural variability. This type of comparison between samples, where density values are held constant across all samples, is based entirely upon changes in vegetation composition and abundance between sampling efforts. A more complex assessment of the influence of environmental conditions on the fountain darter population was incorporated into the statistical model described below. Because these estimates use static estimates of density and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Although vegetation composition is a primary factor in determining fountain darter population abundance, there are many other factors involved, some of which may become increasingly important during low-discharge conditions. In order to assess the relationship between fountain darter abundance and multiple parameters related to their habitat conditions, a statistical model was developed for analysis of the drop net data collected throughout the study. The model was a generalized linear regression model that incorporated multiple parameters for simultaneous evaluation of the relative contribution of each to fountain darter abundance. The model was run using the density of darters captured in each drop net sample (total darters captured / available habitat) as the dependent variable. A square-root transformation was used on the density data to create a linear relationship with most independent variables. Independent variables included dominant vegetation types, season, total discharge in the San Marcos River, depth, flow and standard water quality parameters in the immediate vicinity of the site. In addition the mean and median length of darters collected from each reach were compared over time in the model to determine whether changes in discharge and/or seasonal patterns could be observed in the darter population.

In addition to the detailed analysis of fountain darter data, all other fisheries data collected during drop netting were summarized by total number of each species collected during 2000-2004. These data were used to characterize the community in each reach during each sampling effort using an index of species diversity. Species diversity, which is often related to the ecological stability and environmental "quality" of the community was estimated using the "Shannon-Weaver" index (Margalef 1956). Using this index to evaluate the data provided a meaningful summarization of the fisheries community that could be evaluated over the study period to evaluate trends.

Dip Net Sampling

In addition to drop net sampling for fountain darters, a dip net of approximately 40 cm x 40 cm (1.6-millimeter [mm] mesh) was used to sample all habitat types within each reach. Collecting was generally done while moving upstream through a reach. An attempt was made to sample all habitat types within each reach. Habitats thought to contain fountain darters, such as along the edge of, or within, clumps of certain types of aquatic vegetation, were targeted and received the most effort. Areas deeper than 1.4 m were not sampled. Fountain darters collected by this means were identified, measured, recorded as number per dip net sweep, and returned to the river at the point of collection (except for those retained for refugia purposes under the guidance of Dr. Thomas Brandt, USFWS National Fish Hatchery and Technology Center). The presence of native and exotic snails was also recorded per sweep.

To balance the effort expended across sampling events, a predetermined time constraint was used for each reach (Hotel Reach -0.5 hour, City Park Reach -1.0 hour, IH-35 Reach -1.0 hour). The areas of fountain darter collection were marked on a base map of the reach. Though information relating the number of fountain darters by vegetation type was not gathered by this method (as in the drop net sampling), it did permit a more thorough exploration of various habitats within the reach (particularly shallow shoreline habitat) and often resulted in greater numbers of small fountain darters than drop net sampling in nearby areas. This sample method also permitted comparisons within a site among sampling events.

Dip Net Data Analysis

Dip net data were used to identify periods of fountain darter reproductive activity since this method was more likely to sample small fountain darters (<15 mm) along shoreline habitats. This size-class is indicative of recent reproduction since fountain darters of this size should be <60 days old (Brandt et al. 1993). The dip net data were also useful for identifying trends in edge habitat use by fountain darters since this method focused on that habitat type. In some instances, changes that were observed in fountain darter distribution and abundance in the main channel were not observed in the edge habitat. In that way, the dip net data provided a valuable second method of sampling fountain darters in the same sample reaches as drop netting, that allowed a more complete characterization of fountain darter dynamics in a sample reach. The dip net data were analyzed by visually evaluating graphs of length-frequency distribution for each sample reach.

San Marcos Salamander Visual Observations

Visual observations were made in areas described by Nelson (1993) as habitat for San Marcos salamanders. All surveys were conducted in the headwaters of the San Marcos River and included two areas in Spring Lake and one area below Spring Lake Dam adjacent to the Clear Springs Apartments.

The upstream-most area in the lake was adjacent to the old hotel (known as the Hotel Reach) and was identified as site 2 by Nelson (1993). The other site in Spring Lake was deeper (~6 m) and located directly across from the Aquarena Springs boat dock. This site was identified as site 14 by Nelson (1993). The final sampling area was located just below Spring Lake Dam in the eastern spillway (site 21, Nelson 1993) and was subdivided into four smaller areas for a greater coverage of suitable habitat. San Marcos salamander densities in the four subdivisions below Spring Lake Dam were averaged as one.

SCUBA gear was used to sample habitats in Spring Lake, while a mask and snorkel were used in the site below Spring Lake Dam. In each sample, an area of macrophyte-free rock was outlined using flagging tape, and three timed surveys (5 minutes each) were conducted by turning over rocks >5 cm wide and noting the number of San Marcos salamanders observed underneath. Following each timed search, the total number of rocks surveyed was noted in order to estimate the number of San Marcos salamanders per rock in the area searched. The three surveys were averaged to yield the number of San Marcos salamanders per rock.

The density of suitable-sized rocks at each sampling site was determined by using a square frame constructed out of steel rod to take random samples within the area. Three random samples were taken in each area by blindly throwing the 0.25 m² frame into the sampling area and counting the number of suitable-sized rocks. The three samples were then averaged to yield a density estimate of the rocks in the sampling area.

The area of each sampling area was determined after sampling by using two sets of rope connected 60 cm apart by steel rods. The rods were positioned along marks placed every 60 cm on each rope so that a grid with squares of 60 cm x 60 cm was created over the sampling area. To count the total number of squares in the sampling area, one rod and rope set was placed lengthwise across the sampling area while the other set was placed perpendicular to the first. While the first set of rods and rope remained stationary lengthwise, the second set was moved along the 60-cm intervals. For each placement of the rods and rope along a 60-cm interval, the number of complete squares created by the set of ropes perpendicular to the stationary reference was counted. In addition, a percentage of any incomplete squares was noted. This method effectively allowed for a grid of 60 cm x 60 cm squares to be established across the sampling site in order to determine the total area.

In addition to mapping the sampling areas with the grid system, a GPS with real-time differential correction was used to outline the sampling area and determine the surface area. This was accomplished by attaching the unit to a kayak and paddling/towing it around the flagged sampling area. A comparison of the results of the two methods revealed similar estimates, and the GPS system was adopted for shallower sites where it was more time efficient.

An important note about these San Marcos salamander density estimates is that extrapolating beyond the area sampled into surrounding habitats would not necessarily yield accurate values, particularly in the Hotel Reach. This is because the area sampled was selected based on the presence of silt-free rocks and relatively low algal coverage (compared to adjacent areas) during each survey. Much of the habitat surrounding the sampling areas is usually densely covered with algae and provides a three-dimensional habitat structure that may harbor a different population size. The estimates created from this work are valuable for comparing between trips, but any estimates of a total population size derived from this work should be viewed with caution.

Exotics/Predation Study

In 2000-2001 and during the first three sampling events of 2002 (two Comprehensive sampling events and the summer / high-flow sampling event) surveys of exotic fish species and predator species were conducted. This sampling component was not included in the final sample of 2002 or subsequent Comprehensive sampling efforts since enough data had been collected by that time to determine that few fountain darters or salamanders were being consumed by predators at moderate to high discharge conditions. Had additional low-flow Critical Period sampling events occurred, the exotic/predation sampling would have been included to determine whether impacts may be intensified during low flows.

This sampling was conducted with a 45.7-m (150-ft) experimental gill net with mesh sizes ranging from 1.9 to 7.6 cm (0.75 to 3.0 inches). The net was placed in Spring Lake, in the area previously identified as supporting fountain darters and San Marcos salamanders through SCUBA surveys. All fish collected in the gill net were identified, enumerated, weighed, and measured. The original intention was to retain a few representative individuals of each species within different size classes; however, sample sizes were smaller than anticipated so all fishes were used in the stomach analysis. Fishes collected in the field were stored on crushed ice until transferred to the Texas State University Aquatic Center or the BIO-WEST Nekton Laboratory where the stomachs were removed and contents examined. Although the focus was on fountain darter and/or San Marcos salamander predation by the various species and size classes, all stomach contents were recorded.

Because of the limited sample sizes obtained during Comprehensive sampling events, rod-and-reel sampling was also employed to target larger sunfish and small- to intermediate-sized bass, which are the most likely piscine predators on the fountain darter and San Marcos salamander. In addition, fish trapped in the gill net pose problems unique to that method of capture. Those fish are often partially decomposed if entangled soon after the net is placed; the fish have also been known to regurgitate food items upon entanglement and will continue to digest any remaining food items as long as they are trapped. As a result of incorporating rod-and-reel sampling, sample sizes were much larger and many of the problems with gill net sampling were avoided.

RESULTS

There were a total of 15 Comprehensive (seasonal) samples conducted in the San Marcos Springs/River ecosystem during 2000-20004. Two of those 15 samples also corresponded to high-flow Critical Period samples that occurred shortly after a flood event. One additional high-flow Critical Period sample was conducted in 2001. A high-flow Critical Period sample was triggered in fall 2004, but sampling was limited to a visual assessment and mapping of vulnerable stands of Texas wild-rice due to substantial high-flow data collection over the course of the study. A summary of all data collected from these events is presented below.

Springflow

Springflow in the San Marcos Springs/River ecosystem during 2000-2004 was generally much higher than average conditions (Table 2 and Figures 3-4). The project was initiated during a period of limited recharge during which total discharge in the San Marcos River was at or below 120cfs for 65 days and declined to a low of 108cfs on September 18-20, 2000. Conditions in the fall of 2000 through summer of 2001 were approximately normal based on conditions that have occurred over the period of record, but a substantial rain event in the fall of 2001 (24-hour mean discharge of 1,019cfs in the San Marcos River) resulted in a dramatic increase in aquifer levels (to record conditions). The aquifer levels have remained elevated throughout the remainder of the project. The flooding event in the summer of 2002 raised the aquifer level again and prevented discharge from declining significantly during the late summer and early fall, which has historically provided low recharge. In the first half of 2003, discharge levels in the San Marcos River remained high, but steadily decreased from the peak in July 2002. Discharge in the San Marcos River continued to decline (slightly) through March of 2004, but spring rains increased the aquifer and additional flood events in summer and late fall of 2004 increased the aquifer level again. The flood events in the summer of 2002 and both events in 2004 were particularly influential on aquifer levels and provided extended periods of higher-than-normal flows.

Table 2. Lowest discharge during each year of the study and the date on which it occurred.

Year	Discharge	Date
2000	108	Sept. 18
2001	167	Aug. 19
2002	157	Jun. 28
2003	156	Dec. 29
2004	146	Mar. 8

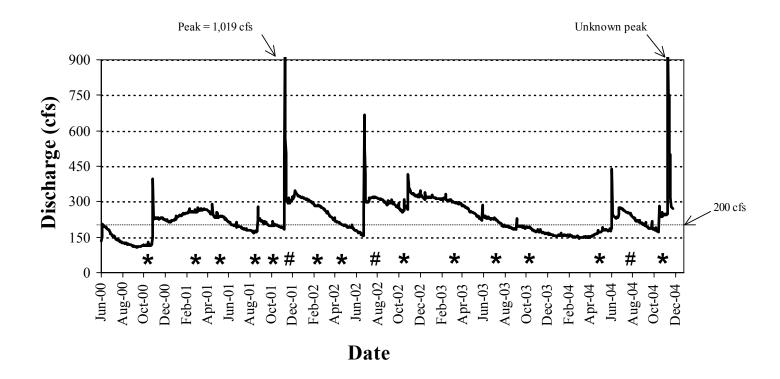


Figure 3. Mean daily discharge in the San Marcos River during the study period; approximate dates for Comprehensive (*) and high-flow Critical Period sampling efforts (#) are indicated.

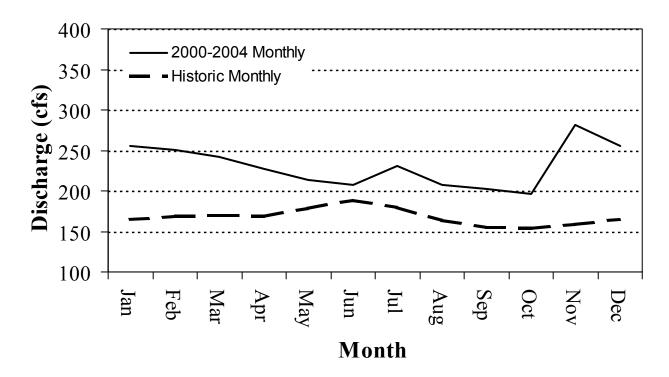


Figure 4. Mean monthly discharge in the San Marcos River during 2000-2004 and during the period of record (1956-2000).

Low-Flow Critical Period Sampling

No low-flow Critical Period sampling events occurred in the San Marcos Springs/River ecosystem in 2000-2004.

High-Flow Critical Period Sampling

Details of the high-flow Critical Period sampling events in 2001 and 2002 can be found in the respective annual reports (BIO-WEST 2002a, BIO-WEST 2003). Findings from those samples, and the 2004 high-flow conditions, are also incorporated into the Results section for each sampling component in this report.

In November 2004, a high-flow period resulted in some changes to Texas wild-rice coverage in some areas, but resulted in relatively minor changes in other areas. Flow overtopped the gage during this time and there are no readings available for the days between the 17th and 23rd. These discharge values resulted in scouring of vegetation in many areas. Although a full-scale sample event was not conducted, mapping of Texas wild-rice in vulnerable (=shallow) areas revealed that some areas were more susceptible than others. For instance, in Sewell Park (just downstream of the University Drive Bridge) there was a net loss of only 40.3 m² out of the 868.7 m² that was observed in the larger stand in the fall The smaller stand actually increased in size during that time. sample (October 13). downstream, in the IH-35 reach (downstream of the Cheatham Street Bridge) there was a dramatic reduction in total areal coverage from 146.9 m² in the fall sample to just 52.4 m² (just 40.5% of the previous total). There was also a dramatic reduction in the two "vulnerable" Texas wild-rice plants in the Thompson's Island Reach. In contrast there was just a slight reduction in the large stand near the IH-35 Bridge crossing and an increase in a smaller vulnerable stand located in the same area. Visual observations in the Spring Lake Dam Reach (upstream of the University Drive Bridge) suggested moderate reductions in total coverage there. Overall, these results suggest that there was a range of impacts on Texas wild-rice associated with high-flow conditions, but overall, there was a net decrease of areal coverage. It is likely that many plants downstream of the IH-35 Bridge were displaced since this has been observed in previous high-flow events during 2000-2003. Also, there seemed to be very few new plants observed during this event compared to previous events. It is anticipated that this event will encourage rapid re-growth of Texas wild-rice in scoured areas as has been observed following past highflow events.

WATER QUALITY

Spring Lake

The nine sample sites in Spring Lake were chosen based on historical locations that had been used during basic limnological sampling conducted at Texas State University (formerly Southwest Texas State University). The sites were as follows:

- Site A was located directly in front of the hotel on Spring Lake in a deep hole
- Site B was located in front of the "submarine" area
- Site C was located across from "The Landing"
- Site D was just upstream from the chute at Joe's Crab Shack
- Site E was located just upstream of the dam

- Site F was chosen to represent the mixing of the slough and spring arms
- Site G was located behind the softball fields and under a powerline in the slough,
- Site H was located downstream of the road crossing
- Site S was in Sink Creek

These water quality sampling sites can be grouped into Spring Arm, Slough Arm, and Sink Creek sites. Spring Arm sites include A through E. Site A was closest to the spring and E was closest to the dam. Slough Arm sites include F through H. Site F was closest to the dam while H was closest to the Sink Creek. Site S was located in Sink Creek, which can go dry during the late summer months.

Standard physico-chemical water quality parameters (Table 3) and conventional water chemistry parameters (Table 4) were collected in each of these sites in 2000-2002. As described above, these measurements were conducted in 2000-2002; since then no low-flow Critical Period events have occurred to trigger a water quality sampling effort. A detailed assessment of these data are presented in the 2002 annual report (BIO-WEST 2003) and summarized below.

Table 3. Summary of Spring Lake physico-chemical water quality measurements, 2000 to 2002.

SITE	TEA		рН		DISSOLVED OXYGEN (mg/L) ²			CONDUCTIVITY (mmhos/cm) ^b			TURBIDITY (NTU) ^c			ALKALINITY (meq/L) ^a				
	Mean Min Max		Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
А	21.71	21.46	22.36	7.07	6.80	7.26	5.45	4.50	6.80	565	530	596	1.2	0.9	1.4	4.72	3.99	5.26
В	21.93	21.52	22.44	7.10	6.83	7.26	6.05	4.74	8.15	560	524	584	1.1	0.9	1.4	4.72	3.99	5.22
C	22.16	21.85	22.73	7.09	6.81	7.28	6.36	5.66	7.34	560	527	581	1.2	1.0	1.6	4.92	4.17	5.66
D	22.51	21.78	23.96	7.13	6.83	7.33	8.19	6.39	12.01	561	527	582	1.2	0.9	1.6	4.92	4.05	5.99
Ε	22.54	21.92	23.11	7.20	6.87	7.56	8.36	6.68	9.96	561	526	583	1.2	1.0	1.4	4.87	3.39	6.32
F	24.04	22.12	26.89	7.32	7.12	7.61	9.16	2.86	13.80	552	507	600	1.5	0.9	1.9	5.01	4.12	6.98
G	23.26	18.45	26.35	7.46	7.18	8.00	7.86	4.44	11.99	550	489	607	1.7	1.1	2.2	4.84	3.34	7.31
Н	22.78	16.68	28.73	7.55	7.11	8.25	9.39	4.22	18.72	568	519	615	1.9	1.4	2.6	4.98	4.00	7.64
S	21.11	15.60	23.59	7.45	6.84	7.83	6.53	2.30	15.05	637	564	773	3.8	1.7	7.7	5.54	4.62	6.96

a milligrams per liter; b micromhos per centimeter; c nephelometric turbidity units; d milliequivalents per liter.

Table 4. Summary of Spring Lake water chemistry measurements, 2000 to 2002.

SITE	SOLUBLE REACTIVE PHOSPHORUS (ugP/I) ²			TOTAL PHOSPHORUS (ug/l) [»]			AMMONIUM (ug/L)			NITRATE (mg/L) ^c			TOTAL NITROGEN (mg/L)			TOTAL SUSPENDED SOLIDS (g/L) ^d		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Α	14.93	2.72	54.25	19.01	2.66	36.96	39.35	4.00	139.00	1.43	0.93	2.62	1.62	0.83	2.46	0.055	0.000	0.427
В	17.58	2.21	45.54	44.37	9.13	171.97	33.97	8.00	97.76	1.36	0.93	1.61	1.71	0.96	3.13	0.046	0.000	0.343
C	13.18	2.90	36.83	17.15	6.10	29.55	20.26	1.00	61.44	1.55	1.23	1.81	1.94	1.27	3.07	0.026	0.000	0.200
D	14.97	3.58	48.33	27.25	9.21	76.10	7.93	1.00	25.12	1.63	1.12	1.93	2.06	1.36	3.90	0.047	0.001	0.343
E	13.84	1.70	49.89	37.89	8.17	155.41	21.90	2.00	95.49	1.69	1.29	2.29	2.00	1.12	3.28	0.016	0.000	0.104
F	17.24	2.55	40.32	23.31	6.79	39.56	48.80	2.30	166.00	0.96	0.25	1.43	1.21	0.05	1.81	0.011	0.001	0.068
G	22.55	0.17	48.53	49.96	9.99	117.96	48.50	3.90	102.00	0.74	0.27	1.17	1.00	0.05	1.60	0.047	0.002	0.324
Н	17.80	1.19	39.45	24.48	6.10	56.50	41.00	3.20	116.00	0.65	0.15	1.25	0.99	0.03	1.69	0.090	0.003	0.295
S	35.49	11.58	88.71	70.29	29.26	129.86	48.33	27.00	68.00	0.40	0.05	1.40	1.10	0.10	1.89	0.199	0.004	0.410

a micrograms of phosphorus per liter; b micrograms per liter; c milligrams per liter; d grams per liter.

There does not appear to be much influence on water quality from surface water inflow to the Spring Arm of the lake, but influences from the immediate watershed may impact Sink Creek and the Slough Arm of the lake.

Dissolved oxygen concentrations recorded at sites A and B (nearest the springs) and at the Slough Arm sites did not always meet the high water quality standard of 6.0 milligrams per liter (mg/l) for dissolved oxygen for the Upper San Marcos River Segment No. 1814 (BIO-WEST 2003, Appendix B). The lowest mean value occurred in Site A, which is most directly influenced by springflow. Dissolved oxygen concentration varied the most in the Slough Arm sites of Spring Lake where the highest and lowest values were recorded.

Total suspended solids values were low at all sites in Spring Lake. The highest values for sites A through H were recorded during the August 2002 sampling event. During the 2001 sampling events, Sink Creek had the highest TSS values, which was probably due to the extremely high plant and algae growth that occurs in this creek. Conductivity did not vary substantially among sites within the lake but values were much higher in Sink Creek. During the August 2002 sampling event, TDS values at each of the Slough Arm sites approached or met the water quality standard value of 400 milligrams per liter (mg/l).

Soluble Reactive Phosphorus concentrations and TP concentrations in Spring Lake were well below the Texas Commission on Environmental Quality (TCEQ; formerly TNRCC)'s screening values of 0.1 and 0.2, respectively. The SRP and TP values fluctuated from season to season and site to site throughout the 2-year sampling period. For example, the lowest SRP concentrations were recorded in October 2001 and the highest concentrations were recorded in October 2000. The Slough Arm sites and Sink Creek generally had higher concentrations of SRP than the Spring Arm sites (BIO-WEST 2003, Appendix B).

Nitrate values exceeded the water quality standards screening level of 1.0 mg/l in most cases, whereas ammonium values were well below the screening level of 1.0 mg/l (BIO-WEST 2003, Appendix B). Similarly, TN concentrations in Spring Lake consisted of a very high percentage of nitrate but a very low percentage of ammonium. Nitrate values at the Spring Arm sites were fairly constant among sites and throughout the year (BIO-WEST 2003, Appendix B); nitrate concentrations in the Slough Arm sites and Sink Creek were much lower than in the Spring Arm sites for most sampling events and fluctuated more throughout the year. These lower concentrations may be due to uptake of nitrate by the abundant plants and algae in the Slough Arm and Sink Creek.

Temperatures recorded for the quarterly sampling events on Spring Lake did not show the same extreme low and high values as the continuous data collected with thermistors (BIO-WEST 2003, Appendix B). Temperatures collected by Hydrolab in Sites A through E were very similar, while temperatures in the Slough Arm sites generally had higher mean and maximum values and lower minimum values than the Spring Arm sites (BIO-WEST 2003, Appendix B). A thermistor located in the Slough Arm of the lake fluctuated between 10°C and 30°C between October 2000 and October 2002, while one in the Spring Arm consistently stayed near 22°C.

San Marcos River

The nine sample sites in the San Marcos River in 2000-2002 were chosen based on historical locations that had been used during basic limnological sampling conducted at Texas State University. The sites were as follows:

- Chute (CS) was located directly downstream of the chute at Joe's Crab Shack
- Dam (DS) was located just downstream of the true dam on Spring Lake
- Sessom's Creek (SC) was at Aquatic Biology building, before confluence with San Marcos River
- City Park (CP) was located within the City Park/Lions Club Reach
- Rio Vista Park (RVP) was located near the far channel at Rio Vista
- IH-35 (IH35) was located just upstream of the IH-35 highway crossing
- Thompson's Island artificial (TIA) was located upstream of the falls on artificial channel
- Thompson's Island natural (TIN) was located upstream of state fish hatchery outflow
- Animal Shelter (AS) was located directly behind the San Marcos animal shelter

Standard physico-chemical water quality parameters (Table 5) and conventional water chemistry parameters (Table 6) were collected in each of these sites in 2000-2002. As described above, these measurements were conducted in 2000-2002; since then no low-flow Critical Period events have occurred to trigger a water quality sampling effort. A detailed assessment of these data are presented in the 2002 annual report (BIO-WEST 2003) and summarized below.

Table 5. Summary of physico-chemical water quality measurements taken on the San Marcos River, 2000 to 2002.

SITE	TEMPERATURE (Celsius)			рН			DISSOLVED OXYGEN (mg/L) ^a			CONDUCTIVITY (µmhos/cm)			TURBIDITY (NTU) ^c			ALKALINITY (meq/L) ^a		
	Mean	Min	Max	Mean	Min	Мах	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Мах
AS	22.15	20.24	22.80	7.59	7.36	7.88	9.05	7.75	10.39	570	531	593	2.70	1.70	4.60	4.61	1.70	4.60
TIA	22.09	20.09	22.81	7.57	7.27	7.91	8.82	6.63	11.47	571	537	596	2.33	1.20	3.80	4.70	1.20	3.80
TIN	22.31	20.60	22.97	7.67	7.37	7.89	9.15	7.99	9.83	572	537	596	2.23	1.50	3.70	4.78	1.50	3.70
IH35	22.55	21.04	23.24	7.57	7.26	7.78	9.59	8.21	10.88	571	537	596	1.75	1.30	2.30	4.81	1.30	2.30
RVP	22.55	21.66	23.28	7.51	7.29	7.62	10.28	8.81	12.32	572	537	596	1.71	1.30	2.20	4.78	1.30	2.20
CP	22.67	21.84	23.28	7.43	7.17	7.66	9.28	8.25	11.60	572	538	595	1.49	1.10	2.00	4.83	1.10	2.00
SC	22.75	21.78	23.70	7.42	7.22	7.53	7.48	6.77	8.63	593	553	611	1.61	0.90	2.20	4.70	0.90	2.20
DS	22.66	22.23	23.34	7.43	7.29	7.52	8.47	7.20	10.91	572	537	598	1.19	0.90	1.40	4.72	0.90	1.40
CS	22.59	22.30	23.01	7.35	7.04	7.47	8.60	7.75	10.61	572	538	596	2.28	1.00	8.20	4.82	1.00	8.20

a milligrams per liter; b micromhos per centimeter; c nephelometric turbidity units; d milliequivalents per liter.

Table 6. Summary of water chemistry measurements taken on the San Marcos River, 2000 to 2002.

SITE	SOLUBLE REACTIVE PHOSPHORUS (µgP/L) ²			EACTIVE PHOSPHORUS (110/L)					AMMONIUM (µg/L)			NITRATE (mg/L) ^c			L EN)	TOTAL SUSPENDED SOLIDS (g/L) ⁴		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Мах	Mean	Min	Мах	Mean	Min	Max
AS	10.55	1.57	30.83	18.00	1.57	33.99	21.01	0.06	56.90	1.27	1.01	1.65	1.51	1.10	1.96	0.021	0.002	0.121
TIA	11.72	3.83	49.46	16.02	3.83	52.49	35.16	0.07	89.00	1.34	0.67	1.89	1.55	0.99	2.35	0.016	0.004	0.090
TIN	6.18	1.74	16.89	12.66	1.74	18.52	32.21	0.04	81.00	1.30	1.05	1.62	1.54	1.35	1.71	0.022	0.004	0.136
IH35	6.86	1.22	19.85	14.43	1.22	31.62	28.07	0.03	127.27	1.40	1.12	1.79	1.57	1.12	2.26	0.015	0.002	0.091
RVP	5.86	0.70	16.54	12.83	0.70	18.36	46.94	0.00	127.27	1.49	1.22	2.12	1.91	1.41	2.82	0.010	0.001	0.059
CP	10.01	4.96	21.07	20.13	4.96	29.90	36.74	0.02	115.92	1.41	1.12	1.68	1.62	1.30	1.90	0.015	0.002	0.094
SC	7.10	4.23	11.49	16.12	4.23	21.77	14.73	0.02	104.57	1.63	1.26	2.26	1.76	1.09	2.71	0.004	0.002	0.015
DS	7.69	4.09	15.67	12.02	4.09	19.58	50.13	0.02	118.00	1.41	1.17	1.76	1.75	1.40	2.33	0.011	0.001	0.064
CS	7.41	4.65	11.15	13.23	4.65	32.24	15.49	0.03	43.28	1.33	0.51	1.90	1.54	0.80	2.20	0.012	0.000	0.080

a micrograms of phosphorus per liter; b micrograms per liter; c milligrams per liter; d grams per liter.

There was no distinct upstream-to-downstream pattern in water quality values observed in the San Marcos River during 2000-2002. Values of most parameters remained fairly constant throughout the system or fluctuated only minimally from site to site. Even water temperature was very consistent among sites although water temperatures tended to decrease more in the winter in Sessom's Creek and at the sites furthest downstream of the springs. Winter minimum temperatures occasionally dropped as low as 14° C in these sites; the most significant of these water temperature decreases coincided with cold rainfall events (Appendix B). The only two sites that differed from the others were the two immediately downstream of Spring Lake, which had much narrower temperature ranges. temperatures fluctuated by less than 2°C daily and by approximately 1°C seasonally in these two sites, which were located in the eastern (chute) and western (dam) spillways (Figure 5). Further downstream in the City Park and IH-35 reaches (Figure 6) temperatures varied by greater than 2°C daily and approximately 2.5°C seasonally and the Thompson's Island and Animal Shelter (upstream of wastewater treatment outfall) sites varied seasonally by only slightly more (Figure 7). The maximum deviation occurred in the City Park site and was approximately 7°C from the average value in the late summer (observed in 2001) however, these data are suspect since no other site had similar readings. The only instances of exceeding the water quality standards value (26.67°C) occurred in the City Park site for several days in August 2001 and in Sessom's Creek, which reached a maximum temperature of 26.68°C on two occasions (also in summer 2001). On two days in August of 2001, water temperatures were greater than 29°C in the City Park reach; the lethal limit for the fountain darter is 34.8°C (Brandt et al. 1993). Higher flow conditions in 2003-2004 resulted in lower fluctuation in water temperature. To compare water temperatures with air temperatures and precipitation, see Appendix B.

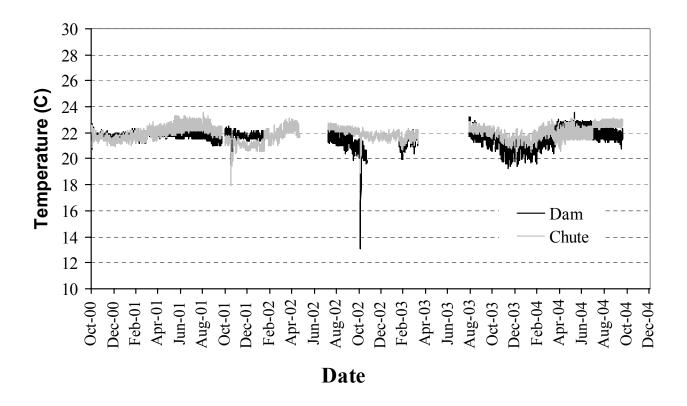


Figure 5. Thermistor data from the dam and chute tailrace areas below Spring Lake.

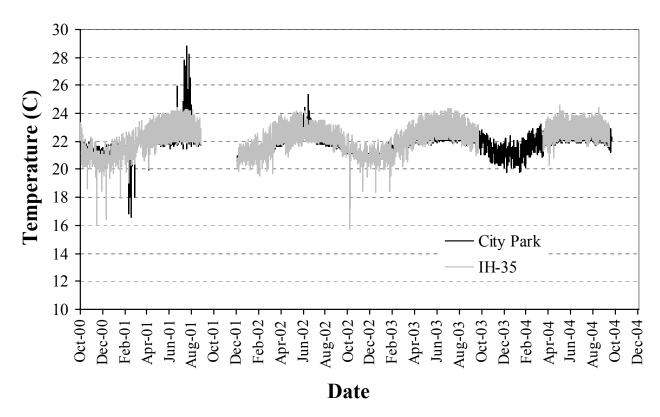


Figure 6. Thermistor data from the City Park and IH-35 Reaches.

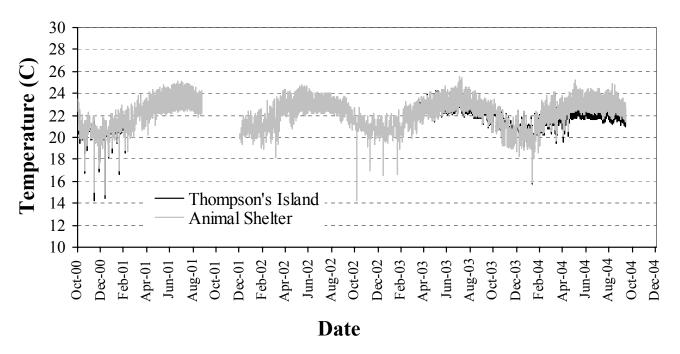


Figure 7. Thermistor data from the Thompson's island artificial canal and animal shelter sites.

Among other water quality parameters, dissolved oxygen concentrations met the high water quality standard of 6.0 mg/l for dissolved oxygen in all samples from the San Marcos River (BIO-WEST 2003, Appendix B). Dissolved oxygen values were, on average, higher in the San Marcos River than in the Comal River. Measurements of pH and specific conductivity did not vary appreciably among sites within the San Marcos Springs/River ecosystem within this study and were always well below limits at which biological impacts may occur. Turbidity and TSS values were also low at all sites during each sampling event. The highest turbidity value of 8.2 nephelometric turbidity units (NTU) was recorded in August 2001 at the Chute site on the San Marcos River below Spring Lake when construction was occurring on Spring Lake Dam. Higher turbidity values of 3.7 to 4.6 NTU were recorded at the downstream-most sites in October 2000. The SRP concentrations and TP concentrations on the San Marcos River were well below the TCEQ's screening values of 0.1 mg/l and 0.2 mg/l respectively (BIO-WEST 2003, Appendix B).

Total dissolved solids (TDS) is one parameter that may be important to monitor during low-discharge conditions. TDS was measured using a conductivity-to-TDS conversion of 0.65 for a comparison with the TDS standards for the San Marcos Springs/River ecosystem. As in the Comal River, the TDS values at each San Marcos River sampling site during the August 2001 sampling event exceeded the water quality standard values of 400 mg/l. The high TDS values recorded in August 2001 may be related to relatively low-flow conditions in the river. The TDS value will need to be monitored closely during future low-flow sampling efforts. If future monitoring reveals additional exceedences, the TCEQ will need to investigate the appropriateness of the water quality standard for this river segment. No previous mention of exceedences has been indicated by the TCEQ, which suggests that this water quality parameter is not a concern.

Nitrate values exceed the water quality standards screening level of 1.0 mg/l in most cases, whereas ammonium values are well below the screening level of 1.0 mg/l (Table 6). Nitrate values in the San

Marcos River are fairly constant throughout the river and throughout the year (BIO-WEST 2003, Appendix B). In contrast, ammonium concentrations vary throughout the sampling period and among sites and the values were very low. The TN values for the San Marcos River are influenced by the high nitrate concentrations. Because the median concentration of nitrate in the Edward's Aquifer ranges from 1.4 to 1.7 mg/l (Bush et al. 1998), it is spring flow and not anthropogenic inputs to the immediate surface waters that affected this parameter.

Hydrolab measurements of water quality at each drop net sample site generated a large dataset across a range of sites in the San Marcos Springs/River ecosystem. There were 240 sites in the San Marcos Springs/River ecosystem during 2000-2004 and the vast majority of the data suggested superior water quality conditions. Only two point measurements of dissolved oxygen were below the water quality standard of 6.0 milligrams per liter (mg/l) during 2000-2004. Dissolved oxygen concentrations were similar between the two San Marcos Springs/River sample reaches with average readings of 8.5 to 9.3mg/l between 2000-2004. Water temperature measurements at drop net sites were also similar among reaches and specific conductivity and pH measurements remained within very narrow ranges at all drop net sample locations.

Aquatic Vegetation Mapping

Maps of the aquatic vegetation observed during each 2004 sample effort can be found in the Appendix A map pockets; maps from previous years were included with the respective annual reports. The maps are organized by individual reach with successive sampling trips ordered chronologically. It is difficult to make sweeping generalizations about seasonal and other trip-to-trip characteristics since most changes occurred in fine detail; however, some of the more interesting observations from 2000-2004 are described below. The relationship between the vegetation abundance and fountain darter abundance is explored in the Discussion section of this report.

Spring Lake Dam Reach

The reach between Spring Lake Dam and the University Drive Bridge was added to those mapped for aquatic vegetation in 2002 and was mapped in all subsequent sample efforts. The reach includes a range of vegetation types, but it is dominated by *Hydrilla*, *Potamogeton*, and a mix of the two species. Since this reach was first mapped, Texas wild-rice has also become a dominant species. The western spillway of Spring Lake Dam is deep (~15 ft) and the turbulence of the spill limits suitable conditions for vegetation growth. The eastern spillway of the dam is shallow, largely shaded, and in places has very swift currents; these conditions support patchy vegetation coverage. The large area in the middle of the reach (downstream of the confluence of the two spillways) contains a dense coverage of *Potamogeton* with a *Potamogeton/Hydrilla* mix in some areas. Sessom's Creek enters the river on the western shore, and little vegetation persists in this area because of the tendency of the creek to scour the area following even moderate rain events and the development and movement of a large gravel bar.

The rapid increase in Texas wild-rice that occurred in 2002 in this reach resulted in a substantial area of total coverage for that species in 2003 and 2004. Throughout that time period, the area continued to have frequent occurrence of newly established plants and rapid growth of existing plants. The total coverage was reduced during high-flow events, but over the 2002-2004 period the coverage of Texas wild-rice in this reach increased from just over 100 m² to nearly 275 m². One of the most impacted areas was the channel between the western spillway and the main river channel where Texas wild-rice

has become dominate despite having only a few small plants prior to the first mapping (BIO-WEST 2002a).

Flooding had distinct impacts on the vegetation in this reach. Although the Texas wild-rice plants were reduced in total coverage after each of these events, that species was often the only one remaining in areas where water was funneled through relatively narrow channels. This was the case in the eastern spillway and in the downstream portion of the reach near the University Drive Bridge. Large areas of *Potamogeton*, *Hydrilla*, and a mix of the two species were scoured during each event. As a result the vegetation composition in 2002-2004 has been constantly changing as the dominant species were scoured and re-grew in approximately the same distributions. Some less common species also grew more quickly after scouring and became relatively abundant for short periods. Overall, this reach is highly susceptible to flooding impacts due to the narrow channels and turbulence of water coming over the spillway. The lower portion of this reach is also highly impacted by sediment inputs from Sessom's Creek.

Recreational impacts were distinct in this reach during 2000-2004. Public access is allowed on the western shoreline between Sessom's Creek and the western spillway and, at times, this is a heavily used access point to the River. In 2002-2004, there was a large area of vegetation scoured (including *Hydrilla*, *Potamogeton*, and a mix of the two species) at the public access point during the summer. A clear line in the vegetation is obvious on the summer 2003 and summer 2004 maps where people used the same path to access deeper water. Recreation may have also contributed to the loss of a moderate-sized patch of Texas wild-rice between the spring 2003 and summer 2003 that had occurred along this pathway. Recreation also appeared to affect the western spillway where vegetation became more patchy in the summer of 2003 despite favorable flow conditions. A similar trend occurred in 2004, but a high-flow event prior to that sample probably also contributed to the sparse vegetation in that area. There was also a distinct pathway in the downstream section of this reach where the water was shallow enough to wade. The loss of vegetation via recreation, though not proportionally high, potentially affects the fountain darter population in this area by reducing total available habitat. Between the summer and fall sample in 2003 and 2004, much of the vegetation began to re-grow in areas that appeared to be affected by recreation, but many areas remained free of vegetation.

This reach also has a great deal of *Hydrocotle* mixed in among the other vegetation types. It is unknown what level of habitat quality this plant type provides to fountain darters since it is not sampled. It does grow close to the substrate and may provide favorable cover, but it tends to grow in areas with higher water velocities than those that typically support high densities of fountain darters and it also typically grows in mixed stands of vegetation (e.g., with *Hydrilla*, *Potamogeton* or *Hygrophila*). *Hydrocotle* does not grow in much abundance in the areas sampled for fountain darters in this study (i.e., City Park and IH-35 Reaches).

Another interesting observation during the summer of 2003 and again in the spring of 2004 was a substantial increase in *Eichornia* (Water Hyacinth). There was more water hyacinth observed in the field than that depicted on the 2003 summer map because one large patch in the middle of the western spillway was moved to accurately map the vegetation (primarily Texas wild-rice) beneath it. This occurred as flows were decreasing (but still above average) and other vegetation became increasingly patchy in the western spillway. By the summer 2004 sampling event, high flows had moved nearly all water hyacinth out of this reach and none was mapped in fall 2004. The water hyacinth observations have little influence on fountain darter habitat since the species presumably does not use this floating vegetation type for habitat, but the water hyacinth does cover Texas wild-rice plants and may reduce the

health of stands over time. This may also indirectly affect fountain darters by decreasing the health of other plant types used for habitat. Overall, however, the impact to fountain darters is minimal since the water hyacinth accumulates in only a few localized areas, but these areas do tend to have Texas wildrice plants in them.

City Park Reach

This reach contains a wide variety of vegetation types including many species combinations. The three dominant types are *Potamogeton* sp., *Hydrilla* sp., and *Hygrophila* sp., with *Potamogeton* sp. mixing with each of the latter two in large stands. This reach is relatively uniform in width but depth varies from shallow in the upper one-third of the reach to much deeper in the lower parts. There is also significant deposition of fine sediments along the river-left in the lower half of this reach. The areas with deep sediments tend to have a mixture of *Potamogeton* and *Hygrophila*, whereas deeper areas with faster current and larger substrates tend to have *Hydrilla* and a mix of *Potamogeton* and *Hydrilla*.

This reach was relatively buffered from scouring impacts during flood conditions. In certain areas there were large patches of vegetation that were sparse after flooding and there were a few areas that were scoured completely at times, but these impacts were much less severe than in the other two San Marcos Springs/River sample reaches. All flooding impacts were quickly ameliorated in this reach as vegetation re-grew shortly after flooding. In the downstream half of this reach there was some scour of *Hydrilla* along the margins and a shift in vegetation composition in one area from a mixture of *Potamogeton* and *Hygrophila* to only *Potamogeton* that occurred in the fall of 2002 and has persisted through 2004. Since pure stands of *Potamogeton* were not sampled with drop nets, it is uncertain whether that vegetation type provides the same quality of habitat as *Hygrophila* or the mixture of the two. Structurally it does not have the same complexity at the substrate level as *Hygrophila*, which may affect its suitability.

Similar to the Spring Lake Dam Reach, this reach had some influence from recreation during 2000-2004. The large patch of *Hydrilla* in the middle of the reach was reduced along the edges and a pathway maintained between the two shorelines in each year of the study. It is not likely that the impacts from recreation are significant; however, the high frequency of recreation in this reach could become a more serious problem during lower flow conditions. There have also been a few instances of Texas wild-rice plants settling along margin areas in the heavily recreated areas that disappeared shortly after they were first mapped.

At times, decreasing flows led to buildup of vegetation mats in the middle of this reach, primarily over *Hydrilla* and a mixture of *Hydrilla* and *Potamogeton*. These vegetation mats can have substantial impacts on the vegetation below them since they often remain for extended times and severely reduce the sunlight for the plants. When the mats cover *Hydrilla*, the impact is negligible so fountain darter habitat loss as a result of the mats is minimal. However, the impacts can be severe to Texas wild-rice plants that are covered for extended periods of time. There was also a fallen tree in the upper portion of the reach along the western shoreline that covered *Hygrophila* and some *Hydrilla* in the summer of 2003 through all of 2004. An extensive vegetation mat developed around the tree and has probably resulted in the loss of these areas as habitat for fountain darters. The loss of *Hygrophila* would be slightly more consequential to fountain darters than losing *Hydrilla*, but the total loss is still minimal.

There was a decrease in Texas wild-rice coverage in this reach in 2003, but there was a sharp increase during 2004. Part of this difference is due to the increased number of plants in the summer of 2003 despite the overall decrease in coverage at that time. These "new" plants grew rapidly in late 2003 and

early 2004 such that there was increase of 72% over the fall 2003 total. It is unclear what caused the decrease in coverage and increase in total plant number between the spring and summer of 2003 since discharge was decreasing progressively during that time. However, observations of Texas wild-rice plants floating downstream during winter and spring sampling throughout the study suggests mechanical (human, dog, etc.) displacement was occurring and several plants must have settled into the reach and set down roots.

IH-35 Reach

This reach differs from the other two San Marcos reaches in that *Cabomba* sp. occurs there and is relatively abundant. It occurs in this reach because of suitable conditions along the outside bends in the river (lentic backwaters, deep silty substrates). This vegetation type is important because it provides the highest-quality fountain darter habitat (of those sampled quantitatively) in the San Marcos River, but it is also highly susceptible to scour during flood events. After the flood in the fall of 2001, *Cabomba* decreased substantially in this reach. Seasonal variation in growth has been observed in *Cabomba* to a greater degree than other plant types with total growth decreasing substantially during winter. Thus, the effects of the fall 2001 flood persisted through the winter sample before growth increased again in the spring. However, the summer 2002 flood reduced the total coverage again to ~25% below the coverage remaining after the fall 2001 flood. Despite the repeated flushing flow, recovery of *Cabomba* was rapid and it returned to the same area in a similar quantity within a few months.

The effects of flooding were more distinct in this reach than in the other two San Marcos Springs/River reaches. In addition to the effects of the flushing flows on the *Cabomba*, the fall 2001, summer 2002 and summer 2004 events all modified the physical characteristics of this reach. Much of this section of river is constricted to a narrow channel with a dense riparian corridor and in some places, steep banks. This combination resulted in extensive scouring of substrate, leaving the deeper progressively deeper in some areas and depositing large quantities in other areas. The flooding also removed some riparian vegetation (overhanging branches) and in-stream woody debris.

Other vegetation types were also strongly affected by the flooding events. *Hydrilla*, *Hygrophila*, and Texas wild-rice were all substantially reduced in this area after each flood event. Although each of the vegetation types were reduced substantially during these events Texas wild-rice was more resistant than the other vegetation types. Most plants were removed completely in areas with strong current, but the Texas wild-rice, while reduced in total coverage, was able to persist in even the most highly impacted areas. The plants have a dense root structure that anchors the plants more effectively than other types and retains sediment in areas that are otherwise deeply scoured. Despite the intense acute affects of flushing flows in this reach, there was rapid re-growth of all affected vegetation after the high-flow events. In 2003, relatively stable flows led to stable conditions among vegetation types.

Texas Wild-Rice Surveys

Maps generated from the 2004 summer survey of the entire San Marcos River (downstream of Spring Lake) can be found in Appendix A. This mapping effort occurred shortly after the relatively high flows in June of 2004. In 2001 and 2002, the calculation by the BIO-WEST project team was nearly identical to the estimate of the total coverage of Texas wild-rice in the entire San Marcos River by the TPWD (Table 7). However, in 2003 and 2004 the BIO-WEST estimate differed slightly from the TPWD estimate.

Table 7. Total coverage of Texas wild-rice (m²) in the San Marcos River as measured by the TPWD for 1994-2003 and BIO-WEST in 2001-2004.

YEAR/EVENT	1994	1995	1996	1997	1998	1999	2000	2001 ^a	High-Flow ^a	2002	2003	2004
TPWD	1,456.3	1,624.0	1,652.1	1,584.2	1,949.0	1,644.9	1,791.1	1,895.6		1916.3	2776.0	3390.0
BIO-WEST								1,901.2	1,765.9	1913.2	2560.7	3145.3

^aTotal coverage values obtained in this study are included for the summer and high-flow events in 2001.

During the project (2000-2004) there has been a striking increase in total coverage of Texas wild-rice in the San Marcos River including an expansion of coverage into areas not previously occupied by Texas wild-rice plants. This trend has coincided with the period of relatively frequent flooding events, which may be a strong influence. Flooding conditions reduced the total coverage of Texas wild-rice temporarily and had a greater impact in certain areas (e.g., the IH-35 reach), but there was rapid regrowth after these events. As mentioned in the vegetation mapping section (IH-35 reach) the Texas wild-rice has a very dense root structure, which allows it to resist displacement during flooding more than other plant types in the San Marcos River. In addition, there were many observations of "new" plants settling into areas after having been presumably dislodged from upstream stands and relocated downstream. Because of the size of many of these new plants it is unlikely that most of them grew from seeds; however, it is possible that some of the smaller plants were a result of seed germination. In most cases the new plants appeared after flooding, but some were observed during periods of relatively stable flow in 2003, which suggests that human-caused mechanical removal (i.e., recreation) is enough to result in transplanted individuals. The growth rate of plants found in new locations has been very high in most instances throughout the study.

The timing of flood events probably also has an influence on the ability of Texas wild-rice to withstand scouring flows. Although Texas wild-rice remains relatively dense throughout the year, many of the other vegetation types vary in areal coverage by season. Thus, the amount of other vegetation immediately surrounding the Texas wild-rice plants, vegetation that may provide some protection, will vary among seasons. Also, residual impacts of a previous flood may result in more risk to Texas wild-rice plants that might otherwise be protected. The total coverage after flooding appears to be largely influenced by the opportunity for displaced Texas wild-rice plants from upstream locations to settle into an area.

Despite the overall increase in Texas wild-rice and apparent benefits of higher flows on displacing plants and allowing those plants to settle into new areas, there has been a trend of decreasing coverage of Texas wild-rice in the section of river downstream of the IH-35 bridge. This area down to the lower boundary of the population appears to be the most vulnerable to the high-flow events based on our data and that of TPWD (2001); pre-1998 flood maps of Texas wild-rice revealed a greater number of stands in these lower reaches. This is a trend in which plants are displaced during significant flooding but not replaced by plants from upstream locations. In most areas above the IH-35 bridge, plants lost through displacement were generally replaced (in different locations) by plants from further upstream (frequently, individual plants are displaced from large stands in the upstream-most reaches, but the majority of the stand remains intact).

Texas Wild-Rice Physical Observations

Observations of vulnerable Texas wild-rice stands (see Methods section for definition of vulnerable) conducted in each sample reach during 2000-2003 are detailed in the respective annual reports. Observations on trends in areal coverage of these vulnerable stands in 2004 (Table 8) are discussed by reach below. Throughout the study many stands have joined together and some small plants have been displaced so there is some variation in the areal coverage data associated with these inconsistencies.

Graphs displaying observations of emergence, root exposure, herbivory, and stand depth in each reach during 2000-2004 are found in Appendix B. There were some overall patterns observed with these data. Emergence was typically lowest in the Thompson's Island sample area, which is indicative of conditions experienced by most Texas wild-rice plants downstream of the IH-35 Bridge. The river is fast moving in this area and there is little shelter from the current velocity; the shallowest portion of the stands is typically covered by 1-2 feet of water. The greatest emergence in that area occurred in the spring of 2002 when total discharge in the San Marcos River was approximately 200 cfs. In other areas, emergence was usually high during the time of year with the lowest flows and was lowest after high-flow events. Emergence was not strongly seasonal, although there tended to be a higher proportion in spring and fall than other seasons. Emergence may have also been stimulated by high flows since the highest proportion in the Sewell Park and Thompson's Island areas occurred in the Spring 2002 event after the significant flood in the fall of 2001. Emergence was high again in the Sewell Park area in the fall of 2002 after the higher flows in the summer of 2002. In the IH-35 Reach, emergence was high in the spring of 2002 as in the other reaches.

Root exposure was another parameter that appeared to vary in direct response to high-flow conditions. Root exposure increased significantly immediately after the high-flow events in the fall of 2001 and summer of 2002. Root exposure was consistently low during 2003 during stable flows. The relative amount of root exposure prior to a high-flow event may also be significant since it was higher in the sample event immediately preceding the summer 2002 flood compared to the fall 2001 flood and the former resulted in a greater reduction of total coverage of Texas wild-rice plants.

Herbivory of Texas wild-rice plants appeared strongly correlated with plant emergence and discharge conditions. Herbivory was consistently highest in the Sewell Park area where the greatest total emergence occurred. In the IH-35 and Thompson's Island areas very little evidence of herbivory was observed in 2000-2004.

The depth of all Texas wild-rice plants in vulnerable areas was greater than the 0.5 feet critical threshold in nearly every measurement during 2000-2004. There was a moderate proportion of the total area in Sewell Park and in the IH-35 Reach during the fall of 2000. Since that time, there have been only three sample dates in which a small amount of Texas wild-rice was in such shallow water and all occurrences were in the IH-35 Reach. During the summer of 2003 two small plants settled into the shallow habitat along the shore of the IH-35 reach and the 100% and 60% proportion of these plants that were in less than 0.5 feet of water skewed the total estimate to appear more significant than they were. The same occurred in the fall of 2003 with slightly higher numbers and the spring of 2004 with much lower numbers, but these two plants disappeared by the summer of 2004.

Table 8. Texas wild-rice areal coverage (m2) for each stand by sampling period (2004 only).

REACH-STAND NO. ^a	Spring 2004	Summer 2004	Fall 2004	High-Flow 2004
Sewell Park-1	42.4	35.6	40.0	46.4
Sewell Park-2	42.4	25.6	48.8	46.4
Sewell Park-3	92.5	100.1	112.1	106.9
Sewell Park-4	12.0	6.0	7 -	12.0
Sewell Park-5	13.9	6.9	7.5	12.9
Sewell Park-6				
Sewell Park-7	754.8	614.9	707.8	675.1
Sewell Park-8				
Total Area	903.6	747.4	876.1	841.3
IH-35-1			0.2	0.3
IH-35-2		0.3	0.4	0.4
IH-35-3	4.2	3.2	3.1	
IH-35-4				
IH-35-5	41.2	35.9	41.1	27.6
IH-35-6	5.2	6.4	5.3	7.7
IH-35-7	27.8	31.2	29.2	32.1
IH-35-8	164.1	155.1	158.9	146.9
Total Area	242.5	232.1	238.1	215.0
Thompson's Island - 1				
Thompson's Island - 2	5.8	3.5	4.5	4.2
Thompson's Island - 3	1.1	0.7	1.1	0.8
Total Area	6.9	4.2	5.6	5.0

Many stands grew together to form individual stands after the first sampling period (SP-1, SP-2; SP-4, SP-5; SP-6, SP-7, SP-8; IH-35-6, IH-35-7 [winter 2001]).

Sewell Park Reach

In this reach, the average areal coverage of the eight Texas wild-rice stands considered to be in vulnerable locations was 842 m² compared to 620 m² in 2003 and 493 m² in 2002. The greatest increase in total coverage occurred between the Fall 2003 (626 m²) and Spring 2004 (903 m²) sample events. The high-flow event in the fall of 2004 had only minor affects on total coverage of Texas wild-rice in this reach.

During 2004, all Texas wild-rice plants in this reach remained deeper than 0.5 ft in the water column. Emergence of leaves and flowering parts was relatively low in the summer and fall of 2004, but high in the spring. The higher proportion of emergence was during a period of relatively stable flows and may indicate that a seasonal peak in emergence would occur if flows remained stable during this time.

Continuing a trend that has occurred since the fall of 2002, root exposure remained very low in all reaches during most of 2004. There was an increase in root exposure in all reaches during the spring of 2004, despite the consistent flows during that time. Although we have clearly observed the scouring effects of flooding on root exposure during this study there is little data for the potential effects of low-flow conditions on this parameter.

Evidence of herbivory has increased in this reach since the spring of 2003 and remained relatively high during all of 2004. The continual deposition of sediments along the eastern shoreline in this area has created a very shallow shelf of Texas wild-rice plants in low velocity water. These conditions have created a dramatic increase in density of Texas wild-rice plants in the area, but the conditions also make the plants more susceptible to herbivores.

IH-35 Reach

The average areal coverage of the eight Texas wild-rice stands considered to be in vulnerable areas in this reach declined slightly in 2004 (238 m²) compared to 2003 (264 m²) and 2002 (262 m²). The total area has remained relatively consistent throughout the study in this area, but some variation has been observed in the individual plants. This reach was severely impacted during the high-flow event in November 2004. The large stand of Texas wild-rice in the middle of the reach was scoured more severely than during other high-flow events in 2000-2004. The total coverage did not decrease dramatically however, because the large stand down near the IH-35 Bridge was relatively unaffected by the flood.

There was one small plant that had a small percentage of its total area shallower than 0.5 ft during the Spring 2004 sample, but there were no other instances of plants in shallow water in 2004. As described in the 2003 annual report (BIO-WEST 2004a) there have been observations of "new" plants in very shallow areas along the western shoreline at the upstream edge of this reach (near Cheatham Road). There has also been scouring, re-growth and appearance of "new" plants just downstream on the inside of the large bend. The higher flows in 2003 and 2004 likely contributed to these changes, but by the latter half of 2004, there were no plants in the shallow shoreline habitat.

As in the other reaches, root exposure was very low in the summer and fall of 2004 but relatively high in the spring. Herbivory was low in this reach in 2004. These observations are likely a result of higher flows. Unlike in the Sewell Park reach, growth was not substantial enough in Texas wild-rice plants in the IH-35 reach during 2000-2004 to allow leaves to reach the water surface and become more accessible to herbivores.

Thompson's Island Reach (Natural)

As noted in the 2001 annual report (BIO-WEST 2002a), one of the three Texas wild-rice stands disappeared by the early sampling efforts of this study. The average coverage of the remaining two stands was 5.3 m² in 2003 compared to 5.1 m² in 2003, 4.7 m² in 2002, and 3.8 m² in 2001. The two plants in this reach decreased in total coverage after the high-flow conditions that occurred early in the summer but increased again by the fall sample event. These plants appear to be moderately affected by flooding, but have withstood several high-flow events during 2000-2004 without being displaced. There was little evidence of impact in this reach associated with the high-flow event in November 2004.

As in the other two reaches, emergence was relatively high in spring of 2004, but lower during the summer and fall. Root exposure followed the same pattern, but herbivory was low in all seasons in

2004. The plants in this reach have remained in very good condition throughout this study. These plants appear to be the least affected by minor changes in conditions, but were somewhat impacted by the major flooding in 2002. However, it remains important to monitor these plants to evaluate the impact of low-flow conditions on plants in the downstream range of the population and on those that have shown minimal variability to habitat changes under moderate- to high-flow conditions.

Fountain Darter Sampling

Drop Net Data

The number of drop net sites and vegetation types sampled per reach in 2004 is presented in Table 9. The drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sampling event; previous drop net site locations were indicated on maps provided with each annual report. The 2004 data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. High flow conditions during the fall 2004 sampling event prevented drop net sampling during that effort.

Table 9. Drop net sites and vegetation types sampled per reach.

CITY PARK REACH	IH-35 REACH	
Bare Substrate (2)	Bare Substrate (2)	
Hygrophila (2)	Hygrophila (2)	
Hydrilla (2)	Hydrilla (2)	
Potamogeton/Hygrophila (2)	Cabomba (2)	
Total (8)	Total (8)	

<u>Importance of aquatic vegetation</u>

One of the primary observations during 2000-2004 was the wide range of suitability (as fountain darter habitat) of the various vegetation types found in both the Comal and San Marcos Rivers (Figure 8; Comal 2004 Annual Report). Compared to the Comal Springs/River ecosystem, the San Marcos Springs/River ecosystem had fewer habitat types available to fountain darters during 2000-2004 and a narrower range of suitability among these habitat types (Figure 8; Comal 2004 Annual Report). The densities of fountain darters sampled in the San Marcos Springs/River ecosystem ranged from 2.3 per m² in the abundant *Hydrilla* to 4.9 per m² in *Cabomba* (sampled only in the IH-35 Reach). The other two dominant habitat types, *Hygrophila* and a mix of *Hygrophila* and *Potamogeton* supported densities of 3.7 and 4.5 fountain darters per square meter respectively. Also, the occurrence of fountain darters over bare substrates was very uncommon. In a total of 56 drop net samples over bare substrate in the San Marcos Springs/River ecosystem, only 2 fountain darters have been sampled.

Abundance Estimates

Estimates of fountain darter population abundance (Figure 9) were based on the changes in vegetation composition and abundance and the average density of fountain darters found in each, as described in the methods section. Estimates were based on the City Park and IH-35 Reaches. Data from the Spring Lake Dam Reach was not included in these estimates because drop net sampling was not conducted there and because the conditions in this reach are unique and not representative of other areas in the river (there is abundant vegetation similar to the City Park Reach, but the turbulence of the dam provides

more dynamic currents). Because there was little difference in the suitability of various vegetation types, and no one particular type that varied substantially (like the bryophytes in the Comal Springs/River ecosystem) there was no vegetation type that had a dramatic influence on these estimates. As in the Comal Springs/River ecosystem, the influence of high-flows resulted in less vegetation and lower population estimates, but in each sample that followed a high-flow sample, the population estimate increased.

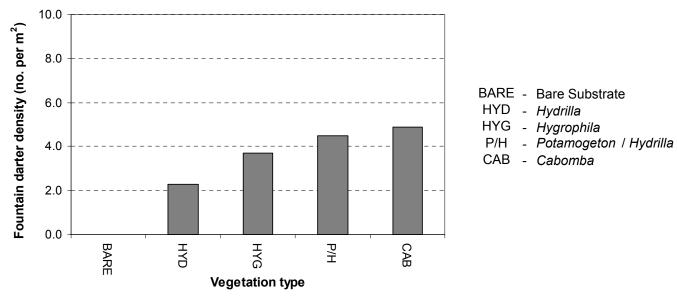


Figure 8. Density of fountain darters collected by vegetation type in the San Marcos Springs/River ecosystem (2000-2004).

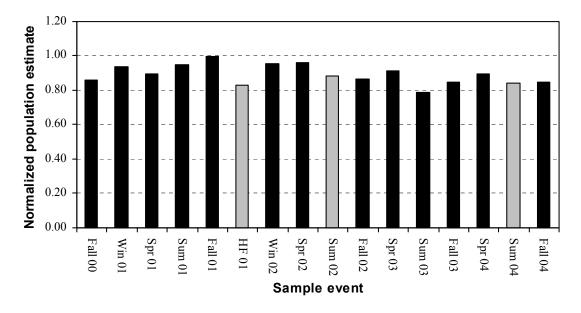


Figure 9. Population estimates of fountain darters in both San Marcos Springs/River sample reaches combined (2000-2004); values are normalized to the maximum sample. Light-colored bars represent high-flow Critical Period sampling events.

Population Modeling

An initial evaluation of a model that incorporated all habitat parameters (Table 10) revealed that there were significant problems with multiple parameters varying together (multicollinearity) which limited the value of a single model to evaluate changes in fountain darter abundance. As an example of multicollinearity, substrate type and water velocity at 15cm above the substrate were highly correlated since lower velocity habitats primarily consisted of silt and sand and higher velocity habitats had much larger substrates. One of the more significant pairs of highly correlated parameters is season and discharge. Fountain darters appeared to have some seasonal variation in density as discussed in previous annual reports, but the high correlation between season and discharge makes it difficult to differentiate which parameter may be the cause of these differences. Additional data at a range of discharges (especially lower flows) may result in a data set that has a greater capability to differentiate effects associated with these two parameters. As a result of the multicollinearity problem, only a few variables could be feasibly incorporated into a model. Therefore, variables had to be evaluated individually or in smaller subsets. In order to evaluate the three variables that are considered to be most critical (total discharge in the San Marcos River, vegetation, and location/reach) a series of linear regressions were conducted on the relationship between fountain darter density (transformed with square-root equation) and discharge in each combination of vegetation and reach. These regressions allowed us to evaluate whether the range of discharge conditions resulted in a significant change in fountain darter density in each reach x vegetation combination. Additional variables (Table 10) were evaluated independently with similar regression analysis.

Individual regressions revealed that many of the relationships are not significantly different from baseline because of the small sample size, but there were some trends that suggest potential relationships that may develop with additional data. Only two reach x vegetation combinations had a significant relationship (p<0.05) with discharge using the data collected through 2004, and one other was nearly statistically significant (p=0.067); all three occurred in the City Park Reach (Figure 10). The two significant relationships were in *Hygrophila* and a mixture of *Potamogeton* and *Hygrophila* and the near-significant relationship was in *Hydrilla*. All three of the relationships indicated a higher number of fountain darters collected at lower discharge. In each case the proportion of variation in density explained by discharge (\mathbb{R}^2) was relatively low (0.11 – 0.23) but that is typical of ecological data where multiple variables affect a population simultaneously and random error is often very high.

Table 10. Parameters collected at drop net locations that were incorporated into initial population model.

dominant vegetation type	substrate
season	pH near substrate
total discharge in the Comal River	dissolved oxygen near substrate
water depth	specific conductivity near substrate
mean water velocity	water temperature near substrate
water velocity at 15cm above substrate	

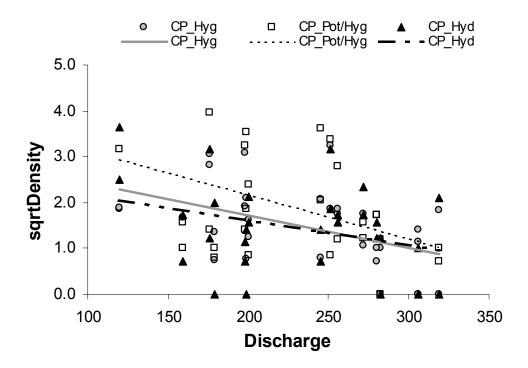


Figure 10. Relationships between discharge and fountain darter density (square-root transformed) were significant (p<0.05) for *Hygrophila* and a mixture of *Potamogeton* and *Hygrophila* in the City Park Reach and nearly significant (p=.067) for *Hydrilla* in the City Park Reach. Genus names of the vegetation types are abbreviated: Hyg=*Hygrophila*, and Pot=*Potamogeton*; Hyd=*Hydrilla*.

Unlike in the Comal Springs/River ecosystem, all individual variables did not have significant relationships with fountain darter density. Density did not differ significantly across the range of two variables, depth and pH. Among significant variables, all but one had linear relationships; dissolved oxygen had a quadratic relationship (Figure 11). The significant relationships suggest that there were differences in fountain darter density across the range of each variable in all samples from the San Marcos Springs/River ecosystem, but does not necessarily mean that each variable has a direct influence on the distribution and abundance of fountain darters. Also unlike in the Comal Springs/River ecosystem where all individual variables had low R^2 values, two parameters (substrate and water velocity at 15 cm above the substrate) explained a moderate amount of the variation in fountain darter densities observed across samples in the San Marcos Springs/River ecosystem ($R^2 = 0.203$ and .277, respectively; Table 11).

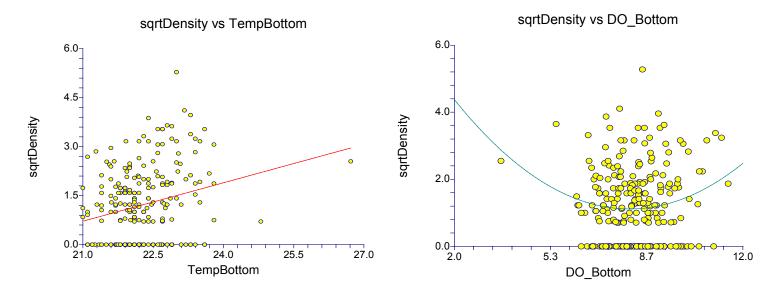


Figure 11. Examples of relationships between individual water quality variables and fountain darter density (square-root transformed) including (a) a significant (p<0.001) linear relationship and (b) a significant (p=0.013) quadratic relationship.

Table 11. Type of relationship (linear or quadratic) between individual variables and fountain darter density during 2000-2004 and R^2 value associated with the relationships.

Parameter	Relationship Type	R^2
Discharge	Linear	0.021
Depth	Linear	0.002
Substrate	Linear	0.203
Water Velocity at 15cm	Linear	0.277
Temperature at bottom	Linear	0.061
DO at bottom	Quadratic	0.027
SpCond at bottom	Linear	0.020
pH at bottom	Linear	0.003

The size-class distribution for fountain darters collected by drop net from the San Marcos Springs/River ecosystem from 2000-2004 is a statistically normal distribution that is typical of a healthy fish population (Figure 12). The distribution is shifted towards larger fish than those observed in the Comal ecosystem with a peak between 30 - 32 mm compared to a peak between 22 - 26 mm in the Comal Springs/River ecosystem. This pattern was relatively consistent in the San Marcos Springs/River sites except during 2004 (Figure 13), when a relatively high proportion of smaller individuals were sampled.

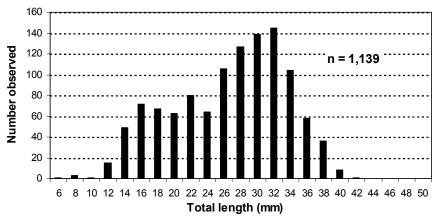


Figure 12. Fountain darter size-class distribution among all drop net sampling events in the San Marcos River during 2004.

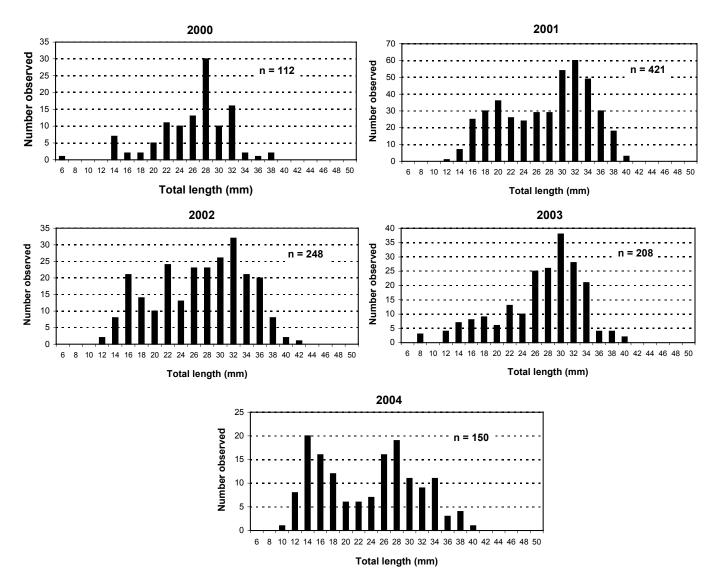


Figure 13. Annual fountain darter size-class distribution among drop net sampling events by sample year in the San Marcos River.

In addition to visually comparing size-class distributions, the mean length of fountain darters was evaluated among discharge levels, vegetation types, and reaches in the same way as density with linear regression. Unlike density, mean length could not have zero values, if no darters were captured in a sample it was not included in this analysis. This analysis revealed that there was not a significant relationship between discharge and mean length of fountain darters overall or within any reach (Appendix B).

Other Species

Including fountain darters, a total of 24 fish species and 7,519 individuals were collected from the San Marcos Springs/River ecosystem in 2000-2004 (Table 12). Of these, 8 species are considered exotic (introduced) but only the sailfin molly (*Poecilia latipinna*) and rock bass (*Ambloplites rupestris*) were relatively abundant (> 40 individuals). An analysis of community composition (Shannon Weaver diversity index) revealed that there was virtually no difference in diversity between the two sample reaches (Figure 14). Examined by reach, there were no discernable patterns in diversity among sample dates (Appendix B).

Among exotic species, the giant ramshorn snail elicits the most concern because of its recent impacts (early 1990s) on aquatic vegetation in the San Marcos River, and to a greater extent, the Comal River. In the fall 2000 sample, 19 giant ramshorn snails were sampled in the San Marcos Springs/River ecosystem, but none were collected during 2001-2003. In 2004, there were 7 collected.

Shannon Weaver Species Diversity

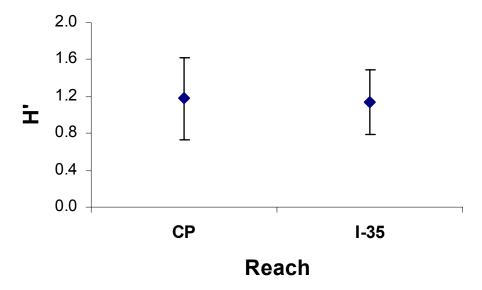


Figure 14. Average value of Index of species diversity (Shannon Weaver) for each San Marcos Springs/River sample reach with +/- one standard deviation indicated.

Table 12. Fish species and the number of each collected during 2000-2004 drop net sampling.

COMMON NAME	SCIENTIFIC NAME	STATUS	TOTAL NUMBER
Rock bass	Ambloplites rupestris	Introduced	206
Black bullhead	Ameiurus melas	Native	2
Yellow bullhead	Ameiurus natalis	Native	50
Mexican tetra	Astyanax mexicanus	Introduced	7
Rio Grande perch	Cichlasoma cyanoguttatum	Introduced	11
Roundnose minnow	Dionda episcopa	Native	17
ountain darter	Etheostoma fonticola	Native	1,026
Gambusia	Gambusia sp.	Native/Introduced	5,400
Suckermouth catfish	Hypostomus plecostomus	Introduced	12
Redbreast sunfish	Lepomis auritus	Introduced	32
Green sunfish	Lepomis cyanellus	Native	4
Varmouth	Lepomis gulosus	Native	17
Bluegill	Lepomis macrochirus	Native	61
ongear sunfish	Lepomis megalotis	Native	3
Spotted sunfish	Lepomis punctatus	Native	394
Sunfish	Lepomis sp.	Native/Introduced	81
argemouth bass	Micropterus salmoides	Native	29
Gray redhorse	Moxostoma congestum	Native	3
Blacktail shiner	Cyprinella venusta	Native	6
exas shiner	Notropis amabilis	Native	13
ron color shiner	Notropis chalybaeus	Native	19
Jnknown shiner	Notropis sp.	Native	4
og perch	Percina caprodes	Native	2
Dusky darter	Percina sciera	Native	12
Sailfin molly	Poecilia latipinna	Introduced	74
Jnknown molly	Poecilia sp.	Introduced	30
Гіlapia	Tilapia sp.	Introduced	4

Dip Net Data

The boundary for each section where dip net collection efforts were conducted is depicted on Figure 15. Section numbers are included to be consistent with the USFWS classification system for the San Marcos River. Data gathered using dip nets in the headwaters of the San Marcos River are graphically presented in Figure 16 and the other two sample areas are presented in Appendix B. Using dip nets, fountain darters were collected from every section during every sampling event during 2000-2004.

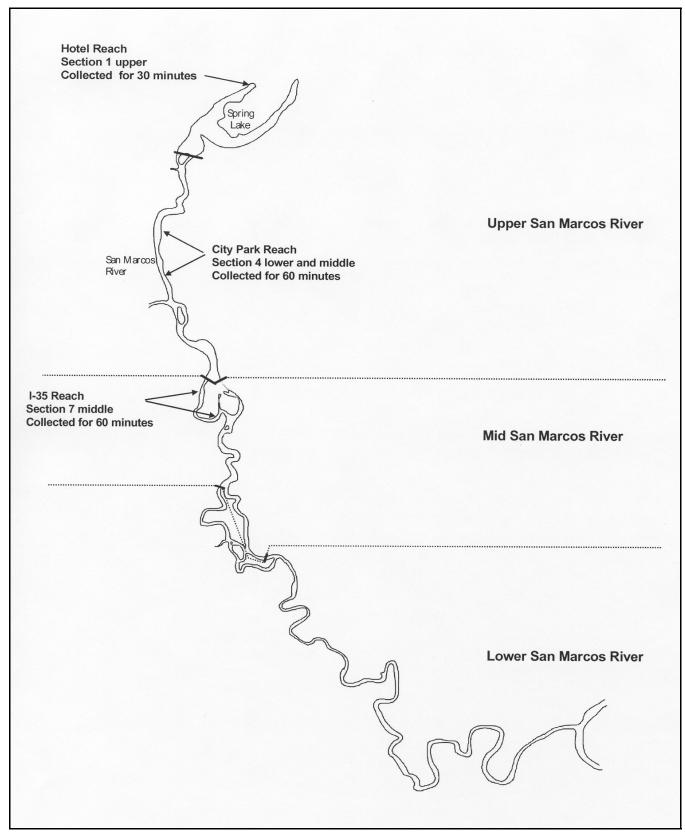


Figure 15. Areas where fountain darters were collected with dip nets, measured, and released in the San Marcos River.

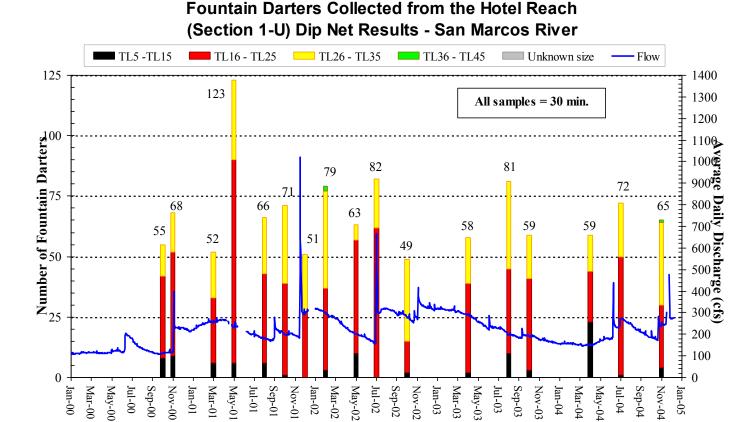


Figure 16. Number of fountain darters collected from the Hotel Reach (section 1 upper) using dip nets.

The Hotel Reach samples, in the headwaters of the San Marcos River, had individuals in the lowest size class (5-15 mm) during nearly every sample effort in 2000-2004 (Figure 16). This size class represents fountain darters <58 days old (Brandt et al. 1993) and their presence in all seasons suggests year-round reproduction. This reach also had high numbers of fountain darters in the next larger size class (16-25 mm) in each sample. Evidence of recent reproduction was much less common in dip-net samples from the City Park or IH-35 Reaches, but when it occurred, it was primarily restricted to the spring. Reproduction also seemed to occur in the City Park and IH-35 reaches in response to flooding. Year-round reproduction was again evident in the higher-quality habitat in Spring Lake in 2004.

San Marcos Salamander Visual Observations

Filamentous algae was abundant in sample areas 2 (hotel reach) and 14 in Spring Lake during 2000-2004. The individual sample areas had to be cleared prior to sampling by moving the algae to areas outside of the perimeter of the delineated sample area. As shown in Table 13, San Marcos salamanders were observed in each sample area during each survey effort.

Sample area 2 has had the highest population densities throughout the study, but the densities have also varied substantially between seasons. For example, the lowest density was observed in winter 2002, but this sample was followed with the highest estimate of density in this area in spring 2002. Data collected

in this site in 2004 was consistent with that observed in previous years. Sample area 14 had the most consistent density estimates from 2000-2004. The lowest numbers in sample area 14 were observed during the fall of 2000 when flows were low (approximately 115 cfs) and after the high-flow event during the summer of 2002. The other high-flow events did not have a noticeable difference in number of San Marcos salamanders observed in this sample area. Prior to 2004, the greatest estimated density of San Marcos salamanders in sample area 21 occurred during the fall 2000 event, which was a low flow period. However, two of the three samples conducted in 2004 had higher estimates than that event. All samples in 2004 were much higher than previous samples in this sample area.

Table 13. San Marcos salamander density per square meter (m²).

SAMPLING PERIOD	SAMPLE AREA 2	SAMPLE AREA 14	SAMPLE AREA 21
Fall 2000	19.4	3.4	5.2
Winter 2001	8.7	Omitted	2.6
Spring 2001	9.4	13.9	0.4
Summer 2001	16.6	11.1	1.5
Fall 2001	10.0	6.7	3.2
High-flow 2001	9.7	8.6	1.0
Winter 2002	6.1	6.5	0.9
Spring 2002	20.2	8.5	0.6
Summer/High Flow 2002	17.7	4.2	0.7
Fall 2002	16.8	8.7	3.0
Spring 2003	7.9	11.9	1.0
Summer 2003	20.1	6.8	2.0
Fall 2003	11.3	9.5	2.7
Spring 2004	14.6	9.9	7.1
Summer 2004	10.9	9.2	7.0
Fall 2004	11.7	13.7	4.5

Exotics/Predation Study

Results of the exotics/predation study can be found in the annual reports for 2000, 2001, and 2002. No sampling was conducted for this study component in 2003 or 2004.

DISCUSSION

Springflow

The much higher-than-normal flow conditions in 2000-2004 have limited the ability to gather data on the low-flow conditions that are the primary focus of the study. However, data collected after individual high-flow events with their significant flushing flows reveal that there are substantial acute impacts on the threatened/endangered species primarily by reducing habitat quality and availability. Because of the acute nature of a flood event and its flushing flows, impacts are more readily characterized than with the gradual impacts that may be associated with a critical low-flow period. Therefore, the data collected following high-flow events provided substantial information on the impacts of these events, but the limited data collected during low-flow conditions during 2000 did not provide nearly enough information to adequately evaluate the effects of such conditions.

Water Quality

Spring Lake

There does not appear to be much influence on water quality from surface water inflow to the Spring Arm of the lake, but influences from the immediate watershed may impact Sink Creek and the Slough Arm of the lake. Overall, water quality was consistently high in the Spring Arm of the lake and fluctuated more in the Slough Arm. Dissolved oxygen concentrations will not consistently meet the high water quality standard of 6.0 milligrams per liter (mg/l) because the concentration is lower from water issuing from the springs. However, the only site that had a lower average than 6.0 mg/l was the site in the headwaters directly adjacent to spring openings. Sites in the Slough Arm had a greater fluctuation. The high photosynthetic rate, caused by the abundance of plants in the lake coupled with the lower water temperatures in winter at the Slough Arm sites, probably accounts for supersaturated oxygen levels during those times. Higher water temperatures in the summer coupled with decomposition of the abundant plant material probably accounts for the low concentrations. This is an important component of the water quality in Spring Lake that may limit habitat usability in this area for some species during certain times, particularly during lower flow conditions. The high temperatures in the Slough Arm are also a concern since the wide fluctuation results in periods of time when fountain darters, and probably most other species, can not use this area.

There was little concern among other water quality parameters. The higher SRP concentrations probably occurred due to recycling of SRP (as plant material decayed) and inputs of phosphorus from the immediate watershed, but these values were not high enough to cause concern. It will be important, however, to monitor this parameter during low flows. Another variable that was high was nitrate, but this was not the result of anthropogenic inputs to the immediate surface waters since the median concentration of nitrate in the Edward's Aquifer ranges from 1.4 to 1.7 mg/l (Bush et al. 1998). Nitrate concentrations in the Slough Arm sites and Sink Creek were much lower than in the Spring Arm sites since these sites receive less direct springflow; however, the values did fluctuate more in the Slough Arm sites throughout the year. Though not a significant concern, these high values could increase eutrophication in the Slough Arm and cause even greater fluctuation in dissolved oxygen during low flow periods.

San Marcos River

The overall assessment of water quality in 2000-2002 supported observations during other studies that there are no parameters of substantial concern in the San Marcos Springs/River ecsystem. Since total discharge remained at or above normal during most of that time there was little evidence to suggest that any parameter provided unfavorable conditions for any of the threatened or endangered species in the San Marcos River. Unlike in the Comal River, there were very limited patterns in any variable, including water temperature and dissolved oxygen. Temperature did not fluctuate much at the two sites located at the outfall of Spring Lake, but all downstream sites were very similar during the study period. In these downstream areas, the continuous record of water temperature collected with thermistors has provided extensive data during 2000-2004 to show that this parameter has remained within the range that allows reproduction of fountain darters (considered to be the most restrictive temperature limitation) the majority of the time. The one instance in which temperatures exceeded the threshold believed to affect fountain darter reproduction (27°C) may not have been an accurate measure of true water temperature since other measurements downstream did not approach these high values. Additional data during low flows should clarify this observation. Sessom's Creek tended to have greater fluctuation in temperature and other water quality parameters, but as with Blieder's Creek in the Comal Springs/River ecosystem, this is a tributary to the San Marcos River that probably provides limited habitat for any of the species of concern. Inputs from the creek probably affect the area immediately downstream of the confluence in the San Marcos River, but these impacts would affect only a very localized area. Water temperature will be one of the most important variables to monitor when low-flow conditions occur and it is important to maintain this continuous record through such conditions.

Although there were no observations that presented a cause for concern with any water quality parameter, there were some observations that warrant additional consideration during low-flow conditions. At the two downstream-most sites (Thompson's Island Artificial Canal and Animal Shelter), and at the Lion's Club site, SRP and TP were higher than in upstream sites during 2000-2002. The higher values at the Lion's Club site may be influenced by urban runoff. The water in the canal at Thompson's Island has a high residence time, which may allow for a build-up of phosphorus in the canal. The higher SRP values in the river upstream of the City of San Marcos wastewater treatment facility are likely caused by point or non-point source loads within the immediate watershed. The only permitted discharge upstream of the last sampling site is the TPWD fish hatchery. Non-point source discharges include the San Marcos urban area as well as agricultural areas. Although values are higher at these sites, these SRP values are still well below the TCEQ's screening levels for surface waters.

Total dissolved solids is another parameter that may be important to monitor during low flows since it exceeded the state water quality value in 2001. However, no previous mention of exceedences by the TCEQ suggests that this water quality parameter is not a concern. The higher turbidity values recorded at the downstream-most sites in October 2000 may have been due to lower flows in the river causing less of a dilution effect from point and non-point source discharges. Thus, turbidity should be carefully monitored during low-flow conditions. Nitrate values also exceeded the water quality standards screening level in most cases, but the values were within the median concentration of nitrate in the Edward's Aquifer. The standard parameters of specific conductivity and pH remained within very narrow ranges over the course of the study; these variables have little direct effect on population dynamics of fish, salamanders or invertebrates except when a substantial deviation occurs.

Dissolved oxygen levels were well above the TCEQ water quality of 6.0mg/l during the comprehensive assessment in 2000-2002 and there were only two observations during drop netting when dissolved

oxygen was below the standard (but still >5.0 mg/L). Dissolved oxygen may become more of a concern overall at lower discharge values in the San Marcos River and should continue to be monitored during those times.

As with other components of this Variable Flow Study, more data are needed to determine the potential impacts of high air temperatures and low flows on water temperature and other water quality components during an extended period of reduced recharge.

Aquatic Vegetation Mapping

Throughout this study it has become clear that vegetation type is an important factor in determining distribution of the fountain darter in the San Marcos Springs/River ecosystem. The difference among vegetation types in the density of fountain darters sampled was not as distinct in the San Marcos Springs/River ecosystem as in the Comal Springs/River ecosystem. In the San Marcos Springs/River ecosystem, the highest quality vegetation type is probably the filamentous algae in Spring Lake. Although the areas containing the algae have not been quantitatively sampled (i.e., with drop nets), direct observations with SCUBA gear and dip netting has revealed an abundance of fountain darters using these habitats. Though they occur in lower densities in areas without filamentous algae and relatively equally among vegetation types, the presence of vegetation is still critical to fountain darters habitat suitability in the San Marcos Springs/River ecosystem.

In general, aquatic vegetation remained abundant in all reaches under the range of flows experienced in the study. However, there were some differences in the relative value of each reach for fountain darter habitat because of differences in vegetation coverage and the response of the vegetation to variable flow conditions. The IH-35 reach tended to be more susceptible to scouring during flooding and also had a more sparse distribution of plants than the City Park and Spring Lake Dam reaches. Scouring tended to have acute, but short-lived impacts on the vegetation in the San Marcos Springs/River ecosystem. When scouring did occur, the vegetation quickly re-grew to occupy the same habitats and same area as before the flushing flows. As in the Comal Springs/River ecosystem, the vegetation type that supported the highest density of fountain darters in 2000-2004 (*Cabomba* sp.) was also the most susceptible to scour during flooding. However, unlike in the Comal Springs/River ecosystem, there was a small difference in the density of fountain darters in that habitat type compared to the others. Additional data during low-flow conditions are needed to evaluate the effects that those conditions have on vegetation.

One threat to the presence of aquatic vegetation is the Ramshorn snail, which was not sampled at all in the San Marcos Springs/River ecosystem during 2000-2003, but seven individuals were sampled in 2004. The population, which occurred in densities as high as 100 per m² (Arsuffi et al. 1993), does not appear to be a concern anymore, but continued monitoring is necessary as there is little understanding about what is currently limiting the snail population and whether another "boom" in the population may occur in the future. The sudden re-appearance of this species after not being sampled during the previous three years of the study may simply be a result of a small population and random chance, but it will be important to monitor observations in 2005. If the population of this species does become a problem again, it will be important to determine which vegetation types are most vulnerable and which reaches are most susceptible to an impact.

Since the different vegetation types in the San Marcos Springs/River ecosystem tend to support similar densities of fountain darters, the relative value of different reaches as fountain darter habitat is determined more by the relative susceptibility of that vegetation to variable flow conditions and less by

vegetation composition. Overall, the differences between reaches were relatively minor under the discharge conditions in 2000-2004 and management goals based on these conditions would not differ between them. However, it will be important to evaluate the relative differences among reaches during lower flow conditions.

The increase in Texas wild-rice has been dramatic in the San Marcos River during 2000-2004 and appears largely influenced by flooding events. The temporary reduction in total coverage of this plant species after a flood is quickly offset with rapid re-growth and expansion of the population into other areas. It seems likely that mechanical displacement (e.g., recreation) of individual plants also allows for settling of individuals into new areas. The one concern for Texas wild-rice was that despite the increase of the population overall, there was a decrease in coverage downstream of the IH-35 bridge. For some reason, conditions are not amenable to plant re-establishment in these lower sections. This may be related to differences in water quality, less vegetation to slow the water down and allow displaced Texas wild-rice plants to settle, or narrower, deeper channel conditions in downstream sections. The findings of the aquatic vegetation laboratory study on response to variable CO₂ conditions (BIO-WEST 2004b) may provide some insight here. It was observed that Texas wild-rice plants devoted more energy to aboveground biomass in low CO₂ conditions (similar to the concentrations found in the lower range of the species' habitat). This is presumably to allow the plants' leaves to reach the surface and gain access to atmospheric CO₂, but the result is a less dense root structure that may allow greater susceptibility to scour during flooding.

Texas Wild-Rice Physical Observations

The change in coverage of "vulnerable" Texas wild-rice plants was similar to observations of the Texas wild-rice coverage in the San Marcos Springs/River ecosystem overall. Individual plants were subject to scouring during high flows, with those found in the shallowest locations generally more susceptible. In between scouring flows during 2000-2004 these plants also grew vigorously and "connected" into larger stands than were observed when the project was initiated. This made comparisons of total coverage difficult. Nonetheless, an overall pattern of increasing coverage was observed during periods of stable flow (e.g., 2003) and reductions immediately after flooding.

Emergence of flowering parts of the Texas wild-rice plants in the San Marcos Springs/River ecosystem is less common in the lower sections of the study area. The two plants considered to be in vulnerable habitat in the Thompson's Island area had a small percentage of emergence during most samples in 2000-2004. It appears that the current was rarely conducive to permit emergence in that area; lower flow conditions may be necessary for emergence and flowering of these plants and other plants in similar habitats in the lower reaches of the species' habitat range. As might be expected, emergence was generally higher in the spring and fall of each year, but the trends were not distinct and emergence was observed during each season. The high-flow conditions probably influenced the seasonal patterns and appeared to stimulate emergence after the flooding that occurred in the fall of 2001. Based on these observations, it seems likely that lower flow conditions will result in greater emergence of flowering parts in Texas wild-rice plants, but other concerns such as herbivory, water depth, and buildup of vegetation mats may be important when flows decrease to very low levels.

Exposure of the roots of Texas wild-rice plants appeared strongly related to flooding and scour, as might be expected. While the exposure of roots may not necessarily translate into any significant physiological impact to the plants, it is possible that the relative proportion of roots exposed prior to a flooding event may have a strong influence on the proportion of plants that are scoured during that event. In addition, the lack of low-flow data does not allow for an accurate assessment of the frequency or potential impacts of root exposure during those conditions. Although the proportion of exposed roots was much higher after high-flow events, the exposure tended to be short-lived during 2000-2004. These data, combined with information on total coverage of Texas wild-rice during 2000-2004 suggest that high-flow events have acute impacts on the species, but long-term benefits of these events may be more substantial. Nonetheless, there is little information on root exposure during low-flows and gathering that information is important to make a complete assessment of root exposure under variable flow conditions in the San Marcos Springs/River ecosystem.

Herbivory and stand depth observations provide little reason for concern under the flow conditions in 2000-2004. Evidence of herbivory was rare and water depth was less than 0.5 feet over any Texas wildrice plants in only a few instances. These variables may become more of a concern during lower flows when a greater portion of the Texas wild-rice plants will be in shallow water and a greater portion of the plants exposed to herbivores.

Fountain Darter Sampling

Drop Net Data

Importance of aquatic vegetation

Vegetation is clearly a key factor in the abundance of fountain darters within any area, as evidenced by the rarity of sampling the species over bare substrates. However, the quality of habitat available to fountain darters is not as high in the San Marcos Springs/River ecosystem as in the Comal Springs/River ecosystem. Also, there is no substantial difference in suitability among vegetation types in the San Marcos Springs/River ecosystem as opposed to the wide variation observed in the Comal Springs/River ecosystem. There was some difference in suitability between the abundant (exotic) Hydrilla and the much less common (native) Cabomba; but the latter occurs only in areas that have a specific set of physical requirements (minimal flow, depositional areas with deep, silty substrates) and will never be abundant in the river. From the perspective of vegetation as a primary factor in the habitat requirements of fountain darters, it is not as important to maintain the highest quality vegetation types or focus on certain reaches as higher quality in the San Marcos River as in the Comal River. In the San Marcos Springs/River ecosystem, habitat requirements for the fountain darter appear to include sufficient flows to maintain vegetation, regardless of the type, in all reaches in order to maintain usable habitat. The variable flows that occurred during 2000-2004 did not substantially alter the abundance or composition of vegetation in the City Park Reach and changes in the other two reaches were short-lived with rapid regrowth occurring in scoured areas. The lack of available data on low-flow conditions; however, has not allowed an assessment of whether there may be a greater disparity in suitability among vegetation types during these conditions or whether filamentous algae may increase and affect these species/habitat relationships. It is also possible that other components of habitat for the species (i.e., water quality parameters) may become more important in defining the suitability of areas as habitat for the fountain darter during low-flow conditions. These considerations will be explored with additional data collected during future low recharge events.

Abundance Estimates

As described above, the variable flows during 2000-2004 did not have a dramatic influence on the total amount of vegetation in the San Marcos Springs/River ecosystem and population estimates based on this

data were relatively consistent over time. There were slight decreases in the population estimates for the two sample reaches (City Park and IH-35 Reaches) combined after each high-flow event, but the regrowth of vegetation was rapid and population estimates returned to pre-flood conditions rapidly. However, one important consideration is that these estimates are dominated by the vegetation composition of the City Park Reach. Relatively substantial changes occurred in the vegetation composition of the IH-35 Reach. Although these were short-lived due to rapid re-growth of the vegetation, there were acute changes in the vegetation that clearly affected the fountain darter population in this reach. The IH-35 Reach is representative of the patchy habitat (vegetation) that occurs from the Rio Vista dam downstream to the lower boundary of the fountain darter range, whereas the City Park Reach is representative of the abundant habitat (vegetation) that occurs upstream of the Rio Vista dam to the University Drive Bridge. Overall, fountain darter abundance may be more variable in areas downstream of Rio Vista Dam, but a much higher proportion of fountain darter habitat occurs upstream of the dam in areas that are less dramatically influenced by high flows. More data are needed to evaluate the influence of low flows on these relationships.

Population modeling

This modeling effort will ultimately provide a greater ability to assess changes in the fountain darter population in response to changes in discharge than estimates of population abundance based solely on vegetation. The major shortcoming to the model results is the limited data during low discharge. All of the significant relationships observed between fountain darter density and total discharge in the San Marcos Springs/River ecosystem were largely influenced by just a few data points from low discharge conditions (approximately 120 cfs).

The slopes of each of the significant and near-significant relationships suggest that the fountain darters will use these habitats more commonly when discharge decreases. This observation suggests that habitat is more favorable for fountain darters at lower flows (though only down to the minimum flows observed in this study). This is probably a result of the vegetation being able to grow more vigorously and provide denser habitat for fountain darters, which presumably provides greater protective cover and may allow greater availability of food. It is important to note that only a few data points were collected at these lower-flow conditions, and that they occurred during the early part of this study, before the frequent flooding conditions that have occurred from 2000-2004. These higher flow conditions have repeatedly scoured the vegetation and may have also temporarily reduced the fountain darter populations. This fluctuation may not have allowed the population to return to levels that occurred during the lower (relatively stable) flows that occurred early in the study. In addition, these data suggest that conditions for the fountain darter may be more suitable at lower flows in the San Marcos Springs/River ecosystem down to some threshold point, below which conditions decline. Since there is little data at low discharge, that possibility can not be evaluated at this time. More data are needed to fully explore all of these relationships before hypotheses are developed and evaluated.

Although most of the other variables that were evaluated individually indicated significant differences in fountain darter density, there was substantial variability in the density values and most had very low R² values. Unlike in the Comal Springs/River ecosystem, there were two parameters that had relatively high R² values, substrate composition and water velocity at 15 cm above the substrate. This suggests that fountain darters are more sensitive to these parameters in the San Marcos Springs/River ecosystem and their distribution is more substantially affected by these parameters. Nonetheless, most variables did not greatly influence the fountain darter distribution. Thus, we believe that a large part of the reason for the low R² values is that during normal-to-high discharge conditions, such as those experienced in 2000-2004, the distribution and abundance of fountain darters is largely influenced by the availability of

high quality aquatic vegetation. When discharge declines to levels below those experienced during 2000-2004, certain variables such as temperature and dissolved oxygen may become more important in determining the distribution and abundance of the fountain darter in the San Marcos Springs/River ecosystem. As more low-flow data are collected, these relationships and potentially additional models to incorporate multiple variables, will be explored further. The low-flow data will be critical to improve our understanding of which variables contribute the most to fountain darter distribution and abundance.

Size-class distribution

The distribution of fountain darters in the San Marcos Springs/River ecosystem is shifted towards larger fish than those observed in the Comal Springs/River ecosystem with a peak between 30 – 32 mm compared to a peak between 22 – 26 mm. This difference may be a function of lower quality habitat in the two sampled reaches compared with the Comal Springs/River ecosystem reaches. Also, currents tend to be stronger in the San Marcos River reaches and may contribute to finding larger, but fewer, fish. Dip-net data from Spring Lake suggests that smaller individuals are present in high numbers there. This high-quality fountain darter habitat in the headwaters of the San Marcos River may be more comparable to sites in the Comal River than the two downstream reaches that were sampled with drop nets. If drop net sampling were possible in Spring Lake and data collected added to that from the other two reaches, it would likely yield a size-class distribution similar to that of the Comal River ecosystem (based on the range of sizes observed during SCUBA surveys of San Marcos salamanders).

Other Species

There is a wide range of species present in the San Marcos River and most of the introduced species are relatively uncommon. Diversity is consistent between the two sample reaches and did not differ in any discernable pattern over time. Patterns of community composition will be examined more closely at lower discharge to determine whether species diversity is altered under certain flow conditions.

The exotic giant ramshorn snail was only sampled in the first sample in the San Marcos Springs/River ecosystem during relatively low flows in 2000 and again in 2004, but the total number was very low. By all indications the densities of giant ramshorn snails observed in the San Marcos Springs/River ecosystem during the study period pose no serious threat to the aquatic vegetative community. However, because of the impact that this exotic species can have at higher densities, close monitoring of this should continue into the foreseeable future.

Dip-Net Data

One of the greatest values of the dip net sampling is the ability of this gear to sample small fountain darters, which can be an indicator of recent reproduction. The observation of these small fountain darters during all samples in the Hotel Reach (headwaters of the San Marcos River) indicate that some level of reproduction can occur year-round. In contrast, it appears that availability of suitable habitat may be important in stimulating reproduction in the City Park and IH-35 Reaches and that reproduction is event-driven and stimulated by certain conditions. There may be little influence of season on reproduction of fountain darters in the San Marcos River. Data from 2001-2002 suggest that the proper conditions at any time of year may stimulate reproduction since evidence of recent reproduction was observed in those samples following flooding. During and immediately after flooding, shoreline habitat is inundated with the higher flow and appears to provide a refuge for fountain darters (based on qualitative observations by Dr. T. Brandt, USFWS). These conditions may stimulate reproduction.

According to BIO-WEST project team observations, the algae present in the Hotel Reach (Spring Lake) provides the highest quality habitat for the fountain darter within the San Marcos River by supplying excellent cover and an abundance of food (visual observations only). Although drop net sampling is not conducted in the Hotel Reach, dip-net sampling and observations during every San Marcos salamander SCUBA survey supports this statement. The City Park and IH-35 Reaches both maintain lower quality habitat, and the number of fountain darters collected with dip nets in each reach was less than in Spring Lake, despite greater collection times. Also, fountain darters >35 mm in length are rare in Spring Lake (two were collected in 2002, none in 2003, and 3 in 2004) while fountain darters of this size-class were commonly collected in the City Park (except one) and IH-35 Reaches. This supports the hypothesis that the higher currents in these habitats select for larger-bodied individuals. Like the apparent relationship between habitat quality and reproduction, the correlation between habitat quality and fountain darter size is an interesting observation that will be explored in greater detail as more low-flow data are gathered to evaluate the dynamics of the relationship.

San Marcos Salamander Visual Observations

The abundance of algae in sample areas within Spring Lake may have affected density estimates of San Marcos salamanders in these habitats because the area had to be cleared prior to sampling activities. This disturbance may have startled salamanders and caused them to move or may have alerted the San Marcos salamanders to the presence of the divers and caused some individuals to retreat into deeper cavities within the rocks. It is also possible that a significant portion of the San Marcos salamander population that would have been found under rocks was instead occupying the algae over top of the rocks during these times. Nonetheless, San Marcos salamanders continue to be abundant under rocks despite the presence of this algae and a thriving San Marcos salamander population has been observed in the two Spring Lake sample areas throughout the study to date. The algae is probably very important habitat (though difficult to sample quantitatively) and potentially provides increased three-dimensional habitat for the salamanders to disperse into.

The observation of higher numbers of San Marcos salamanders in sample area 21 (the eastern spillway of Spring Lake dam) during the fall of 2000 was likely a result of sampling methodology rather than a substantial difference in the San Marcos salamander population at that time. Sampling during lower-flow conditions is much easier than during higher flows when turbulence reduces visibility and the observer's effectiveness. The higher numbers observed in all 2004 samples in that site appeared to be a legitimate increase in the population in the area. There have been changes in the physical characteristics in this area in 2000-2004, which may have influenced these changes. Rocks piled in one area have been deflecting most of the current around one part of this site that has changed the habitat condition. Also, there has been an increase in coverage of Texas wild-rice part of the reach. It will be important to monitor this habitat in 2005 to determine whether the increase is temporary or will persist.

Overall, the estimated population densities of San Marcos salamander have been consistent throughout 2000-2004. A lack of substantial low-flow data precludes discussion of potential influences of lower flows on the population at this time.

Exotics/Predation Study

Discussion of the exotics/predation component of this study can be found in the annual reports for 2000, 2001, and 2002. In summary, the data reveal limited predation on any threatened or endangered species under the discharge conditions that occurred during 2000-2002. There remains the possibility that a period of low discharge may result in greater susceptibility of fountain darters or salamanders to predation, therefore predator diets will be examined when low-flow sampling is triggered.

REFERENCES

- American Public Health Association (APHA). 1992. standard methods for the examination of water and wastewater, 18th edition. American Public Health Association, Washington D.C.
- Arsuffi, T.L. 1993. Status of the Comal Springs riffle beetle (*Heterelmis comalensis*), Peck's Cave amphipod (*Stygobromus pecki*), and the Comal Springs dryopid beetle (*Stygoparnus comalensis*). Final Report to the U.S. Fish and Wildlife Service. 25 pp.
- Brandt, T. M., K. G. Graves, C. S. Berkhouse, T. P. Simon, and B. G. Whiteside. 1993. Laboratory spawning and rearing of the endangered fountain darter. Progressive Fish-Culturist 55: 149-156.
- BIO-WEST, Inc. 2002a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2001 Annual Report. Edwards Aquifer Authority. 33 p.
- BIO-WEST, Inc. 2002b. Fountain darter laboratory study: reproductive response to parasites and temperature fluctuations. Executive summary. Edwards Aquifer Authority. 2 p.
- BIO-WEST, Inc. 2003. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2002 Annual Report. Edwards Aquifer Authority. 42 p.
- BIO-WEST, Inc. 2004a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos Springs/River Aquatic Ecosystem. 2003 Annual Report. Edwards Aquifer Authority. 30 p.
- BIO-WEST, Inc. 2004b. Aquatic vegetation laboratory study: Phase 1: Observations of water quality changes and plant growth under various flows. Phase 2: Effects of carbon dioxide level on aquatic plants found in the Comal and San Marcos Springs/River Ecosystems. Final Report. Edwards Aquifer Authority. 23 p.
- Bush, P.W., A.F. Ardis, L. Fahlquist, P.B. Ging, C.E. Hornig, and J.L. Lanning-Rush. 1998. Water Quality in South Central Texas, Texas 1996-98. U. S. Geological Survey, Circular 1212.
- Crumpton, W.G. T.M. Isenhart, P.D. Mitchell. 1992. Nitrate and organic n analyses with second-derivative spectroscopy. Limnol. Oceanogr. 37(4): 907-913.
- Holmes, R.M., A. Aminot, R Kerovel, B.A. Hooker, and B.J. Peterson. 1999. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. Can. J. Fish. Aquat. Sci. 56(10): 1801-1808.
- Margalef, R. 1956. Informacion y diversidad especifica en las comunidades de organismos. Inv. Pesq., 3:99-106.
- Nelson, J. 1993. Population size, distribution, and life history of *Eurycea nana* in the San Marcos River. Thesis, Master of Science, Southwest Texas State University. 43 pp.

- Strickland, J.D.H. and T.R. Parsons. 1972. A practical handbook of seawater analysis, 2nd edition. Fisheries Research Board of Canada, Ottawa.
- Texas Parks and Wildlife Department (TPWD). 2001. An evaluation of spring flows to support the Upper San Marcos River Spring Ecosystem, Hays County, Texas. Resource Protection Division, Texas Parks and Wildlife Department, Austin, Texas.
- U.S. Fish and Wildlife Service (USFWS). 1996. San Marcos and Comal Springs and Associated aquatic ecosystems (revised) recovery plan. 121 pp.
- U.S. Geological Survey (USGS). Provisional data for Texas. Location: http://tx.waterdata.usgs.gov/niwis/help/provisional.

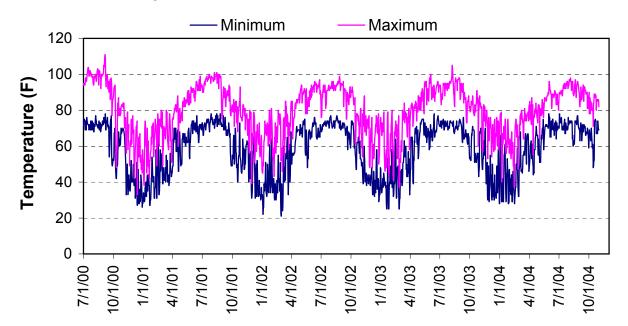
APPENDIX A: AQUATIC VEGETATION MAPS

(separate file)

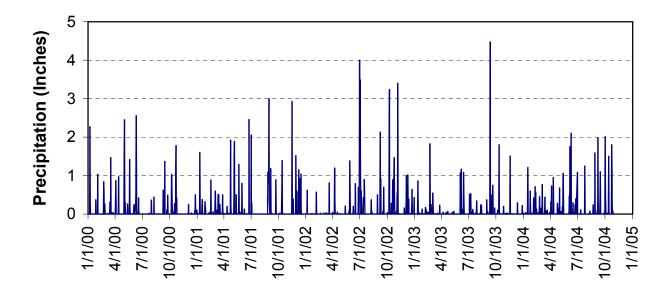
APPENDIX B: DATA AND GRAPHS

Water Quality Data and Thermistor Graphs

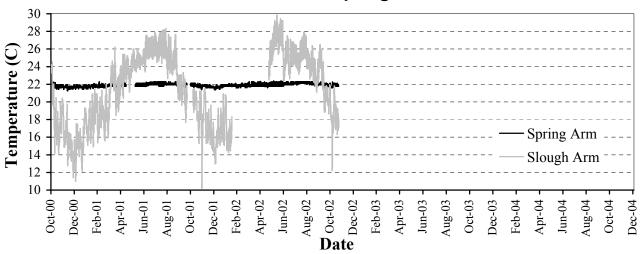
Daily Air Temperature Data for San Marcos, Texas



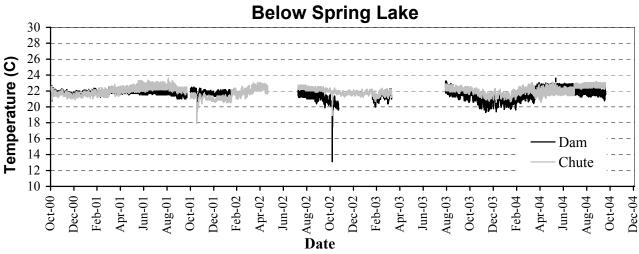
Daily Precipitation Data for San Marcos, Texas



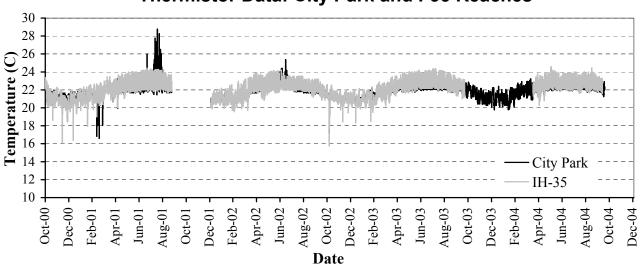
Thermistor Data: Spring Lake Sites



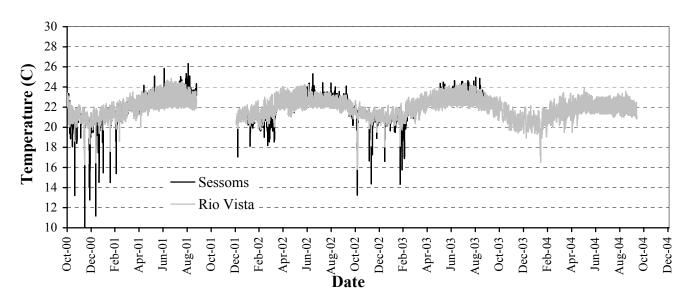
Thermistor Data: Dam and Chute



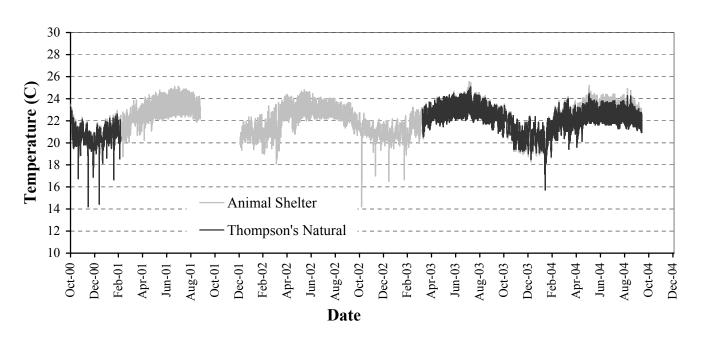




Thermistor Data: Sessoms Creek and Rio Vista Dam

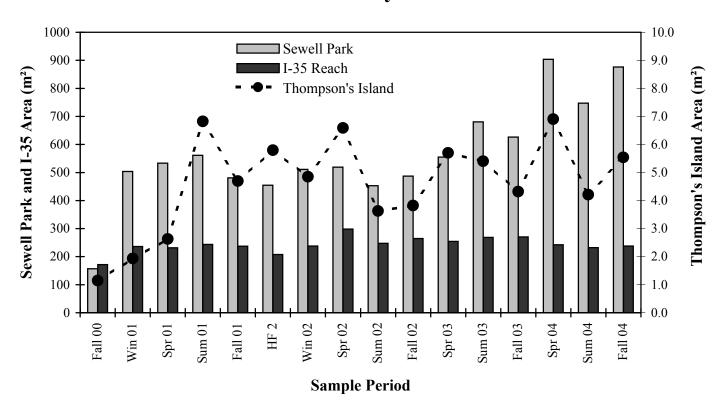


Thermistor Data: Thompson's Island and Animal Shelter

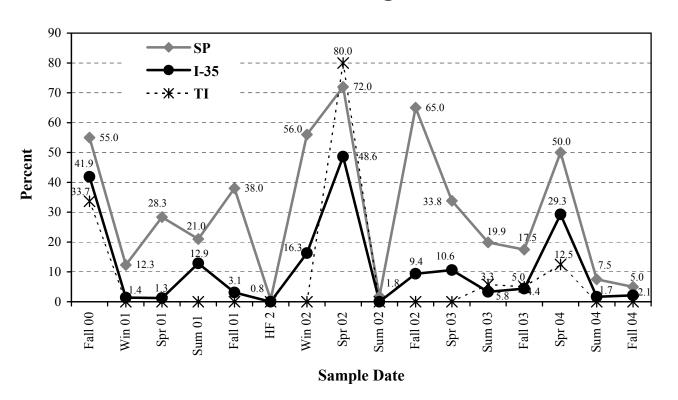




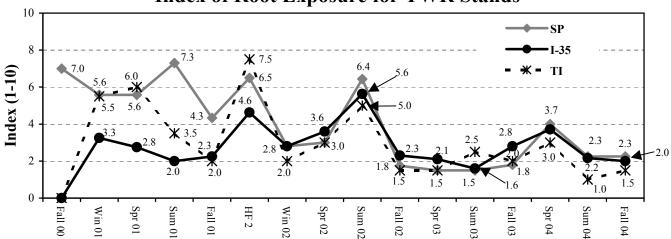
TWR Area by Season



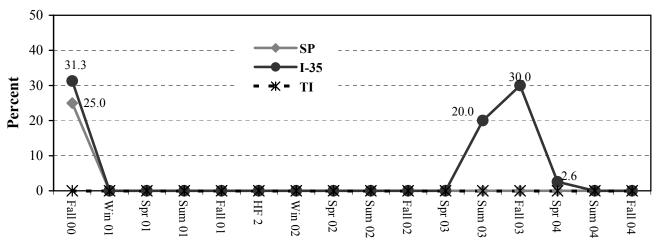
Percent Emergent TWR



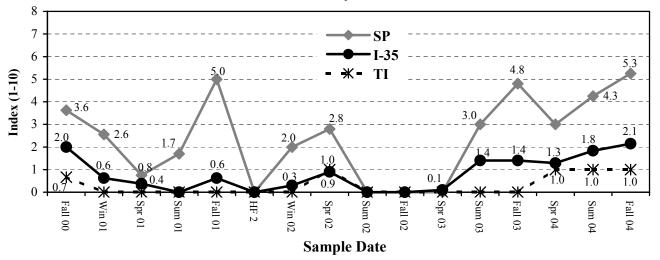
Index of Root Exposure for TWR Stands



Percent of TWR Stands < 0.5 Feet



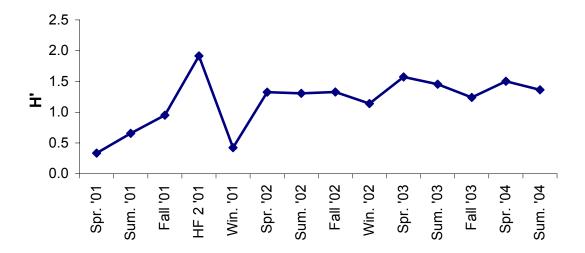
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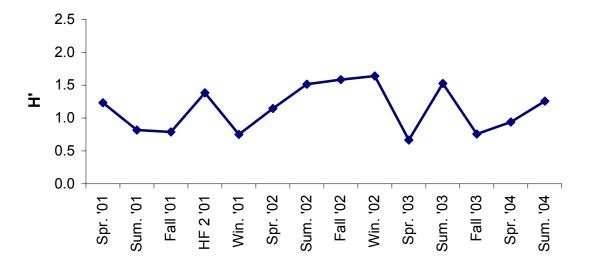
Drop Net Data:

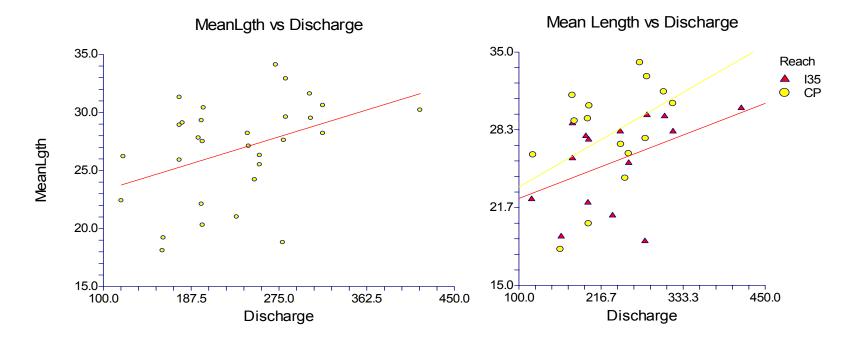
Species Diversity and Fountain Darter Mean Length

City Park Reach

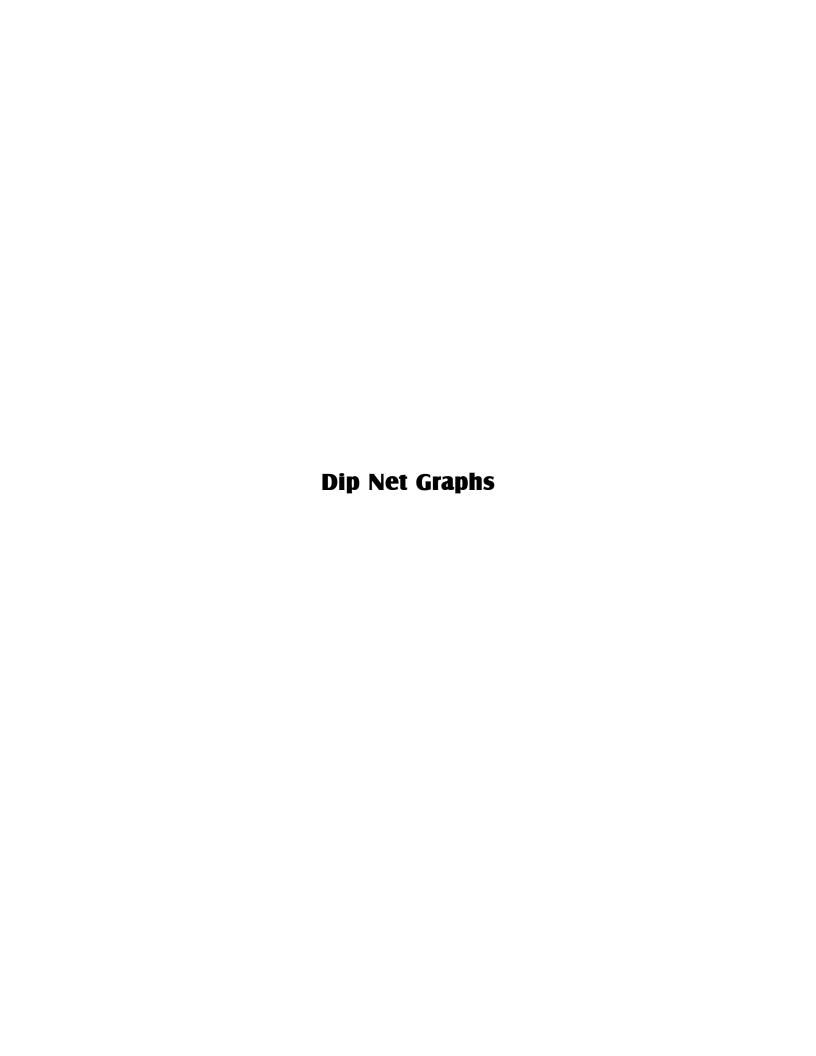


IH-35 Reach

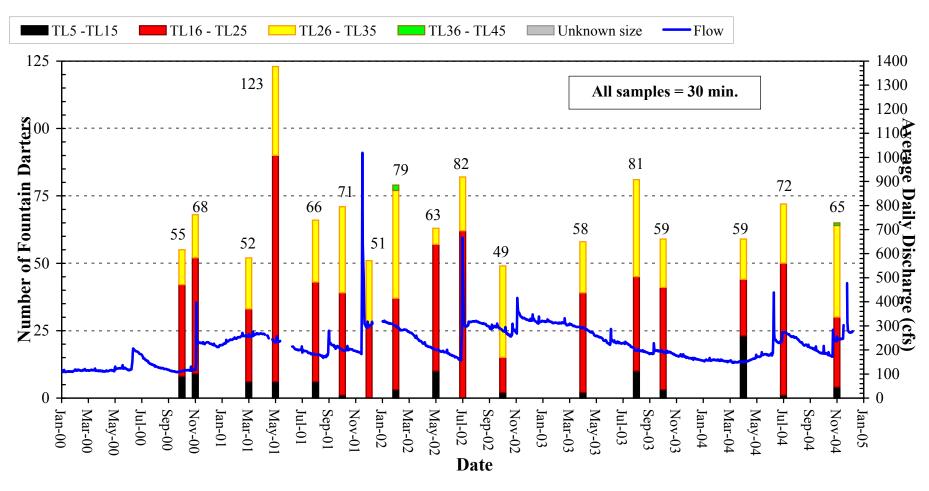




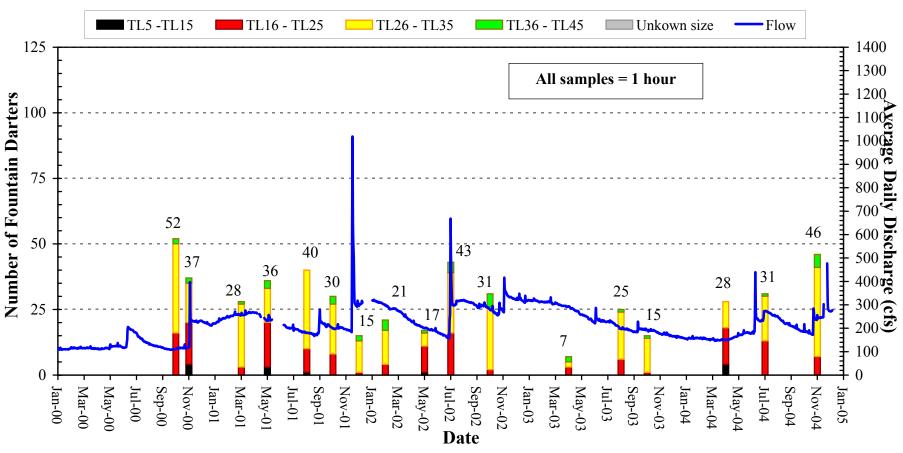
The relationship between mean length of fountain darters and total discharge in the San Marcos River (2000-2004) was significant (a) in all reaches combined (p=0.025) and (b) in each of the two sample reaches individually.



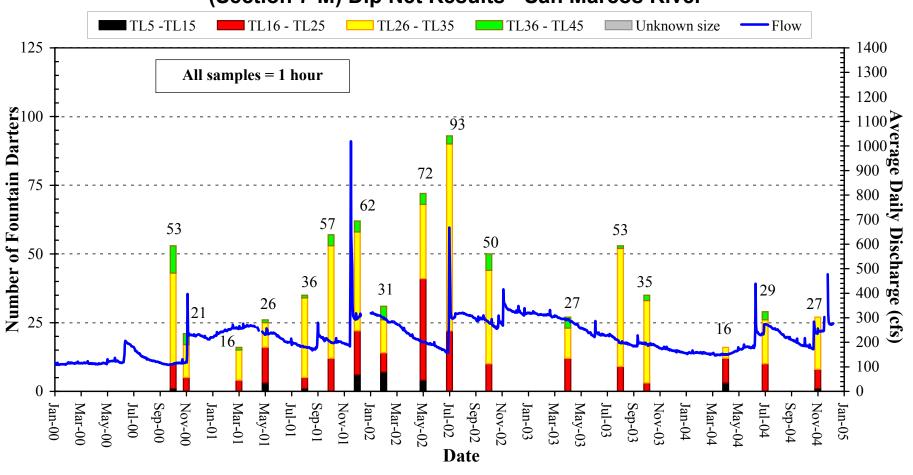
Fountain Darters Collected from the Hotel Reach (Section 1-U) Dip Net Results - San Marcos River



Fountain Darters Collected from the City Park Reach (Section 4L-M) Dip Net Results - San Marcos River



Fountain Darters Collected from the I-35 Reach (Section 7-M) Dip Net Results - San Marcos River



APPENDIX C: DROP NET RAW DATA

(not available online)

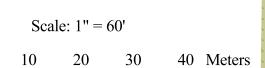
APPENDIX A: AQUATIC VEGETATION MAPS

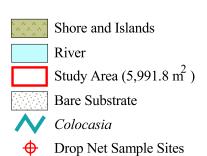


San Marcos River Aquatic Vegetation

City Park - Spring

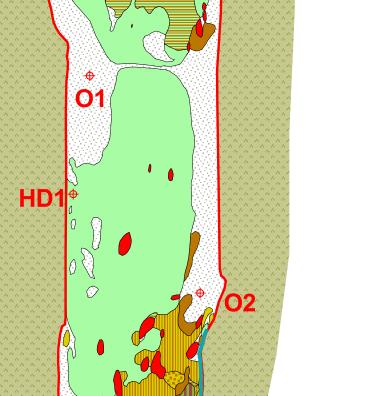
April 13, 2004





Meters ²
2,074.6
1.9
142.8
226.8
29.1
3.8
136.9
388.2
1,475.7
66.9
50.2
22.9

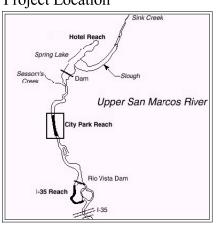
/Sagittaria

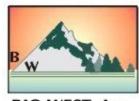


HD2



Project Location



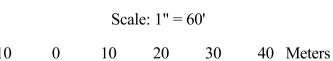


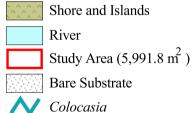
BIO-WEST, Inc.

San Marcos River Aquatic Vegetation

City Park - Summer

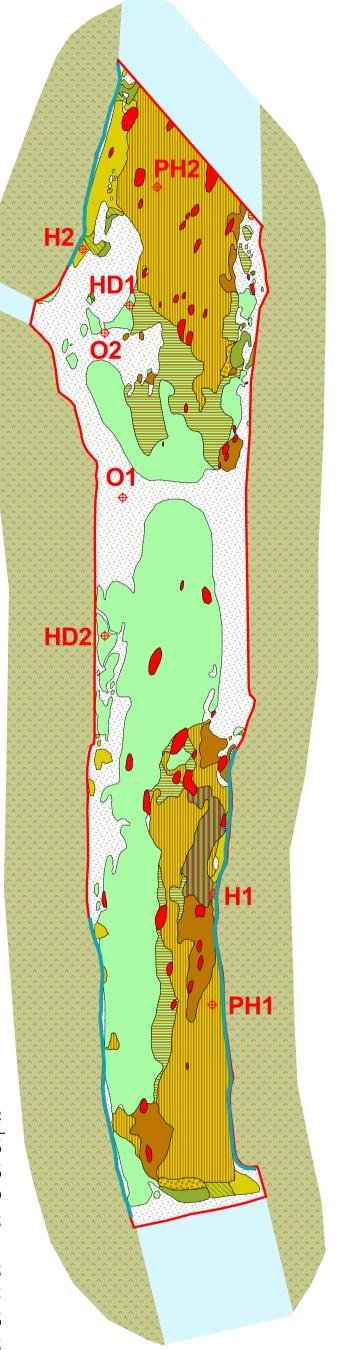
July 26, 2004





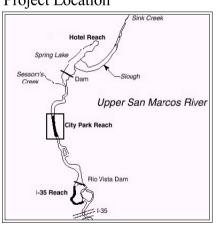
Drop Net Sample Sites

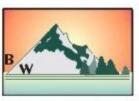
	Meters ²
<i>Hydrilla</i>	1,783.6
	154.6
Potamogeton	242.9
Sagittaria Sagittaria	51.8
Vallisneria Vallisneria	3.1
Zizania	131.8
Hydrilla / Potamogeton	432.5
Hygrophila / Potamogeton	1,413.6
Hygrophila / Sagittaria	29.8
Potamogeton / Sagittaria	97.8
Hygrophila / Potamogeton / Sagittaria	24.3





Project Location



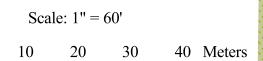


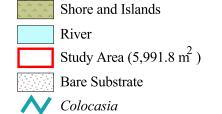
BIO-WEST, Inc.

San Marcos River Aquatic Vegetation

City Park - Fall

October 11, 2004





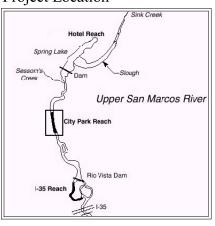
• Drop netting not conducted

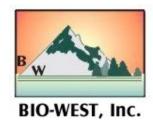
	Meters ²
<i>Heteranthera</i>	0.4
Eichonia	108.1
Hydrilla	1,651.2
<i>Hydrocotle</i>	1.9
Hygrophila	192.4
Potamogeton	306.6
Sagittaria Sagittaria	38.2
Vallisneria Vallisneria	1.5
Zizania	140.3
Hydrilla / Potamogeton	446.5
Hygrophila / Potamogeton	1,391.1
Hygrophila / Sagittaria	14.3
Potamogeton / Sagittaria	95.9
Hygrophila / Potamogeton	24.6

/Sagittaria

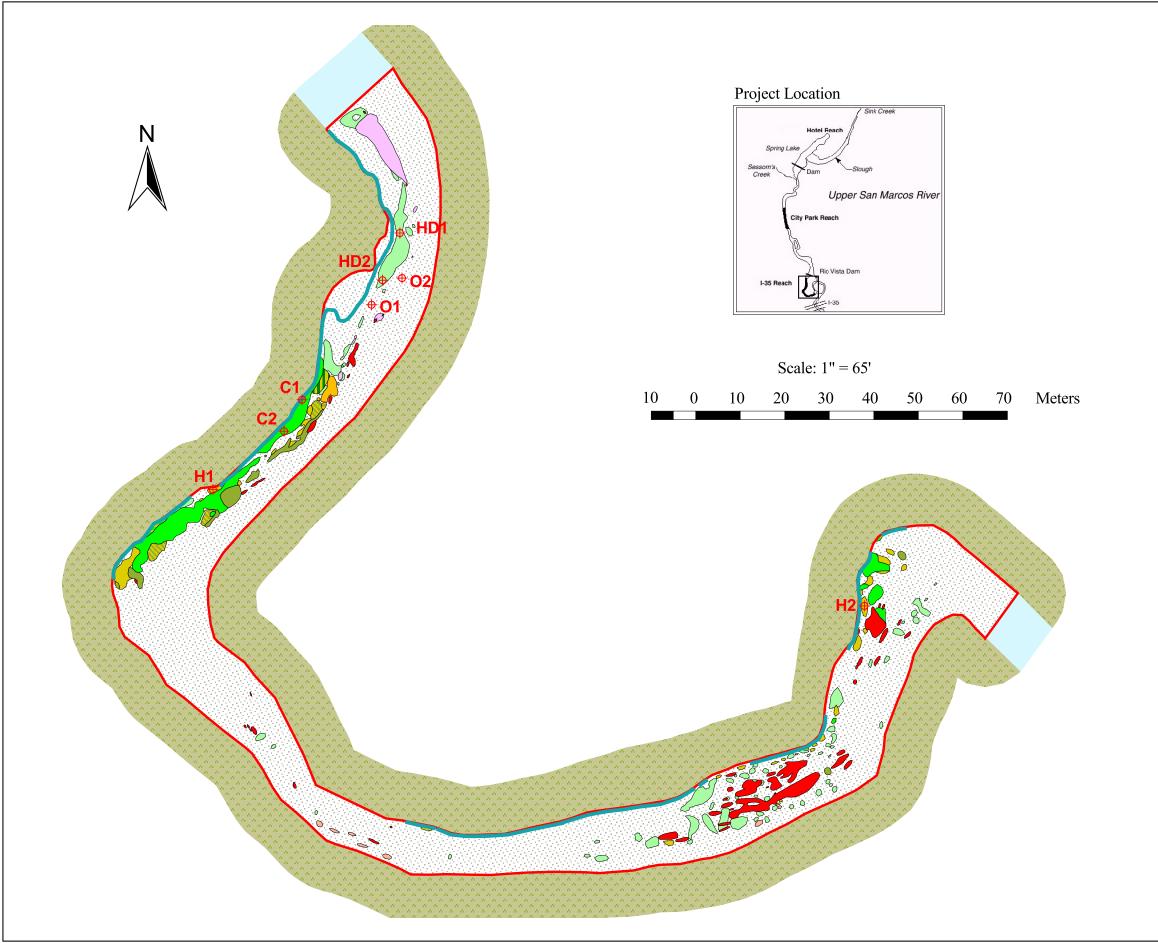


Project Location









San Marcos River Aquatic Vegetation I-35 Reach - Spring

April 14, 2004

0.00	C1	1	T 1	1
60.00	Shore	and	ICI21	าปร
	DITOLO	ana	DIG	IUO

River

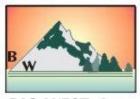
Study Area (4,616.3 m²)

Bare Substrate

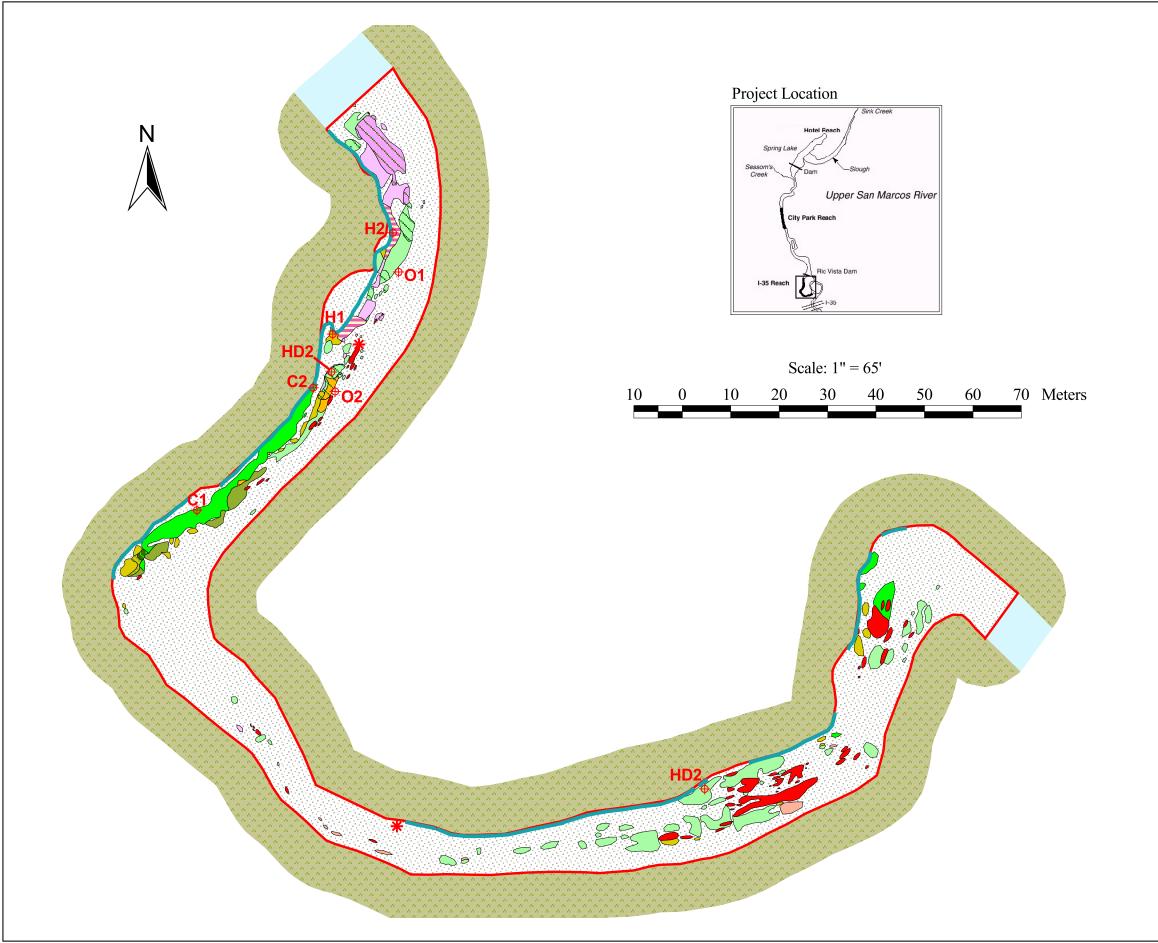
N Colocasia

Drop Net Sample Sites

	Meters ²
Algae	0.5
Cabomba	157.3
Heteranthera	6.9
Hydrilla	189.4
	57.3
J usticia	52.4
Ludwigia	10.7
Sagittaria Sagittaria	45.6
Vallisneria	0.3
Z izania	129.8
	21.3
=== Sagittaria / Ludwigia	1.2
Nydrilla / Algae	0.5
IIIII Hydrilla / Hygrophila	7.9
Cabomba / Hygrophila	5.0



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San Marcos River Aquatic Vegetation I-35 Reach - Summer

July 27, 2004

Shore and Islands

River

Study Area (4,616.3 m²)

Bare Substrate

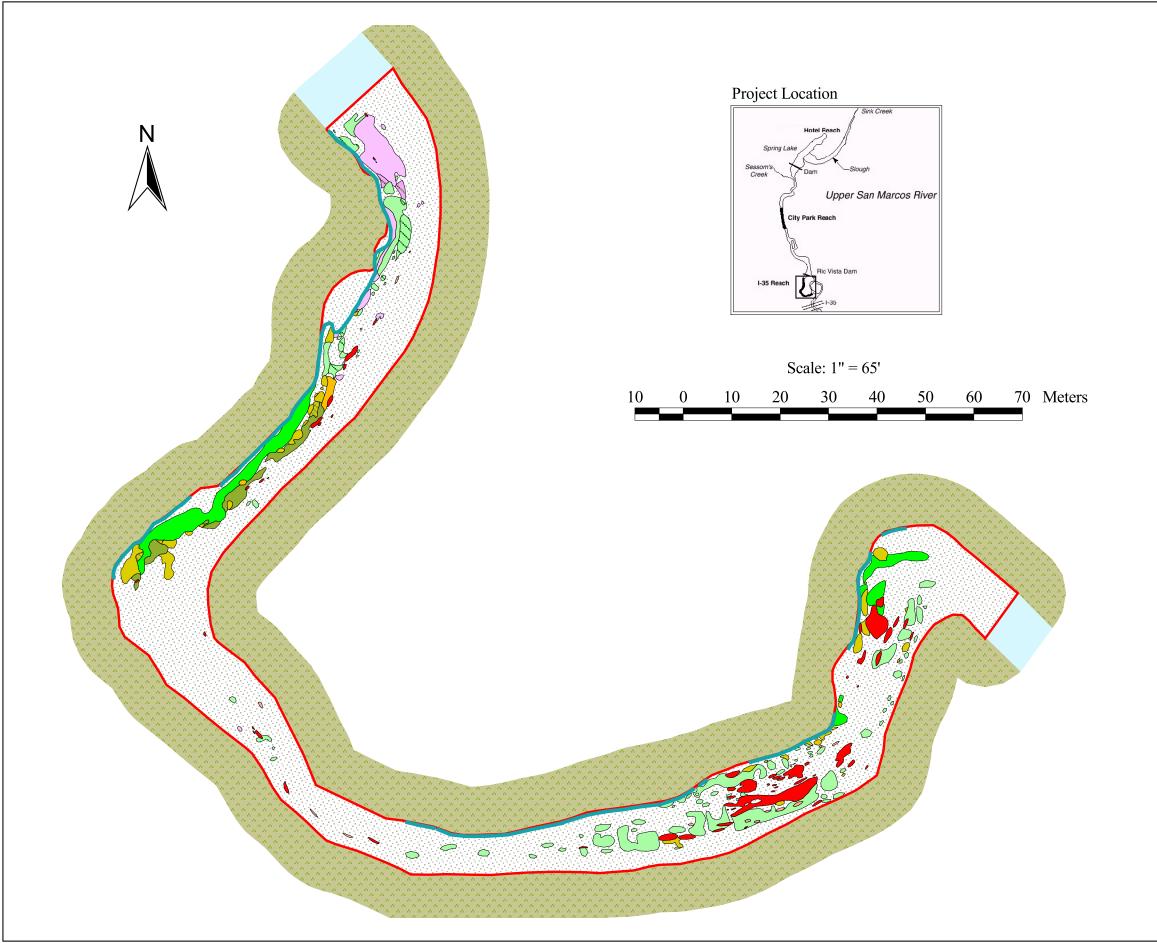
N Colocasia

Drop Net Sample Sites

	Meters ²
Algae	2.3
Cabomba	154.9
Heteranthera	13.3
Hydrilla	272.9
	50.8
Justicia	56.5
Ludwigia	5.2
Sagittaria	38.5
Z izania	118.0
	4.0
∭ Hydrilla / Algae	38.2
Nygrophila / Algae	1.6
🚃 Hygrophila / Justicia	29.0
📉 Justicia / Algae	62.3
🚫 Ludwigia / Algae	6.9



BIO-WEST, Inc.



San Marcos River Aquatic Vegetation I-35 Reach - Fall

October 12, 2004

Shore and Islands

River

Study Area (4,616.3 m²)

Bare Substrate

N Colocasia

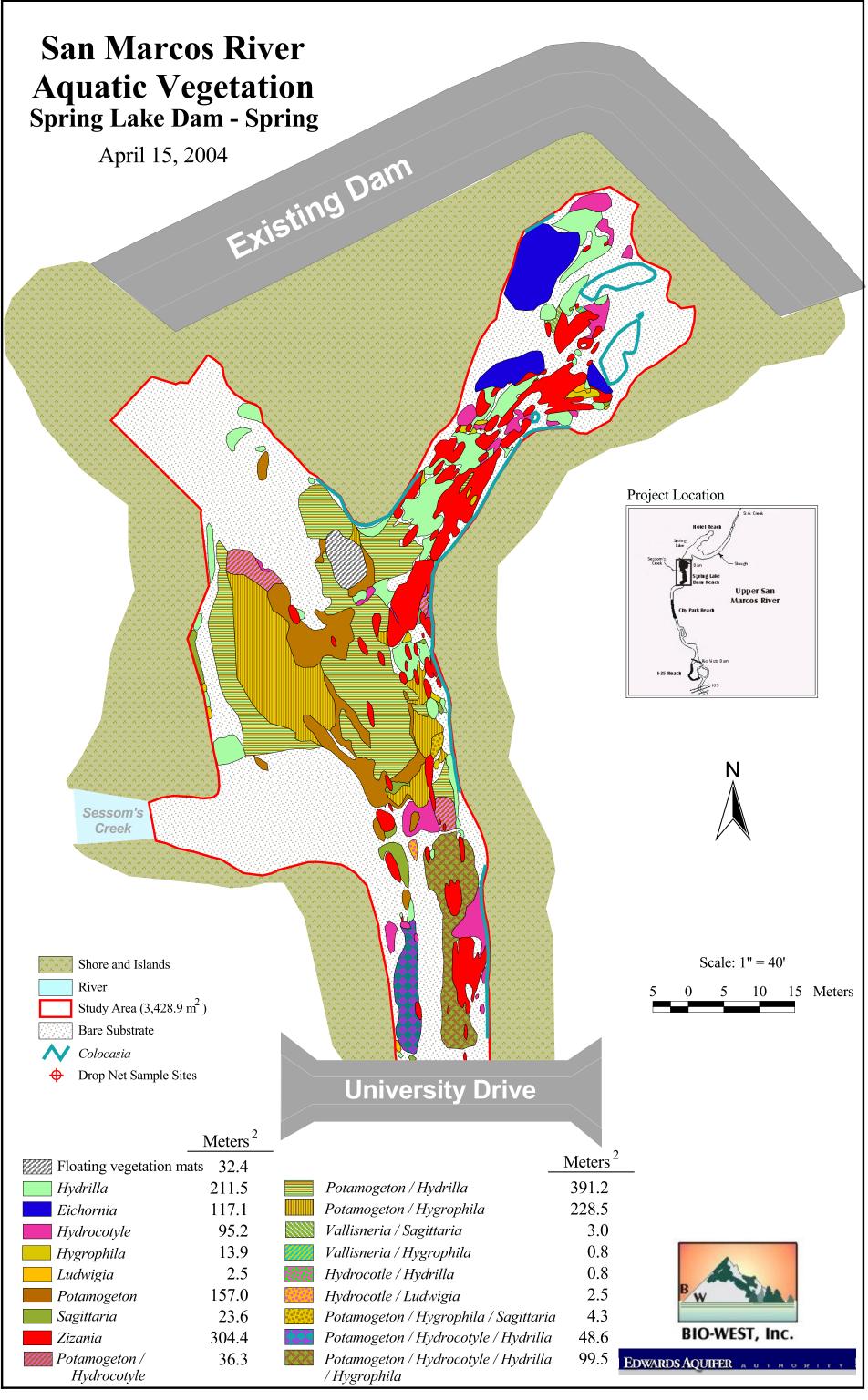
Drop Net Sample Sites

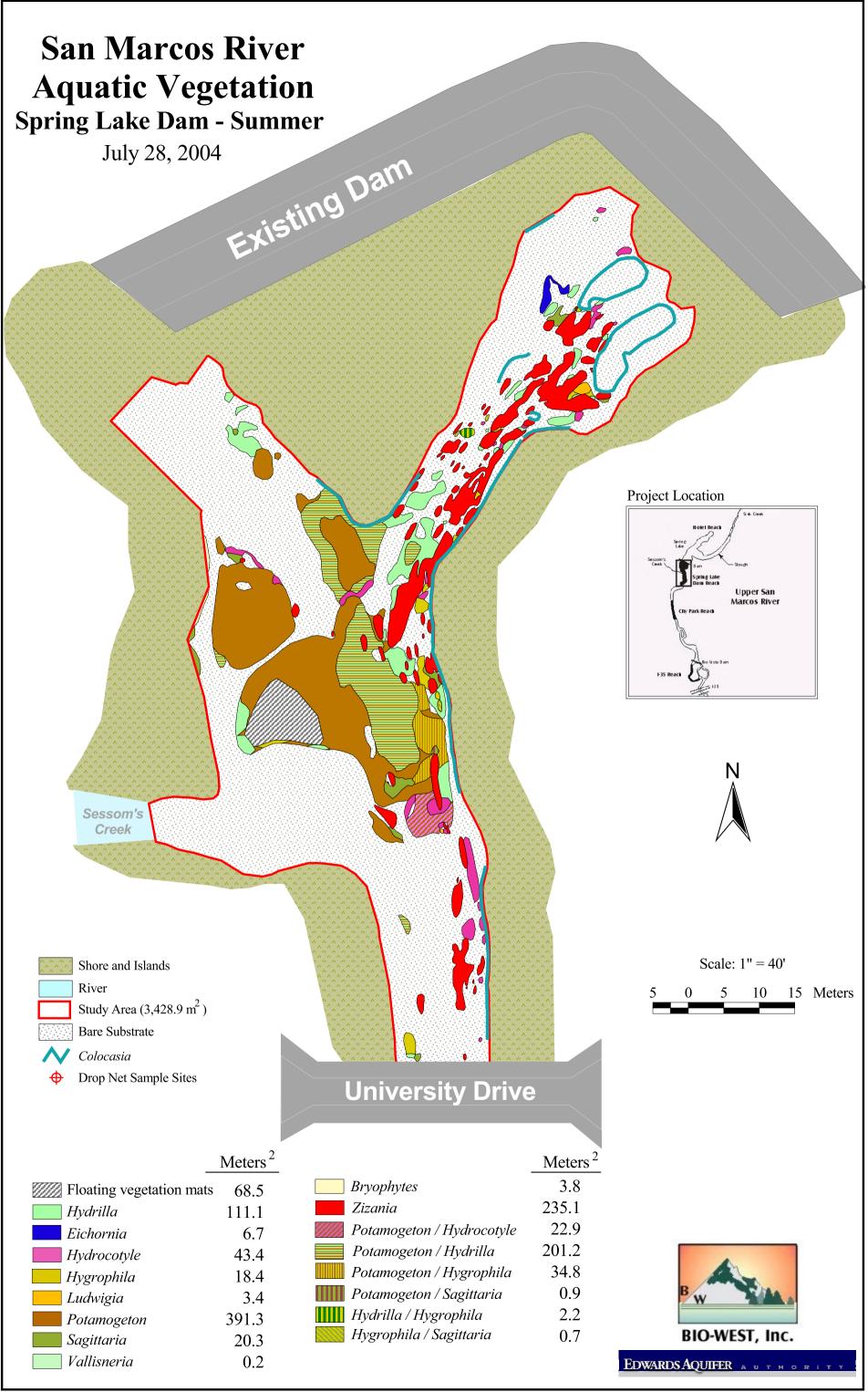
	Meters ²
Cabomba	182.5
— Heteranthera	6.2
	295.3
	90.7
Justicia — — — — — — — — — — — — — — — — — — —	98.9
Ludwigia	10.9
Sagittaria Sagittaria	61.2
Z izania	130.6
	6.2
Nydrilla / Algae	34.8
📉 Justicia / Algae	7.8
🔣 Hydrilla / Hydrocotle	0.4

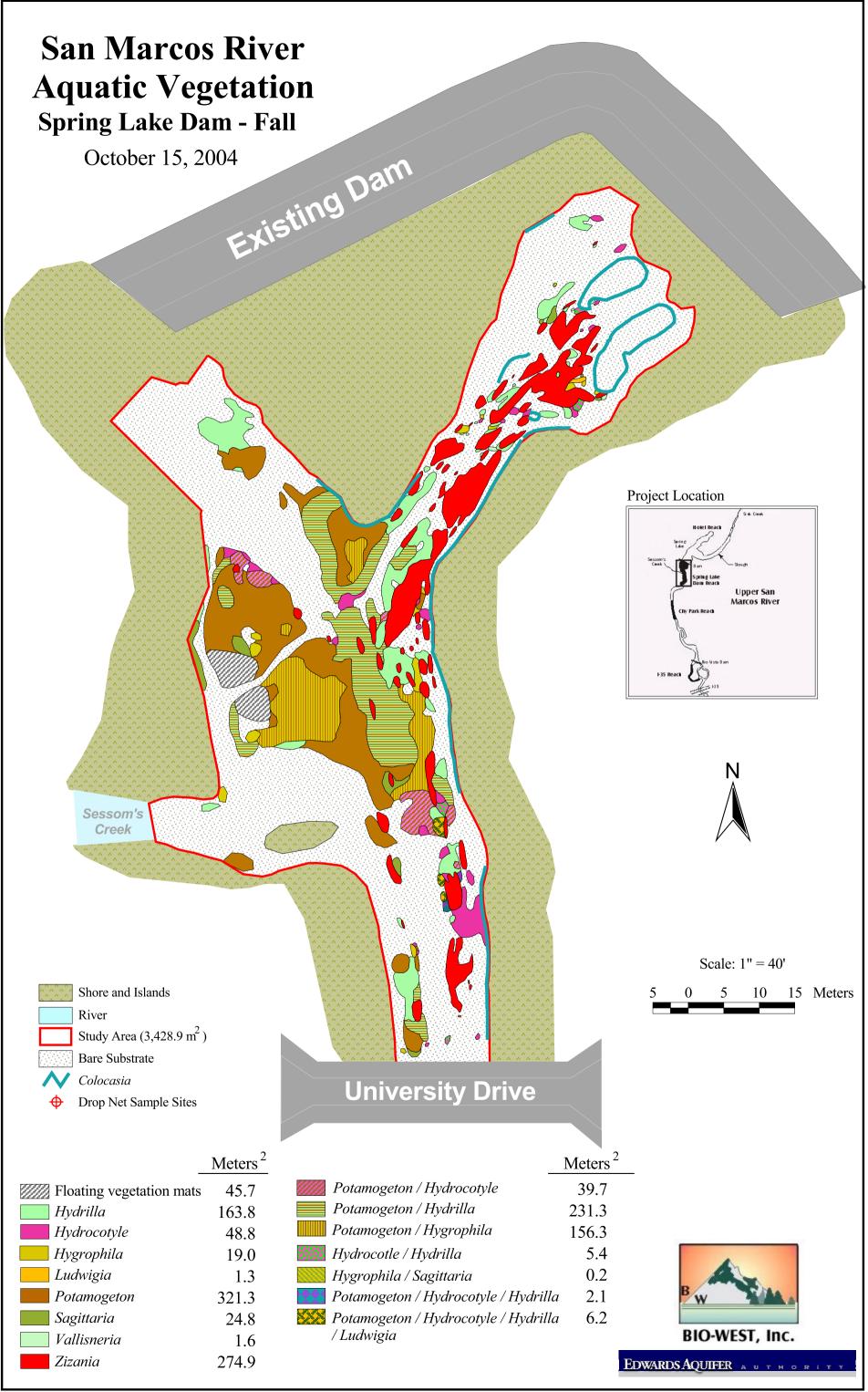


BIO-WEST, Inc.

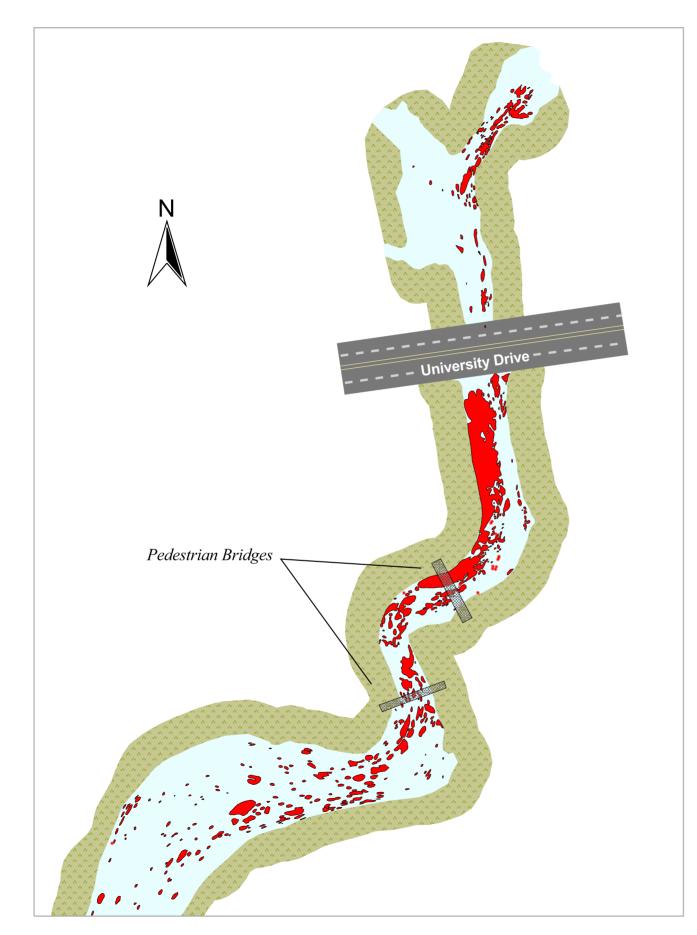












(Zizania texana)

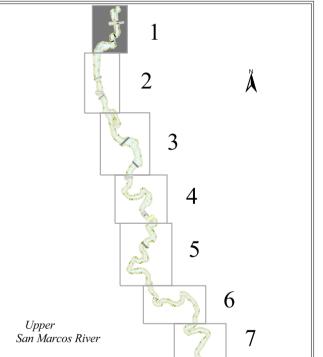
Summer 2004 - Map 1 of 7

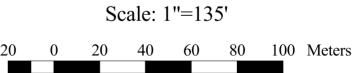
July 19 - July 21, 2004

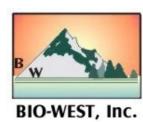
 $Zizania \qquad \frac{\text{Map 1 (m}^2)}{1,896.2} \quad \frac{\text{Total Population (m}^2)}{3,145.3}$

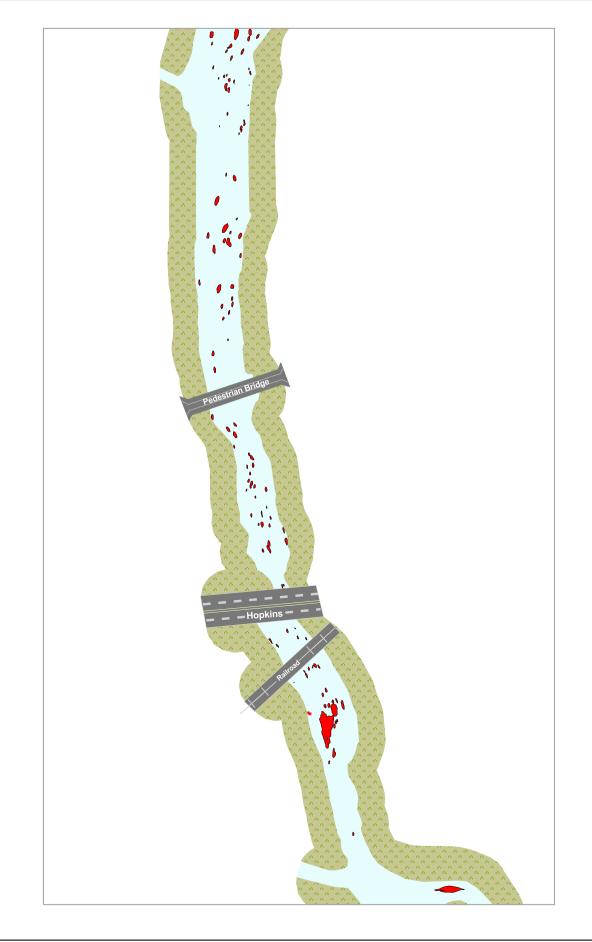
* small Zizania plants

Project Location









(Zizania texana)

Summer 2004 - Map 2 of 7

July 19 - July 21, 2004



Scale: 1"=175'

80 100 120 140 160 Meters

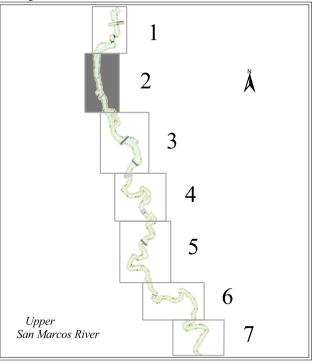
40 60

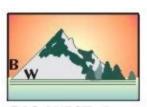
Zizania

 $\frac{\text{Map 2 (m}^2)}{374.9} \frac{\text{Total Population (m}^2)}{3,145.3}$

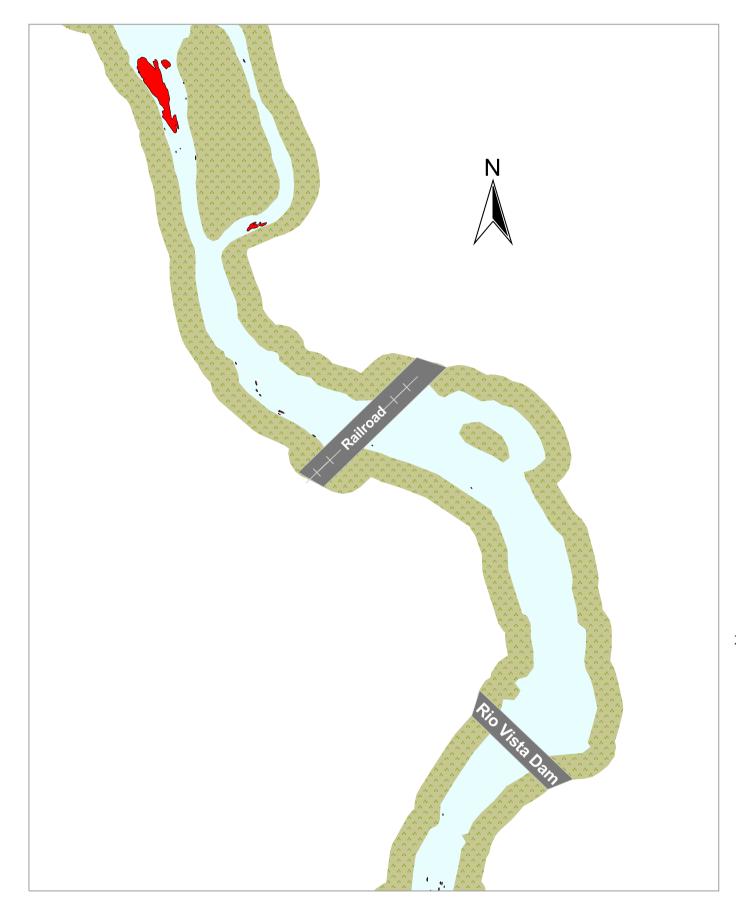
* small Zizania plants

Project Location





BIO-WEST, Inc.



(Zizania texana)

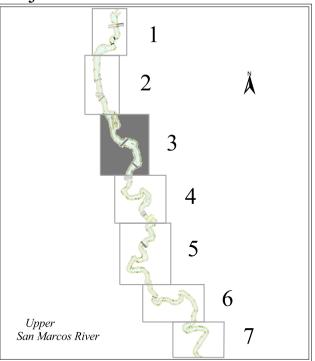
Summer 2004 - Map 3 of 7

July 19 - July 21, 2004

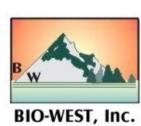
 $Zizania \qquad \frac{\text{Map 3 (m}^2)}{392.8} \quad \frac{\text{Total Population (m}^2)}{3,145.3}$

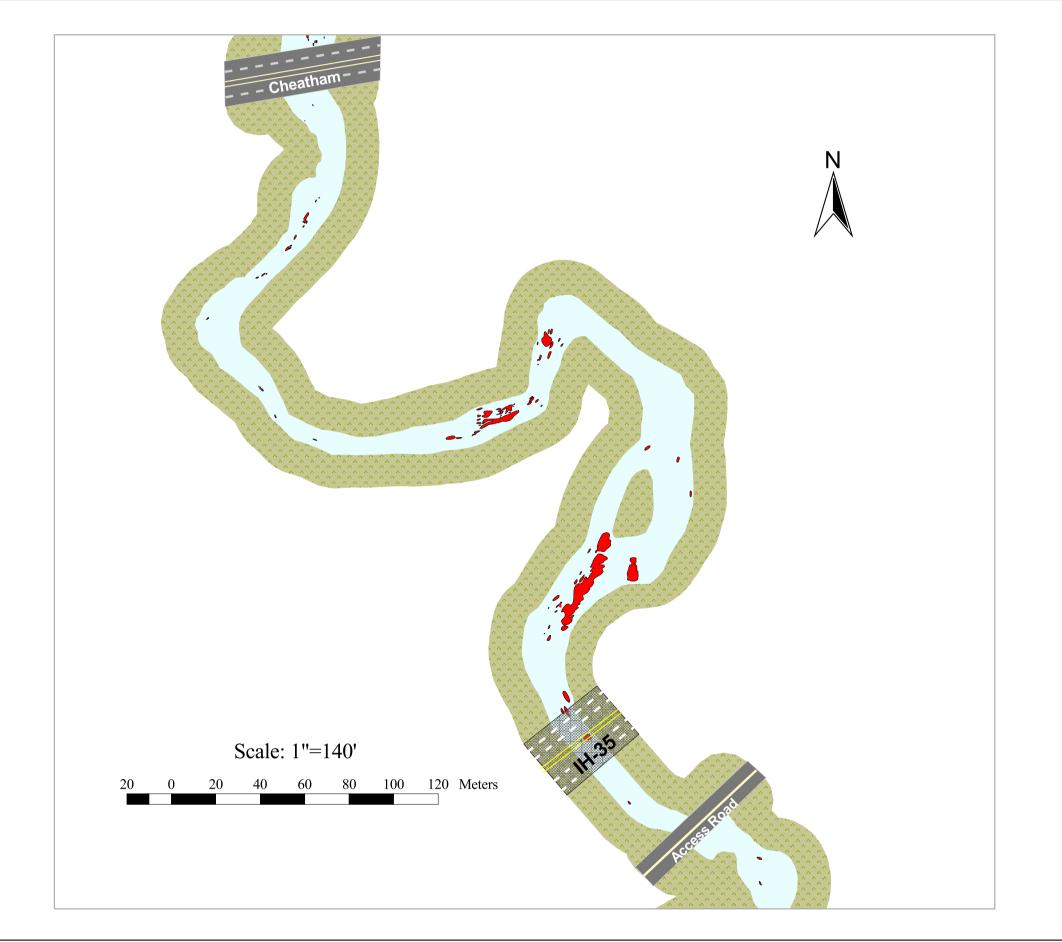
* small Zizania plants

Project Location



Scale: 1''=180'
20 0 20 40 60 80 100 120 140 Meters





(Zizania texana)

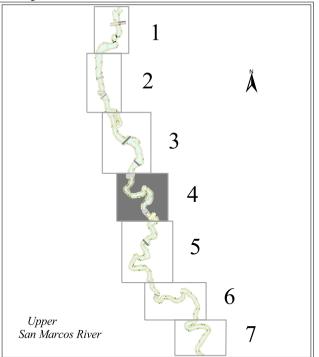
Summer 2004 - Map 4 of 7

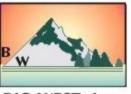
July 19 - July 21, 2004

 $Zizania \qquad \frac{\text{Map 4 (m}^2)}{403.3} \quad \frac{\text{Total Population (m}^2)}{3,145.3}$

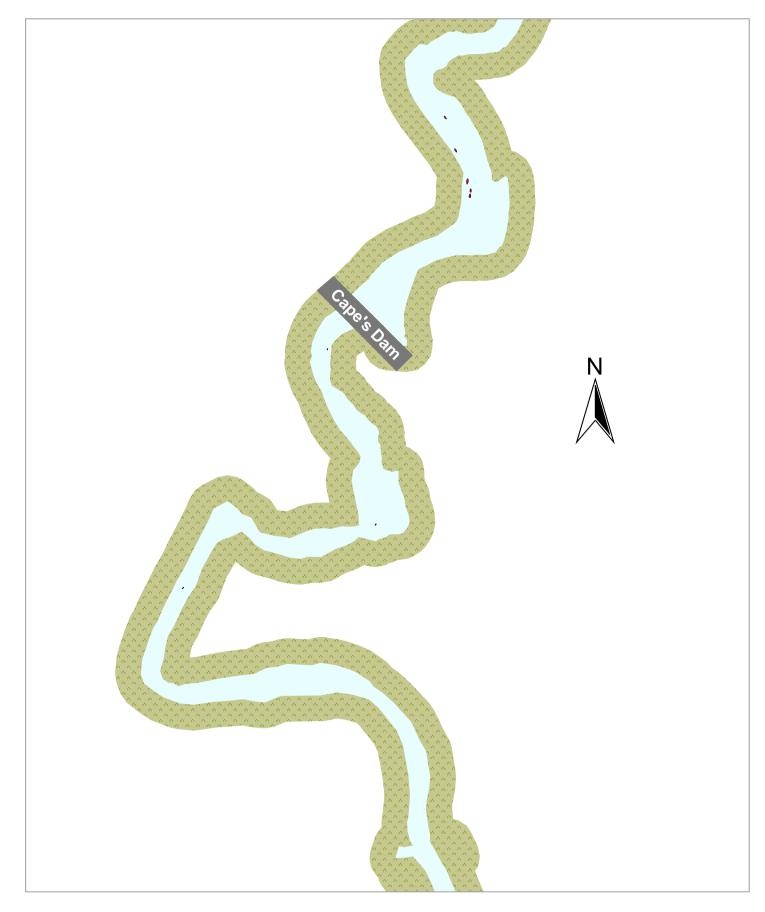
* small Zizania plants

Project Location





BIO-WEST, Inc.



(Zizania texana)

Summer 2004 - Map 5 of 7

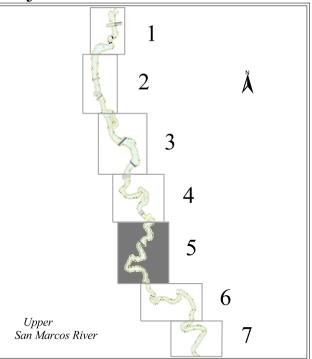
3,145.3

July 19 - July 21, 2004

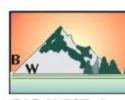
Map 5 (m²) Total Population (m²) Zizania 8.4

***** small *Zizania* plants

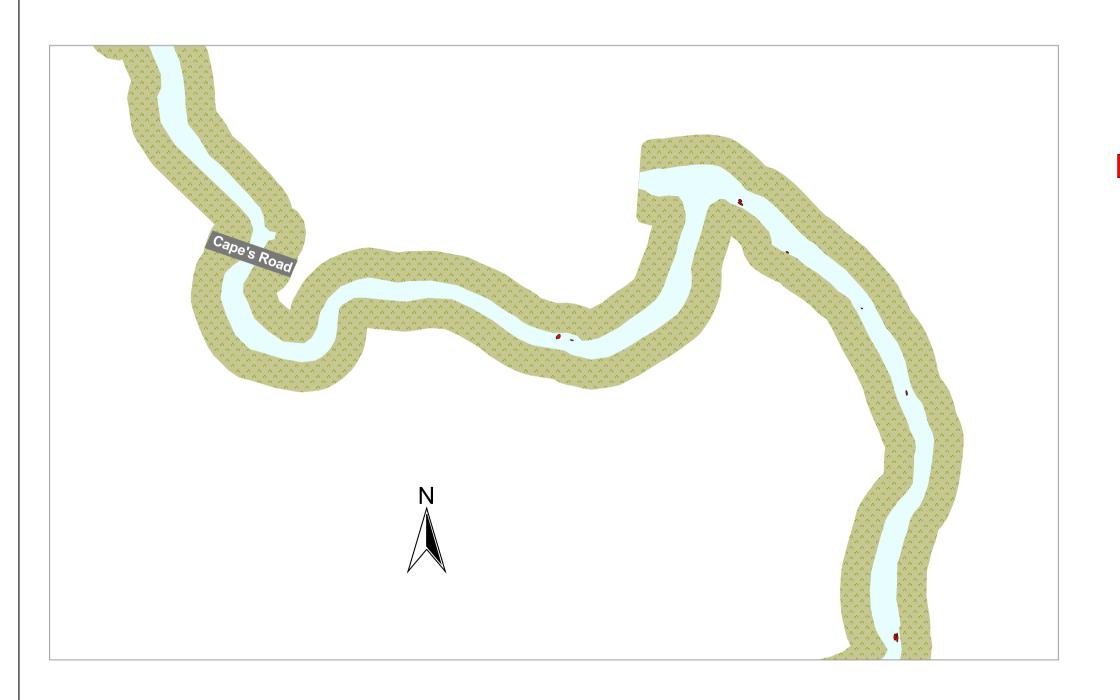
Project Location



Scale: 1"=180' 40 60 80 100 120 140 Meters



BIO-WEST, Inc.



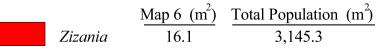
Scale: 1"=160' 40 60 80 100 120 140 Meters

San Marcos River Texas wild-rice

(Zizania texana)

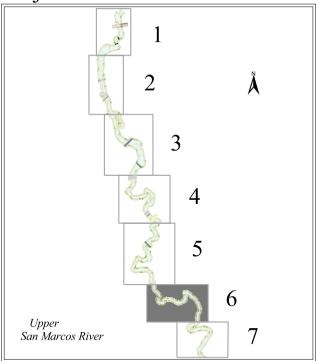
Summer 2004 - Map 6 of 7

July 19 - July 21, 2004

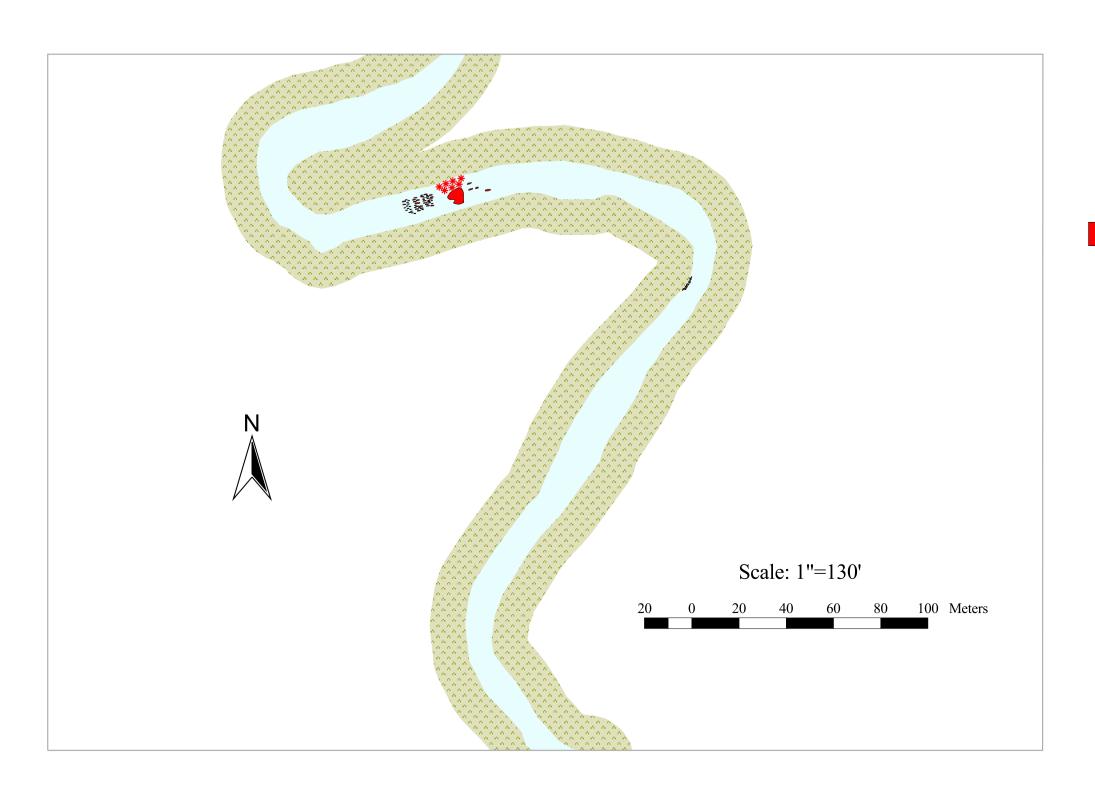


* small Zizania plants

Project Location



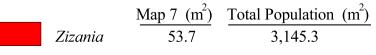




(Zizania texana)

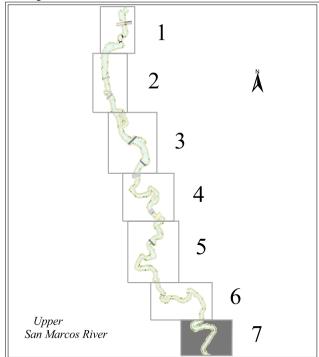
Summer 2004 - Map 7 of 7

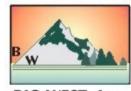
July 19 - July 21, 2004



* small Zizania plants

Project Location





BIO-WEST, Inc.