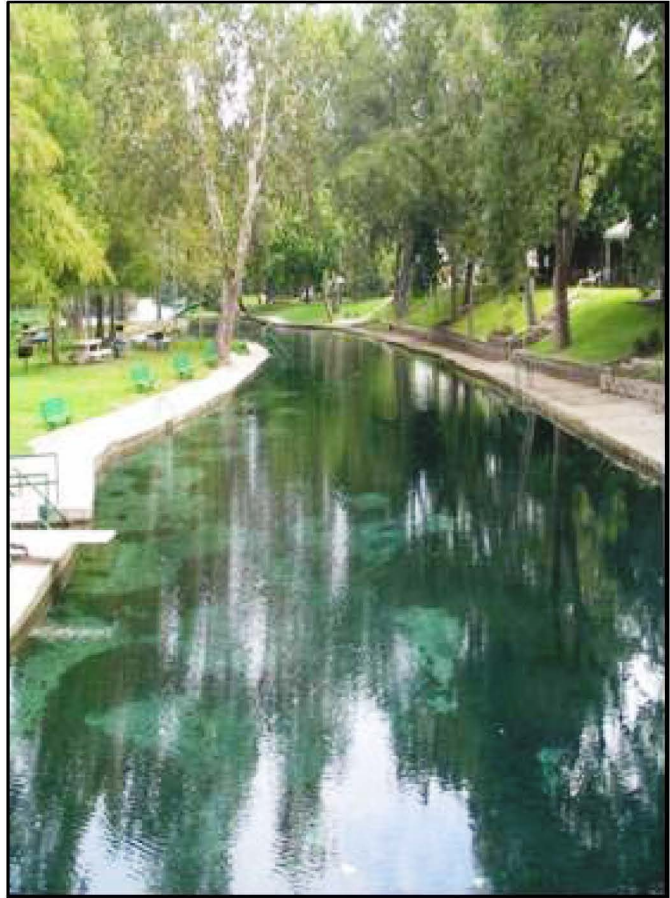


# **Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem**

## **Final 2006 ANNUAL REPORT**



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# TABLE OF CONTENTS

EXECUTIVE SUMMARY	1
METHODS	4
Springflow	4
Low-flow sampling	4
High-flow sampling	5
Water Quality	5
Aquatic Vegetation Mapping	7
Fountain Darter Sampling	7
Drop Nets	7
Drop Net Data Analysis	8
Dip Nets	9
Dip Net Data Analysis	9
Dip Net Techniques Evaluation	9
Visual Observations	10
Gill Parasite Evaluation	10
Comal Springs Salamander Visual Observations	11
Macroinvertebrate Sampling	12
Master Naturalist Observations	13
Exotics / Predation Study	14
OBSERVATIONS	15
Springflow	15
Water Quality	18
Aquatic Vegetation Mapping	19
Upper Spring Run Reach	19
Landa Lake Reach	20
Old Channel Reach	21
New Channel Reach	21
Fountain Darter Sampling	22
Drop Nets	22
Dip Nets	29
Dip Net Techniques Evaluation	31
Visual Observations	33
Gill Parasite Evaluation	33
Comal Springs Salamander Visual Observations	34
Macroinvertebrate Sampling	36
Drift Net Sampling	36
Comal Springs Riffle Beetle	39
Master Naturalist Observations	40
Exotics / Predation Study	41
REFERENCES	42
APPENDIX A: AQUATIC VEGETATION MAPS	
APPENDIX B: DATA AND GRAPHS	
APPENDIX C: DROP NET RAW DATA	

**LIST OF TABLES**

Table 1. Distribution of 50 dip net sites among four reaches and five vegetation types.	10
Table 2. Components of 2006 sampling events.	15
Table 3. Lowest discharge during each year of the study and the date on which it occurred.	15
Table 4. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, and 3 and Old Channel reach during each sample effort in 2005-2006.	17
Table 5. Proportion of total discharge in the Comal River (USGS data) that each Spring Run Contributed and proportion that traveled down the Old Channel during each sample effort In 2005-2006.	17
Table 6. Drop net sites and vegetation types sampled per reach in 2006.	23
Table 7. Fish taxa and the number of each collected during 2006 drop-net sampling.	28
Table 8. The number of fountain darters observed in Landa Lake per grid/sampling event.	33
Table 9. Total number of Comal Springs salamanders observed at each survey site during each sample period.	35
Table 10. Total number of troglobitic and endangered species collected in drift nets during April and November, 2006. Federally endangered species are designated with (E). A = adult beetles. L = larvae.	37
Table 11. Results of water quality measurements conducted in 2006 during drift net sampling efforts at Comal Springs.	38
Table 12. Total numbers of Comal Springs riffle beetles ( <i>Heterelmis comalensis</i> ) at each survey site during each sampling period.	39

**LIST OF FIGURES**

Figure 1. Comal River water quality and biological sampling areas.	6
Figure 2. Weekly water quality/recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers in 2006.	14
Figure 3. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (*), low (+) and high-flow (#) samples are indicated.	16
Figure 4. Mean monthly discharge in the Comal River during the 1934-2001 period of record.	16
Figure 5. Thermistor data from Spring Run 1.	18
Figure 6. Density of fountain darters collected by vegetation type in the Comal Springs/ River ecosystems combined (2000-2006).	24

Figure 7. Population estimates of fountain darters in all four sample reaches combined (2000-2005); values are normalized to the maximum sample. Light-colored bars represent high-flow critical sampling events.	25
Figure 8. Length frequency distribution of fountain darters among all drop-net sample events in the Comal River in 2006..	26
Figure 9. Length frequency distributions of fountain darters collected in spring and fall 2006 in the Old Channel and Upper Spring Run Reaches.	27
Figure 10. Density of giant ramshorn snails by vegetation type (averaged across all sites and all years).	28
Figure 11. Density of giant ramshorn snails collected in drop net sampling in each year across all vegetation types.	29
Figure 12. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.	29
Figure 13. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.	30
Figure 14. Percent of sites (n = 50) with fountain darters present during each Presence/Absence dip net sample.	32
Figure 15. Percent of sites within each vegetation type with fountain darters present in 2006.	32
Figure 16. Drift net sampling results for each species (except <i>Stygobromus</i> spp.) combined across all sample sites.	38
Figure 17. Average carbon dioxide concentrations from weekly samples taken at five sites in the Comal Springs/River ecosystem from July through November 2006.	40
Figure 18. Average pH from weekly samples taken at five sites along the Comal River from July through November 2006.	41

## EXECUTIVE SUMMARY

This annual summary report presents a synopsis of methodology used and an account of sampling activities, including sample conditions, locations and raw data obtained during two quarterly sampling events (Comprehensive Monitoring Effort) conducted on the Comal Springs/River ecosystem in 2006. Although discharge was considerably lower in 2006 than previous years, no Critical Period sampling events were triggered for this system. For ease of comparison, the data are reported here in an annual report format similar to previous reports (BIO-WEST 2001-2006).

The lack of significant precipitation in 2006 caused the aquifer to drop to levels not observed in recent years. As a result, springflows decreased throughout the region, which led to reduction in discharge in the Comal River. Average monthly discharge in the river dropped below historic flows in May and stayed below historic averages for the rest of the year. The lowest discharge of 202 cubic feet per second (cfs) occurred in August. While total discharge in all springs was well below that measured in 2005, the proportions coming out of each spring were nearly identical. Thermistor data continues to display thermal uniformity throughout the Comal system despite wide-ranging discharge conditions. In many places the temperature remained nearly constant due to nearby spring inputs, while other locations (typically further away from spring influences) were more substantially influenced by atmospheric conditions. The Heidelberg site in the Upper Spring Run Reach, the eastern channel at Spring Island, Landa Lake, and the Spring Runs maintained a narrow range of temperatures that never exceeded 26°C. In contrast, Blieders Creek followed a distinct seasonal trend because of the lack of spring influence, with temperatures exceeding 30°C in August when air temperatures were highest. Temperature and flow data collected during lower than average discharges in 2006 provided valuable information on the temperature regime of the Comal Springs/River ecosystem.

Aquatic vegetation in the four sample reaches was mapped twice in 2006 to better understand discharge to vegetation growth relationships, and the relationship between fountain darter (*Etheostoma fonticola*) populations and their habitat. Overall, growth in vegetation was variable among the sites during 2006. In contrast to previous above-average discharge years, growth of several aquatic vegetation species was prevalent in the New Channel Reach in 2006. Plants were able to gain a firm foothold in this reach in 2006 because there were few flushing events that would normally scour this highly channelized reach. *Cabomba*, *Ludwigia*, and bryophytes were all able to establish in 2006 in this reach. Total area of *Cabomba* plants increased 35x by fall with most of the plants establishing in the downstream end of the reach. *Hygrophila* area more than doubled in 2006 with several smaller plants growing together. *Hygrophila* plants also grew together over 2006 in the eastern side of the Upper Spring Run Reach. As such, the Upper Spring Run Reach experienced an increase in total vegetation area from 2005 to 2006. Bryophytes also continued to expand during these less than average discharge conditions. Large, erect pillars were observed in much of the site creating three-dimensional structure for fountain darters. This is one of the most important vegetation types in this reach and the Comal River for supporting populations of fountain darters. Fountain darters are able to occupy this habitat type in much higher densities than other vegetation types because it provides substantial food and cover.

*Vallisneria* continued to be the dominant vegetation type in the Landa Lake Reach even though it decreased slightly from 2005. Another important vegetation type for fountain darters, *Ludwigia* (native plant), increased slightly in 2006 as a few stands grew together. In contrast, *Hygrophila* (a non-native plant) began to fragment in the lower portion of Landa Lake. Total area of *Cabomba* increased from 2005, but much of this growth occurred in areas too deep to sample, so it is difficult to assess any

change in fountain darter usage of this vegetation type. Bryophytes in the Landa Lake Reach also appeared to be unaffected by the lower than average discharge, but still reflected seasonal variation. Although filamentous algae are still a preferred habitat type for fountain darters, limited area was found in the Old Channel Reach in 2006. In addition, *Ludwigia* decreased considerably in the Old Channel Reach as *Hygrophila* continued to infiltrate pure *Ludwigia* stands. *Ceratopteris* (a non-native plant) grew substantially in the lower end of the reach in places previously occupied by *Hygrophila*. This area must continue to be closely monitored to understand the interaction between native (once prevalent), and non-native plants that seem to be gaining a foothold in the Old Channel Reach.

Direct sampling of the fountain darter occurred in the same reaches using aquatic vegetation to stratify random sample locations. Filamentous algae and bryophytes continued to be the most important vegetation types to fountain darters (26.8 and 22.9 darters / 1.0 m<sup>2</sup>, respectively). *Ludwigia* and *Cabomba* also had higher numbers (12.7 and 11.0 darters / 1.0 m<sup>2</sup>, respectively). Size-class distributions of fountain darters in reaches furthest removed from spring influence demonstrated that larger fountain darters were more abundant in fall, and smaller darters were more numerous in spring indicating a spring reproductive peak. In the reaches closer to spring influence, a second peak of small darters was also seen in fall indicating that year-round reproduction does occur in certain areas.

Last year, an effort was made to establish a rapid method for assessing changes in fountain darter population abundance between sample efforts, especially during Critical Periods (high- and low-flow events). This dip-netting protocol was continued in 2006 at all four sites. In 2006, darters were found in 70% of dips across all vegetation types in the spring, and 60% in the fall. Using this method, the highest percentage of darters was found in *Cabomba*. This sampling method continues to be important because if correlation with seasonal drop netting data is observed, this method could serve as a surrogate technique to provide continuous monitoring, especially during critical low-flow periods. Exotic species continue to be present in much of the Comal River. Suckermouth catfish (*Hypostomus* sp.) and giant ramshorn snails (*Marisa cornuarietis*) have been documented as causing large impacts in aquatic systems. Though these snails had a serious impact on the vegetation in the Comal River in the 1990's, densities since 2001 have been minute, and no live specimens were collected during 2006 sampling efforts. However, since these snails can have severe impacts at high densities, their populations will continue to be monitored and remain a key focus during critical low-flow periods.

All SCUBA/snorkel surveys revealed the presence of Comal Springs salamanders along the lake bottom and in each sampled Spring Run in 2006. Though lower than average discharge was present in 2006, salamander populations remained consistent in the spring runs. At the Spring Island sites *Amblystegium* were more prevalent possibly due to the lack of flushing flows, which resulted in higher numbers of salamanders being observed.

A total of 144 hours of sample time occurred among the three drift net sites at Comal Springs in 2006 and 11 species were captured. Population changes of species in 2006 were similar to previous years. Species of the genus *Stygobromus* and *Lirceolus* continued to be most abundant at all sites. *Stygobromus pecki* (Peck's cave amphipod) was the dominant amphipod (among identifiable individuals) at all sites, though most were a few millimeters in length indicating that smaller individuals are more susceptible to expulsion from the aquifer. In 2006, there appeared to be no seasonal nor discharge related influences on invertebrates, as total numbers were similar to that of last year. In November 2006 a new species of blind amphipod was discovered in Spring Run 3 and the upwelling sites along the western shore of Landa Lake. Two organisms were collected, and this undescribed

species is in the *Parabogidiella* genus. This species is similar to *Artesia subterranea*, but none of these were collected in 2006. *Parabogidiella* and *Artesia* are the only bogidilleads reported north of Mexico.

Initial observations of the gill parasite *Heterophyid cercariae* show that the Elizabeth Street site (Old Channel) has the highest abundance in the water column compared to the other two sites. In addition, concentrations of the parasite appeared to increase as discharge decreased. Since the intensive parasite evaluation began in 2006, there have been a total of thirteen rounds of parasite sampling, and an additional fifteen rounds will be completed before the study is completed in June 2007.

The Texas Master Naturalist Program is a partnership among the Texas Cooperative Extension, Texas Parks and Wildlife Department, and numerous local partners designed to provide natural resource education, outreach, and other services through volunteer efforts. In 2006, several members volunteered their time to record point water quality and recreation data weekly from July through November at five sites on the Comal River. Water quality data showed that carbon dioxide concentrations were highest in areas near spring outflows (Houston St. and Gazebo) and decreased downstream. Recreation data collected by volunteers shows the most intense recreation at sites near popular tubing areas (the New Channel and Union Avenue). These dedicated volunteers have assisted in filling in some key data gaps and have contributed valuable information to this project.

Overall, the most concerning observation in the Comal Springs / River ecosystem is that the Old Channel Reach is not nearly as productive as it had been during the early years of this study. The variable and often times elevated discharge conditions in this reach over the past few years had led to drastic shifts in the aquatic vegetation community. The filamentous algae (once abundant in this reach) were replaced by the native *Ludwigia* and non-native *Hygrophila*. In recent years, the non-native *Hygrophila* and *Ceratopteris* have out-competed the native vegetation and currently dominate this reach. As such, fountain darter populations within this reach have decreased substantially.

Though discharge in the Comal River was lower than the previous several years, no Critical Period events were triggered in 2006. Therefore, the data in this report remain preliminary, with data from periods of low-flow (particularly from an extended period of low-flow) necessary to fully evaluate the biological risks associated with future Critical Periods (high or low-flow). Fortunately, the Authority's Variable Flow monitoring program includes an extensive data-collection protocol for periods of critical flow that should address this current limitation. In the interim, efforts to evaluate response mechanisms of the threatened and endangered species to low-flow conditions either via laboratory investigation (as conducted in previous years under this program) or via field (*in situ*) experiments as proposed with the "intensive management areas" concept would provide valuable information for management decisions. Data collected during natural low-flow conditions (Critical Period sampling) is essential to verify any interim results and gather additional information to further evaluate low-flows.

Though the comprehensive portion of the study has been reduced to two annual samples (plus a limited summer effort), it is still adequate to maintain a continuous record of conditions. Maintaining this continuous record is vital since antecedent conditions influence community-level response to reduced discharge conditions. Sampling only during a low-flow event simply does not provide the necessary context to adequately assess changes that occur during such conditions. As such, comprehensive monitoring continues in the Comal Springs / River ecosystem in 2007.

## METHODS

As in 2005, two full comprehensive sampling efforts were conducted (spring and fall) in 2006 with additional summertime dipnetting by the project team, and some volunteer assistance (new in 2006) on the Comal system. A full comprehensive event includes the following sampling components and volunteer activities:

### Water Quality

- Thermistor Placement
- Thermistor Retrieval
- Fixed Station Photographs
- Point Water Quality Measurements

### Salamander Observations

### Macroinvertebrate Sampling

- Drift Nets
- Comal Springs Riffle Beetle Surveys

### Aquatic Vegetation Mapping

### Recreation Observations

- Weekly Recreation Counts

### Fountain Darter Sampling

- Drop Nets
- Dip Nets
- Visual Observations
- Gill Parasite Evaluation

## Springflow

Total discharge data for the Comal River were acquired from United States 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 2006). The discharge data for the Comal ecosystem was taken from USGS gage 08169000 from the Comal River at New Braunfels. This site represents the cumulative discharge of the springs that form this river system.

In addition to these cumulative discharge measurements, which are used to characterize the Comal Springs ecosystem during sampling, spot water velocity measurements were taken during each sampling event using a Marsh McBirney model 2000 portable flowmeter. Discharge was also measured in Spring Runs 1, 2, and 3 and in the Old Channel during each sampling effort to estimate the contribution of each major Spring Run to total discharge in the river and to estimate the relative proportion of water flowing in the Old and New Channels.

## Low-Flow Sampling

Discharge was considerably lower than average in the Comal River in 2006 with the lowest reported flow of 202 cfs occurring on August 25<sup>th</sup>. Though reduced discharge triggered Critical Period events in the San Marcos River/Springs, critical period sampling on the Comal River is triggered at 200 cfs and thus, no critical period sampling events were conducted on the Comal system.



## High-Flow Sampling

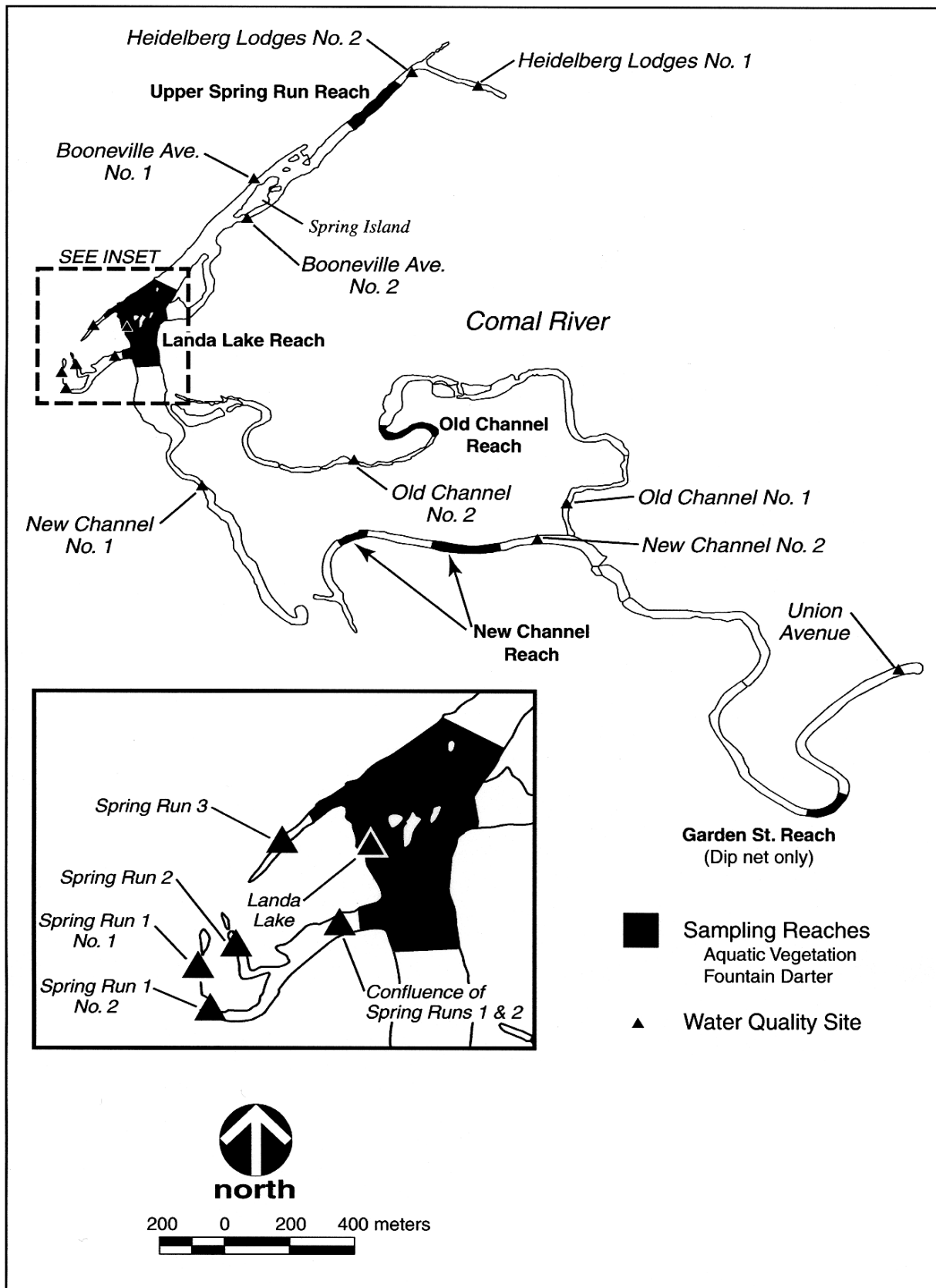
There were no high-flow events in 2006.

## Water Quality

The objectives of the water quality analysis are: delineating and tracking water chemistry throughout the ecosystem; monitoring controlling variables (i.e., flow, temperature) with respect to the biology of each ecosystem; monitoring any alterations in water chemistry that may be attributed to anthropogenic activities; and evaluating consistency with historical water quality information. The water quality component of this study was reduced in 2003, but the two components necessary for maintenance of long-term baseline data, temperature loggers (thermistors) and fixed station photography, were continued in 2006. In addition, conventional physico-chemical parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen, water depth at sampling point, and observations of local conditions) were taken at the surface and near the bottom in all drop-net sampling sites using a Hydrolab Quanta. When conditions trigger low-flow sample events in the future, the full range of water quality sampling parameters will be employed, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

Thermistors were placed in select water quality stations along the Comal River and downloaded at regular intervals to provide continuous monitoring of water temperatures in these areas. The thermistors were placed using SCUBA in deeper locations within Landa Lake and set to record temperature data every 10 minutes. The thermistor locations will not be described in detail here to minimize the potential for thermistor tampering.

In addition to the water quality collection effort, a long-term record of habitat conditions has been maintained with fixed station photography. Fixed station photographs allowed for temporal habitat evaluations and included an upstream, a cross-stream, and a downstream picture; these were taken at each water quality site depicted in Figure 1.



**Figure 1. Comal River water quality and biological sampling areas.**

## Aquatic Vegetation Mapping

Aquatic vegetation mapping was conducted using a Trimble Pro-XH global positioning system (GPS) unit with real-time differential correction capable of submeter accuracy. The Pro XH receiver was linked to a Trimble Recon Windows CE device with TerraSync software that displays field data as it is gathered and improves efficiency and accuracy. The GPS unit was placed in a 10-meter (m) Perception Swifty kayak with the GPS 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 that measured between 0.5 and 1.0 m in diameter were mapped by recording a single point. Vegetation stands less than 0.5 m in diameter were not mapped.



*Hygrophila* in the Old Channel Reach

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium*; primarily in the upper Spring Run and Landa Lake) were included in all 2006 sampling events. Difficulties with mapping these vegetation types (patchiness, bryophytes were easily obscured by filamentous algae, etc.) precluded them from early samples; however, these vegetation types were clearly important fountain darter habitat and were included in all sample events beginning in the summer of 2001.

## Fountain Darter Sampling

### Drop Nets

A drop net is a type of sampling device previously used by the USFWS to sample fountain darters and other fish species. The design of the net is such that it encloses a known area (2 square meters [ $\text{m}^2$ ]) and allows a thorough sample by preventing escape of fishes occupying that area. A large dip net (1  $\text{m}^2$ ) 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, a drop net was placed in randomly selected sites within specific aquatic vegetation types. The vegetation types used in each reach were defined at the beginning of the study as the dominant species found in that reach. Sampling sites were randomly selected per dominant vegetation type for each sampling event from a grid overlain on the most recent map (created with GPS-collected data during the previous week) of that reach.

At each location the vegetation type, height, and aerial 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 aerial 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. When collected, all live giant ramshorn snails (*Marisa cornuarietis*) 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 2000-2006 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 due to 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. Because these abundance estimates use the same density values across sites and seasons, 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).

## **Dip Nets**

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 recorded per sweep.

To balance the effort expended across samples, a predetermined time constraint was used for each reach (Upper Spring Run - 0.5 hour, Spring Island area - 0.5 hour, Landa Lake - 1.0 hour, New Channel - 1.0 hour, Old Channel - 1.0 hour, Garden Street - 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. Also, spending a comparable length of time sampling the entirety of each reach allowed comparisons between data gathered during each sampling event.

## **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, which 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.

## **Dip Net Techniques Evaluation**

In 2005, an effort was made to establish a rapid method for assessing changes in fountain darter population abundance between sample efforts, especially during Critical Periods (high- and low-flow events). While drop netting provides quantitative data of fountain darter populations, it is somewhat labor-intensive and destructive to vegetation that is valuable habitat especially in low-flow periods. Dip netting, as it is currently used, provides information on the relative abundance of fountain darters between samples. However, sample sites are selected in high quality channel edge habitat and are not distributed among available vegetation types. In addition, this method yields one data point (a single timed survey) for a given reach. Therefore, it does not result in data that may be used to determine a statistical difference among samples, or account for possible habitat shifts and clumping under low-flow conditions. Objectives of this portion of the study were to assess the viability of an alternative dip netting method designed to gather presence/absence data at multiple sites within each reach and thereby increase the number of data points that may be collected, reduce the time necessary to collect data at all

sites, and reduce habitat disturbance. Although presence/absence data provides no means of calculating fountain darter abundance, repeated sampling provides a quick and less labor intensive way to monitor trends in the fountain darter population. This technique was thoroughly evaluated on the Comal River in 2005 (BIO-WEST 2006) and determined to be a repeatable way to examine trends in the fountain darter population between sites.

In 2006, presence/absence dip netting was conducted on the Comal River during the spring and fall sampling events. During each sample, fifty sites were distributed among the 4 sample reaches based on total area, diversity of vegetation, previous fountain darter abundance estimates, and overall biological importance of each reach (Table 1). In most cases, sites were randomly selected from a grid overlain on the most recent vegetation map of that reach. However, occasionally, where certain vegetation types exhibited limited coverage, sites were chosen to fall within the proper vegetation type. After each dip, presence or absence of fountain darters was noted and the entire contents of the net were placed into a plastic tub with river water to avoid recapturing organisms. After all dips were completed at a site, all organisms were released and time of day was recorded.

**Table 1. Distribution of 50 dip net sites among four reaches and five vegetation types.**

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
<i>Hygrophila</i> (3)	<i>Cabomba</i> (3)	<i>Hygrophila</i> (6)	Filamentous Algae (2)
Bryophytes (3)	<i>Hygrophila</i> (8)		<i>Hygrophila</i> (8)
	<i>Ludwigia</i> (3)		<i>Ludwigia</i> (6)
	Bryophytes (8)		
<b>Total (6)</b>	<b>Total (22)</b>	<b>Total (6)</b>	<b>Total (16)</b>

## **Visual Observations**

Visual surveys were conducted using SCUBA in Landa Lake to verify the continued fountain darter and Comal Springs salamander (*Eurycea sp.*) use of habitat in deeper portions of the lake. The locations of these time-constrained surveys were deeper than drop net or dip net methods for the darters would allow. Observations were conducted in the early afternoon for each effort. An additional component to these surveys was a grid (0.6 m x 13.0 m) added in summer 2001, and subsequent sampling. The grid was used to quantify the number of fountain darters using these deeper habitats. To sample the area, all fountain darters within the grid were counted. Time constraints limited the sampling to just one grid. A much more labor-intensive effort would have been required to develop an estimate of the true population size in the sample area, but the data were useful in providing an indication of the relative abundance of the fountain darters that are found in areas similar to those sampled. This method also allowed some insight into trends in population dynamics that may occur over time.

## **Gill Parasite Evaluation**

The objectives of the intensive parasite evaluation were to examine the variation in spatial and temporal concentration of the trematode parasite, *Heterophyid cercariae* in the Comal Springs/River ecosystem. Specifically, the prime objective was to determine if cercarial abundance is dependant upon discharge,

temperature, current velocity, or dissolved oxygen. If abundance is dependant upon discharge then the trematode may intensify potential impacts during drought conditions. The preliminary work for the intensive evaluation began in January 2006 in which six sites on the Comal River were selected and cross section sampling of cercariae was conducted using the filtration method. The filtration method is a technique that allows for the cercarial stage of the life cycle to be pumped out of the water column and collected on filters. The numbers of cercariae are then counted for each five liter sample. All five liter water samples were fixed with a 0.1% formalin solution after collection and prior to filtration to increase the percentage of parasites recovered from the sample. Thirty samples were taken at each of these six sites. Statistical analysis was conducted to determine the minimum number of samples needed to represent each cross section. The preliminary sampling and statistical exercises were conducted in order to streamline operations and better represent parasite populations and their effects on fountain darter populations. Upon completions of preliminary activities, the intensive evaluation was initiated in June 2006. Three of the six preliminary sites were selected to be included in the intensive evaluation. These sites included Liberty Avenue, Houston Street, and Elizabeth Avenue. Each site was sampled every two weeks, weather permitting. Since June 2006, there have been a total of thirteen rounds of sampling, and an additional fifteen rounds will be completed before the intensive evaluation is completed in June 2007. Data analyses are currently being conducted and will be completed in 2007.

## **Comal Springs Salamander Visual Observations**

In addition to the visual observations made in the deeper portions of Landa Lake for fountain darters and Comal Springs salamanders, the BIO-WEST project team performed presence/absence surveys for the Comal Springs salamanders in the spring reaches located at the head of the Comal River during both 2006 sampling events. Surveys were conducted in Spring Run 1, Spring Run 3, and the Spring Island area (Figure 1) and performed by two people in each spring reach. Each survey began at the downstream-most edge of the sampling area and involved turning over rocks located on the substrate surface within the Spring Run while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. The Comal Springs salamander locations were noted, along with time and water depth. In order to maintain consistency between samples, all surveys were initiated in the morning and terminated by early afternoon.

Within Spring Run 1, surveys were conducted from the Landa Park Drive Bridge up to 9-m below the head spring orifice. Spring Run 3 was surveyed from the pedestrian bridge closest to Landa Lake up to 9-m below the head spring orifice. In the Spring Island area, surveys were conducted within the entire spring reach including approximately a 15-m radius from each Spring Run outfall. These two areas include the spring outfall on the east side of Spring Island (closest to Edgewater Drive) and the area north of Spring Island (upstream).





Salamander survey in Spring Run 1

## Macroinvertebrate Sampling

From an overall ecosystem health perspective, benthic (bottom-dwelling) macroinvertebrates are reliable indicators of localized alterations in stream conditions (Gore 1977, Corrarino and Brusven 1983, Rosenberg and Resh 1992) because differential habitat requirements make it possible to assess water quality and water quantity issues in stream ecosystems. Regular sampling of the benthic macroinvertebrate community in the Comal Springs/River ecosystem is being conducted to provide the information to make such assessments. Additionally, the macroinvertebrate sampling in Spring Runs 1, 2, and 3 is an integral component of this study because of the presence of the three endangered macroinvertebrate species; Comal Springs riffle beetle (*Heterelmis comalensis*), Comal Springs dryopid beetle (*Stygoparnus comalensis*), and Peck's cave amphipod (*Stygobromus pecki*). At the outset of this project, drift nets were used in the middle of Spring Runs 1-3 to explore the movement of organisms downstream of the spring openings since drifting downstream is a primary means of dispersal by benthic invertebrates (Smock 1996). The focus was to determine whether a single species might be used to serve as an indicator for measuring community response to changes in springflow. That portion of the study, completed in 2002 (BIO-WEST 2003) yielded drift rates, densities, and patterns of selected aquatic invertebrates in the Spring Runs. In 2003, there was a shift of focus to the spring openings in order to evaluate the frequency with which the three primarily spring-adapted (troglobitic) endangered species are expelled from the aquifer. In 2003-2006, there were also sampling efforts targeted at the Comal Springs riffle beetle distribution and relative abundance among the three areas identified during a 2001 survey (BIO-WEST 2002) as having the largest concentrations of individuals and under various discharge conditions within each site.



In 2006, drift nets were placed in spring openings during the spring and fall comprehensive sampling efforts. Drift nets were placed over the openings of Comal Spring Runs 1 and 3 and a moderate-sized spring upwelling along the western shoreline of Landa Lake. The nets were anchored into the substrate directly over the spring opening, with the net face perpendicular to the direction of flow of water. The nets had a 0.45-m by 0.30-m rectangular opening and mesh size of 350 micrometers ( $\mu\text{m}$ ). The tail of the net was connected to a detachable 0.28-m-long cylindrical bucket (300- $\mu\text{m}$  mesh). The buckets were removed at 4-hour intervals, and the cup contents were sorted in the field. Except for voucher specimens of Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle, all organisms of these three species were identified and returned to their spring of origin. Voucher specimens included fewer than the 20 living specimens (identifiable in the field) of each species. All other invertebrates were preserved in 70% ethanol for later identification. Water quality measurements (temperature, pH, conductivity, dissolved oxygen, and current velocity) were taken at each drift net site using a Hydrolab multiprobe and DataSonde (model 2) and a Marsh McBirny portable water current meter (model 201D).

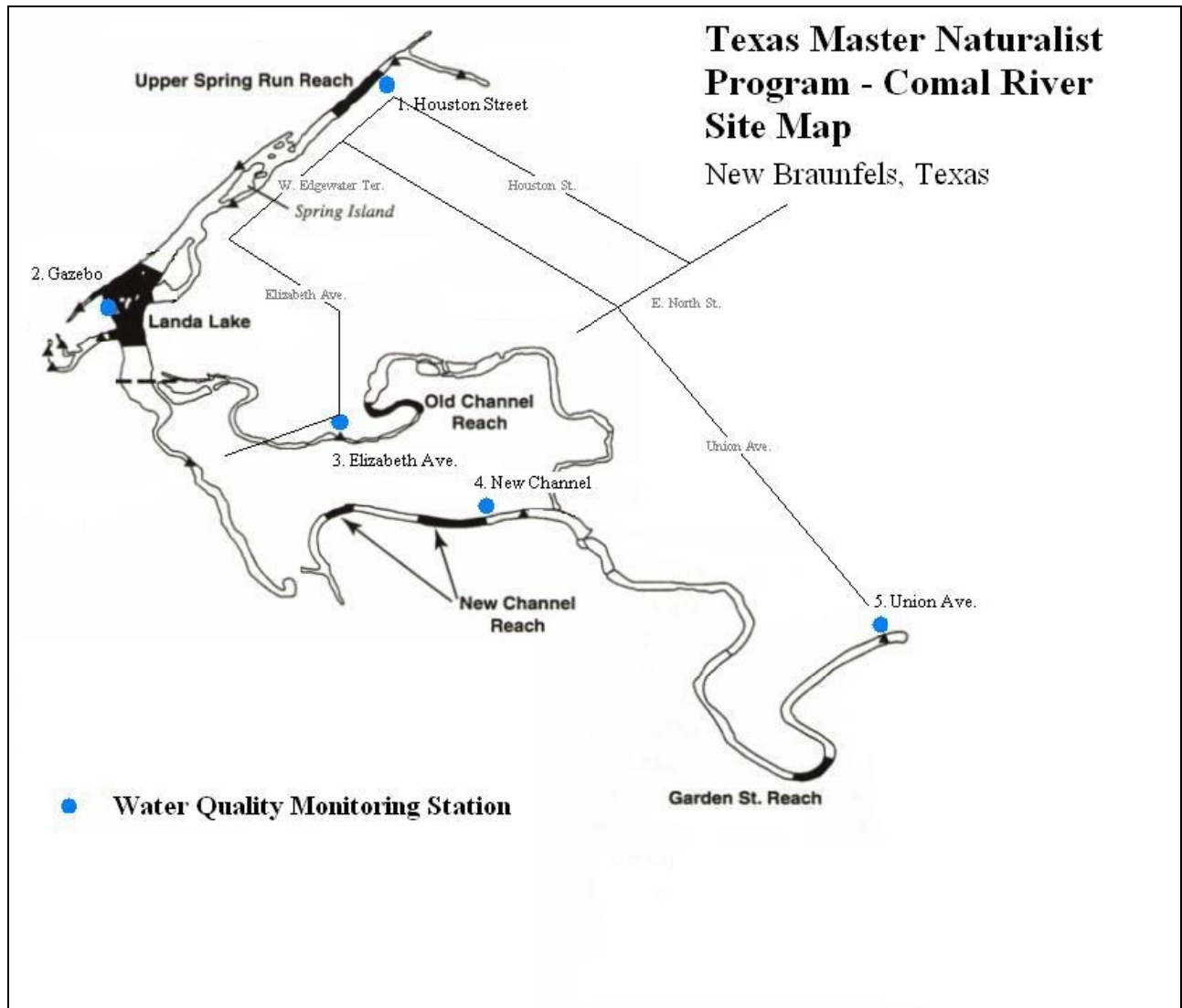
In addition to drift nets placed over spring openings, surveys of the endangered Comal Springs riffle beetle, were conducted in the two comprehensive sampling efforts in 2006 (May and November). These samples were conducted in three disjunct areas of Landa Lake on the Comal River, in locations that were previously identified (BIO-WEST 2002) to have the highest densities of Comal Springs riffle beetles. The three sites included Spring Run 3, the western shoreline of the lake, and upstream of Spring Island. Samples were collected in 2006 using the same methodology as in previous years (see BIO-WEST 2006) for a description of earlier sampling technique). Bed sheets (60% cotton, 40% polyester) were cut into 15-cm x 15-cm squares. At each of the three study sites, 10 springs found in potential habitat were selected and sampled using this method. Depth (ft), current velocity (m/s), and landmark distance measurements were taken at each spring. Each square had the corners folded inward and placed in the spring with rocks loosely stacked over top to keep it in place. Approximately four weeks later, squares were located and removed followed by depth and current velocity measurements. Beetles were identified, counted, and returned to their spring of origin. Other spring invertebrates collected on the squares were also noted. These included two other riffle beetle genera (*Microcyllloepus* and *Stenelmis*), Comal Springs dryopid beetles, Peck's cave amphipods, and blind isopods (*Lirceolus* near pilus).

## Master Naturalist Observations

The Texas Master Naturalist Program is a partnership among the Texas Cooperative Extension, Texas Parks and Wildlife Department, and numerous local partners designed to provide natural resource education, outreach, and other services through volunteer efforts. To become a Master Naturalist, an individual must complete an approved training course and complete at least 40 hours of volunteer service per year. The program currently supports over 2,750 volunteers across the state of Texas (<http://masternaturalist.tamu.edu>).

In 2006, volunteers from the Master Naturalist program assisted BIO-WEST by collecting water quality and recreation data on the Comal Springs/River ecosystem. Volunteers collected data at five sites (Figure 2) on a weekly basis from July through November. At each site, an Oakton Waterproof pHTestr 2 was used to assess pH, and a LaMotte Carbon Dioxide Test Kit was used to calculate carbon dioxide ( $\text{CO}_2$ ) concentrations. In addition to water quality measurements, recreational use data was collected at

each site by counting the number of tubers, kayakers, swimmers, anglers, etc. Photos were taken at each sampling event and any other notes on recreational use or condition of the river were recorded. Most sampling took place on Friday afternoon in order to get an accurate estimate of recreational use.



**Figure 2. Weekly water quality/recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers in 2006.**

## Exotics / Predation Study

This sampling component was not included in 2006 as no critical period sampling efforts were triggered.

## OBSERVATIONS

The BIO-WEST project team conducted the 2006 sampling events as shown in Table 2.

**Table 2. Components of 2006 sampling events.**

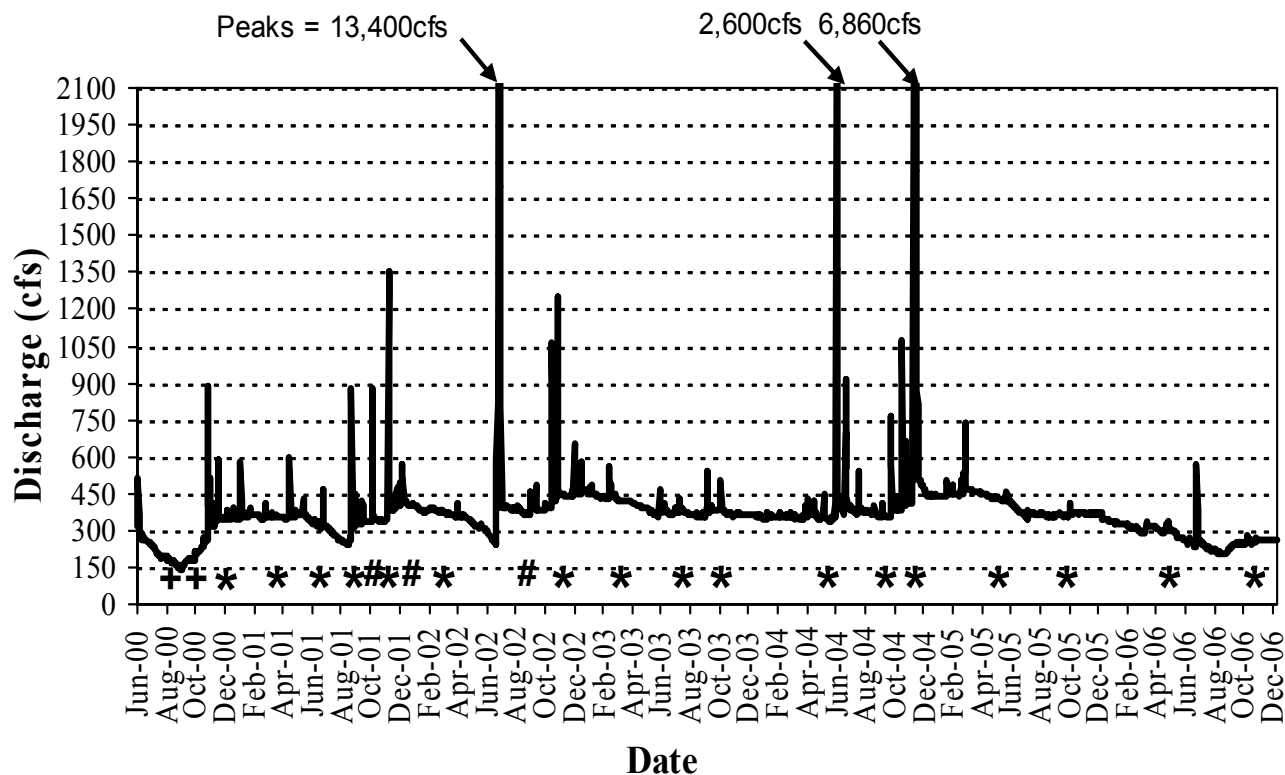
EVENT	DATES	EVENT	DATES
<b>Spring Sampling</b>		<b>Fall Sampling</b>	
Vegetation Mapping	Apr 24 - 27	Vegetation Mapping	Nov 7, 13 - 16
Fountain Darter Sampling	Apr 27, May 2 - 4	Fountain Darter Sampling	Nov 14 - 17
Comal Salamander Observations	May 12	Comal Salamander Observations	Nov 14
Macroinvertebrate Sampling	Apr 26 - 28	Macroinvertebrate Sampling	Nov 2 - 4
<b>Summer Sampling</b>			
Fountain Darter Sampling	July 19		

## Springflow

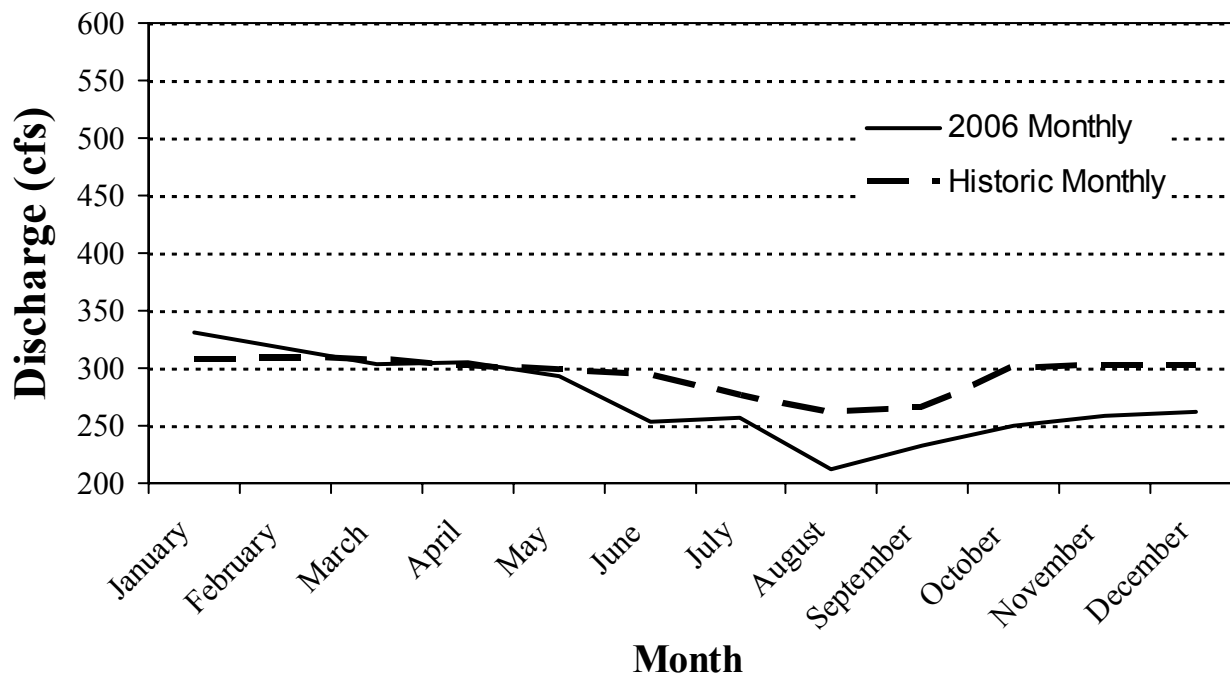
Discharge continued to decrease from late 2005 into 2006. The lowest discharge of the year was recorded in August, and was the lowest flow recorded since 2000 (Table 3). Only one significant rain event occurred in the watershed in early July, which pushed the flow in the Comal River to 566 cfs (Figure 3). From January through April 2006, mean monthly discharge was above average, but starting in May, discharge was considerably below the historic average (Figure 4). After August, discharge began to increase with scattered rain events, but remained below historic averages.

**Table 3. Lowest discharge during each year of the study and the date on which it occurred.**

Year	Discharge	Date
2000	138	Sept. 7
2001	243	Aug. 25
2002	247	Jun. 27
2003	351	Aug. 29
2004	335	May 28
2005	349	July 14
2006	202	Aug. 25



**Figure 3. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (\*), low (+), and high-flow (#) sampling events are indicated.**



**Figure 4. Mean monthly discharge in the Comal River during the 1934-2006 period of record.**

Table 4 shows the discharge measured in each of the Spring Runs (including one upstream and one downstream site in Spring Run 3) and the Old Channel. Table 5 shows the proportion that each spring contributed to the total Comal River discharge and the proportion of total discharge that traveled down the Old Channel during each sample effort. Discharge was well below 2005 levels for all sites and dates in 2006. While discharge continued to drop at all sites, the proportions of the total discharge changed little and in some cases increased. Spring runs 1 and 3 made up 21.6% of the total discharge in spring 2006, a 0.5% decrease from 2005, but had increased to 22.5% in fall 2006. Spring run 2 continues to make up a small fraction (1.3%) of the total discharge in the Comal River. Interestingly, the proportion did not change as discharge continued to decrease in 2006. Discharges at each of the springs will continue to be closely monitored and a key focus during limited discharge conditions.

**Table 4. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, and 3 and Old Channel reach during each sample effort in 2005 - 2006.**

Location	Discharge (cfs)			
	Spring 2005	Fall 2005	Spring 2006	Fall 2006
Total Discharge Comal River (USGS)	446	368	295	259
Spring Run 1	64.2	37.7	27.4	24.5
Spring Run 2	10.5	5.0	3.7	3.3
Spring Run 3 (upstream)	45.6	33.8	28.2	14.6
Spring Run 3 (downstream)	58.8	43.9	36.4	33.6
Old Channel	77.3	65.4	51.6	42.5

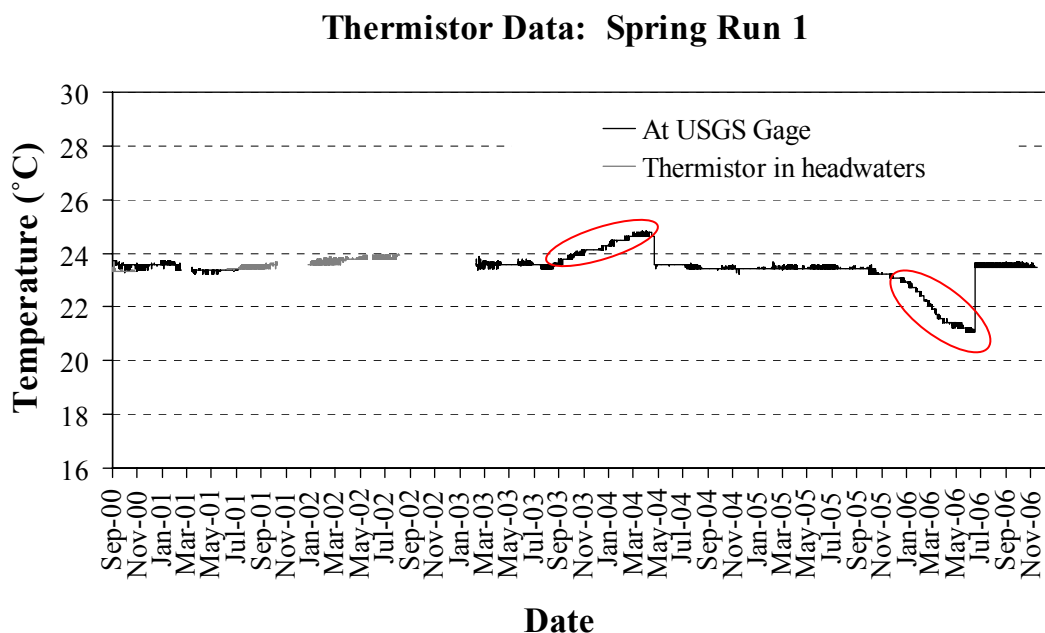
**Table 5. Proportion of total discharge in the Comal River (USGS data) that each Spring Run contributed and proportion that traveled down the Old Channel during each sample effort in 2005 - 2006.**

Location	Proportion of Total Discharge			
	Spring 2005	Fall 2005	Spring 2006	Fall 2006
Spring Run 1	14.4%	10.2%	9.3%	9.5%
Spring Run 2	2.4%	1.4%	1.3%	1.3%
Spring Run 3 (upstream)	10.2%	9.2%	9.6%	5.6%
Spring Run 3 (downstream)	13.2%	11.9%	12.3%	13.0%
Old Channel	17.3%	17.8%	17.5%	16.4%

## Water Quality

The continuously sampled water temperature data have provided a good view of the conditions experienced by fountain darters and other species throughout the Comal Springs ecosystem from 2000-2006. In many places the temperature remains nearly constant due to nearby spring inputs, while other locations (typically further away from spring influences) are more influenced by atmospheric conditions. At times, precipitation can have acute impacts (typically very cold rainfall) in some locations, but these are generally short-lived and the overall relationship at these sites is more directly associated with air temperature (air temperatures also strongly influence precipitation temperatures).

A representative graph of thermistor data for the Comal Springs/River ecosystem is presented in Figure 5; additional graphs can be found in Appendix B. The perceived increase in temperature during spring 2004 and the apparent decrease during spring 2006 at Spring Run 1 are a result of faulty thermistors. Thermistor failure is also evident in data collected from Landa Lake, the Hiedleberg site, and Spring Run 3 (Appendix B). As thermistors lose battery power temperature measurements begin to drift. Once thermistors are downloaded and drift is noted in the data, they are immediately replaced.



**Figure 5. Thermistor data from Spring Run 1. Red circles indicate thermistor “drift”.**

As has been observed in past years, the range of water temperatures in several sites is around 1°C or less, except for a few acute peaks/troughs (Spring Run 1, Figure 5). The Heidelberg site in the Upper Spring Run Reach, the eastern channel at Spring Island, Landa Lake, and the Spring Runs maintained a narrow range of temperatures that never exceeded 26°C (Appendix B). The Spring Island western channel receives most of its flow from springs located farther upstream, and thus fluctuates in temperature to a much greater extent than the eastern channel which receives most of the spring flow from the spring in the center of the island. Similarly, temperature data from the New Channel, the Old Channel, and the Other Place are affected more by ambient air temperature and exhibit more seasonal fluctuations (Appendix B). These fluctuations are usually the greatest at the Other Place site because it is located

farthest from spring inputs. However, during 2006 the highest temperatures observed were in the New Channel downstream reach. Here temperatures exceeded the TCEQ water quality standards value of 26.67 on three separate days during summer 2006, reaching a maximum value of 27.32.

In contrast to other sites, Blieder's Creek (upstream of the Spring Runs and Landa Lake) followed a distinct seasonal trend because of its lack of spring inputs. In fact, the greatest fluctuation in water temperature throughout the Comal River system occurred in Blieder's Creek, where the temperature increased to 31°C during late summer 2006 as a result of hot summertime temperatures and limited flow in the creek. While the wide fluctuations in temperature found in Blieder's Creek do not appear suitable to provide habitat for fountain darters during a portion of the year, the effects of these water conditions are highly localized. The lower end of Blieder's Creek is typically a stagnant pool that does not flow into the Comal River and temperatures observed in the Upper Spring Run Reach (immediately downstream of Blieder's Creek) were within a much narrower range (Heidelberg site; Appendix B).

Data collected during lower than average discharge in 2006 provides valuable information on the temperature regime of the Comal Springs/River ecosystem. However, as with other components of this study, additional data are needed to determine the potential impacts of high air temperatures and extremely low discharge during an extended period of reduced recharge.

## Aquatic Vegetation Mapping

Maps of the aquatic vegetation observed during each sample effort can be found in the Appendix A map pockets. Vegetation growth in many of the reaches was substantial due to the lower than average flows prevalent in the Comal River system in 2006. 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 are described below.

### Upper Spring Run Reach

Vegetation area continued to increase as discharge decreased in the Upper Spring Run Reach from fall 2005 (3,018.4 m<sup>2</sup>) to spring 2006 (3,640.4 m<sup>2</sup>). In previous years, flushing flows had scouring effects that often scoured vegetation and then stimulated growth in this reach, but these scouring events did not occur in 2006.

*Cabomba* declined in early 2006 (0.6 m<sup>2</sup>) to only one small plant in the lower section of the reach. The plants that had previously existed near *Hygrophila* at the top of the reach were no longer present. Little changed by fall (0.8 m<sup>2</sup>) with the only *Cabomba* plant growing slightly. These plants are typically found in deep, heavily silted portions of the river, a condition that is not common in this reach. *Sagittaria* continued to increase in total area from 2005 to 2006 (494.2 and 552.0 m<sup>2</sup>, respectively). A few plants grew together by spring leading to this increase in area. *Sagittaria* supports a low density of fountain darters; thus, the fluctuations in total coverage of that habitat type are less important to the fountain darter population. *Sagittaria* continued to increase in area over 2006 (639.4 m<sup>2</sup>) with plants filling in along the western side of the river. *Ludwigia* only increased slightly with no new plants found in the Upper Spring Run Reach in spring 2006. These native plants continued to grow with lower than average discharge through 2006.

*Hygrophila* decreased substantially from fall 2005 to spring 2006 (468.2 and 148.3 m<sup>2</sup>, respectively). Previously it appeared that this non-native plant was beginning to successfully re-establish in this reach, but mechanical disturbance (paddle boats) attributed to this loss in spring. When the reach was sampled in spring it appeared that large portions of the plant had been torn out along the river left (eastern) side of the reach. Often, homeowners will rip out vegetation in order to make a more pleasant swimming experience near their home, as was evidenced by large pieces of the *Hygrophila* plants lying along the banks in this section. Though *Hygrophila* is not preferred habitat of the endangered fountain darter, they are still found in these plants, and may be an important secondary habitat when more desirable vegetation is not available. By fall 2006, these plants had rebounded and begun to colonize along the eastern side of the river.

With the lack of flushing flows occurring in early 2006, bryophytes were able to increase substantially in this reach. Large, erect pillars of bryophytes were found in much of the upper section of the reach. While these mosses were much closer to the substrate in the lower section of the reach, they were still ubiquitous. Bryophytes became patchier by November 2006, likely due to rain event driven scouring in the fall. Bryophytes are not secured to the substrate like plants found in this reach, so they are more likely to be removed during large rain events. This is one of the most important vegetation types in this reach and the Comal River for supporting populations of fountain darters. Fountain darters are able to occupy this habitat type in much higher densities than other vegetation types because it provides substantial food and cover. Interestingly, there appears to be a lag time of several months between vegetation increase and darter exploitation of the new habitat.

### **Landa Lake Reach**

While the Landa Lake Reach is filled with a myriad of aquatic vegetation, *Vallisneria* is clearly dominant. Total area of this plant decreased slightly from fall 2005 (13,015.8 m<sup>2</sup>) to spring 2006 (12,604.1 m<sup>2</sup>) with larger patches of bare substrate present. Several suckermouth catfish (*Hypostomas* sp.) were seen near these bare patches in the lake, but it is unknown if they are affecting these plants. In addition, a large area near the southern tip of the southernmost island was reduced after being covered by a large, floating vegetation mat that formed. By fall 2006, *Vallisneria* area had increased (13,243.7 m<sup>2</sup>) to numbers comparable to 2005, largely due to the vegetation mat breaking up and floating downstream. *Hygrophila* area increased substantially by spring 2006 (785.9 m<sup>2</sup>), but by November (520.3 m<sup>2</sup>) it had fragmented noticeably in the downstream portions of the stand. This fragmentation made it more difficult to find suitable *Hygrophila* patches for fountain darter sampling. Mechanical disturbance from paddleboats is a possible cause, but *Ludwigia* plants in the same area did not exhibit this fragmentation. It is important to note that one or more *Nutria* were seen in the area around the islands in the middle of Landa Lake, and may be feeding on certain vegetation types preferentially. Though *Ludwigia* patches are scarce in Landa Lake, the few still present increased slightly in area from spring to fall in 2006 (38.1 and 41.0 m<sup>2</sup>, respectively). The water is relatively shallow where the *Ludwigia* plants are found, and large rain events can scour them out. Since discharge was below average, and scouring precipitation events few, these plants were able to increase in 2006. As previously mentioned, *Ludwigia* plants are considered important fountain darter habitat.

Another important plant to darters, *Cabomba*, decreased slightly from fall 2005 (300.2 m<sup>2</sup>) to spring 2006 (231.9 m<sup>2</sup>), but then increased by fall 2006 (332.4 m<sup>2</sup>). Most of the increase came in deep areas in the middle of the lake, where fountain darter populations could not be assessed. *Cabomba* colonized small areas that were previously dominated by *Vallisneria*. Green algae that were present in significant



numbers in spring 2006 (599.7 m<sup>2</sup>), were non-existent by fall. Seasonal die off and some flushing likely led to this decline.

Though discharge decreased substantially over the course of 2006, it seemed to have little effect on the normal cycle of bryophyte growth. Bryophyte area doubled in size from fall 2005 to spring 2006 (1,055.4 and 2,114.2 m<sup>2</sup>, respectively), but decreased substantially again by fall (928.8 m<sup>2</sup>). Bryophytes are often scoured out and consequently growth is stimulated after significant rain events. In 2006, there were few of these events, but one did occur in July with more limited events in the fall which may explain why bryophytes were less abundant by the fall sampling period. BIO-WEST hypothesized that higher CO<sub>2</sub> levels resulting from greater discharge stimulates bryophyte growth, and the lab study confirmed that growth of the two bryophytes (*Riccia* and *Amblystegium*) was clearly related to CO<sub>2</sub> concentration (BIO-WEST 2004). Therefore, CO<sub>2</sub> concentration is likely a key factor in the spatial and temporal bryophyte abundance in the Comal system. The bryophytes remain an important vegetation type in Landa Lake because they support the greatest densities of fountain darters in that reach (of the vegetation types sampled).

### **Old Channel Reach**

Until 2003, the Old Channel Reach maintained the most stable aquatic vegetation community with a structure (culvert) that regulates flow through this section. Later, the USFWS reconstructed this culvert to increase its capacity which resulted in increased and more variable discharge that has affected aquatic communities downstream.

*Hygrophila* continued to increase by spring 2006 (1,443.7 m<sup>2</sup>) filling in areas of bare substrate in both the downstream and upstream sections of the reach. In the deeper waters of this reach, *Hygrophila* continued to grow higher in the water column. In fall 2006, *Hygrophila* had begun to fragment in this deeper area (1,292.3 m<sup>2</sup>). *Ludwigia* exhibited considerable declines in 2006 as *Hygrophila* infiltrated and created many mixed stands of the two plants. *Ludwigia* decreased by 153 m<sup>2</sup> from spring to fall 2006, with much of this loss attributed to *Hygrophila* growing into previously pure stands of *Ludwigia*. With *Hygrophila* dominating more of this reach, fountain darter populations may decrease because this vegetation type provides lower quality habitat than the native vegetation it replaced.

*Ceratopteris* grew substantially by fall 2006 when smaller plants appeared to grow together. This plant is non-native and was likely introduced from aquariums, and can thrive in the constant temperatures of the Comal River. Filamentous algae continued to be scarce in 2006 with only one small area near the northern bank present all year. Filamentous algae is considered high-quality habitat for the endangered fountain darter. It will be important to closely monitor this reach to understand the interaction between native plants (once prevalent) and non-native plants that seem to be gaining a foothold in this reach.

### **New Channel Reach**

Of all the reaches sampled in the Comal River in 2006, the New Channel Reach appeared to benefit the most from the lower than average discharge. The steep, concrete walls and solid bedrock substrate in this section facilitates large scouring impacts when flows are high. In addition, the river is deep in this section making it difficult for plants to receive adequate sunlight. However, as a result of lower than average discharge and limited flushing events, *Cabomba*, *Ludwigia*, and bryophytes were able to at least temporarily establish in this reach.

Three new *Cabomba* plants appeared in spring 2006, though they were small (3.8 m<sup>2</sup> total area). However, by fall 2006, total area of *Cabomba* had increased by more than 35x to 144.1 m<sup>2</sup>. Large plants established in several areas especially along the north side of the reach. New *Ludwigia* plants also appeared in spring (11.3 m<sup>2</sup>), but had decreased slightly by fall (9.6 m<sup>2</sup>).

Bryophytes made their first appearance since study inception (fall 2000) in this reach in spring 2006 (344.0 m<sup>2</sup>) likely benefiting from the non-existent flushing events over winter. By fall however, they had decreased substantially to 121.0 m<sup>2</sup>, exhibiting a seasonal variability similar to the Landa Lake and Upper Spring Run reaches. *Hygrophila* continued to flourish in this reach with the lack of flushing flows. It increased to 310.1 m<sup>2</sup> in spring, and more than doubled by fall 2006 (715.4 m<sup>2</sup>). *Hygrophila* established large plants along the southern bank, and the downstream end of the reach.

Lower than average discharge was prevalent throughout 2006 in the Comal River. However, isolated small rain events and demand reduction activities were able to keep recharge high enough so that a Critical Period event was not triggered. Regardless, some trends in aquatic vegetation growth were observed in 2006. Vegetation growth occurred at all reaches during these conditions, but in certain reaches non-native vegetative growth excelled over native plant establishment. Bryophyte growth appeared to be linked to seasonal trends rather than discharge, but this observation was confounded by precipitation events in 2006. Bryophytes are an important habitat for fountain darters, and will continue to be closely monitored to understand what drives their growth cycles. The New Channel Reach appeared to benefit most from lower than average discharge and limited flushing events because higher-flow conditions and flashy rainfall causes extensive scouring in this channelized reach.

## **Fountain Darter Sampling**

### **Drop Nets**

A total of 540 drop net samples were conducted during 2000-2006 in the Comal Springs/River ecosystem. Twenty-two of these samples were conducted in spring 2006 and 22 were conducted in fall 2006. The number of drop net sites and vegetation types sampled per reach is presented in Table 6. Drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sample event, and data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. There were some changes over the course of the study including a shift from sampling two bare substrate sites during each sampling event in the Upper Spring Run and Landa Lake in 2000-2001 to sampling two bryophytes sites in those reaches beginning in the summer of 2001. In 2004, there was a change in the sample design for the Old Channel Reach in response to the dramatic shift from a vegetation community dominated by filamentous algae and *Ceratopteris* to one dominated by *Hygrophila* and *Ludwigia*. Also, the New Channel Reach was removed from the drop net sampling effort as vegetated areas were often too deep to sample.

**Table 6. Drop net sites and vegetation types sampled per reach in 2006.**

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes <sup>a</sup> (2)	Bryophytes <sup>a</sup> (2)	None <sup>b</sup>	<i>Ludwigia</i> (2)
<i>Sagittaria</i> (2)	<i>Hygrophila</i> (2)		<i>Hygrophila</i> (3) <sup>c</sup>
<i>Hygrophila</i> (2)	<i>Cabomba</i> (2)		Filamentous Algae (1) <sup>d</sup>
	<i>Vallisneria</i> (2)		
	<i>Ludwigia</i> (2)		
<b>Total (6)</b>	<b>Total (10)</b>	<b>Total (0)</b>	<b>Total (6)</b>

<sup>a</sup> Switched from Open to Bryophytes, summer 2001.

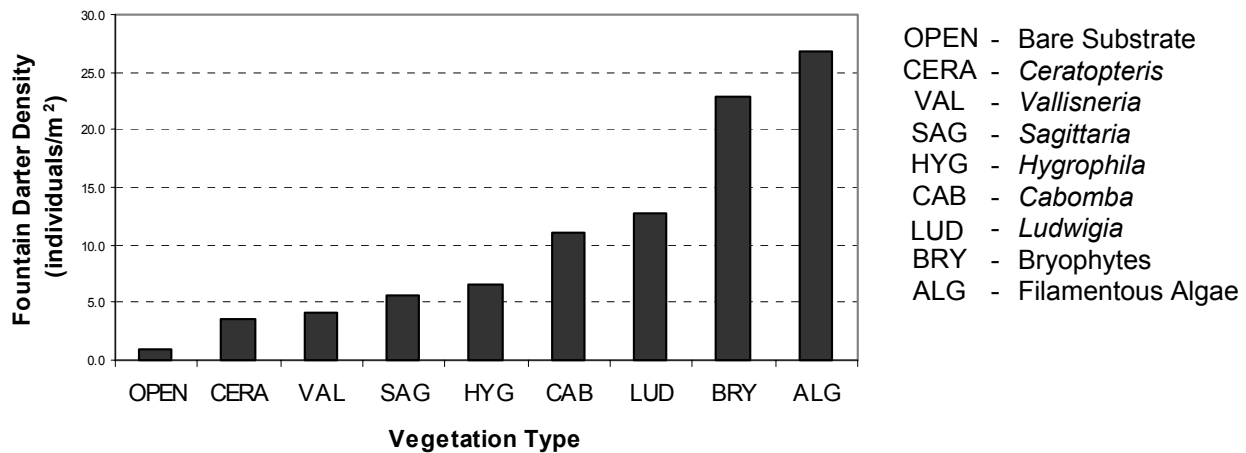
<sup>b</sup> Areas with vegetation were too deep to sample in spring and fall 2006.

<sup>c</sup> Three *Hygrophila* sites were sampled in 2006 sample to make up for one filamentous algae site.

<sup>d</sup> Only one filamentous algae site was sampled in 2006 due to limited coverage.

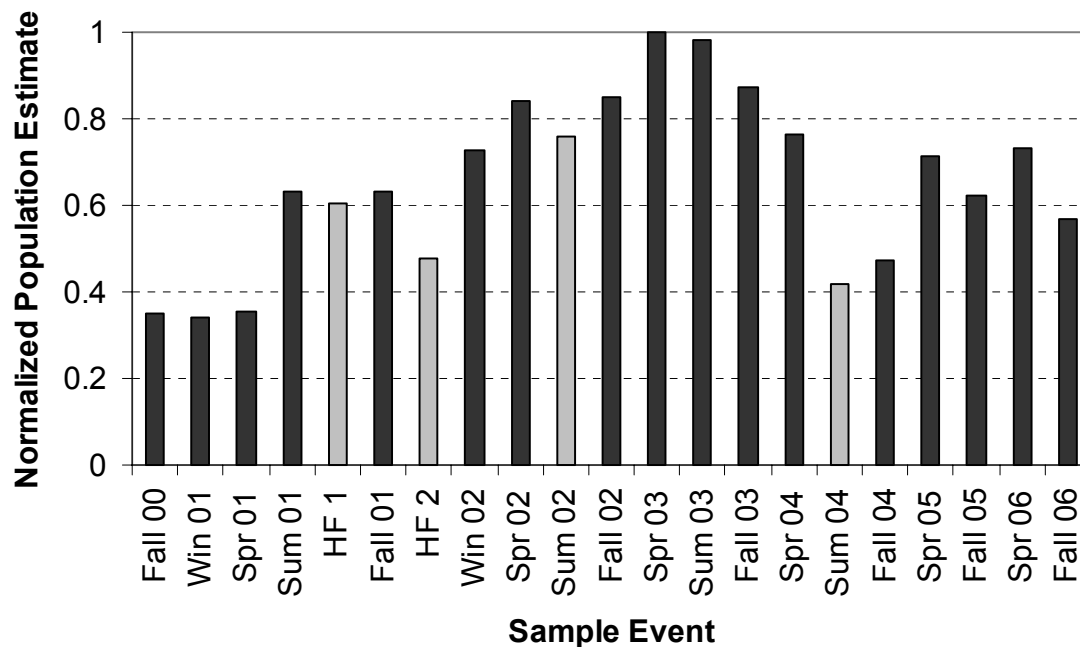
The diversity of aquatic vegetation found in the Comal Springs/River ecosystem provides a great range of available habitat for fountain darters covering a wide level of suitability. During 2000-2006, densities of fountain darters in the various vegetation types ranged from 3.6 per 1.0 m<sup>2</sup> in *Ceratopteris* to 26.8 per 1.0 m<sup>2</sup> in filamentous algae (Figure 6). The bryophytes also contained high numbers of fountain darters (22.9/m<sup>2</sup>), followed by *Ludwigia* (12.7/m<sup>2</sup>), and *Cabomba* (11.0/m<sup>2</sup>). Although filamentous algae contained the most fountain darters per unit area, it is rather uncommon and occurs mainly in a few small patches in the Old Channel Reach. Coverage of filamentous algae has decreased in the Old Channel Reach in recent years, and this area is now dominated by *Hygrophila* and *Ludwigia* (see aquatic vegetation mapping section). Although fountain darter densities are slightly less in bryophytes than in filamentous algae, bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake reaches, and thus make up a significant portion of the available habitat. Filamentous algae and bryophytes have high densities of fountain darters because they provide cover at the substrate level and high numbers of amphipods (an important food item). However, they are also the most easily impacted by scouring during high-flows.

Although darter densities are relatively high in *Ludwigia* suggesting that it is high quality habitat, it is also susceptible to scouring and has shown large fluctuations in coverage during the study period. *Hygrophila*, an exotic species similar to *Ludwigia*, is abundant within all four reaches; however, it provides substantially lower quality habitat. *Cabomba*, which grows in relatively stable locations with some seasonal variation, provides similar fountain darter habitat suitability (based on density) as *Ludwigia*. *Sagittaria*, *Vallisneria*, and *Ceratopteris* exhibited relatively poor habitat suitability. Of these, densities in *Sagittaria* were the highest mostly as a result of its association with *Riccia*. *Sagittaria* is on the low end of habitat suitability for fountain darters, except when *Riccia* is abundant and settles on and within this vegetation type resulting in a sharp increase in fountain darter densities. Although *Ceratopteris* was sampled with drop nets in previous years, its coverage was drastically reduced in 2005 and it has not been sampled since. The wide range in suitability and relative importance of the various aquatic vegetation types makes the composition and abundance of aquatic vegetation a critical factor in monitoring the fountain darter population.



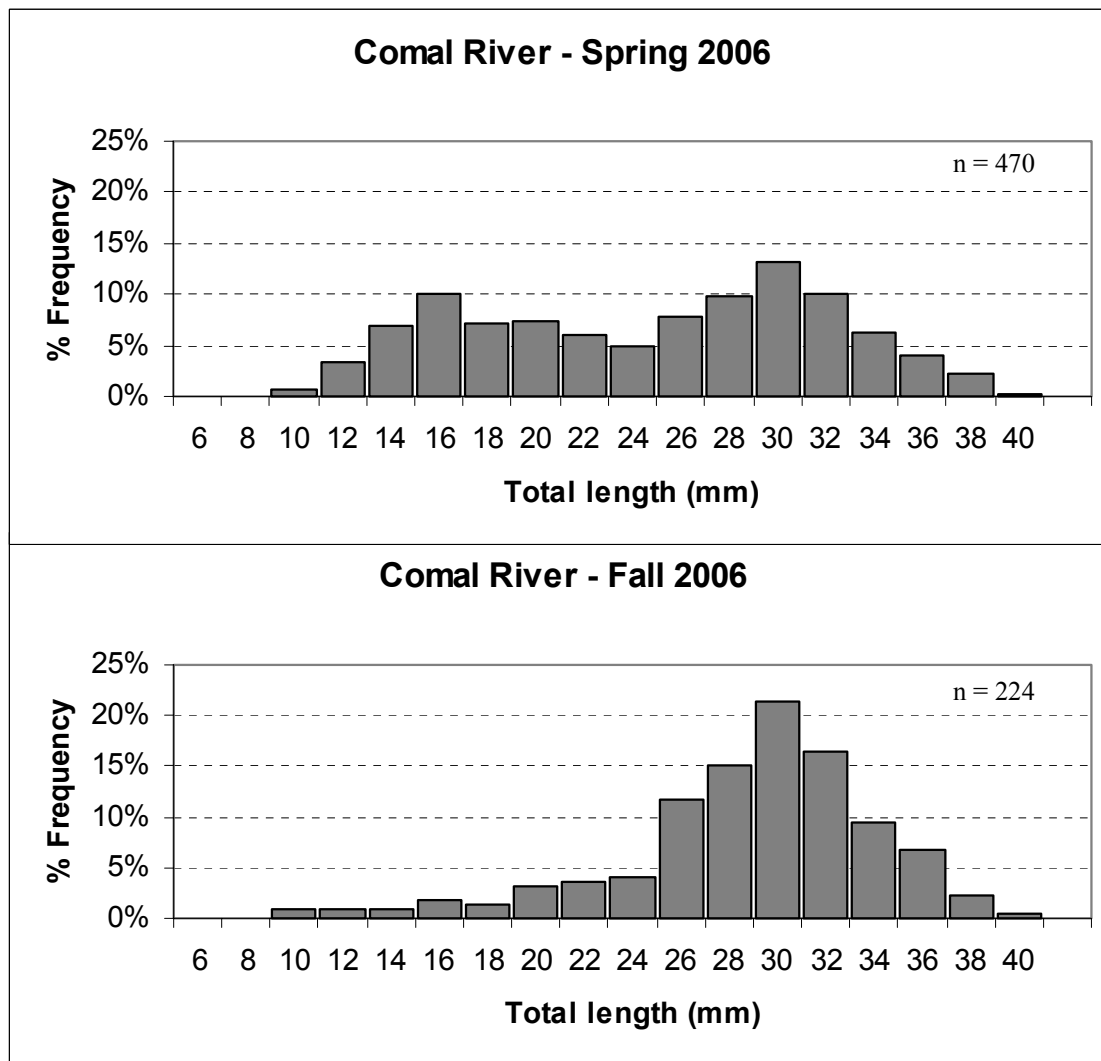
**Figure 6. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem from 2000-2006.**

Estimates of fountain darter population abundance in all reaches (Figure 7) 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. The vegetation type that had the greatest influence on these estimates during 2000-2006 was the bryophytes because of the size of the Landa Lake Reach (where most of the bryophytes were mapped) and the density of fountain darters found there. Estimates of population abundance were highest in spring 2003 when coverage of bryophytes peaked in Landa Lake (Figure 7). Population estimates for spring and fall 2006 were similar to those observed in 2005. Although they did not approach the highest estimates observed in spring 2003, they fell within the range of variation observed during the study period. Population estimates in fall 2000, winter 2001, and spring 2001 are low because mapping at the time did not include algae in the Old Channel Reach or bryophytes in the Landa Lake Reach. All four high-flow Critical Period samples during the study period showed a decrease in the population estimate relative to the previous sample, although there was an increase in the subsequent sample each time. This is most likely related to scouring of important vegetation types resulting in fountain darters becoming more scattered at high flows.

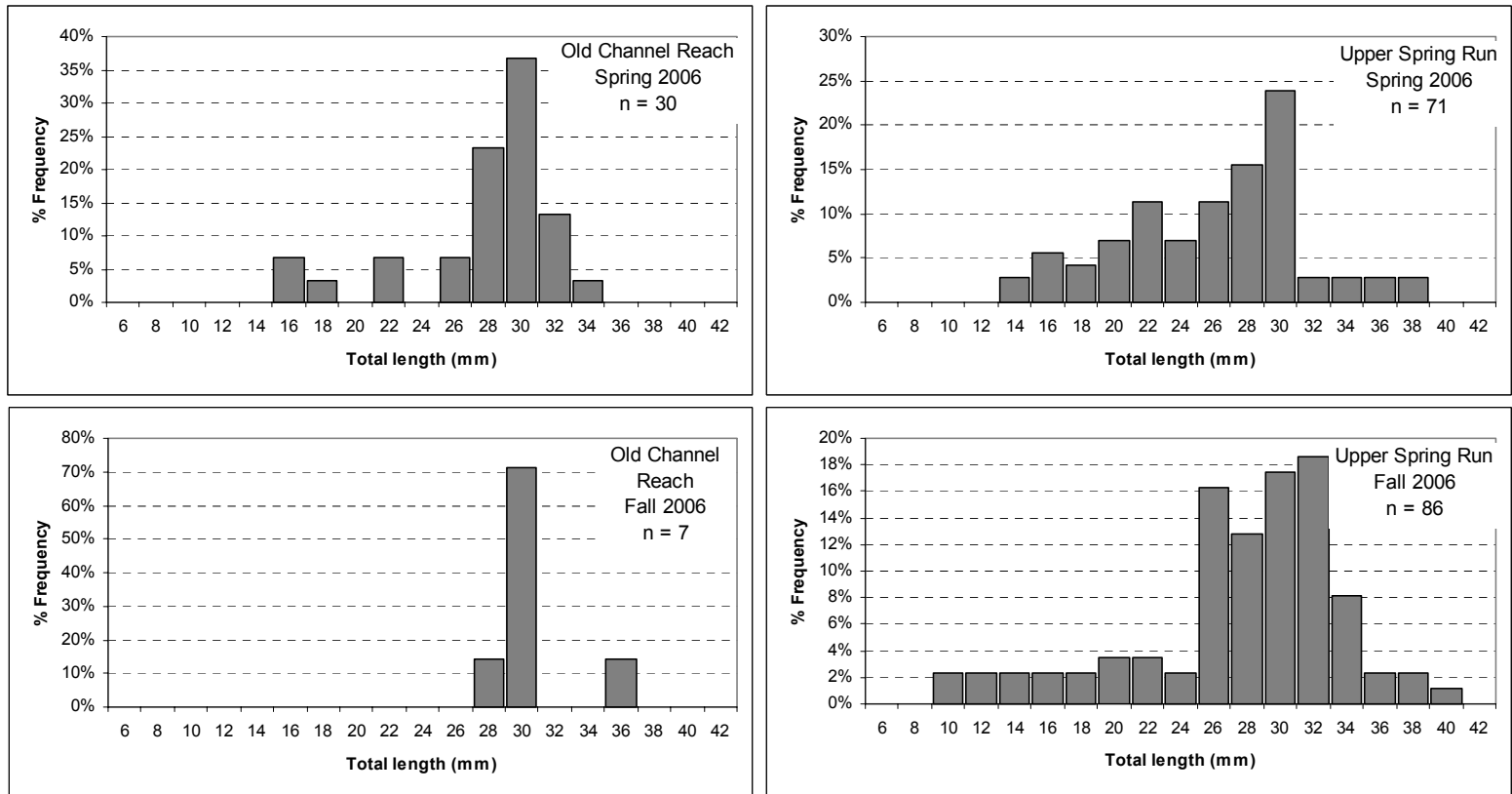


**Figure 7. Population estimates of fountain darters in all four sample reaches combined (2000-2006); values are normalized to the maximum sample. Light-colored bars represent high-flow Critical Period sampling events.**

Drop netting efforts in 2006 resulted in collection of 694 fountain darters in the Comal River/Springs ecosystem. The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem during each sample period in 2006 is presented in Figure 8 (all data collected in previous years is presented in Appendix B). Smaller fountain darters are more abundant in the spring sample suggesting a peak in reproduction during this time. However, in some reaches reproduction seems to occur year around. For example, small darters were present in both spring and fall samples in the Upper Spring Run Reach (Figure 9) suggesting year-round reproduction. Higher abundance of fountain darters in the Upper Spring Run Reach compared to the Old Channel Reach is most likely a result of differing vegetation communities in these sections. Bryophytes are abundant in the Upper Spring Run Reach, while the Old Channel Reach is dominated by less suitable *Hygrophila*.



**Figure 8. Length frequency distribution of fountain darters among all drop-net sample events in the Comal River in 2006.**



**Figure 9. Length frequency distributions of fountain darters collected in spring and fall 2006 in the Old Channel and Upper Spring Run Reaches.**

In addition to fountain darters, 73,313 specimens representing 22 other fish taxa have been collected by drop netting from the Comal Springs/River ecosystem during the study period; of these, seven are considered exotic or introduced (Table 7). Although several of these species are potential predators of fountain darters, previous data collected during this study suggested that predation by both native and introduced predators is minimal during average discharge conditions. The impact of predation is to be further evaluated under low discharge.

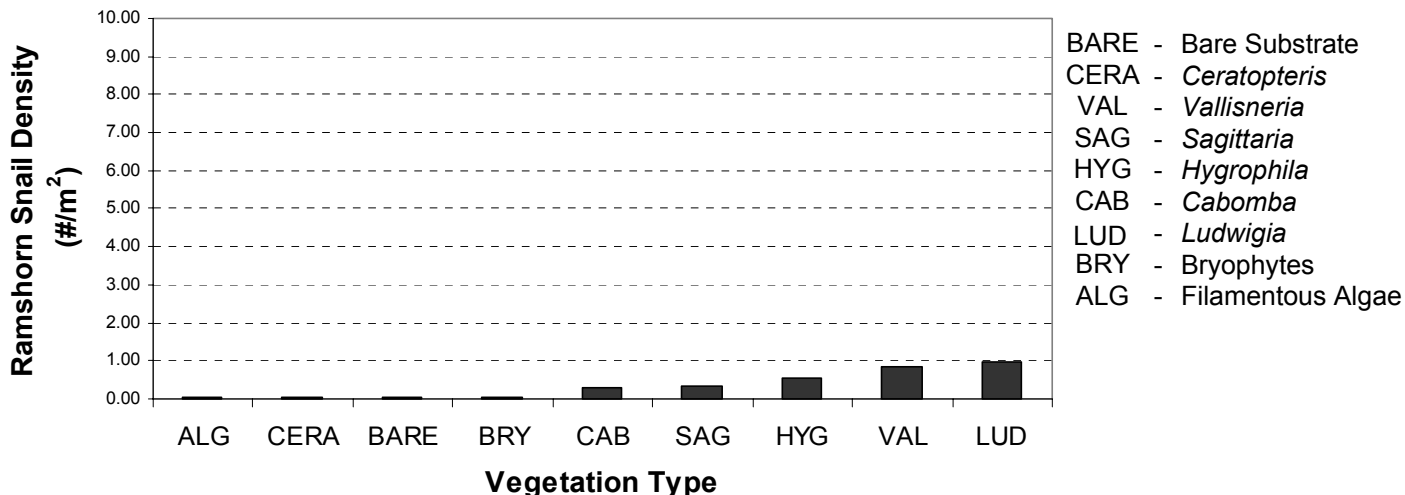
Other potential impacts of exotic fish species include effects of herbivorous species such as the suckermouth catfish (*Hypostomus sp.*) on algae and vegetation communities that serve as fountain darter habitat. Although these fish are rarely captured in drop nets, based on visual observations they are extremely abundant in the system. This species has the potential to affect the vegetation community and thus impact important fountain darter habitats and food supplies.

Although not a fish, one exotic species which has had considerable impact on the vegetation community in the Comal Springs/River ecosystem in the past is the giant ramshorn snail. In the early 1990s, giant ramshorn snails became very dense and caused substantial destruction to the vegetation community in the Comal River. Ramshorn snail densities averaged across all sites and years show that the snails are most common in *Ludwigia*, *Vallisneria*, and *Hygrophila*, and are rare in vegetation types where fountain darter densities are highest (filamentous algae, bryophytes) (Figure 10). Densities of giant ramshorn snails have declined throughout the study period (Figure 11). In fact, no giant ramshorn snails were

collected from the Comal Springs/River ecosystem during drop net sampling in 2006. However, because this exotic species can have considerable impacts at higher densities, close monitoring of their populations will be continued.

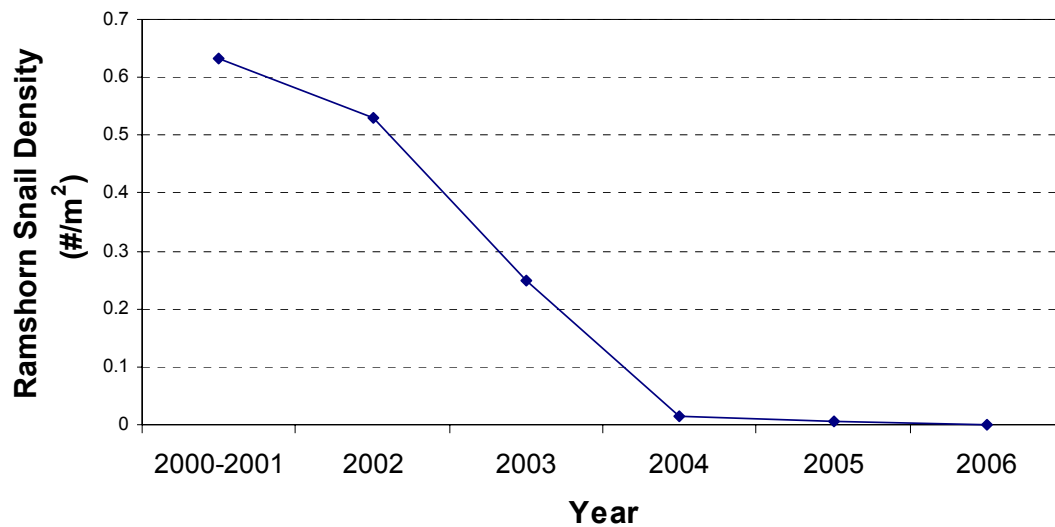
**Table 7. Fish taxa and the number of each collected during drop-net sampling.**

COMMON NAME	SCIENTIFIC NAME	STATUS	NUMBER COLLECTED	
			2006	2001-2006
Rock bass	<i>Ambloplites rupestris</i>	Introduced	0	18
Black bullhead	<i>Ameiurus melas</i>	Native	0	1
Yellow bullhead	<i>Ameiurus natalis</i>	Native	8	81
Mexican tetra	<i>Astyanax mexicanus</i>	Introduced	9	251
Central stoneroller	<i>Campostoma anomalum</i>	Native	0	1
Rio Grande cichlid	<i>Cichlasoma cyanoguttatum</i>	Introduced	25	331
Guadalupe roundnose minnow	<i>Dionda nigrotaeniata</i>	Native	2	259
Fountain darter	<i>Etheostoma fonticola</i>	Native	694	9421
Greenthroat darter	<i>Etheostoma lepidum</i>	Native	10	53
Gambusia	<i>Gambusia sp.</i>	Native	9509	66830
Suckermouth catfish	<i>Hypostomus plecostomus</i>	Exotic	2	59
Redbreast sunfish	<i>Lepomis auritus</i>	Introduced	3	119
Green sunfish	<i>Lepomis cyanellus</i>	Native	0	10
Warmouth	<i>Lepomis gulosus</i>	Native	0	24
Bluegill	<i>Lepomis macrochirus</i>	Native	0	30
Longear sunfish	<i>Lepomis megalotis</i>	Native	0	36
Spotted sunfish	<i>Lepomis punctatus</i>	Native	128	978
Sunfish	<i>Lepomis sp.</i>	Native/Introduced	46	631
Spotted bass	<i>Micropterus punctulatus</i>	Native	0	1
Largemouth bass	<i>Micropterus salmoides</i>	Native	6	82
Texas shiner	<i>Notropis amabilis</i>	Native	4	33
Mimic shiner	<i>Notropis volucellus</i>	Native	1	1
Sailfin molly	<i>Poecilia latipinna</i>	Introduced	582	3468
Tilapia	<i>Tilapia sp.</i>	Exotic	0	16



**Figure 10. Density of giant ramshorn snails by vegetation type (averaged across all sites and all years).**



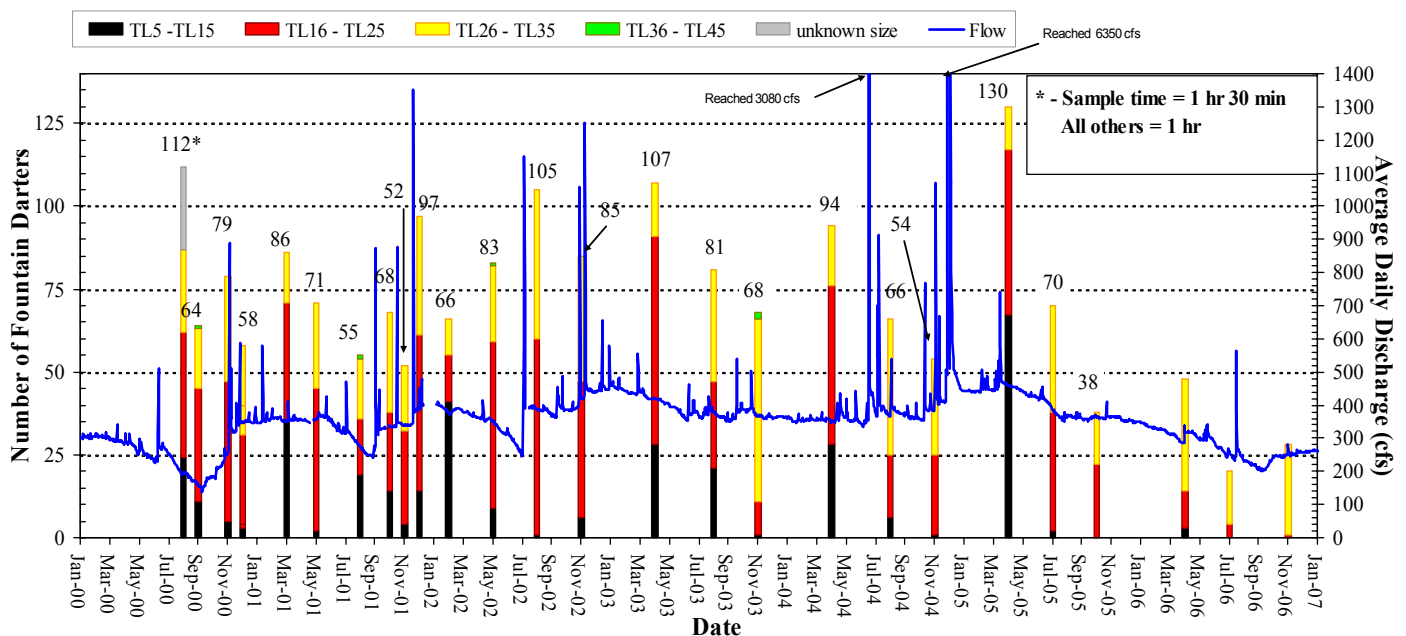


**Figure 11. Density of giant ramshorn snails collected in drop net sampling in each year across all vegetation types.**

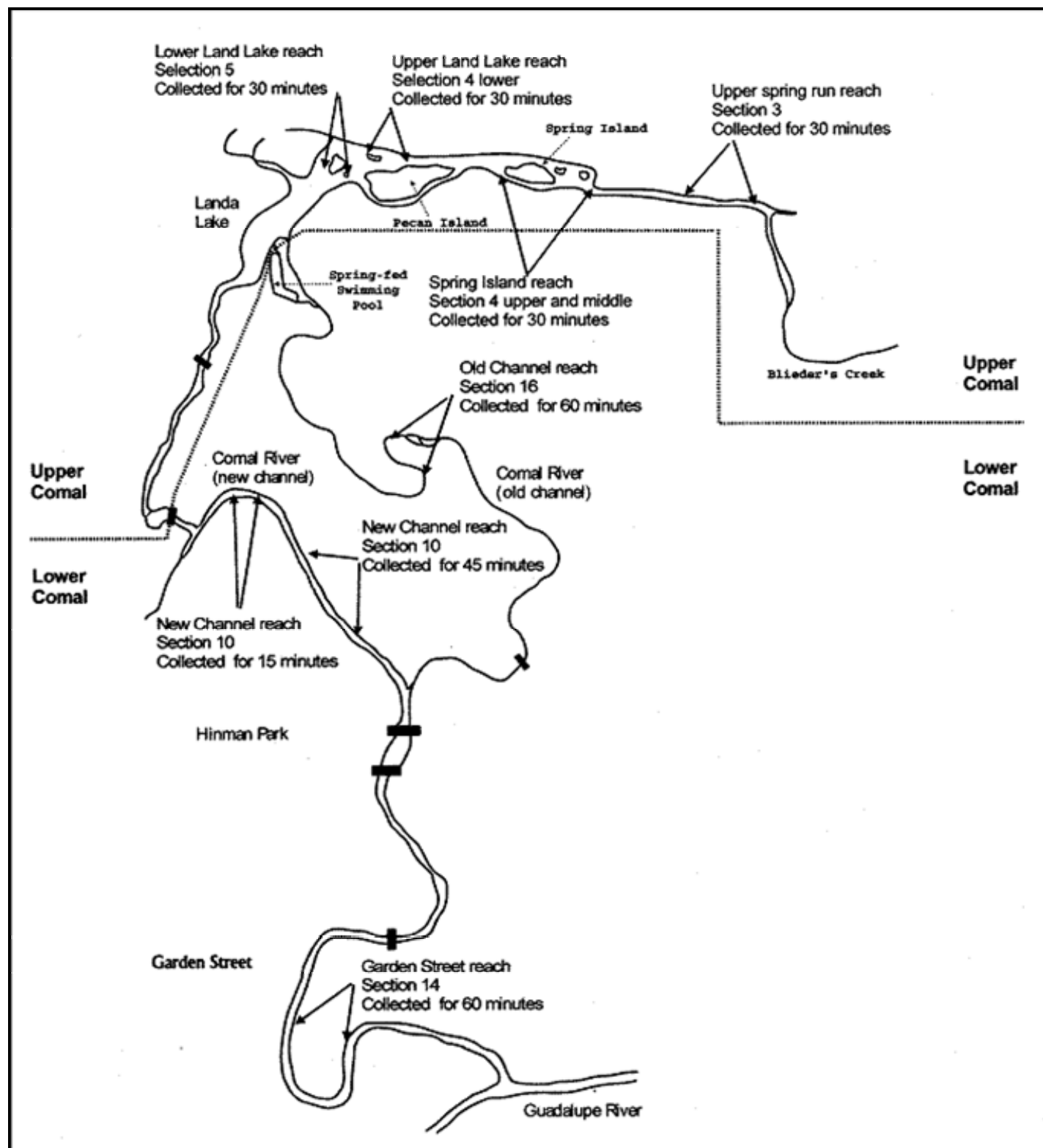
## Dip Nets

Data gathered using dip nets are graphically represented in Figure 12 for the Old Channel Reach and in Appendix B for all other reaches. The boundaries for each section of the dip net collection efforts are depicted in Figure 13.

### Fountain Darters Collected from the Old Channel Reach (Section 16) Dip Net Results - Comal River



**Figure 12. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.**



**Figure 13. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.**

In 2001-2005, the highest numbers of small fountain darters (5-15 mm TL) were usually observed in dip net samples during the spring of each year in all sample reaches (Appendix B). This trend was consistent in 2006 data, suggesting a peak in reproduction in early spring. However, small fountain darters are occasionally captured in summer, winter, and fall sample periods as well. In 2006, three out of seven sample reaches (Upper Spring Run, Spring Island, Landa Lake) contained small fountain darters during all three sample periods. This indicates that there is some reproduction occurring year-round, although perhaps on a limited basis and only in certain areas. These areas are relatively close to spring upwellings and contain large amounts of bryophytes, which provide high-quality fountain darter habitat according to drop net density estimates.

The Old Channel Reach has contained darters of the smallest size group (<15 mm TL) in every sample taken during the study period, except for fall 2005, summer 2006, and fall 2006. This shift in size class distributions has been accompanied by an overall reduction in the number of darters collected during dip net samples, and is likely the result of changes in the vegetation community in the Old Channel Reach. This reach, which previously had good coverage of filamentous algae, has become dominated by the non-native *Hygrophila* in recent years.

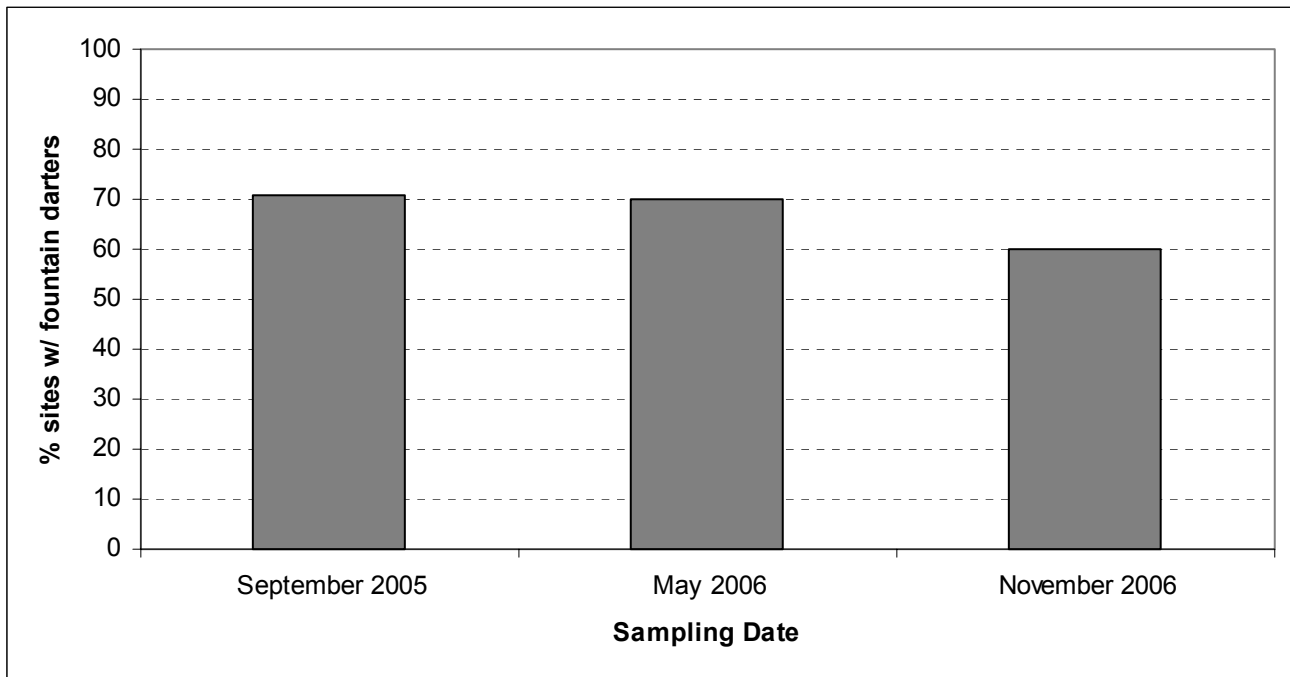
In general, large fountain darters (>25 mm TL) are most abundant in summer and fall dip net samples, and are usually in low abundance in spring samples. Size class distributions of fountain darters from dip netting correlate well with those of drop netting: small fountain darters most abundant in the spring, and larger darters dominating fall samples. Variability in the total number of fountain darters collected by dip netting makes any inference into population trends difficult with this method. Although there was a substantial increase in the number of darters collected from the Upper Spring Run Reach in 2003, this increase is associated with an increase in bryophytes in this reach at approximately the same time. Similarly, vegetation shifts in the Old Channel Reach seem to have resulted in a decrease in the overall numbers of darters collected there since summer 2005.

## **Dip Net Techniques Evaluation**

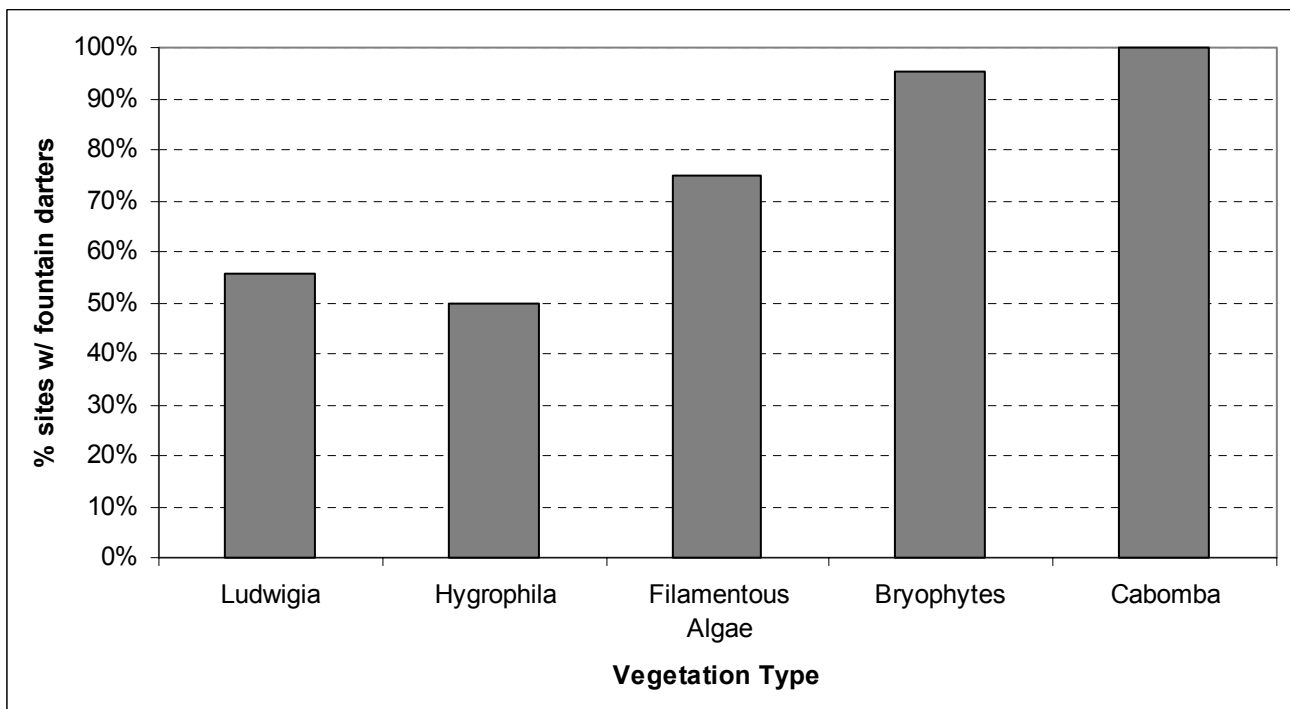
Across all vegetation types, fountain darters were present at 70% of sites during the May sample, and at 60% of sites during the November sample. These percentages are similar to those documented during evaluation of this technique in September 2005, when 71% of sites contained fountain darters (Figure 14). Although fountain darters were slightly less common in November 2006 samples, this is likely due to the variation inherent in a random sampling technique.

Fountain darters were most common in *Cabomba* (100% of sites), Bryophytes (95%), and filamentous algae (75%), and were much less common in *Ludwigia* (56%) and *Hygrophila* (50%) sites (Figure 15). Compared to 2005 presence/absence data, fountain darters were more common in *Cabomba* and less common in filamentous algae in 2006. The former is likely due to changes in the coverage of filamentous algae. Dense stands of filamentous algae which were previously present in the Old Channel Reach exhibited substantial reductions in coverage in 2006. Therefore, samples were taken in relatively sparse stands of algae where darters were less common. Fountain darter density data from drop net sampling confirms that algae and bryophytes represent high quality habitat, however, densities in *Cabomba* were somewhat lower. Given that previous dip netting found fountain darters to be less common in *Cabomba* and that drop netting data shows relatively lower densities in *Cabomba*, the high

percentage of *Cabomba* sites containing fountain darters in 2006 presence/absence data may be a result of variation due to a small number of sites.



**Figure 14. Percent of sites (n = 50) with fountain darters present during each Presence/Absence dip net sample.**



**Figure 15. Percent of sites within each vegetation type with fountain darters present in 2006.**

## **Visual Observations**

Fountain darters were observed in the deepest portions of Landa Lake (depths greater than 2 m) during each sampling event, including all low-flow and high-flow events to date. Due to time constraints in November 2006, only one quantitative sample was performed in Landa Lake in 2006. The quantitative sampling results are limited to a single grid per sampling event; therefore an accurate estimate of the true population size within the sample area is not possible. A much more labor-intensive effort would be required to provide such an estimate. These data simply provide an indication of the relative abundance of the fountain darters that are found in areas similar to that sampled and allow some insight into trends that may be occurring over time. Table 8 shows the number of fountain darters observed in the 7.8 m<sup>2</sup> grid per sampling event.

**Table 8. The number of fountain darters observed in Landa Lake per grid/sampling event.**

SAMPLE DATE	NUMBER OF FOUNTAIN DARTERS	PERCENT RICCIA WITHIN GRID
Summer 2001	24	50
High Flow 1 2001	31	50
Fall 2001	44	65
High Flow 2 2001	39	60
Winter 2002	50	90
Summer/High Flow 2002	21	40
Fall 2002	88	80
Spring 2003	43	85
Summer 2003	51	90
Fall 2003	56	80
Spring 2004	45	60
Summer 2004	12	15
Fall 2004	48	70
Spring 2005	49	90
Fall 2005	65	95
Spring 2006	32	35

These results suggest there is some fluctuation in the use of the deepest areas of Landa Lake by fountain darters. Coverage of bryophytes in spring 2006 was approximately one-third of fall 2005, and as a result the number of darters was cut in half. In summer 2004 and summer 2002, similar bryophyte coverage led to low numbers of fountain darters. Overall, our observations continue to reinforce the hypotheses that Landa Lake is an integral component to the habitat of species found in the Comal Springs ecosystem, and a sizable portion of the fountain darter population is found there.

## **Gill Parasite Evaluation**

Although the intensive parasite evaluation is only at its midpoint, a few preliminary trends were identified in 2006. Observations show that the Elizabeth Avenue site (Old Channel Reach) consistently has the highest trematode abundance, followed by Liberty Avenue and Houston Street sites (both in the Upper Spring Run Reach). While no seasonal trend is evident at this time, discharge and flushing flows do appear to have an effect on parasite concentrations. For example, on July 4, 2006 there was

precipitation that caused the overall discharge of the Comal River to peak at 566 cfs. The river was sampled nine days later, and the abundance of cercariae was the lowest seen to date. Additionally, when the discharge of the river was the lowest, in late summer, higher abundances of cercariae were observed. The sample with the highest cercarial concentration seen thus far was taken from the Liberty Avenue site in October and contained 303 cercariae per 5L sample of river water.

## **Comal Springs Salamander Visual Observations**

All SCUBA/snorkel surveys revealed the presence of Comal Springs salamanders along the lake bottom and in each sampled Spring Run in 2006. No Comal Springs salamanders were observed in any areas with excessive sediment. The total number of Comal Springs salamanders observed at each survey site during each sampling event is presented in Table 9. Four salamanders were found in the Spring Island spring run in 2006, where none had been found in 2005. In 2006, it was observed that larger rocks were more numerous in this area. In addition, there were larger patches of *Amblystegium* present. Immediately adjacent, salamanders in the Spring Island outfall decreased by half from fall 2005 to spring 2006 (16 and 8, respectively). Numbers nearly quadrupled by fall 2006 (29) as discharge remained lower than average. The lack of flushing flows contributed to a higher amount of *Amblystegium* present in the East outfall creating much more habitat for salamanders to hide and feed.

Salamander numbers were at their lowest in spring 2006 (12) since spring of 2002 (10) in Spring Run 1. These numbers only increased slightly by fall (14). Continually decreasing flows in Spring Run 1 in 2006 (Table 4) enabled considerable growth of aquatic macrophytes in this reach. *Bacopa* and *Eleocharis* established in large areas and covered rocks that are easily sampled salamander habitat. The increased vegetation makes surface sampling more difficult likely leading to the slight decrease in salamander numbers in 2006 (only 3 salamanders were found in these plants in 2006).

Salamander numbers in Spring Run 3 were similar to other years, though they nearly doubled from fall 2005 to spring 2006 (7 and 13, respectively). Little change was observed in the habitat here except a small dam was built near the gage creating higher velocity flows near the river right bank. While lower than average discharge was prevalent throughout the system in 2006, the relatively constant spring flow led to little change in salamander numbers. Aquatic vegetation growth has not been an issue in Spring Run 3 as the coarser substrate and shaded conditions are not amenable for vegetation expansion.

**Table 9. Total number of Comal Springs salamanders observed at each survey site during each sample period.**

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
August 2000	9	13	11	1	34
September 2000	5	14	6	5	30
Fall 2000	8	4	4	2	18
Winter 2001	16	9	8	1	34
Spring 2001	20	7	17	6	55
Summer 2001	23	15	4	4	46
High-flow 1 2001	31	12	1	6	50
Fall 2001	11	8	13	7	39
High-flow 2 2001	18	2	6	5	31
Winter 2002	18	9	7	3	53
Spring 2002	10	15	6	5	62
High Flow 2002	18	7	3	16	67
Fall 2002	20	10	8	9	47
Spring 2003	20	21	6	13	60
Summer 2003	25	10	3	13	51
Fall 2003	31	10	3	19	63
Spring 2004	36	14	7	12	69
Summer 2004	27	14	4	14	59
Fall 2004	20	2	2	35	59
Spring 2005	18	10	2	11	41
Fall 2005	22	7	0	16	45
Spring 2006	12	13	2	8	35
Fall 2006	14	11	2	29	56
Average	18.8	10.3	5.4	10.4	44.9

## Macroinvertebrate Sampling

In 2006, sampling around spring openings and regular monitoring of Comal Springs riffle beetles in several locations were designed to assess habitat requirements of the federally listed invertebrate species.

### **Drift Net sampling**

A total of 144 hours of sample time occurred among the three drift net sites at Comal Springs in 2006 and 11 species were captured (Table 10). Table 11 displays the physico-chemical data collected at these sites. Total discharge in the Comal River was approximately 304 cfs during the April sample and 256 cfs during the November sample. Though no Critical Period events were triggered in 2006, lower than average flows offered an opportunity to study these unique populations.

Despite lower than average discharge conditions in 2006, population changes of species were similar to previous years. Species of the genus *Stygobromus* and *Lirceolus* continued to be most abundant at all sites (Table 10, Figure 16). *Stygobromus pecki* (Peck's cave amphipod) was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long, which suggests that smaller individuals may be more susceptible to expulsion from the aquifer. Those individuals that were too small to identify to species were recorded as *Stygobromus* sp. and most likely consisted of both *S. russelli* and *S. pecki*.

An average of 19 *Stygobromus pecki* (other small individuals were unidentifiable to species) were observed during each period, slightly higher than the average from 2005 (14.5, BIO-WEST 2006). Most of *S. pecki* were found in the upwelling along the western shore of Landa Lake. A total of 10 *Heterelmis comalensis* and 2 *Stygoparnus comalensis* were found in the Spring Run sites in 2006. These numbers were both considerably lower than previous years. As in previous years, *H. comalensis* and *S. comalensis* were only found in Spring Runs 1 and 3. One *S. comalensis* was collected in the upwelling site in 2003, but no *H. comalensis* have been discovered there. A possible reason for the lack of these organisms in the upwelling could be due to where the water is upwelling from. The water in Spring Runs 1, 2, and 3 is connected to the shallower recharge zone in the aquifer, while water in the springs in Landa Lake and upstream are connected to the deeper artesian flow system (G. Schindel, pers. comm., 2007). These springs (Landa and upstream) are warmer, and because the water comes from a deeper source, there may not be an ample food source for the beetles. These discoveries may explain why *H. comalensis* has never been found in the upwelling along the western shore of Landa Lake.



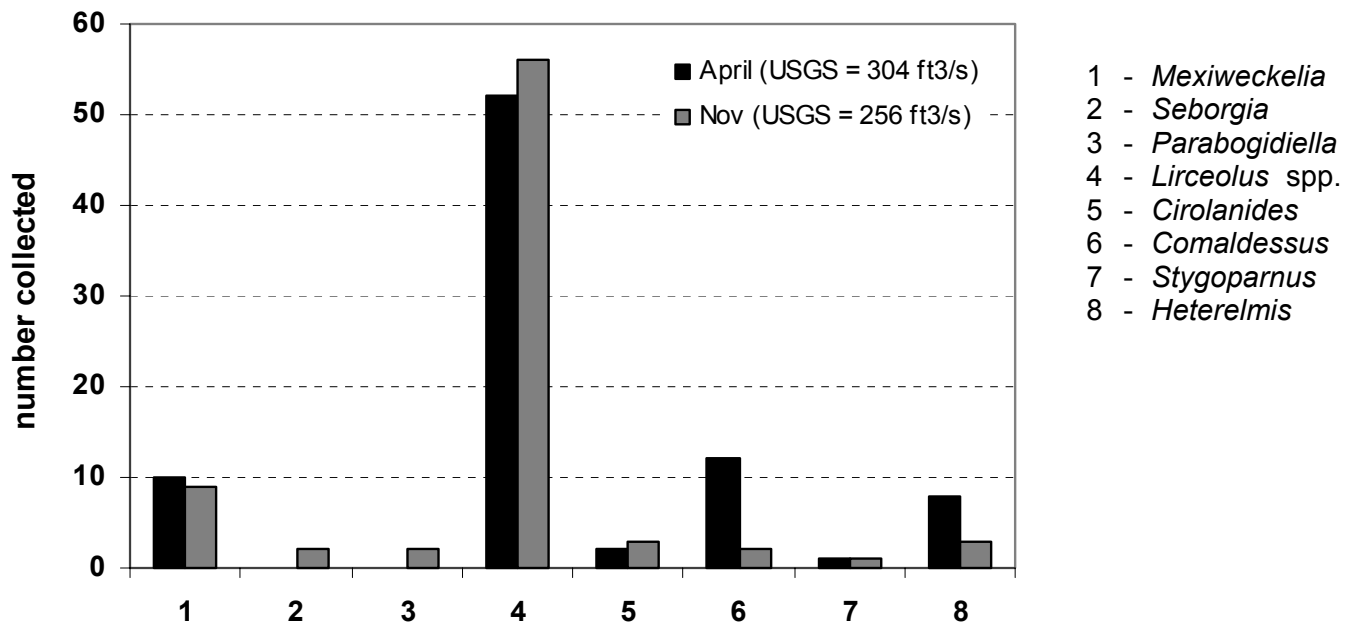
**Table 10. Total numbers of troglobitic and endangered species collected in drift nets during April and November, 2006. Federally endangered species are designated with (E). A = adult beetles. L = larvae.**

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	48	48	48	144
<b>Crustaceans</b>				
Amphipoda				
Crangonyctidae				
<i>Stygobromus pecki</i> (E)	15	11	31	57
<i>Stygobromus russelli</i>	1	1		2
<i>Stygobromus</i> spp.	96	180	443	719
All <i>Stygobromus</i>	112	192	474	778
Hadziidae				
<i>Mexiweckelia hardeni</i>	3	16		19
Sebidae				
<i>Seborgia relict</i> a	1	1		2
Bogidiellidae				
<i>Parabogidiella n. sp. ?</i>		1	1	2
Isopoda				
Asellidae				
<i>Lirceolus</i> (2spp.)	70	36	2	108
Cirolanidae				
<i>Cirolanides texensis</i>		1	4	5
<b>Insects</b>				
Coleoptera				
Dytiscidae				
<i>Comaldessus stygius</i>		14 A		14
<i>Haideoporus texanus</i>		9 (1L, 8A)		2
Dryopidae				
<i>Stygoparnus comalensis</i>	1 L	1 L		2
Elmidae				
<i>Heterelmis comalensis</i>	8 (5L, 3A)	3 L		10

**Table 11. Results of water quality measurements conducted in 2006 during drift net sampling efforts at Comal Springs.**

	Spring Run 1		Spring Run 3		West Shore Upwelling	
	April	Nov	April	Nov	April	Nov
Date						
Temperature (°C)	23.1	23.1	23.3	23.3	23.7	23.6
Conductivity (mS)	0.555	0.560	0.6	0.560	0.557	0.560
pH	6.9	6.9	6.9	7.0	6.9	6.9
Dissolved Oxygen (mg/L)	6.5	5.7	5.6	5.4	5.6	5.1
Current Velocity (m/s)	0.5	0.6	0.4	0.4	0.4	0.2

In 2006, there appeared to be no seasonal influence on total numbers of invertebrates found in the sample sites. Even though discharge continued to decrease in 2006, total numbers remained similar to previous years. Water quality variables remained relatively constant at all sites in 2006, indicating a stable environment for the organisms at the sampled discharges.

**Figure 16. Drift net sampling results for each species (except *Stygobromus* sp.) combined across all sample sites.**

An undescribed species of blind amphipod in the Bogidiellidae family was discovered in the Spring Run 3 and upwelling sites along the western shore of the lake in November 2006. Two organisms were collected, and this undescribed species is in the *Parabogidiella* genus. This species is similar to *Artesia*

*subterranea*, but none of these were collected in 2006. *Parabogidiella* and *Artesia* are the only bogidilleads reported north of Mexico.

## **Comal Springs Riffle Beetle**

The Comal Springs riffle beetle sampling provides basic information on the population dynamics and distribution among sample sites. The total number of beetles collected in 2006 (1,013) were very similar to that of 2005 (1,009) in the Comal River system (Table 12). The proportion of larvae to adults in 2006 (0.26) and 2005 (0.29) were also nearly identical. It is unclear if this disparity is due to sampling bias or behavior of the beetles. Reproductive behavior of the Comal Springs riffle beetle may account for their disparity considering members of the Elmidae family are known to require anywhere from 6 months to 3 years to complete the life cycle from egg to adult (Arsuffi 1993).

**Table 12. Total numbers of Comal Springs riffle beetles (*Heterelmis comalensis*) at each survey site during each sampling period.**

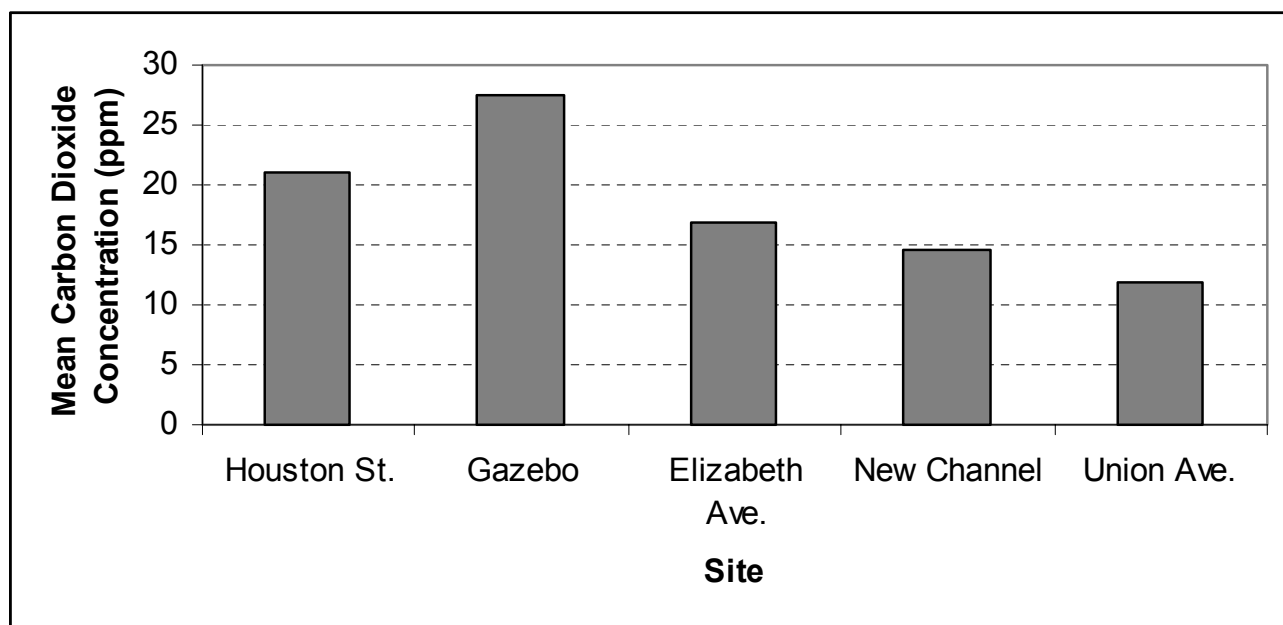
SAMPLE PERIOD	SPRING RUN 3	Western Shore Landa Lake	SPRING ISLAND	TOTAL BY SAMPLE
January 2003	65	7	47	119
March 2003	32	5	10	47
September 2003	10	15	42	67
November 2003	16	9	18	43
May 2004	88	83	122	293
August 2004	169	143	90	402
November 2004	170	175	146	491
April 2005	119	121	121	361
November 2005	262	201	185	648
May 2006	256	195	160	611
November 2006	185	92	125	402
Average	124.7	95.1	96.9	316.7

Though it is currently unclear how discharge affects this sensitive species, numbers of beetles at all sites decreased from spring to fall in 2006. Each of the three sample sites has very different physical characteristics. The Spring Run 3 site has lateral spring flow from the shoreline into the fast moving primary current of the Spring Run, the western shoreline site has lateral spring flow directly into Landa Lake and the Spring Island site has upwelling flow from the bottom of Landa Lake. The Spring Island site had the lowest velocity, primarily because the water tends to diffuse out rather than travel in a defined stream as with the lateral springflow in the other two sites, but also because these were upwelling sites where it is difficult to make velocity measurements. Despite the physical differences between sites, there were minimal differences in abundance among sites in 2006 (as in 2005).

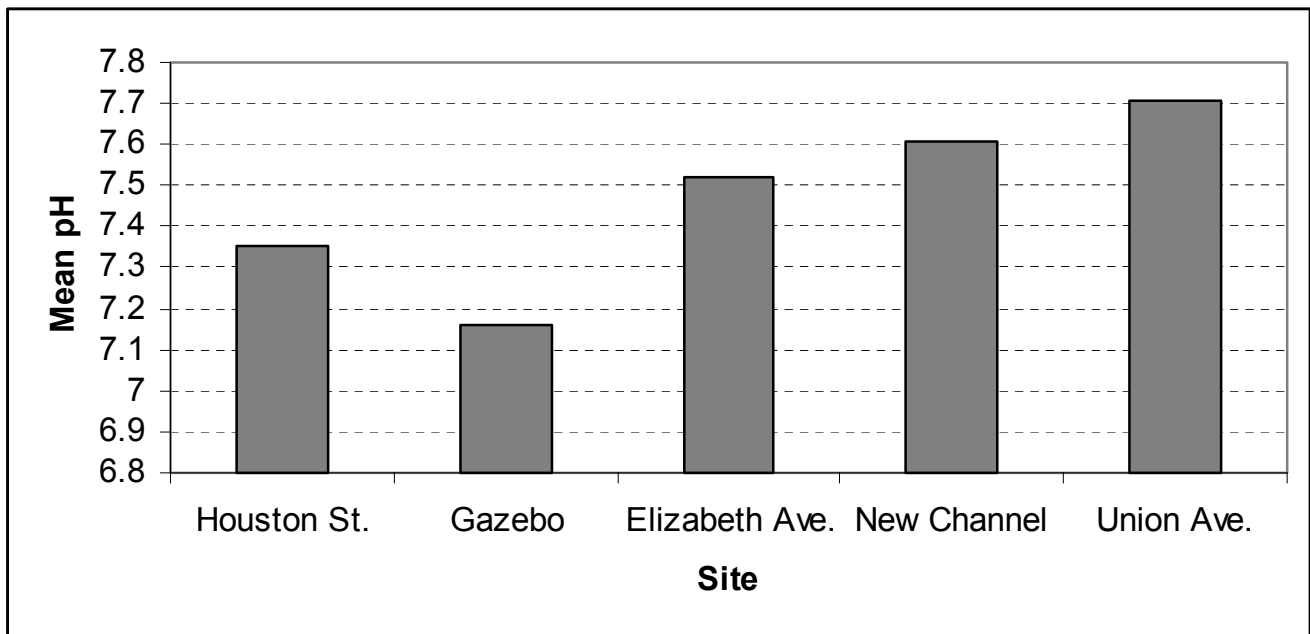
As in previous years, beetles tended to congregate on the western shore (river left) of Spring Run 3. At the Spring Island site, *H. comalensis* were found in a variety of sites, and certain areas within each site tended to have higher numbers than other areas. The beetles (both larvae and adults) tended to be patchily distributed with wide ranges of abundance among samples within a site and season.

## Master Naturalist Observations

Water quality data collected by Master Naturalist volunteers was averaged by site and is presented in Figures 17 and 18. Carbon dioxide concentrations were highest in areas near spring outflows (Houston St. and Gazebo) and decreased downstream. Uptake of carbon dioxide by aquatic plants and through interaction with the atmosphere resulted in a higher pH in downstream areas (Figure 18).



**Figure 17. Average carbon dioxide concentrations from weekly samples taken at five sites in the Comal Springs/River ecosystem from July through November 2006.**



**Figure 18. Average pH from weekly samples taken at five sites along the Comal River from July through November 2006.**

Recreation data collected by Master Naturalist volunteers shows the most intense recreation at sites near popular tubing areas (the New Channel, and Union Avenue). In contrast, there were fewer recreational users at the Houston Street site and near the Gazebo at Landa Lake. No recreation was documented at the Elizabeth Avenue site due to limited public access in the area.



Tubers at the Union Avenue (left) and New Channel (right) sites.

## Exotics / Predation Study

Because there were no Critical Period events triggered in 2006, no samples were made for the exotics / predation component of this study.

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## **APPENDIX A: AQUATIC VEGETATION MAPS**

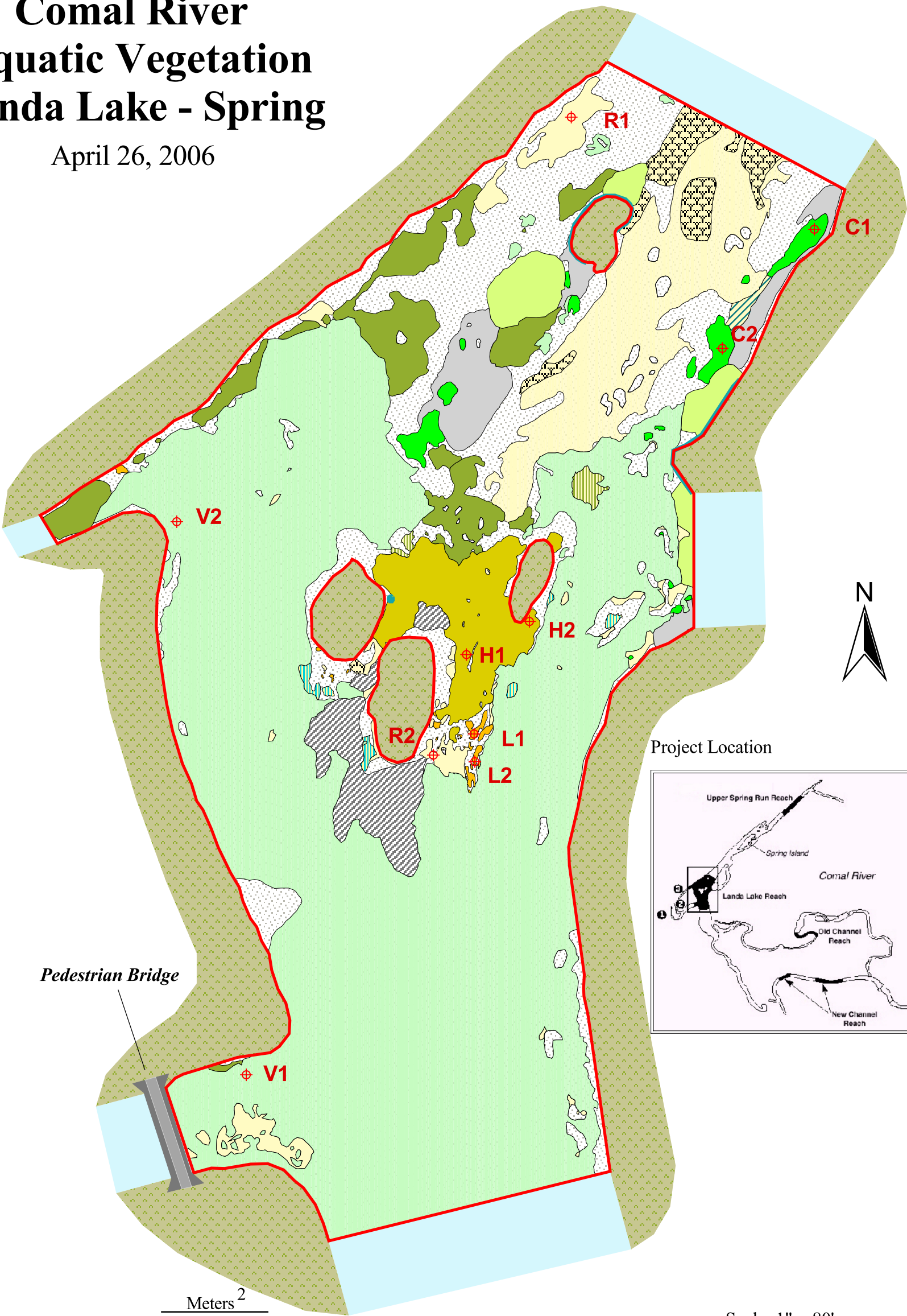
(Included here)

## **Landa Lake Reach**



# Comal River Aquatic Vegetation Landa Lake - Spring

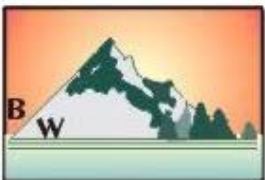
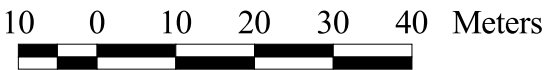
April 26, 2006



	Meters <sup>2</sup>
Floating vegetation mat	547.7
<i>Cabomba</i>	231.9
<i>Hygrophila</i>	785.9
<i>Ludwigia</i>	38.1
<i>Nuphar</i>	462.4
<i>Bryophytes</i>	2,114.5
<i>Sagittaria</i>	1,071.8
<i>Vallisneria</i>	12,604.1
Algae	599.7
<i>Bryophytes</i> < 50%	362.7
<i>Bryophytes</i> / <i>Sagittaria</i>	57.4
<i>Bryophytes</i> / <i>Vallisneria</i>	51.4
<i>Bryophytes</i> / Algae	36.0

- Shore and Islands
- River
- Study Area (22,551.2 m<sup>2</sup>)
- Bare Substrate
- Colocasia*
- Drop Net Sample Sites

Scale: 1" = 80'

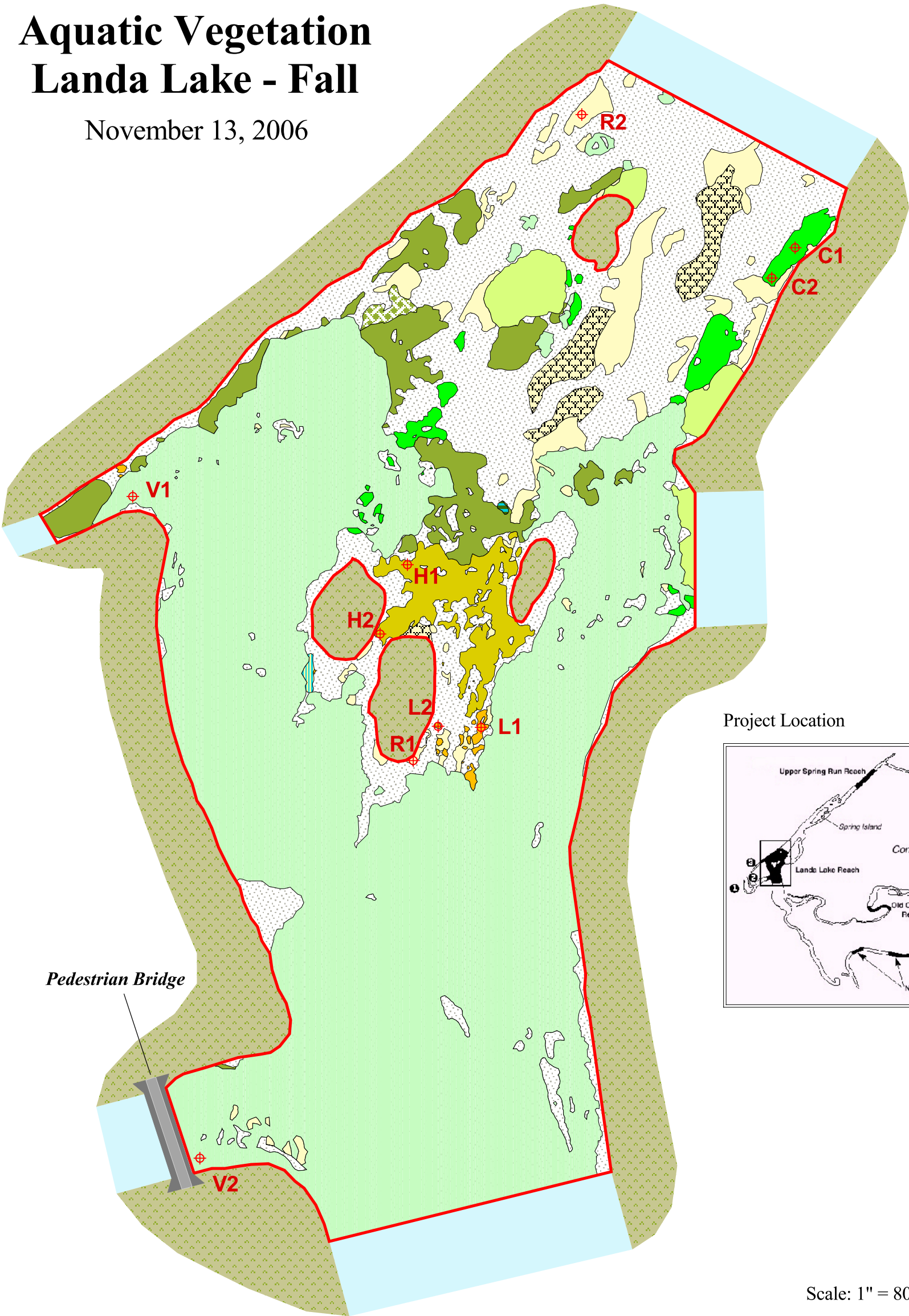


BIO-WEST, Inc.

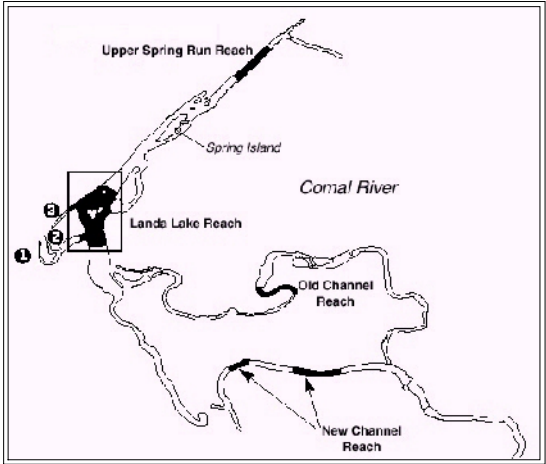
EDWARDS AQUIFER AUTHORITY

# Comal River Aquatic Vegetation Landa Lake - Fall

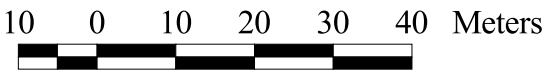
November 13, 2006



Project Location



Scale: 1" = 80'



	Meters <sup>2</sup>
Cabomba	332.4
Hygrophila	520.3
Ludwigia	41.0
Nuphar	457.1
Bryophytes	928.8
Sagittaria	1,096.0
Vallisneria	13,243.7
Bryophytes < 50%	412.6
Sagittaria < 50%	49.8
Vallisneria / Sagittaria	4.4
Bryophytes / Vallisneria	14.6

- Shore and Islands
- River
- Study Area (22,551.2 m<sup>2</sup>)
- Bare Substrate
- Colocasia
- Drop Net Sample Sites



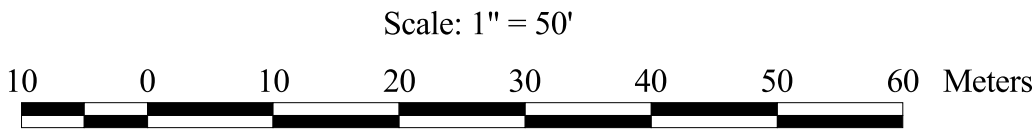
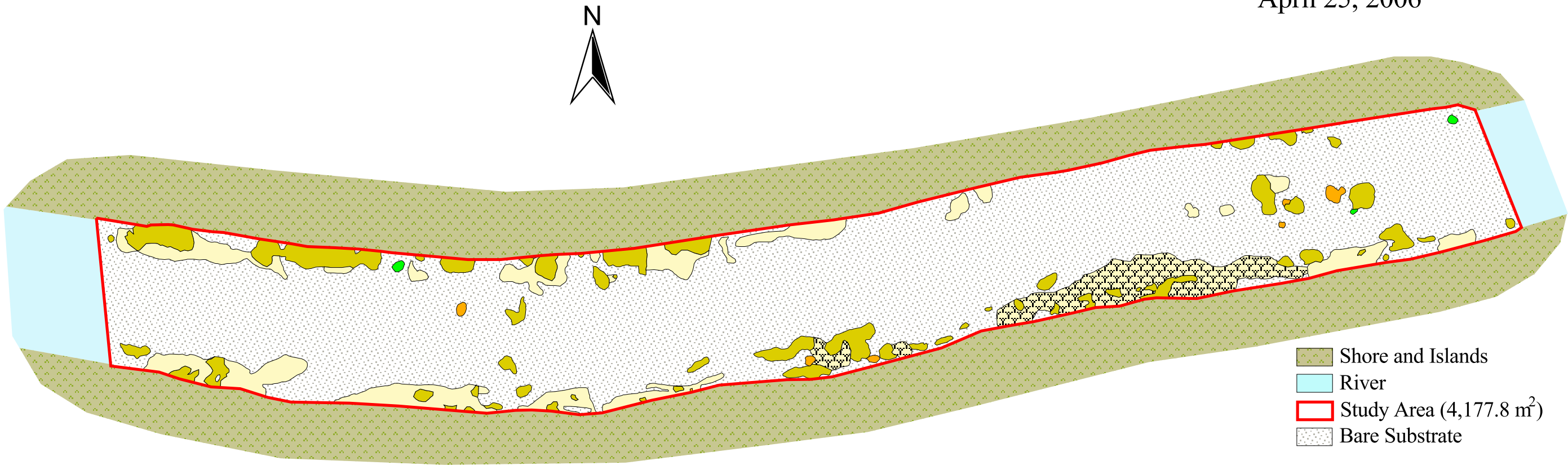
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

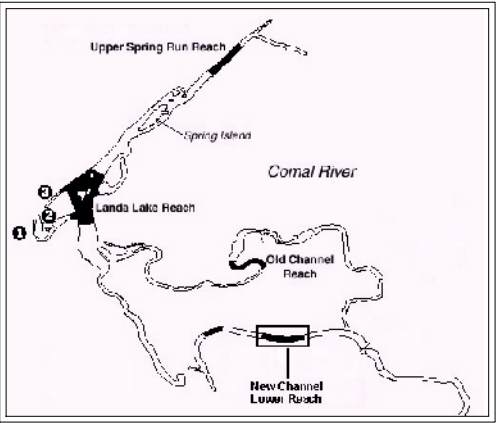
## **New Channel Reach**

Comal River  
Aquatic Vegetation  
New Channel Lower Reach  
Spring

April 25, 2006



Project Location



- Shore and Islands
- River
- Study Area (4,177.8 m<sup>2</sup>)
- Bare Substrate

	Meters <sup>2</sup>
<i>Cabomba</i>	3.8
<i>Hygrophila</i>	310.1
<i>Ludwigia</i>	11.3
<i>Bryophytes</i>	344.0
<i>Bryophytes</i> < 50%	186.2

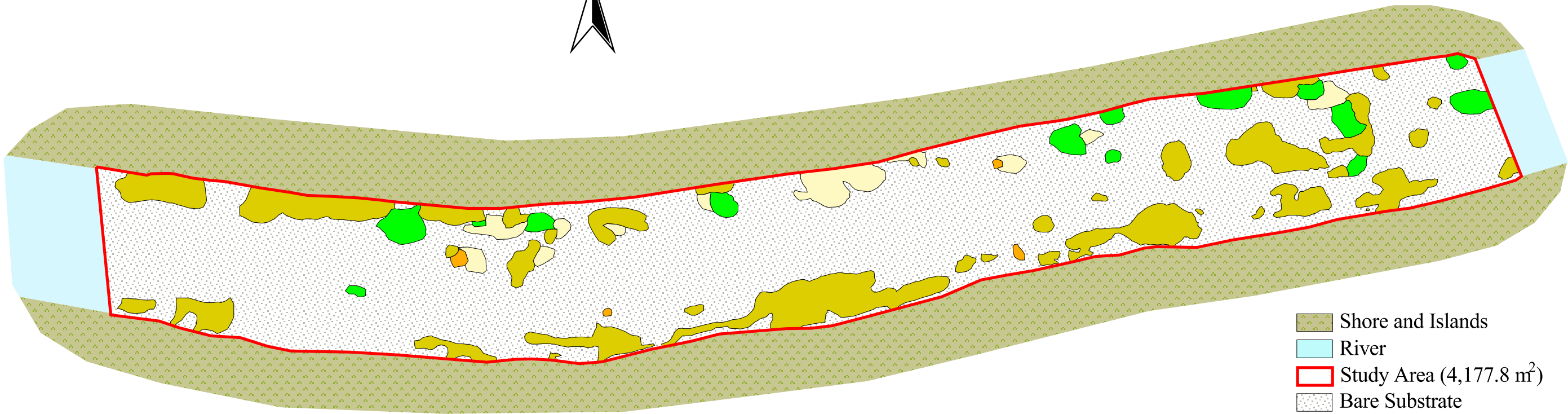


BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

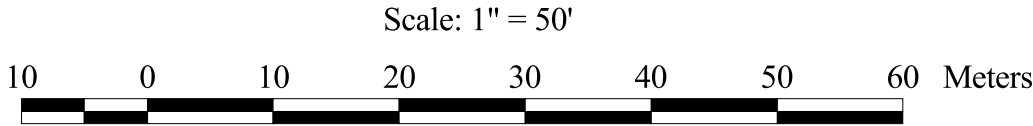
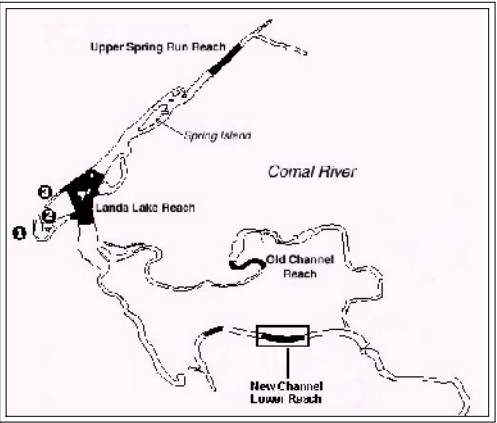
Comal River  
Aquatic Vegetation  
New Channel Lower Reach  
Fall





November 16, 2006



- Shore and Islands
- River
- Study Area (4,177.8 m<sup>2</sup>)
- Bare Substrate

Project Location



	Meters <sup>2</sup>
 <i>Cabomba</i>	144.1
 <i>Hygrophila</i>	715.4
 <i>Ludwigia</i>	9.6
 <i>Bryophytes</i>	121.0



BIO-WEST, Inc.

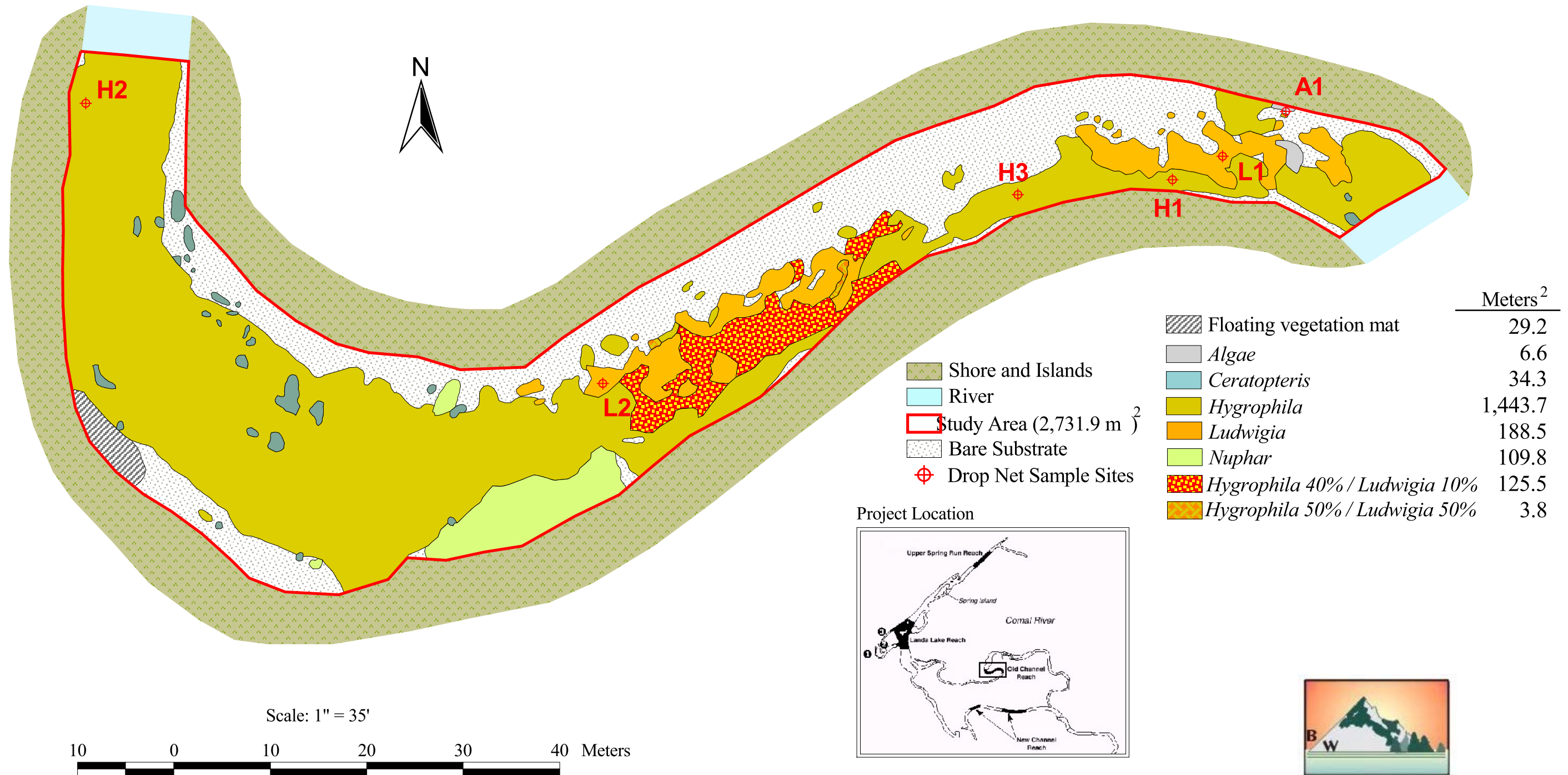
## **Old Channel Reach**

# Comal River

## Aquatic Vegetation

### Old Channel Reach - Spring

April 27, 2006



Scale: 1" = 35'

10 0 10 20 30 40 Meters



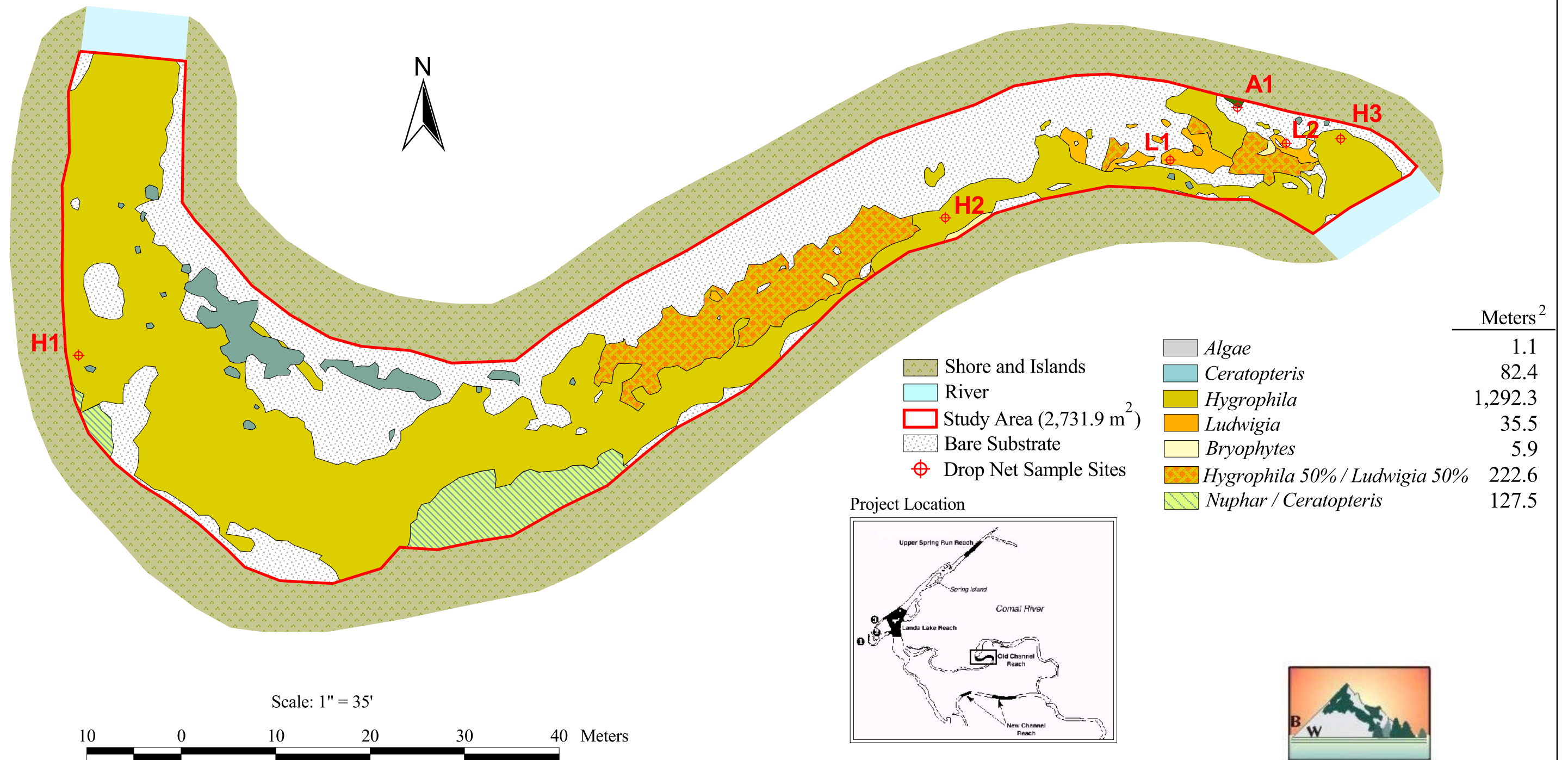
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY



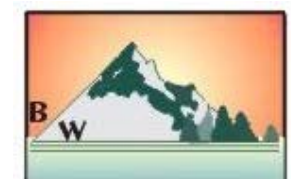
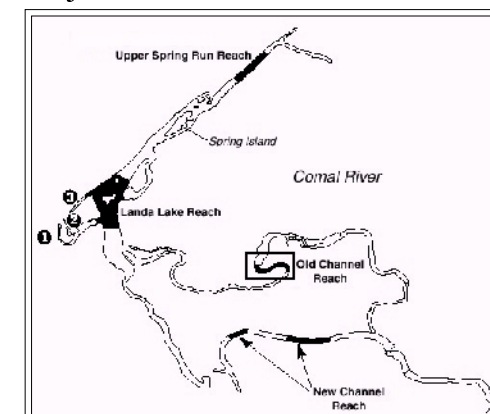
# Comal River Aquatic Vegetation Old Channel Reach - Fall

November 13, 2006



	Meters <sup>2</sup>
Algae	1.1
<i>Ceratopteris</i>	82.4
<i>Hygrophila</i>	1,292.3
<i>Ludwigia</i>	35.5
<i>Bryophytes</i>	5.9
<i>Hygrophila</i> 50% / <i>Ludwigia</i> 50%	222.6
<i>Nuphar</i> / <i>Ceratopteris</i>	127.5

Project Location



BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY



## **Upper Spring Run Reach**

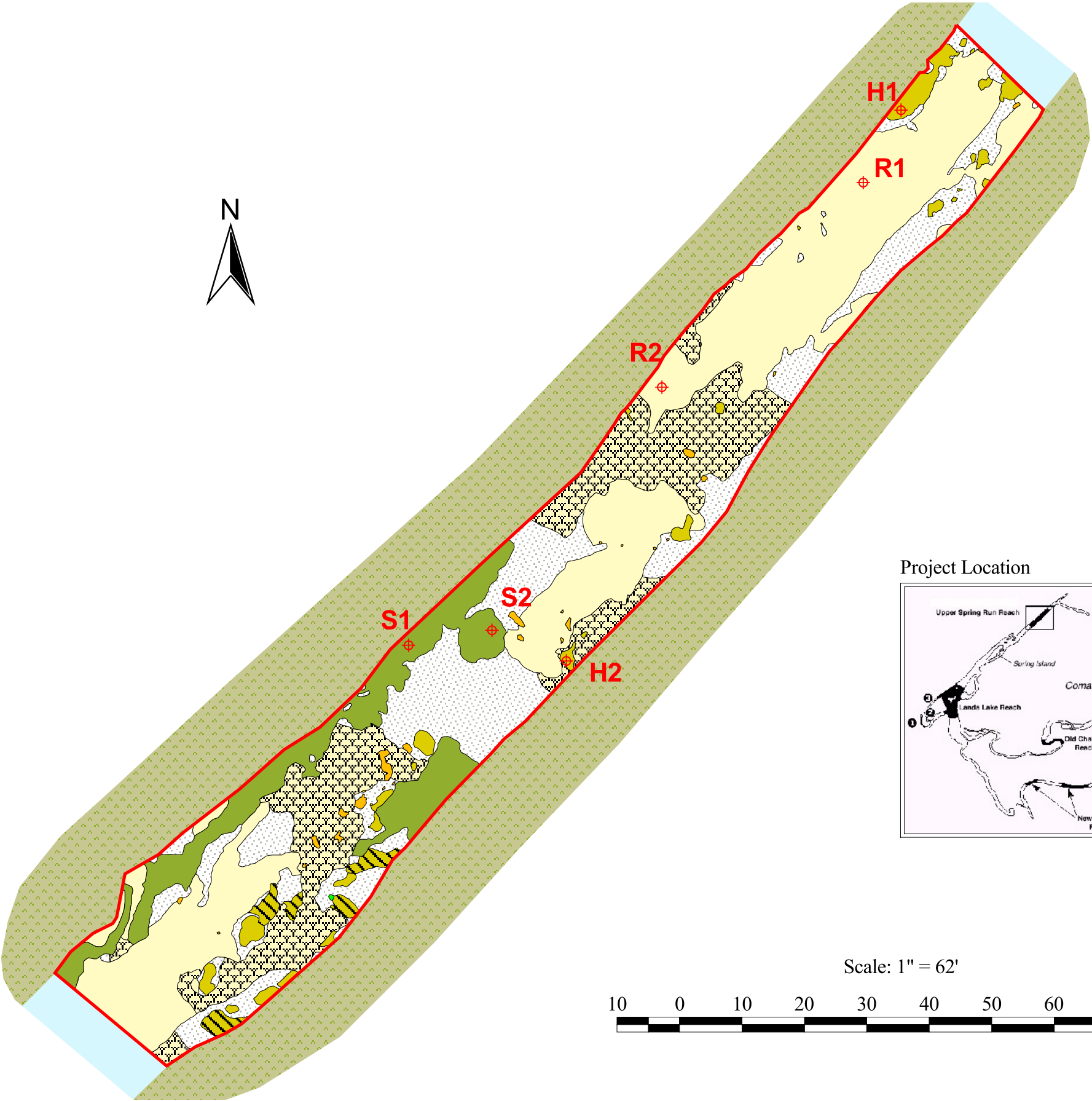
# Comal River

## Aquatic Vegetation

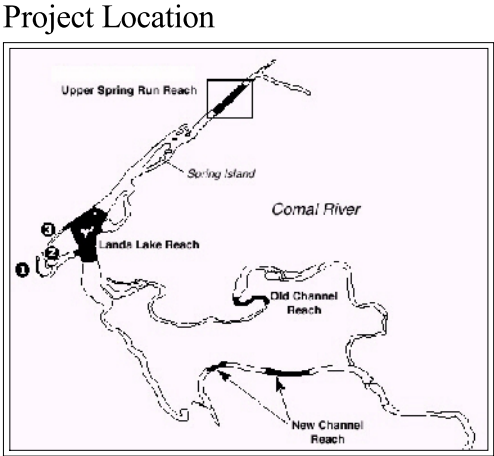
### Upper Spring Channel Reach

Spring

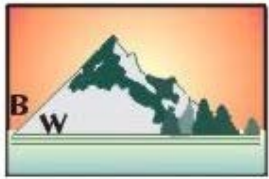
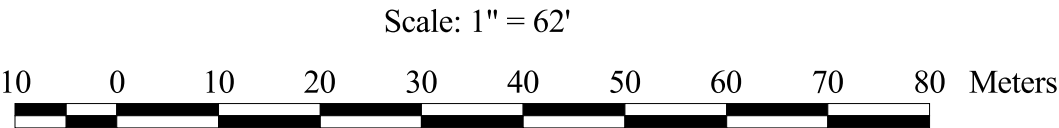
April 24, 2006



- Shore and Islands
- River
- Study Area (4,723.5 m<sup>2</sup>)
- Bare Substrate
- Colocasia*
- Drop Net Sample Sites



	Meters <sup>2</sup>
<i>Cabomba</i>	0.6
<i>Hygrophila</i>	148.3
<i>Ludwigia</i>	25.1
<i>Bryophytes</i>	1,849.4
<i>Sagittaria</i>	552.0
<i>Bryophytes</i> < 50%	989.8
<i>Hygrophila</i> < 50%	75.2



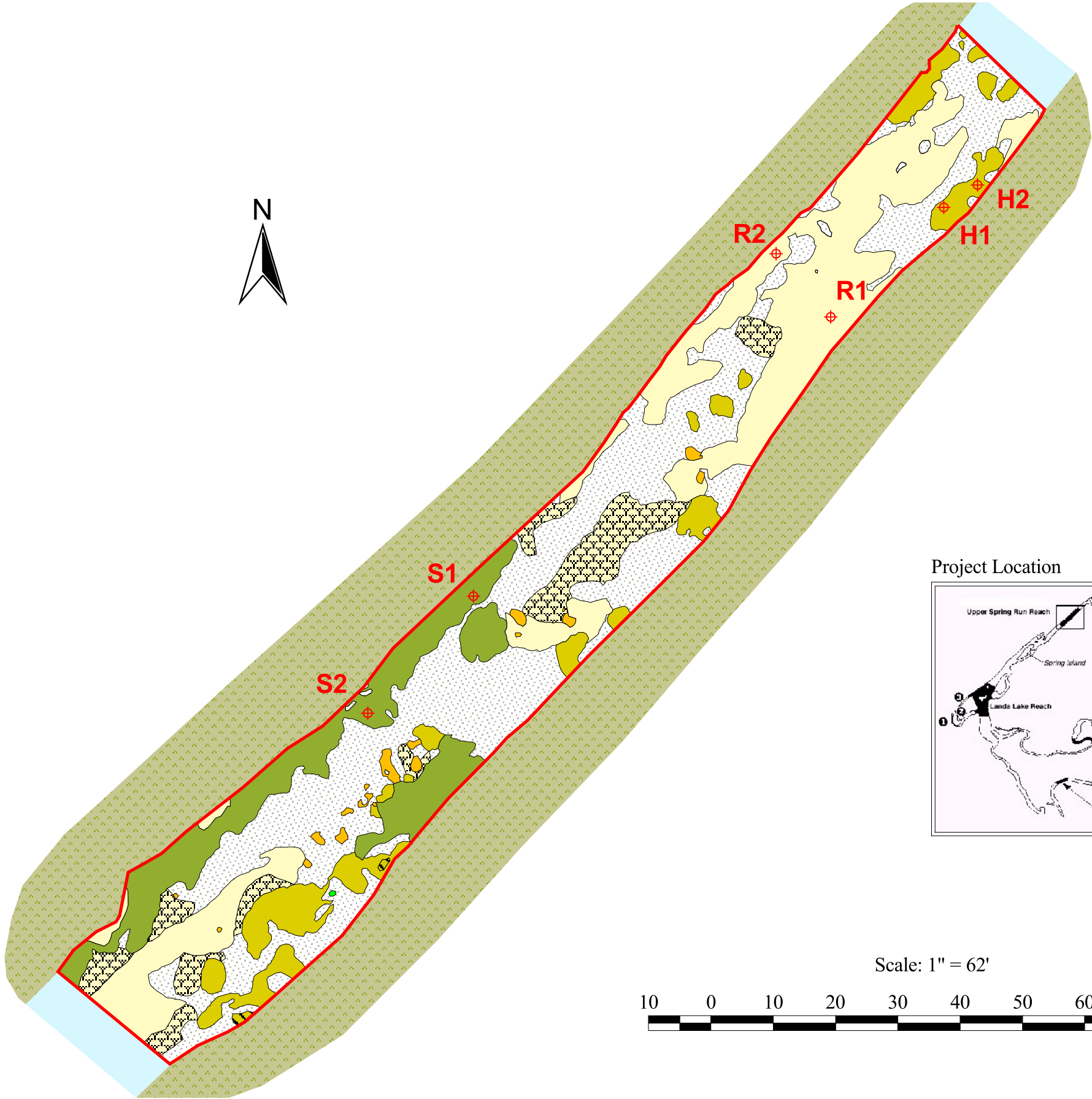
# Comal River

## Aquatic Vegetation

### Upper Spring Channel Reach

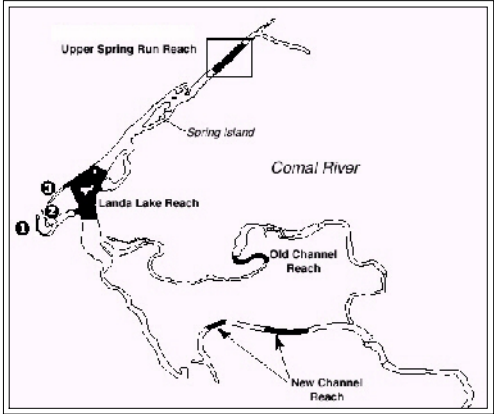
### Fall

November 7, 2006



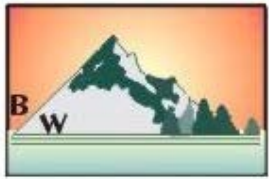
- Shore and Islands
- River
- Study Area (4,723.5 m<sup>2</sup>)
- Bare Substrate
- Colocasia*
- Drop Net Sample Sites

Project Location



	Meters <sup>2</sup>
<i>Cabomba</i>	0.8
<i>Hygrophila</i>	443.4
<i>Sagittaria</i>	639.4
<i>Ludwigia</i>	42.0
<i>Bryophytes</i>	1,250.8
<i>Bryophytes</i> < 50%	389.3
<i>Hygrophila</i> < 50%	6.8

Scale: 1" = 62'



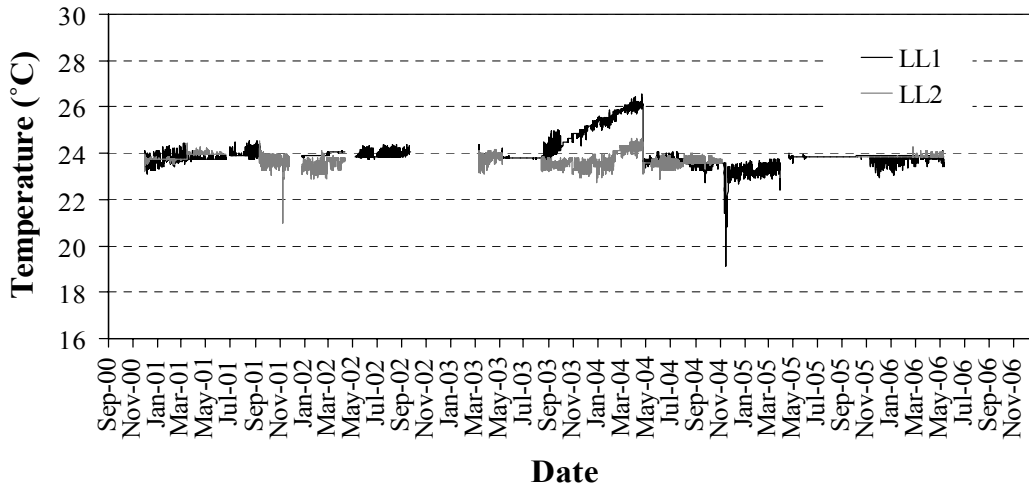
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

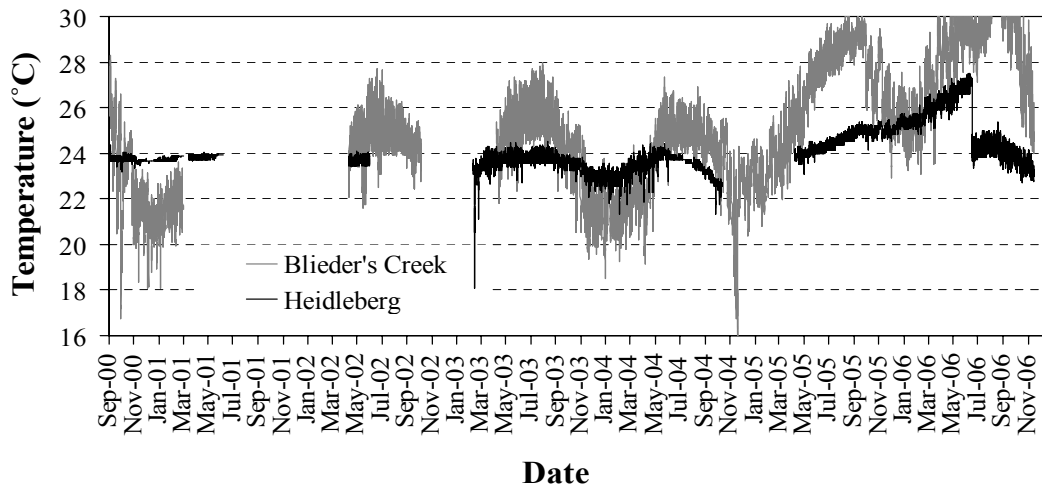
## **APPENDIX B: DATA AND GRAPHS**

# **Water Quality Data and Thermistor Graphs**

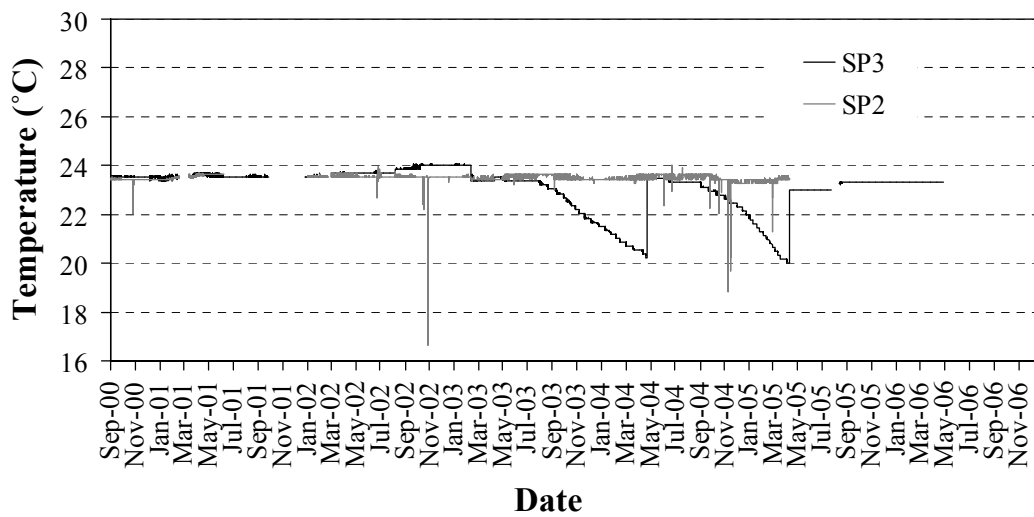
### Thermistor Data: Landa Lake Bottom



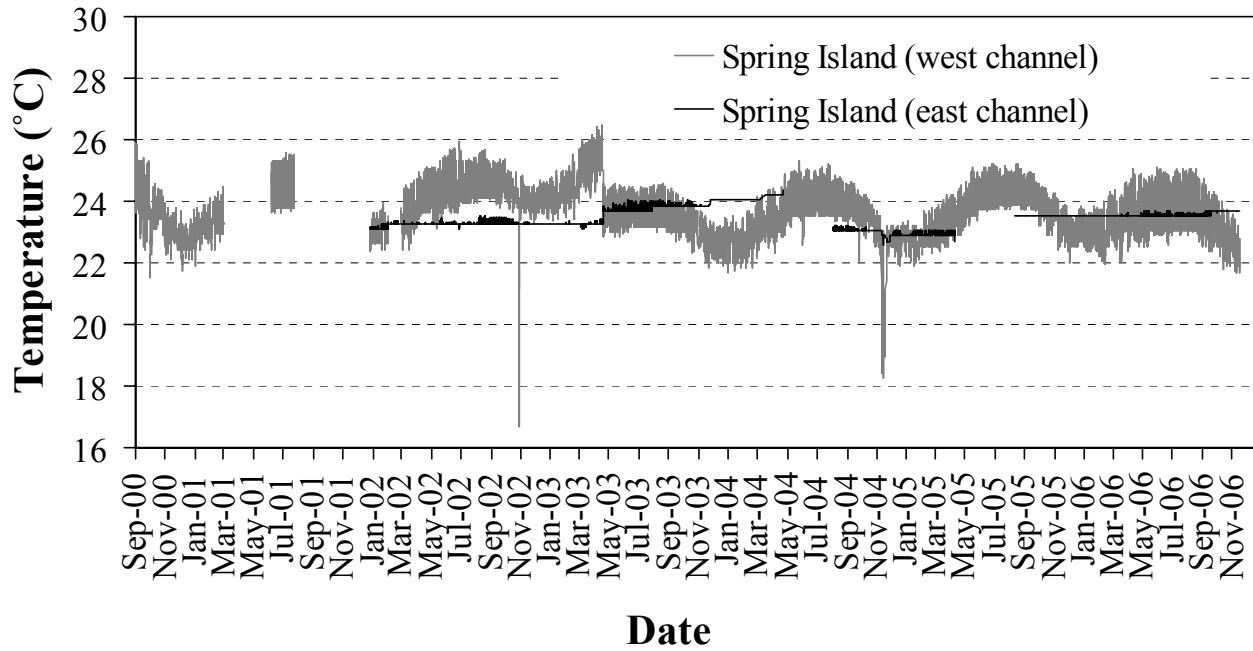
### Thermistor Data: Comal Headwaters



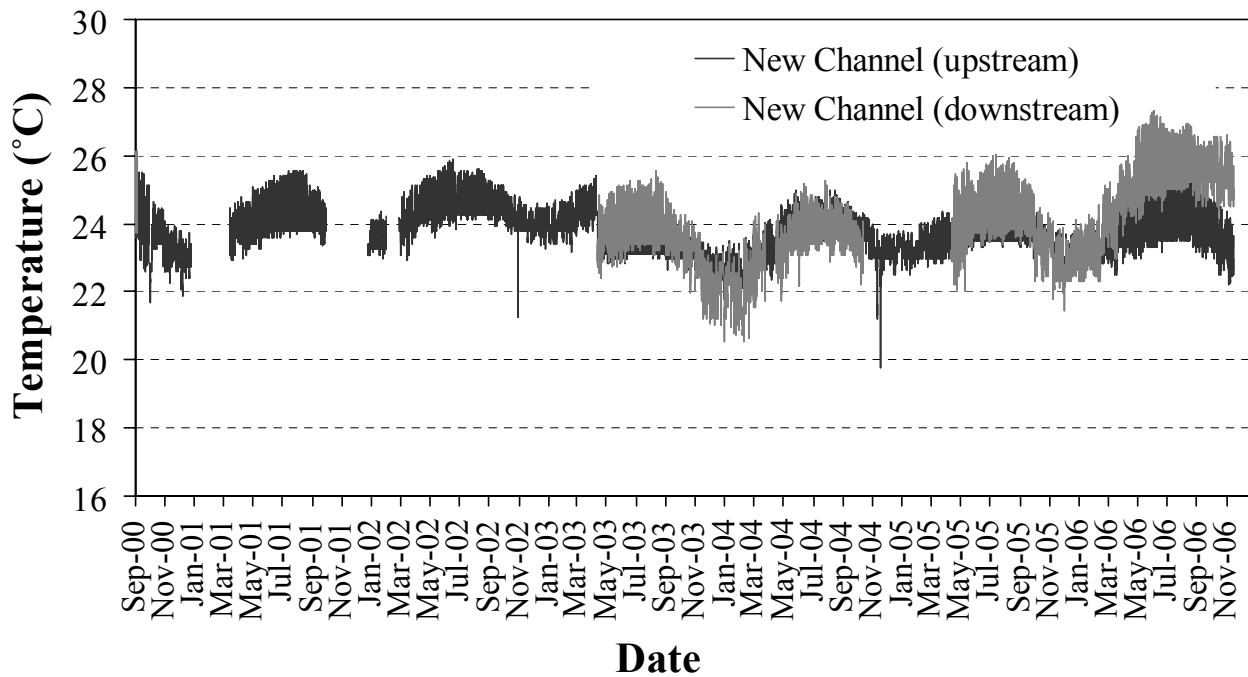
### Thermistor Data: Spring Runs 2 & 3



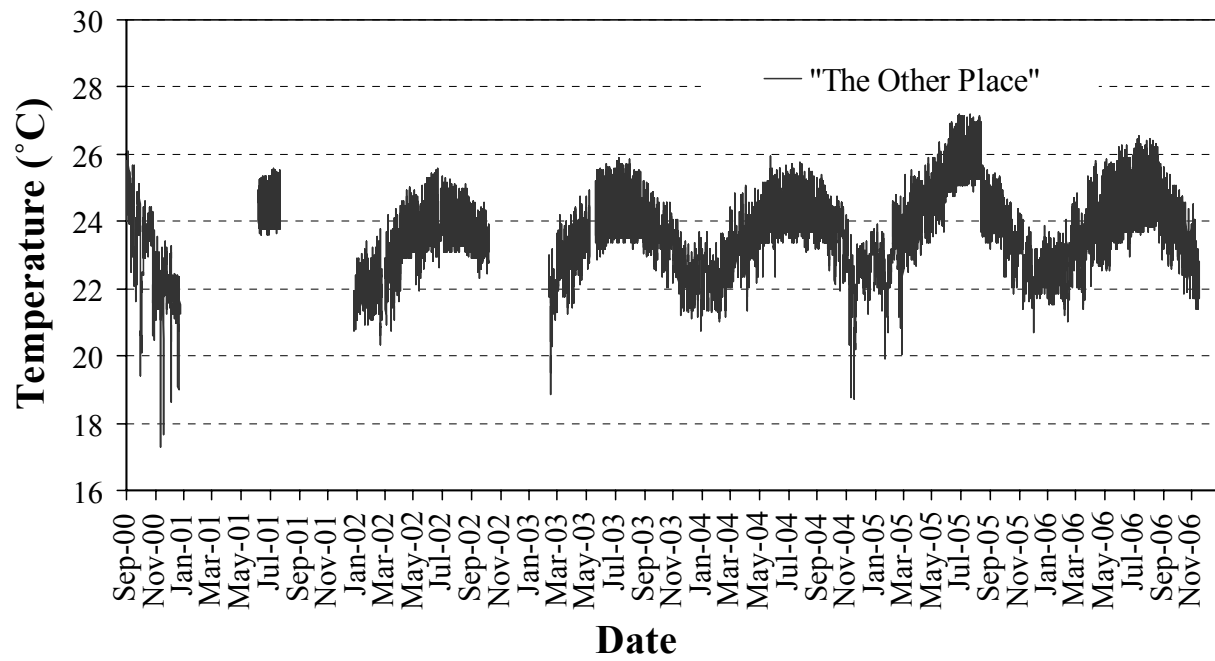
## Thermistor Data: Spring Island



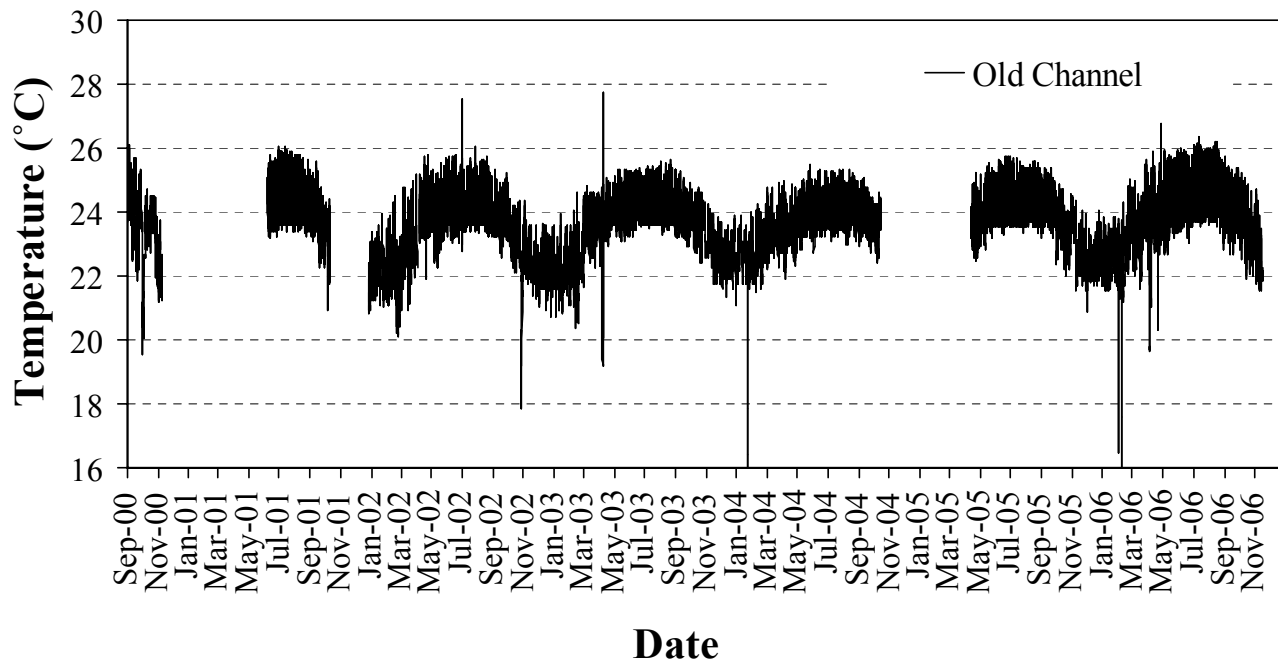
## Thermistor Data: New Channel



## Thermistor Data: The Other Place



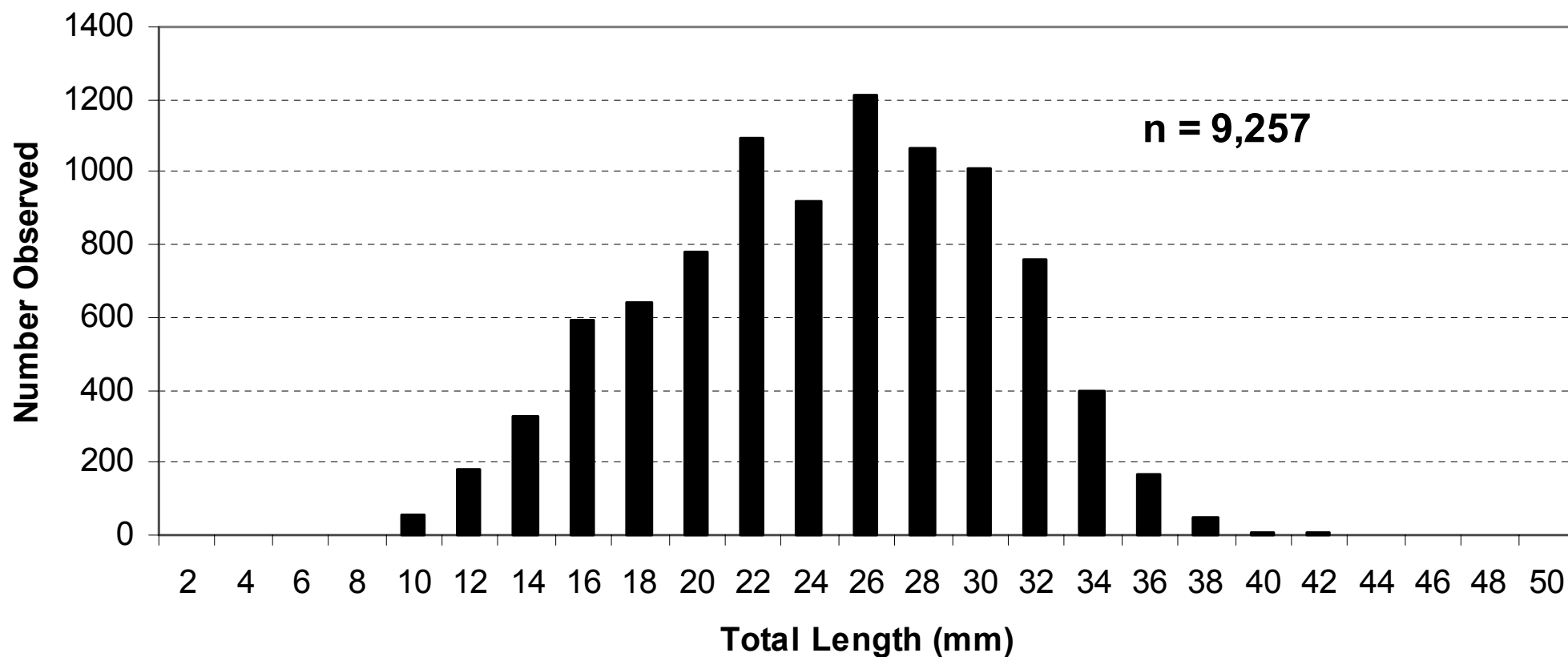
## Thermistor Data: Old Channel





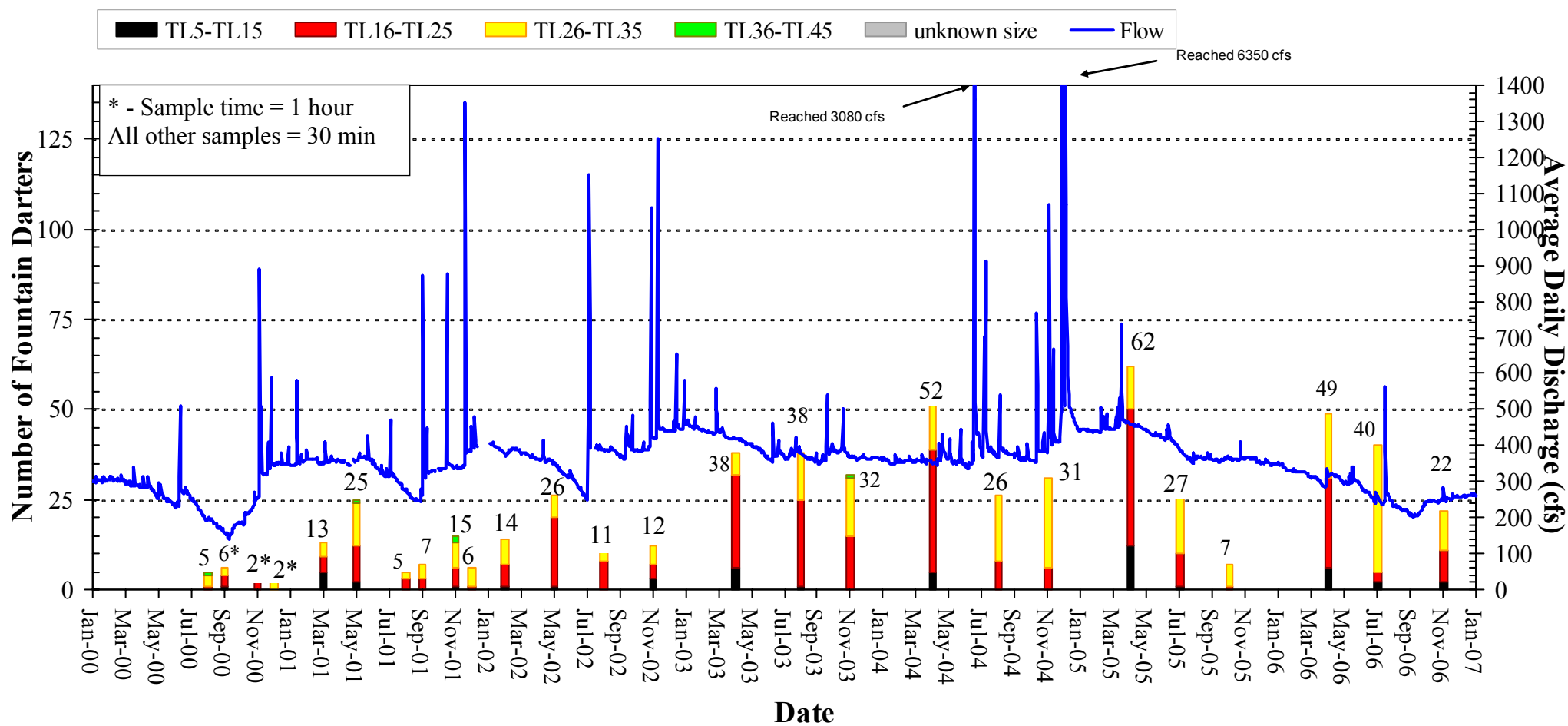
## **Drop Net Graph**

## Drop Net Results 2000-2006 in the Comal River

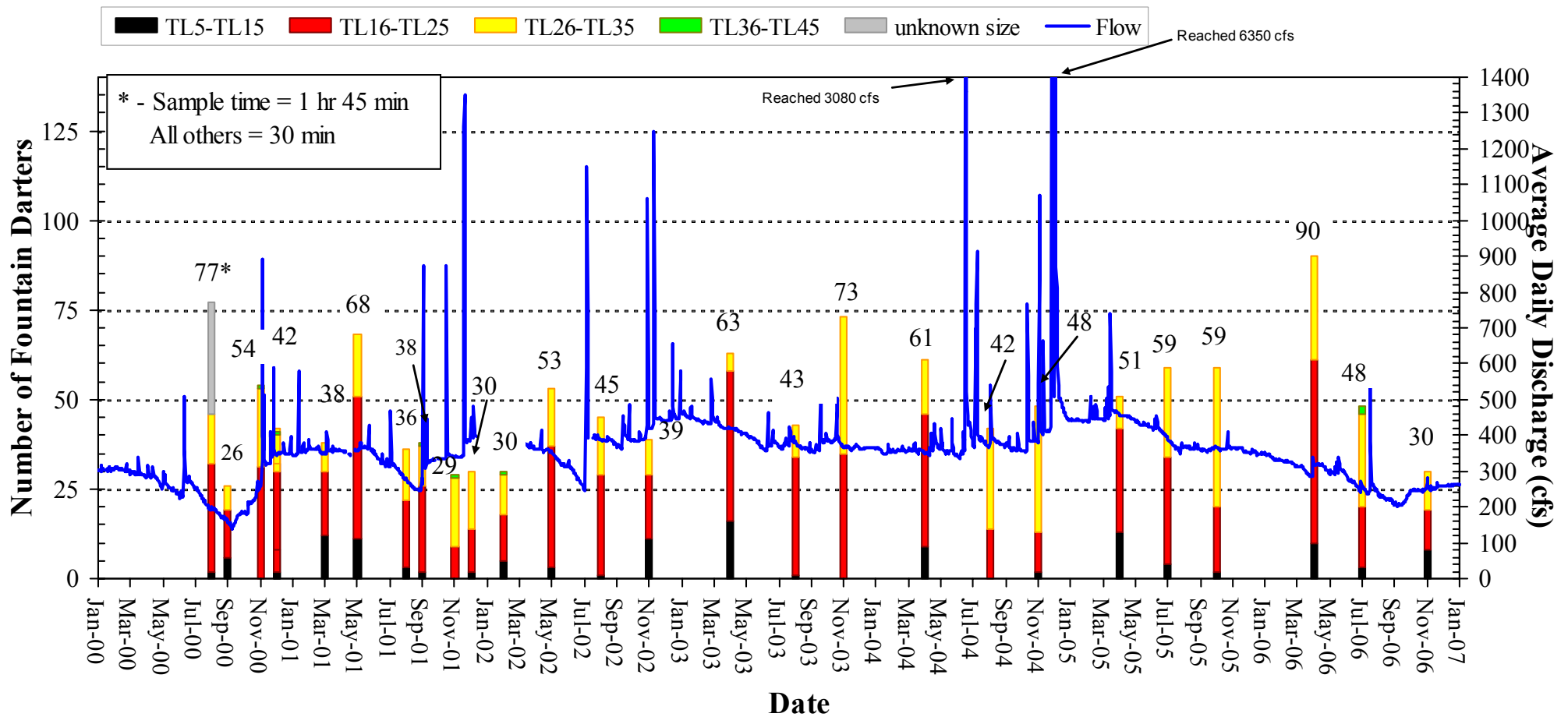


## **Dip Net Graphs**

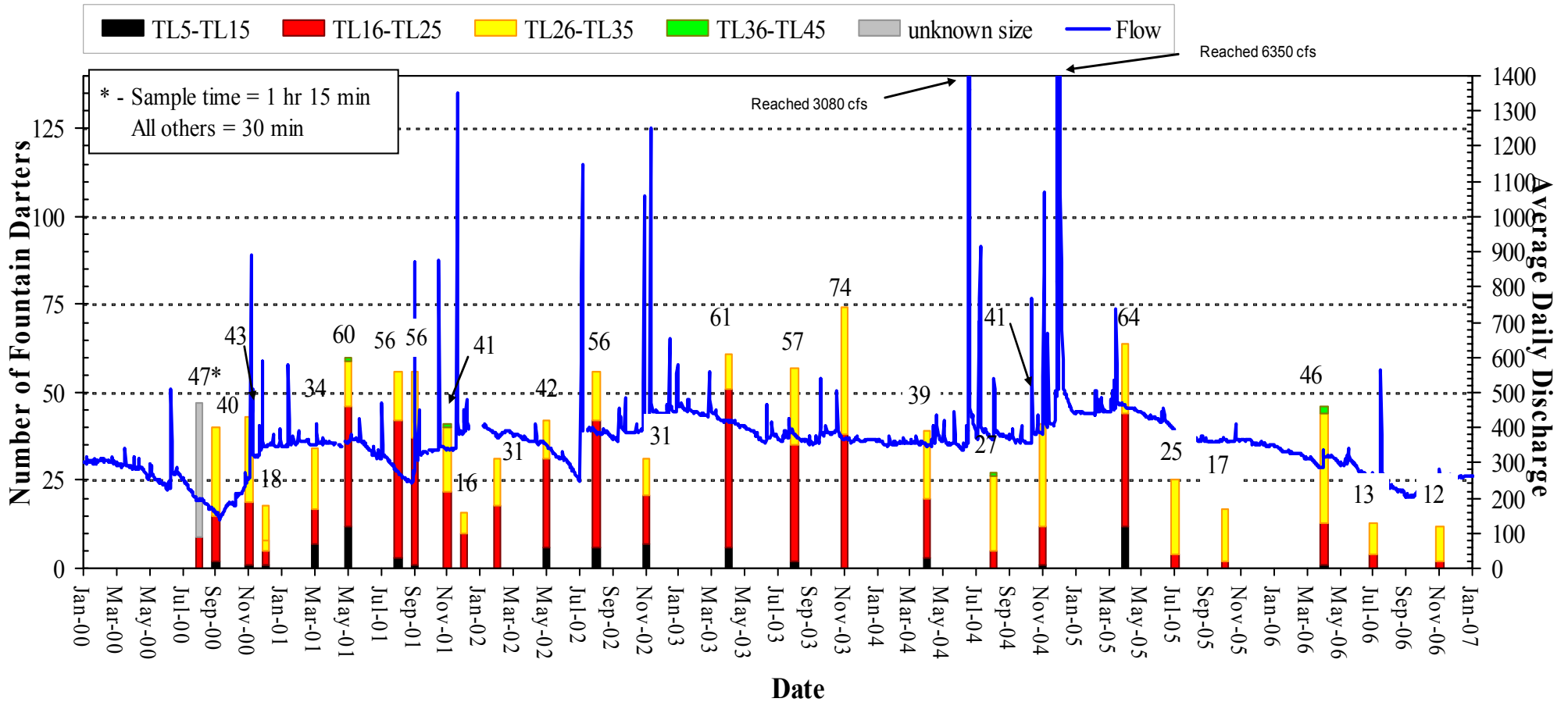
## Fountain Darters Collected from the Upper Spring Run Reach (Section 3) Dip Net Results - Comal River



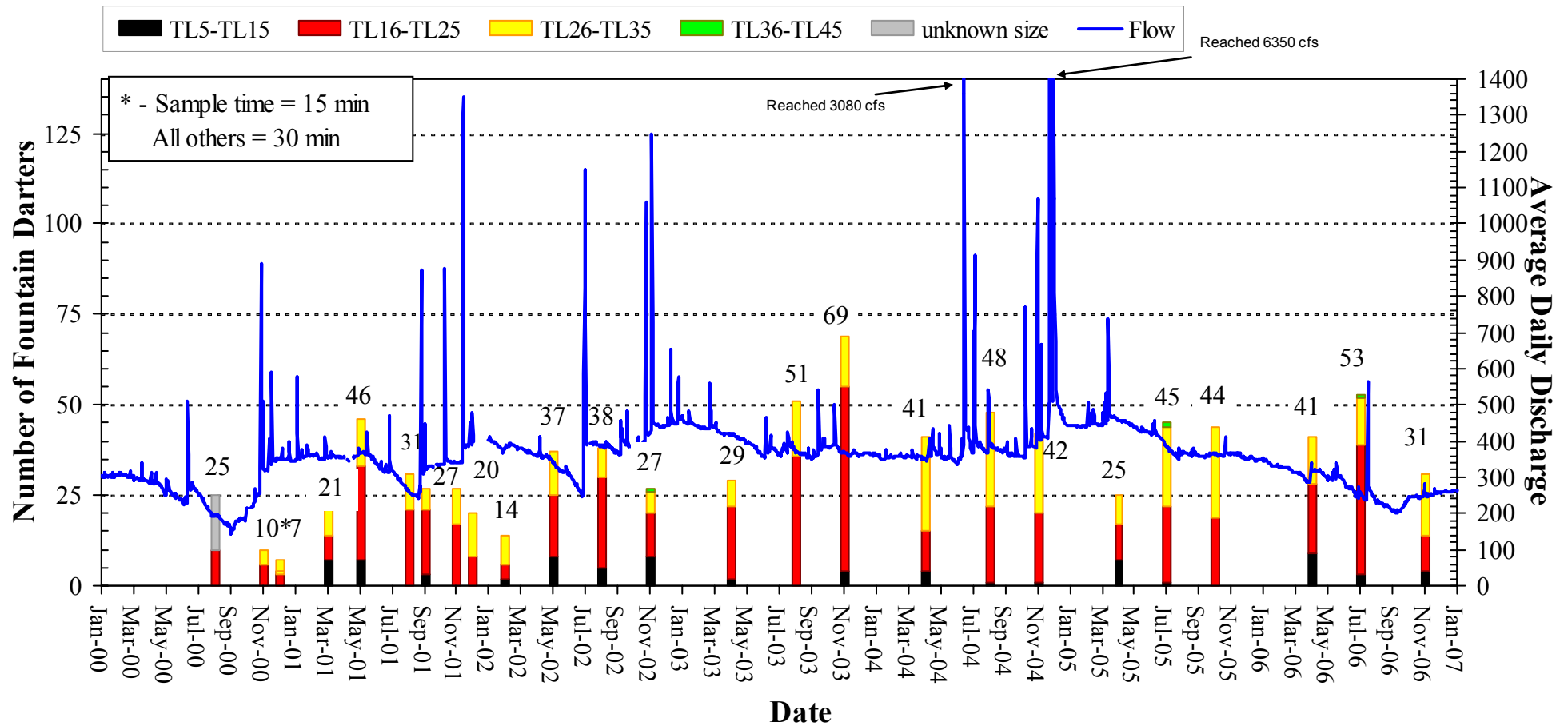
## Fountain Darters Collected from the Spring Island Area (Section 4U-M) Dip Net Results - Comal River



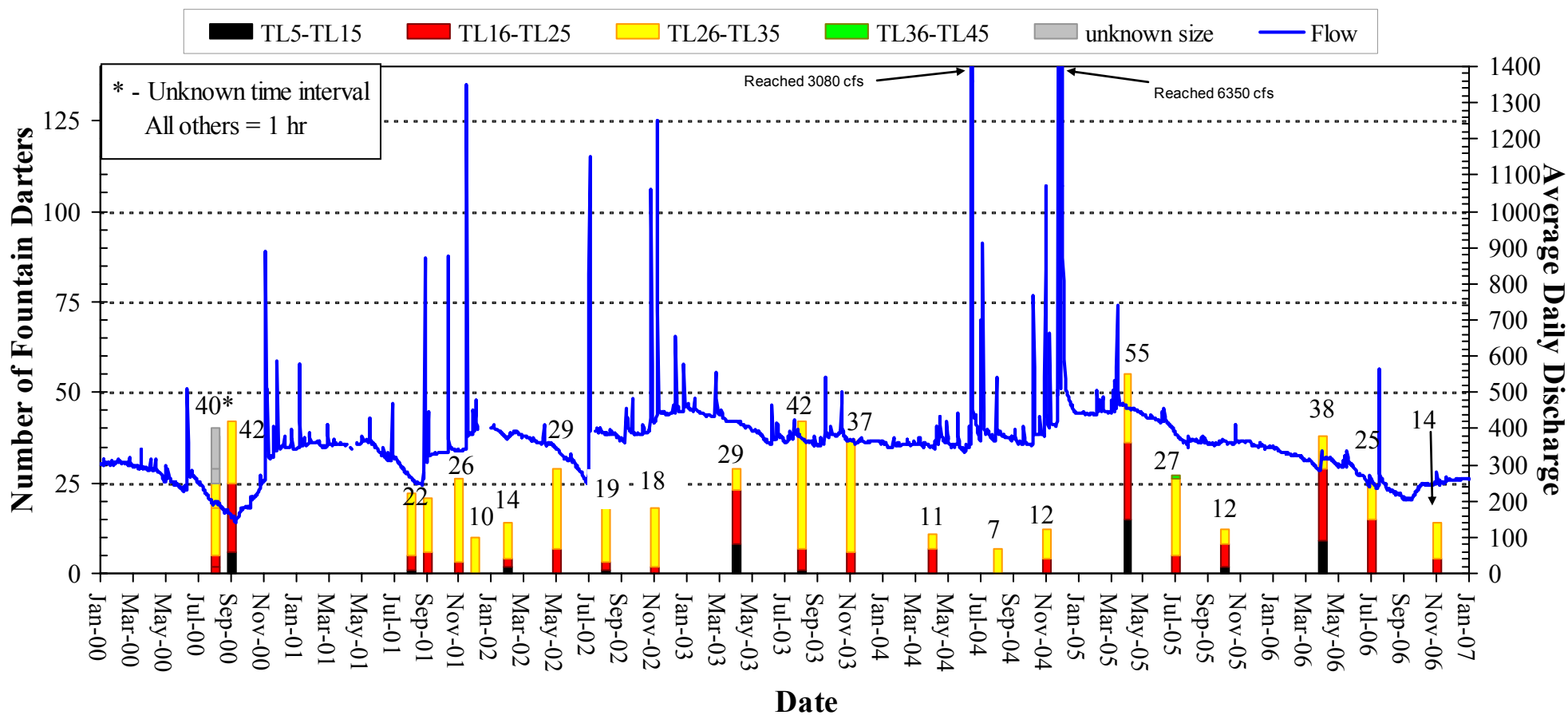
## Fountain Darters Collected from the Landa Lake Reach (Section 4L) Dip Net Results - Comal River



## Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River

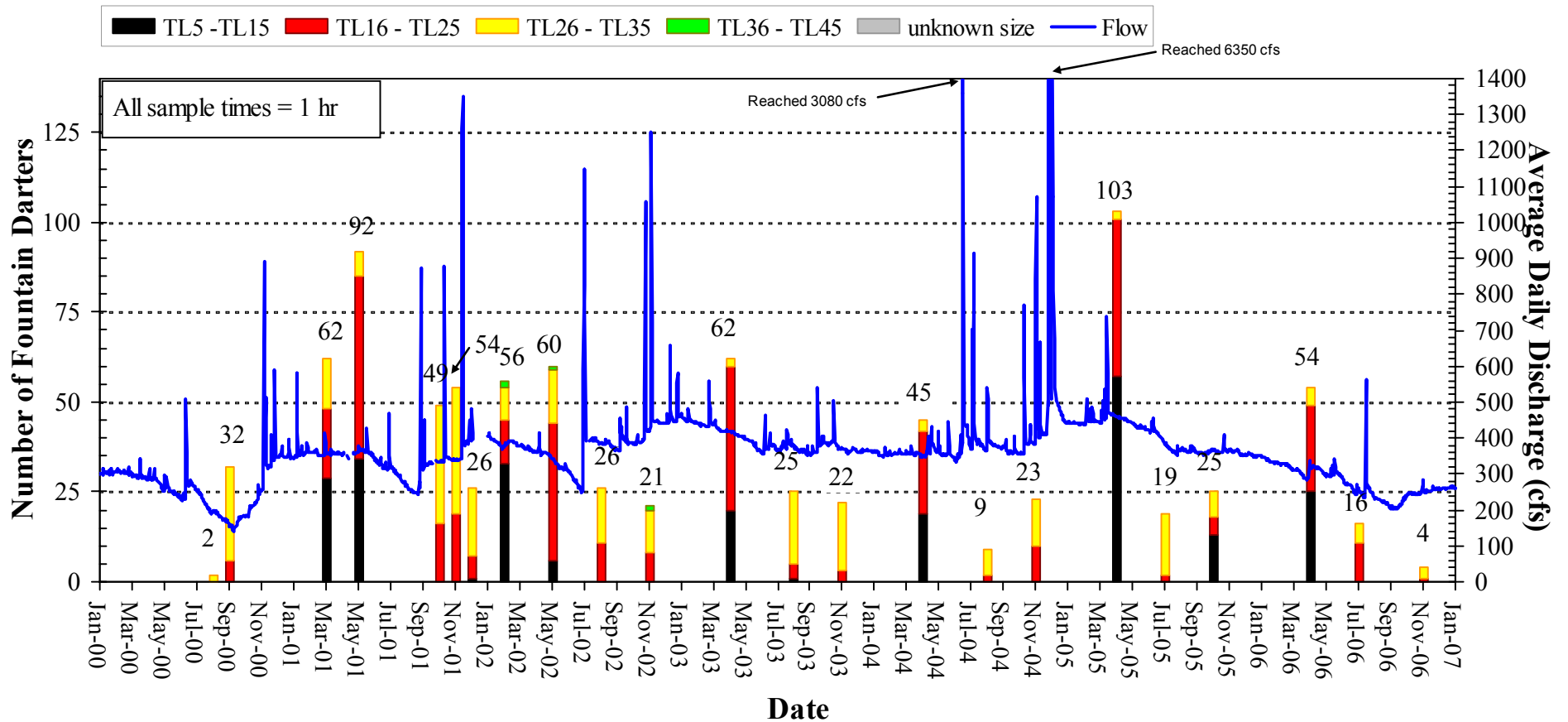


## Fountain Darters Collected from the New Channel Reach (Section 10) Dip Net Results - Comal River





## Fountain Darters Collected from "The Other Place" Reach (Section 14) Dip Net Results - Comal River



**APPENDIX C:**  
**DROP NET RAW DATA**  
**(not available online)**