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

FINAL 2004 ANNUAL REPORT

Prepared for:

Edwards Aquifer Authority
1615 North St. Mary's Street
San Antonio, Texas 78215

Prepared by:

BIO-WEST, Inc
Austin Office
1406 Three Points Road, Suite A-200
Pflugerville, Texas 78660-3155

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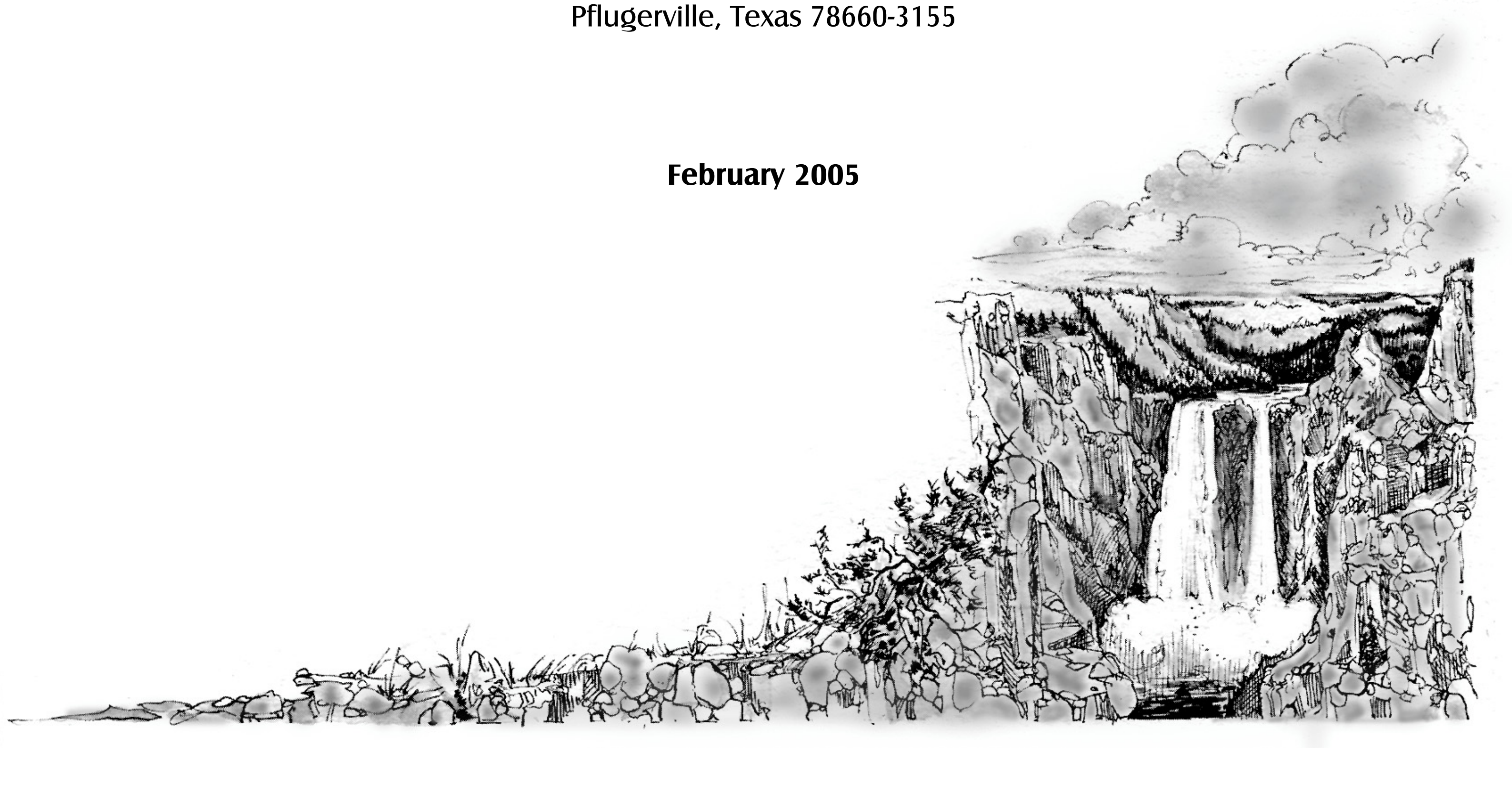

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EXECUTIVE SUMMARY

During this study, valuable information has been gathered on current population dynamics of each listed species found in the Comal Springs/River ecosystem (except those that are primarily subterranean) and their habitat conditions. These data were collected under a Comprehensive Monitoring effort that included regular, quarterly samples to provide baseline information and during Critical Period events that were triggered by infrequent, extremes in discharge that might affect each species. Because above-average flow conditions occurred during most of 2000-2004, there were only two low-flow Critical Period events, but four high-flow Critical Period events in the Comal Springs/River ecosystem during that time.

There were multiple components used to make assessments of population condition of each species and of habitat availability. Overall water quality was assessed quarterly throughout the entire Comal River during 2000-2002 using standard parameters (water temperature, dissolved oxygen, pH, and conductivity) as well as conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids). This resulted in a detailed assessment of baseline conditions, which were then augmented with standard parameters collected during each subsequent sample effort. This assessment suggested that all water quality parameters had relatively narrow ranges that were providing high quality conditions for aquatic life use. A continuous record of water temperature was also maintained through the study in multiple locations within the Comal Springs/River ecosystem during the study using temperature loggers. These loggers revealed that a relatively narrow range of water temperatures occurs near spring openings, but more fluctuation occurs further downstream. Under the discharge conditions experienced during this study, water temperature only reached the threshold of potentially affecting fountain darter reproduction (26.7°C) in Blieders Creek, which is not directly influenced by spring inputs and showed a much larger temperature range than other sites. Fixed station photographs also documented physical changes in habitat at multiple locations throughout the Comal Springs/River ecosystem. Additional low-flow data are vital to improve understanding of water quality conditions that may be expected at various discharges.

For the fountain darter, habitat use is largely influenced by aquatic vegetation and assessments of habitat availability were conducted by mapping this vegetation during each sampling event. Throughout the study, aquatic vegetation remained abundant in most reaches despite the frequent scouring associated with flooding. In most cases, flooding resulted in a temporary reduction in total aquatic vegetation coverage, but many plant types quickly responded with rapid re-growth and expanded to a total coverage that exceeded the pre-flooding condition. The plant types that were the most susceptible were those that are not strongly rooted (algae and bryophytes) and those occurring in areas where flows are constricted and "funneled" through narrow channels. The most highly affected plant types tended to be the vegetation types that support the highest densities of fountain darter. One notable result of the flooding was a substantial reduction in algae, which supports the highest densities of fountain darters, in the Old Channel and subsequent establishment of *Ludwigia* and *Hygrophila*, which support much lower densities of fountain darters than algae. The higher flows during this study appeared to stimulate growth of many vegetation types, primarily the bryophytes, but the higher densities of these plants were not maintained during extended periods of higher-than-average flows. It remains unclear how various vegetation types will respond to low-flow conditions and whether each plant type will support the same densities of fountain darters under such conditions.

Direct sampling of the fountain darter occurred in the same reaches with aquatic vegetation used to stratify random sample locations. The highest densities were found in filamentous algae and bryophytes (*Riccia* sp. and *Amblystegium* sp.). The average densities of fountain darters in all drop net sites in each vegetation type and maps of total vegetation coverage were used to estimate fountain darter abundance in each reach during 2000-2004. These rough estimates were subject to a wide variability in density estimates so results were normalized and presented as a proportion of the maximum. The results show a trend of population increase in Landa Lake during 2000-2003 with a moderate decrease associated with each high-flow event and an overall decline in 2004 resulting from reduced vegetation coverage. These results indicate the strong influence of bryophytes on the total population of fountain darters in the Comal Springs/River ecosystem. The drop net data were also used to develop linear regressions using the three variables of most importance to fountain darter densities (vegetation, reach, and discharge). These results revealed that discharge has had limited influence on fountain darter densities with the discharge that has occurred in 2000-2004. However, the limited data from low-flow conditions did not allow an extensive statistical assessment of fountain darter population dynamics.

Drop net data also provided information on the overall size-class distribution for the Comal Springs/River ecosystem, which continues to indicate a healthy fish assemblage. In addition, species diversity was calculated for the entire fish community in each reach during each sample effort in 2000-2004. Though there were some differences in natural diversity among reaches, there were no discernable patterns of change in diversity at the reach level during 2000-2004. One additional area of concern to fountain darters, the density of giant ramshorn snails (Marisa cornuarietis), was monitored in 2000-2004. By all indications the density of giant ramshorn snails observed in the Comal ecosystem during the study period to date (including the 2000 low-flow events) pose no serious threat to the aquatic vegetative community (i.e., fountain darter habitat). However, because of the impact that this exotic species can have under heavier densities, close monitoring of this species should continue.

Gill parasites varied substantially in abundance among sample sites during this study, but variation among seasons was less pronounced. The greatest number was found in an area (near Spring Island) where water velocities were low and snail density historically high, leading to speculation that these two factors may have an important influence on the parasite population. However, results did not show a significant relationship between parasite abundance in the water column and water velocity and more studies are needed to evaluate the relationships between snails and abundance of parasites. Nonetheless, it is logical that low-velocity areas with abundant snail populations (intermediate host) should be targeted in any efforts to reduce parasite numbers. The weak trend in temporal distribution of the parasites is possibly due to the year-round, constant thermal temperatures.

Observations of Comal Springs salamander have varied in number within and between locations during 2000-2004, but individuals have been observed in each sample location during each sample period. There was no distinct pattern of variation in salamander abundance with changes in discharge; however, there was a trend of increasing numbers of salamanders surveyed in the eastern outfall adjacent Spring Island in 2003 and 2004. In addition, silt appears to affect habitat suitability for Comal Springs salamanders at the substrate surface since none were observed in any areas with excessive silt and fewer salamanders were surveyed in Spring Run 3 each time sediment was washed into that habitat. It is not clear whether there may be a response by the Comal Springs salamander to low-discharge conditions, since there have been only two occasions to sample at low flow during this study. As with other study components, additional data are needed within that critical flow range.

Although the hydrograph in 2004 did not present any opportunities to gather data on low-flow impacts to macroinvertebrates around spring openings, there was a distinct influence of high-flows on the 2004 samples. However, despite the differences in springflow conditions relative to 2003, many of the findings in 2004 were very similar to those in 2003. There was a greater difference in Peck's cave amphipods with substantially more sampled in the western shoreline upwelling in 2004, but there is little indication as to the cause. There was an indication that higher flows in the October sample resulted in greater numbers of most species in the nets, but this only occurred in Spring Run 3. Spring Run 1 and the upwelling on the western shoreline of Landa Lake appeared to be less influenced by these higher flows. It will be important to assess whether there are similar differences in response among sites to low-discharge conditions.

The new sample method for Comal Springs riffle beetles, adopted in 2004, provided a larger sample size which improved the ability to evaluate potential differences in population abundance among sites, seasons and with different depths and flows. Previous sampling indicated that there were differences in population size among the three sample areas, but there were similar numbers of Comal Springs riffle beetles (adults and larvae) observed in all sites in 2004. There were also data to suggest that the species has precise requirements (e.g., physical characteristics or food source) that govern its distribution. This valuable information on microhabitat use and distribution within the three sample sites can benefit management strategies that focus on maintaining suitable habitat conditions in specific areas during a period of low recharge. There were no significant relationships between depth, flow or season and abundance of Comal Springs riffle beetles. Additional sampling during a range of flow conditions (particularly during low flow) will improve our understanding of the relationships between discharge, water velocity at spring openings and the abundance and distribution of Comal Springs riffle beetle.

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

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

INTRODUCTION

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

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

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

During this study, valuable information was gathered on water quality and habitat conditions for each of these species as well as monitoring data on current population dynamics of each. For those species that are primarily subterranean, Comal Springs dryopid beetle and Peck's cave amphipod, information is less extensive due to sampling limitations. There were multiple components incorporated into this study because sampling efforts were unique to each species and for general water quality conditions.

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

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

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

One additional component of the fountain darter portion of this study was an investigation of potential impacts of an exotic trematode parasite, *Centrocestus formanosus*, which has become established in the Comal River since its discovery there in 1996. The parasite completes part of its life cycle within the digestive gland of an exotic snail, *Melanoides tuberculata*, which is locally abundant within the Comal Springs/River ecosystem. After being released from the snail, the parasite infects the fountain darter gills and form cysts that accumulate over time. The initial focus of this study component was to examine variation in spatial and temporal concentration of the parasite in the water column throughout the Comal Springs/River ecosystem. The initial design also focused on comparing three monitoring methods to determine the most cost-effective and reliable means for long-term monitoring of parasite densities. In addition to sampling the water column, this included sampling infection intensity on the gills of caged and resident fountain darters in the Comal Springs/River ecosystem. This effort (Cantu 2003, BIO-WEST 2004a) revealed that sampling parasites in the water column was most effective and should be used for future monitoring efforts. Parasite sampling in 2004 has continued monitoring of parasite concentrations in the water column in the eight study locations.

The Comal Springs salamander was monitored in multiple locations throughout this study to evaluate relative distribution among known habitat locations and to examine population responses to changes in springflow. The sample areas included Spring Runs 1 and 3 as well as the Spring Island area. Timed surveys in each of these areas provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge. Qualitative visual observations in the deeper portion of Landa Lake were also used to verify presence/absence in that area during each sample effort.

Another component of this study was 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 provided the information to make such assessments, but macroinvertebrate sampling in Spring Runs 1, 2, and 3 was an integral component of this study primarily because of the presence of the three endangered macroinvertebrate species (Comal Springs riffle beetle, Comal Springs dryopid beetle, and Peck’s cave amphipod). 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 2003-2004, there was also a sample effort targeted at the Comal Springs riffle beetle relative abundance among the three areas identified during a 2001 survey (BIO-WEST 2002b) as having the largest concentrations of individuals and under various discharge conditions within each site.

The final sample component in the Comal Springs/River ecosystem was to estimate the density of various exotic fish species and to evaluate the potential that predators may be consuming one or more of these threatened/endangered species in large numbers. This was conducted by using gill nets and evaluating stomach contents of captured fish for the presence of any threatened/endangered species (primarily fountain darters and salamanders). Because of limited sample sizes early in the study, rod-and-reel sampling was added to supplement gill net sampling for this study component. Rod-and-reel sampling allowed researchers to target larger sunfish and small- to intermediate-sized bass, which are the most likely piscine predators on fountain darters and salamanders. As a result of using both rod-and-reel and gill net sampling, sufficient baseline data had been collected by the final 2002 sample to discontinue this component except during “Critical Period” events.

In addition to each of the study components described above, several individual research efforts that developed during the course of this study were conducted by BIO-WEST, Inc. (BIO-WEST). The details of each study is not covered in this document but can be found in the individual reports cited here. In 2001, a comprehensive survey of Landa Lake (BIO-WEST 2002b) revealed that the Comal Springs riffle beetle was found in areas outside of the Spring Runs, specifically in one area along the western shoreline of Landa Lake and in the lake around Spring Island. Additional laboratory efforts with the Comal Springs riffle beetle (BIO-WEST 2002c) provided data that suggest Comal Springs riffle beetles oriented toward lateral flow and upwelling flow. A laboratory study of fountain darter reproductive response to parasites and fluctuations in temperature (BIO-WEST 2002d) revealed that parasites did not affect reproductive capability, but reaffirmed earlier work by Brandt et al. (1993) and Bonner et al. (1998) that reproduction in the laboratory is reduced when water temperature reaches 26ºC regardless of daily temperature fluctuations. In 2004, BIO-WEST conducted a laboratory study on the influence of fluctuations in discharge on water quality and determined that carbon dioxide was a potentially limiting factor affecting growth and structural support of several plants in the San Marcos Springs/River ecosystem including Texas wild-rice (BIO-WEST 2004b).

The data for the primary study components were gathered during regular, quarterly "Comprehensive" sample efforts to provide a baseline of information on population dynamics of each species under "normal" discharge conditions. However, the study design also incorporated sampling events that occurred following infrequent, extremes in discharge (Critical Period events) to evaluate the response of each species and its habitat to such events. Unfortunate to the primary goals of this study, the sampling events resulting from the infrequent, extremes in discharge have been primarily high discharge events, but the data have yielded valuable information nonetheless. The scouring effects of flooding had often-dramatic results on aquatic vegetation and population abundance of fountain darters. The two sampling events that were triggered as a result of low discharge conditions occurred in 2000 and the low-flow period was relatively short-lived. Therefore, it is difficult to make direct evaluations of the water quality conditions that would be expected with a repeat of these discharge levels (or lower discharge conditions) or the response of each species' population and its habitat to such events.

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

METHODS

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

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

- Water quality evaluation
  - Standard parameters (DO, pH, specific conductivity, temperature)
  - Conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids)
  - Thermistors for continuous record of water temperature
  - Fixed station photographs for qualitative evaluation of temporal variation
- Detailed mapping of aquatic vegetation (fountain darter habitat) in study reaches
- Fountain darter sampling
  - Drop nets
  - Dip nets (time constrained surveys)
  - Visual observations
  - Gill parasite evaluation
- Comal Springs salamander observations
- Macroinvertebrate sampling
  - Drift nets
  - Comal Springs riffle beetle surveys
- Exotics/Predation evaluation

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

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

There were two other modifications between samples conducted in 2000-2002 and those conducted in 2003-2004. One was the removal of the winter Comprehensive sample. Data collected during the first two years of the study provided sufficient information to adequately describe baseline conditions during this season. Low-flow conditions are most likely to occur during the late summer and early fall, so it was considered to be more critical to have continuous monitoring of conditions immediately preceding and following this timeframe each year. The other modification was to the macroinvertebrate sample efforts. During the first two years of the study, drift nets were placed in the spring runs to collect drifting macroinvertebrates. To maximize the characterization of all macroinvertebrate habitat use, the drift nets have been placed over spring openings since 2003.

Springflow

Total discharge data for the Comal River were acquired from U.S. Geological Survey (USGS) Water Resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2000). The discharge data for the Comal ecosystem were 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 monitoring total discharge in the Comal River using USGS gage data, which are used to characterize the Comal Springs ecosystem during sampling, localized discharge was also measured in Spring Runs 1, 2, and 3 and in the Old Channel during each sampling effort (2003-2004). This information was used 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. Spot water velocity measurements were also taken during each sampling event using a Marsh McBirney model 2000 portable flowmeter.

Low-Flow Critical Period Sampling

This project was initiated during a period of limited recharge in the summer of 2000, which resulted in relatively low total discharge in the Comal River. As a result, the first discharge trigger for a low-flow Critical Period sampling event (<200 cfs total discharge) occurred at that time. This was followed by a discharge trigger for a second low-flow Critical Period sampling event (<150 cfs total discharge), but immediately after that sample, a rainfall event raised the discharge back above these trigger levels and no additional low-flow Critical Period sampling events occurred through the fall of 2004. As a crucial component of the study, more biological data during low-flow periods are essential to adequately assess population-level impacts during these periods. A thorough analysis of all available data is presented in this report, but the continuation of this monitoring and research effort will allow collection of more critical low-flow data.

High-Flow Critical Period Sampling

Although there were few low-flow discharge events in 2000-2004 to adequately evaluate that component of the study, four significant flood events provided valuable information to describe high-flow impacts (Figure 1). These events occurred in August and November 2001, July 2002, and June 2004. The 24-hour mean discharges for the four events were, respectively, 873, 1,350, 13,400, and 3,150 cfs. The largest event, in July 2002, was unique because the "high discharge" estimates were a result of water backing up from the swollen Guadalupe River just downstream. A full sampling event occurred, to the extent possible, in response to each event after a sufficient time elapsed (approximately one week) to allow conditions to stabilize after the flushing flows subsided in order to evaluate post-flooding effects. In two instances these samples corresponded with the summer Comprehensive sampling event (July 2002 and June 2004), whereas the other two samples were additional events. Some components were not incorporated into the additional samples including macroinvertebrate sampling, gill parasite evaluation, and exotic/predator sampling. In some instances, dip net and/or drop net sampling for fountain darters was not possible during these events due to high water. In addition to these four high-flow Critical Period sampling events, a high-flow trigger was reached in November 2004 (24-hr mean discharge on November 22 was 6,860 cfs). However, sufficient data had been collected on high-flow impacts and only a visual assessment (including photographs) was conducted along with mapping of vulnerable stands of Texas wild-rice plants in the San Marcos Springs/River ecosystem.

Figure 1. Photographs of the New Channel of the Comal River two days after total discharge reached 2,600 cfs (November 17, 2004). The river was 18-24 inches higher than normal in these photos and there is evidence of flow overtopping the hand railings.

Water Quality

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

A total of 15 sites were chosen to represent the water quality conditions along the complete length of the Comal River from the headwaters down to an area just upstream of its confluence with the Guadalupe River (Figure 2). In each of the 15 sites a Hydrolab profile was conducted during each Comprehensive and Critical Period sampling event in 2000-2002. A hydrolab profile measured water temperature, conductivity compensated to 25°C, pH, and dissolved oxygen. In addition, the water depth at sampling point and observations of local conditions were noted. These Hydrolab measurements were taken at the surface at all water quality stations. In addition, these same parameters were measured at each fountain darter sample (drop net) location at the surface, mid-depth, and near the bottom when there was stratification.

In addition to hydrolab profiles, water "grab" samples were taken in 12 of the 15 sites in the San Marcos River (Figure 2) to evaluate conventional water chemistry parameters. These sites include:

Heidleberg Lodges No. 1	New Channel No. 1
Heidleberg Lodges No. 2	New Channel No. 2
Booneville Ave. No. 1	Old Channel No. 1
Booneville Ave. No. 2	Old Channel No. 2
Spring Run 1	Union Ave.
Spring Run 2	
Spring Run 3	

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

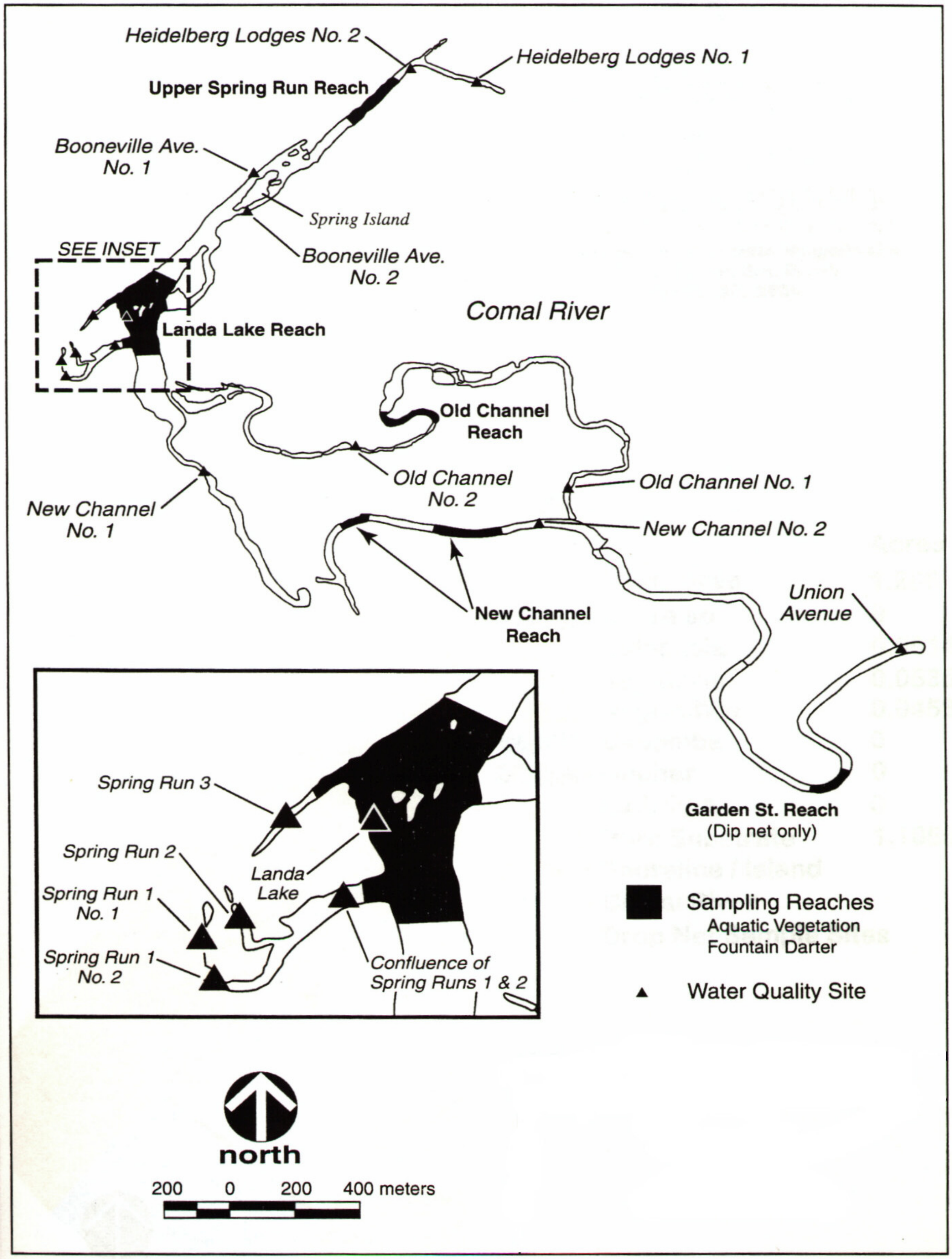


Figure 2. Comal River water quality and biological sampling areas.

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

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

[a] micrograms.

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

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

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

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

Ammonium: The ammonium concentration was determined following the outline of Holmes et al. (1999). The method uses fluorescence of the sample minus background fluorescence and matrix effects against a standard curve. Protocol B was followed for systems with ammonium concentrations generally exceeding 0.5 micromole per liter (µmol/L). The method uses a fluorometer equipped with a

Turner designs optical kit 10-AU, near UV mercury vapor lamp, a 350 nm interference excitation filter with a 25 nm bandpass, a 410-600 nm combination emission filter, and a 1:75 attenuator plate.

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

Aquatic Vegetation Mapping

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

From 2000-2002, five reaches were mapped throughout the Comal Springs/River ecosystem, but in 2003-2004, one of the sites, the upper section of the New Channel was not mapped. The site was dropped from the sample protocol because this section did not increase habitat coverage substantially, but the effort required to map it was extensive. In addition, most of the upper section is too deep to conduct drop net samples for fountain darters. Only *Hygrophila* along the margins could be sampled there and the one sample site conducted during each sample effort in 2000-2002 was typically similar in fish species composition to the two *Hygrophila* sample sites in the lower section.

At the beginning of the study, filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium* primarily in the upper Spring Run and Landa Lake) were not mapped because of various difficulties (patchiness, bryophytes are easily obscured by filamentous algae, etc.). However, drop net sampling clearly identified these vegetation types as important fountain darter habitat and they were included in all mapping efforts beginning in the summer of 2001.

Fountain Darter Sampling

Drop Net Sampling

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

At each location the vegetation type, height, and areal coverage were recorded, along with substrate type, mean column velocity, velocity at 15 centimeters (cm) above the bottom, water temperature, conductivity, pH, and dissolved oxygen. In addition, vegetation type, height, and areal coverage, along with substrate type, were noted for all adjacent 3-m cell areas. Fountain darters were identified, enumerated, measured for standard length, and returned to the river at the point of collection. The same measurements were taken for all other fish species, except for abundant species where only the first 25 individuals were measured; a total count was recorded for a drop net sample beyond the first 25 individuals in such instances. Fish species not readily identifiable in the field were preserved for identification in the laboratory. All live giant ramshorn snails were counted, measured, and destroyed, while a categorical abundance was recorded (i.e., none, slight, moderate, or heavy) for the exotic Asian snails (*Melanoides tuberculata* and *Thiara granifera*) and the Asian clam (*Corbicula* sp.). A total count of crayfish (*Procambarus* sp.) and grass shrimp (*Palaemonetes* sp.) was also recorded for each dip net sweep.

Drop Net Data Analysis

The fisheries data collected with drop nets were analyzed in several ways. Calculations of fountain darter density in the various vegetation types during 2000-2004 provide valuable data on species/habitat relationships. These average density values were also used with aquatic vegetation mapping data on total coverage of each vegetation type by sampling effort to create estimates of the population abundance in each reach (fountain darter density within a vegetation type x total coverage of that vegetation type in the given reach). Because there were generally only two drop net samples in each vegetation type within each reach, density estimates between sampling efforts had great variation and population estimates based on those densities would be greatly influenced by this variation. Part of the variation would be due to changes in environmental conditions (discharge, temperature, etc.) that had occurred since the last sample, but part would be 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. A more complex assessment of the influence of environmental conditions on the fountain darter population was incorporated into the statistical model described below. Because these estimates use static estimates of density and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Although vegetation composition is a primary factor in determining fountain darter population abundance, there are many other factors involved, some of which may become increasingly important during low-discharge conditions. In order to assess the relationship between fountain darter abundance and multiple parameters related to their habitat conditions, a statistical model was developed for analysis of the drop net data collected throughout the study. The model was a generalized linear regression model that incorporated multiple parameters for simultaneous evaluation of the relative contribution of each to fountain darter abundance. The model was run using the density of darters captured in each drop net sample (total darters captured/available habitat) as the dependent variable. A square-root transformation was used on the density data to create a linear relationship with most independent variables. Independent variables included dominant vegetation types, season, total discharge in the Comal River, depth, flow and standard water quality parameters in the immediate vicinity of the site. In addition the mean and median length of darters collected from each reach were compared over time in the model to determine whether changes in discharge and/or seasonal patterns could be observed in the darter population. The model was run using NCSS software (Hintze 2001).

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

Dip Net Sampling

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

To balance the effort expended across 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 (particularly shallow shoreline habitat) and often resulted in greater numbers of small fountain darters than drop net sampling in nearby areas. This sample method also permitted comparisons within a site among sampling events.

Dip Net Data Analysis

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

Visual Observations

Visual surveys were conducted using SCUBA in Landa Lake to make observations of presence/absence of fountain darters and Comal Springs salamanders and to document use of habitat in deeper portions of the lake. The locations of these time-constrained surveys were deeper than drop net or dip net sampling 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 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 because a much more labor-intensive effort would have been required to develop such an estimate. As a result, this method was not used to create 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 this study component were to examine the variation in spatial and temporal concentration of the trematode parasite, *Centrocestus formosanus* in the Comal Springs/River ecosystem. This was assessed initially with a study designed to evaluate these variables using three sampling methodologies. One method sampled parasites drifting in the water column using filtration, another evaluated infection intensity on the gills of "resident" fountain darters captured within the sample site, and the third evaluated infection intensity on the gills of caged fountain darters placed in the sample site. Each of the three methods of estimating parasite infestation was also assessed for viability as a long-term monitoring tool. Data for this study were collected in 2002-2003 and a description of the findings is found in the 2003 annual report (BIO-WEST 2004a) and in Cantu's thesis (2003; Texas State University) entitled: "Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas." In summary, the initial study determined that there were differences in the spatial and temporal distribution of parasites in the Comal Springs/River ecosystem. The study also concluded that monitoring of parasites by filtering a known quantity of water was the most effective and efficient method for long-term monitoring. Since the completion of the initial portion of the study in May 2003, monitoring efforts using the filtration method have been conducted in the same eight sites used during the initial study. Data from monitoring efforts since May 2003 are presented in the Results section of this report. In addition, the Discussion section includes recommendations for future efforts to improve on long-term monitoring efforts.

There were eight sample sites selected for the parasite study, which were dispersed throughout the Comal Springs/River ecosystem. Study sites were selected to represent a broad range of habitat conditions, including Spring Run habitat, backwater conditions in the headwaters, shallow lentic habitat near islands in Landa Lake, edge habitat in the Old Channel, and edge habitat downstream of the Old and New Channel confluence (see Cantu 2003 for map). Samples were conducted quarterly between 2002-2004 in each of these sites. In 2002-2003, parasites were assessed quarterly in each sample site using each of the three methods described in detail in Cantu (2003), but in monitoring efforts in 2003-

2004, only parasites drifting in the water column were measured. All study sites were sampled every other month during the study, but two sites, one in the Old Channel and the other at the confluence of Spring Runs 1 and 2, were sampled monthly in 2004. Cross-section discharge of the river channel was taken during each of the monthly samples to evaluate the relationship between local discharge and parasite concentration.

To measure the concentration of drifting *C. formosanus* in the water column, water was collected and filtered using a filtration apparatus developed by Cantu (2003). An adjustable fiberglass rod (3.5m) attached by a clamp to a 10-Liter (L) bucket was used to collect 3, 5-L samples while standing on the riverbank. A preliminary diel filtration experiment with samples conducted every 3 hours over a 24-hour period revealed that the highest parasite densities occurred between 0930 and 1230 hours; therefore, all study samples occurred during this time period. Another preliminary exercise revealed that "fixing" the sample with a 0.1% formalin solution greatly increases the percentage of parasites recovered during filtration. Thus, all 5-L samples of river water were fixed with a 0.1% formalin solution after being collected and prior to filtration.

Comal Springs Salamander Surveys

In addition to the visual observations of fountain darters and Comal Springs salamanders in the deeper portions of Landa Lake, 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 all 2000-2004 sampling events. Surveys were conducted in Spring Run 1, Spring Run 3 and the Spring Island area (Figure 2) 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).

Macroinvertebrate Sampling

In 2000-2002, drift nets were placed in Comal Spring Runs 1, 2, and 3. Two nets were placed in Spring Run 1 approximately 15 m downstream of Landa Park Drive. Spring Run 2 had one net placed just upstream of the road crossing before the wading pool, and one net was placed in Spring Run 3 approximately 6 m downstream of the last footbridge. Drift nets were anchored into the substrate using rods, with the net face perpendicular to the direction of water flow. The nets consisted of a 0.45-m by 0.30-m rectangular opening, which connected to a 1-m-long net with a mesh size of 600 μm. The tail of the net was connected to a detachable 0.15-m-long cylindrical bucket. The bottoms of the nets were positioned 2-3 cm above the sediment to reduce the possibility of macroinvertebrates crawling into the nets. The depth of the water column entering the net was then measured at three locations across the mouth of the net, and a flowmeter was used to measure water velocity at the mouth of the net. Collecting buckets were then connected to the end of the nets and the time was recorded. The buckets were removed at 3-hour intervals, and the cup contents were washed into seal-able bags. A squirt bottle filled with water was used to remove any macroinvertebrates that were clinging to the inside of the cups. The samples were preserved in 90% ethanol, and the bags were labeled with the date, net number, and the time of the sampling interval. This process was repeated over a 24-hour period and then all samples were transported to the Stream Ecology Lab at Texas State University and stored for analysis. Because the amount of time required to separate samples and identify all organisms was greater than anticipated, samples from Spring Run 2 were collected and saved for future use, but not examined during this study.

The samples yielded estimates of drift rates (the number of drifting organisms per 24-hour-period) and densities (the number of organisms per 100 cubic meters (m^3) of water [see formula below]) for each taxa in all samples. This information was used to evaluate patterns of selected aquatic invertebrates and determined if changes in depth, season, and current velocity affect the composition and abundance of selected indicator insects. The details of this portion of the study are described in the 2002 annual report (BIO-WEST 2003) and summarized in the Results and Discussion sections of this document.

$$\text{Drift Density} = \frac{(N)\,(100)}{(t)\,(W)\,(H)\,(V)\,(3600\ s/h)}$$

Where N is the number of macroinvertebrates in a sample; t, length of time of sampling (hours), W, net width (m); H, height of water column in the net mouth (m); and V is the mean velocity of water at the mouth of the net (m/s). Drift density was expressed as the number of macroinvertebrates drifting per 100 m^3 of water sampled (Smock 1996).

In 2003 and 2004, drift nets that were placed in the Spring Runs in 2000-2002 were moved to spring openings to obtain a more direct observation of movement of organisms out of the aquifer. In particular, the two of the three endangered macroinvertebrate species are more closely associated with spring openings and more likely to be sampled with this method. 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 using rods, with the net face perpendicular to the direction of flow of water. The nets had the same size opening as used previously (0.45-m by 0.30-m rectangular opening) but the mesh size was smaller (350 µm). The tail of the net was connected to a detachable 0.28-m-long cylindrical bucket (300-µm 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 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, *Heterelmis comalensis*, were conducted in 2003-2004 to determine its distribution and relative abundance within and among three disjunct areas of Landa Lake on the Comal River, Texas. The lake was surveyed extensively in 2001 (BIO-WEST 2002b) and these three sites (Spring Run 3, western shoreline of the Lake, and upstream of Spring Island) were found to have the highest densities of Comal Springs riffle beetles. The three sites were surveyed in June, September and November in 2003 and in May, August and October in 2004. In 2003, these surveys were conducted with a 60-cm x 60-cm quadrat, which was placed over the substrate in areas known to have high quality habitat (based on a preliminary survey). The survey consisted of examining all rocks of approximately 1-cm and larger within the quadrat for Comal Springs riffle beetles (rocks along the edge of the quadrat were considered sampled if >50% of the rock was inside the boundary). The sides and bottom of each rock was carefully examined and beetles were identified as either Comal Springs riffle beetles or the similar *Microcylloepus pusillus* using a dissecting microscope. Identified beetles were held until the survey was complete and then returned to the sample area. Each survey included 4 quadrats within the sample area to expend the same effort across surveys. There were 3 sample areas including the shallow edge of Spring Run 3 (northwest shoreline) between the two pedestrian bridges, a small area along the western shoreline of Landa Lake across from the city golf course, and an area just upstream of Spring Island where upwelling springs provide habitat on the bottom of Landa Lake.

In 2004, a different sample method was used to sample the Comal Springs riffle beetle. Previous sampling at Comal Springs during this study (BIO-WEST 2003) and a study by the USFWS (2004) revealed that riffle beetles are attracted to a variety of cotton substrates placed in the spring orifices for several weeks. A quantitative survey method was developed based on this behavior and incorporated into this study. Bed sheets (50% cotton, 50% polyester) were cut into 15cm x 15cm 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 relocated 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 beetles (*Microcylloepus* and *Stenelmis*), Comal Springs dryopid beetles (*Stygoparnus comalensis*), Peck's cave amphipods (*Stygobromus pecki*), and blind isopods (*Lirceolus* near *pilus*).

Because this was a more complex sampling method that resulted in much larger sample sizes than the data collected in 2003, a more detailed analysis of the patterns of population and abundance of the species were possible. In addition to basic information on the population, it was also possible to evaluate whether flow, depth, or season has a significant influence on the distribution of Comal Springs riffle beetles in the Comal River. Non-parametric analyses were used for all tests because the data were not normally distributed, even with various transformations applied to the data set. Comparisons of mean abundance of Comal Springs riffle beetles among sites and among seasons were conducted with the Kruskal-Wallis one-way Analysis of Variance and the Spearman correlation test was used to evaluate potential relationships between abundance of Comal Springs riffle beetles and the depth and flow variables.

Exotics/Predation Study

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

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

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

RESULTS

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

Springflow

Springflow in the Comal Springs/River ecosystem during 2000-2004 was generally much higher than average conditions (Table 2 and Figures 3-4). The project was initiated during a period of limited recharge during which total discharge in the Comal River was at or below 200 cfs for 74 days and declined to a low of 138 cfs on September 7, 2000. Conditions in the fall of 2000 through summer of 2001 were approximately normal based on conditions that have occurred over the period of record, but two substantial rain events in the fall of 2001 (24-hour mean discharges of 873 and 1,350 cfs in the Comal River) resulted in a dramatic increase in aquifer levels (to record conditions). The aquifer levels have remained elevated throughout the remainder of the project. Additional flooding events in the summer of 2002 and again in 2004 prevented the aquifer from declining significantly during the late summer and fall, which has historically provided low recharge. These latter two events, providing 24-hour mean discharges of 13,400 and 3,150 cfs in the Comal River, respectively, were particularly influential on aquifer levels and provided extended periods of higher-than-normal flows. During 2003-2004, total discharge in the Comal River never declined below 335 cfs, whereas the historical mean is 286 cfs. Discharge in 2003 was relatively stable with daily discharge values between 350 cfs and 600 cfs. Discharge in much of 2004 was similar with high flows between 335 cfs and 600 cfs, but there were numerous peaks that were much higher including a mean daily discharge value of 3,130 cfs on June 9, a value of 2,600 cfs on November 17 and a value of 6,860 on November 22.

Table 2. 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

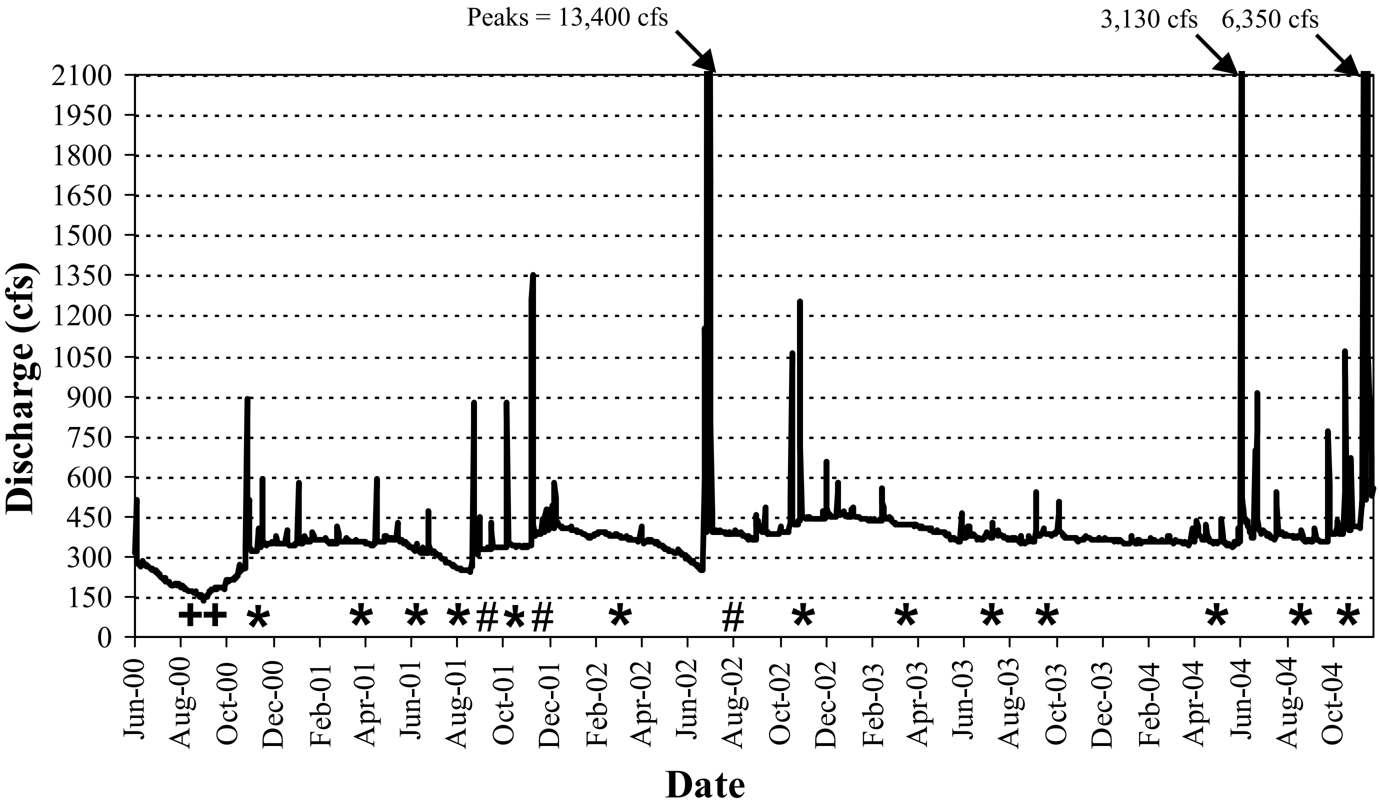


Figure 3. Mean daily discharge in the Comal River during the study period; approximate dates for Comprehensive (*), low (+), and high-flow (#) Critical Period sampling events are indicated.

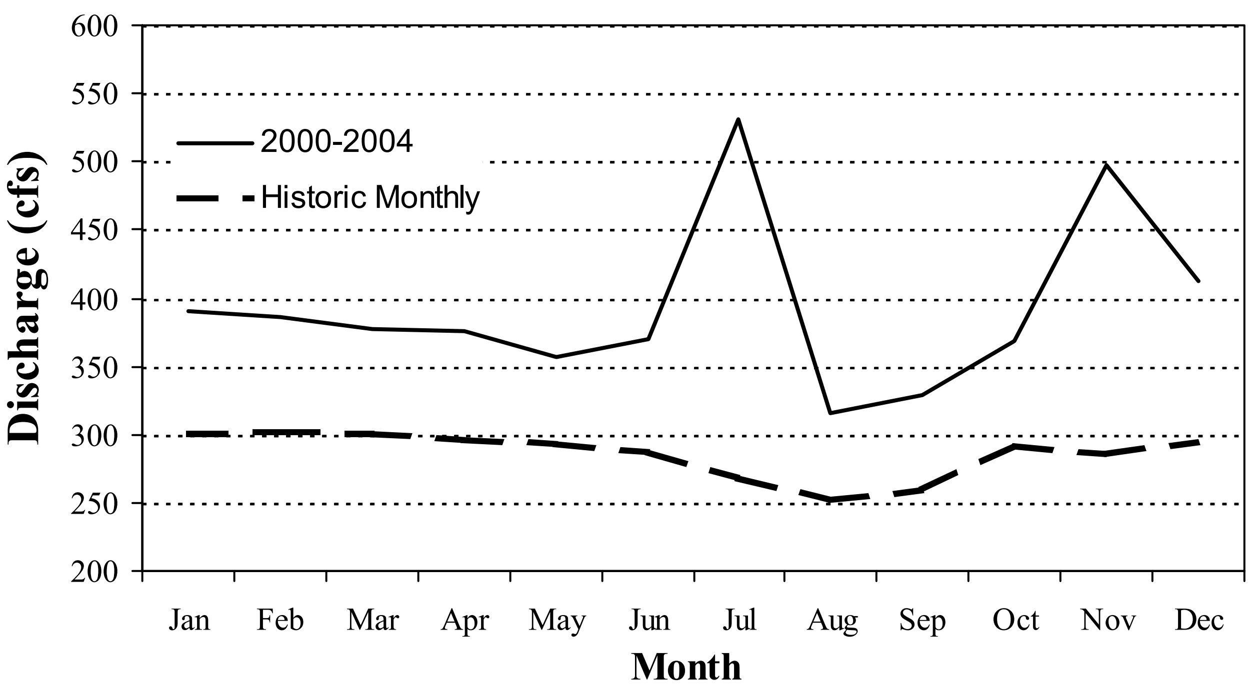


Figure 4. Mean monthly discharge in the Comal River during the project (2000-2004) and during the 1934-2001 period of record.

In 2003, discharge in the Old Channel was increased due to an adjustment in the culvert that regulates flow through this channel (Table 3). Discharge was greater than 90 cfs in each sample, which is significantly higher than the 40 cfs estimate for typical conditions by the USFWS (2000). In 2004, discharge decreased progressively during each sample in the Old Channel to a low of 76.5 cfs in the fall sample. No measurements are available prior to 2003, but conditions were closer to the 40-cfs estimate of typical conditions during that time. The discharge through the upper section of the Old Channel was approximately 25-28% of the total discharge (measured downstream of the confluence of the Old and New Channels) in 2003 and between 18-25% in 2004 (Table 4). Spring Runs 1 and 3 (combined) yielded approximately 25-30% of the total discharge in the Comal River during each sampling event in 2003-2004 while Spring Run 2 contributed approximately 2%. Lateral spring flow (difference between up and downstream measurements) in Spring Run 3 was also an important source of springflow (about 3% of the total discharge) in 2003 and 2004.

Table 3. 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 2003-2004.

	Discharge (cfs)					
Location	Spring 2003	Summer 2003	Fall 2003	Spring 2004	Summer 2004	Fall 2004
Total Discharge Comal River (USGS)	405	361	385	375	377	411
Spring Run 1	48.9	42.6	43.1	43.3	50.1	50.1
Spring Run 2	7.4	6.3	5.9	6.2	8.8	7.2
Spring Run 3 (upstream)	40.0	37.4	37.3	36.8	41.8	42.4
Spring Run 3 (downstream)	53.5	49.3	47.4	49.5	52.5	54.7
Old Channel	114.5	93.2	93.2	91.8	83.8	76.5

Table 4. 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 2003-2004.

	Proportion of Total Discharge					
Location	Spring 2003	Summer 2003	Fall 2003	Spring 2004	Summer 2004	Fall 2004
Spring Run 1	12.1%	11.8%	11.2%	11.5%	13.3%	12.2%
Spring Run 2	1.8%	1.7%	1.5%	1.7%	2.3%	1.8%
Spring Run 3 (upstream)	9.9%	10.4%	9.7%	9.8%	11.1%	10.3%
Spring Run 3 (downstream)	13.2%	13.7%	12.3%	13.2%	13.9%	13.3%
Old Channel	28.3%	25.8%	24.2%	24.5%	22.2%	18.6%

Low-Flow Critical Period Sampling

Details of the two low-flow Critical Period sampling events that occurred in 2000 can be found in the reports for that year. Findings from those samples are also incorporated into the Results section for each sampling component in this report.

High-Flow Critical Period Sampling

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

Water Quality

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

Table 5. Summary of Comal River ecosystem physico-chemical water quality measurements, 2000 to 2002.

SITE	TEMPERATURE (Celsius)			pH			DISSOLVED OXYGEN (mg/L)[a]			CONDUCTIVITY (μmhos/cm)[b]			TURBIDITY (NTU)[c]			ALKALINITY (meq/L)[d]		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Blieder's Creek	23.45	20.87	25.38	7.27	6.87	7.66	6.05	3.19	10.50	524	481	640	1.83	1.20	3.00	4.03	3.61	4.34
Heidleberg, Main Channel	24.16	23.54	26.56	7.17	6.88	7.41	5.91	4.48	6.86	532	492	667	1.74	1.10	2.10	4.15	3.94	4.52
Island Park, Far Channel	23.62	22.57	24.11	7.21	6.88	7.37	6.62	5.88	7.88	532	495	672	1.44	1.10	1.70	4.26	3.97	4.51
Island Park, Near Channel	23.74	22.85	24.36	7.15	6.82	7.36	6.21	4.81	8.40	531	491	671	1.23	1.00	1.50	4.26	3.97	4.50
Spring Run 1	23.39	23.18	23.72	7.14	6.87	7.34	5.79	5.10	6.84	531	491	667	0.83	0.07	1.20	4.17	3.83	4.59
Spring Run 2	23.47	23.14	23.62	7.15	6.81	7.37	5.56	5.02	6.24	531	491	665	0.88	0.09	1.20	4.18	3.94	4.54
Spring Run 3	23.44	23.17	23.58	7.15	6.82	7.42	5.82	5.23	6.84	529	492	665	0.87	0.09	1.20	4.22	4.04	4.54
New Channel, upstream	23.99	22.61	25.47	7.33	6.93	7.70	8.43	7.17	9.68	531	491	670	1.47	1.10	2.00	4.22	3.94	4.54
New Channel, downstream	23.94	22.02	25.11	7.56	7.20	7.77	9.39	7.81	10.05	529	490	665	1.48	1.00	2.10	4.28	4.00	4.56
Old Channel, upstream	23.54	20.39	25.17	7.55	7.15	7.87	7.98	6.12	9.85	537	492	664	1.86	1.40	2.80	4.32	4.00	4.60
Old Channel, downstream	23.68	21.63	26.05	7.58	7.36	7.84	8.44	6.58	9.90	533	496	665	2.07	1.60	2.80	4.29	4.07	4.66
The Other Place (Iverness)	23.73	21.22	25.51	7.67	7.25	7.89	8.94	6.70	9.75	530	491	662	2.44	1.10	3.20	4.30	4.00	4.60

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

Table 6. Summary of Comal River ecosystem water chemistry measurements, 2000 to 2002.

SITE	SOLUBLE REACTIVE PHOSPHORUS (ugP/L)[a]			TOTAL PHOSPHORUS (ug/L)[b]			AMMONIUM (ug/L)			NITRATE (mg/L)[c]			T NITROGEN (mg/L)			TOTAL SUSPENDED SOLIDS (g/L)[d]		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Blieder's Creek	9.0	2.2	22.0	21.1	8.2	42.0	115.5	43.0	283.9	1.74	1.32	2.23	1.95	1.36	2.44	0.018	0.006	0.026
Heidleberg Main Channel	7.6	2.3	15.7	16.2	7.8	24.3	93.2	20.6	199.9	1.91	1.65	2.21	2.07	1.28	2.38	0.020	0.005	0.028
Island Park, Far Channel	6.8	2.6	13.3	19.5	10.7	47.8	46.2	10.0	188.6	1.91	1.56	2.24	1.96	0.92	2.40	0.018	0.004	0.049
Island Park, Near Channel	7.4	1.9	11.4	18.4	7.1	34.0	53.7	13.8	288.4	2.31	1.46	5.97	1.91	0.62	2.37	0.012	0.005	0.019
Spring Run 1	8.1	2.8	17.0	19.4	9.5	37.1	69.7	25.0	165.9	2.06	1.69	2.48	2.07	1.24	2.76	0.007	0.004	0.018
Spring Run 2	8.0	2.6	13.0	18.0	12.4	26.5	48.9	19.0	138.6	2.01	1.79	2.25	1.94	1.06	2.32	0.007	0.000	0.022
Spring Run 3	7.0	3.3	13.1	14.6	8.5	22.0	70.9	21.0	159.1	2.01	1.74	2.37	2.08	1.21	2.52	0.009	0.000	0.021
New Channel, upstream	7.9	1.5	25.1	17.4	6.5	45.4	52.3	11.0	168.0	1.94	1.73	2.19	2.15	1.71	2.39	0.017	0.003	0.038
New Channel, downstream	7.9	2.1	14.4	18.5	8.9	23.4	54.2	29.0	106.8	1.85	1.26	2.25	2.03	1.28	2.34	0.016	0.004	0.044
Old Channel, upstream	10.7	3.2	26.7	20.0	9.2	35.1	44.1	11.5	131.8	1.83	1.61	2.15	1.94	1.38	2.32	0.033	0.004	0.164
Old Channel, downstream	7.6	2.4	14.3	37.4	14.3	188.9	44.4	9.0	134.1	1.96	1.64	2.22	1.97	0.63	2.42	0.053	0.002	0.366
The Other Place (Iverness)	8.0	1.4	16.5	16.9	2.7	29.6	71.1	20.6	202.0	2.37	1.62	5.83	2.14	1.00	2.56	0.016	0.005	0.029

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

Overall, water quality does not appear to be influenced by surface water inflow from either runoff or outfalls. According to the 1996 State of Texas 305(b) Water Quality Inventory, three domestic outfalls are permitted to release a total of 1.05 MGD, and one industrial outfall is permitted to release a total of 0.06 MGD on the Comal River. The standard physico-chemical parameters and water chemistry values of the water as it exits the springs tend to be maintained within the study area with little degradation.

Among all water quality parameters measured in this study, only water temperature and dissolved oxygen tended to have an upstream to downstream pattern during the study. Values for the other parameters remained fairly constant throughout the system or fluctuated only minimally from site to site. Temperature was generally higher in downstream sites and those areas not near spring inputs (e.g., Blieder's Creek). Data collected with thermistors (discussed below) was substantially more valuable than point measurements taken during the quarterly water quality samples, which do not capture the full range of water temperatures, daily fluctuation, or extreme values. Dissolved oxygen was lowest near spring openings because of the characteristics of the water as it emerges from the aquifer. However, all samples met the water quality standard of 5.0 milligrams per liter (mg/l) in all Comal River samples except on two occasions at the Heidleberg Lodges No. 2 site (in August 2000 and May 2001) and during three summer sampling events in Blieder's Creek. Measurements of pH and specific conductivity did not vary appreciably among sites within the Comal Springs/River ecosystem within this study and were always well below limits at which biological impacts may occur. Turbidity and TSS values were also low at all sites during each sampling event. The SRP concentrations and TP concentrations on the Comal River were well below the TCEQ's screening values of 0.1 mg/l and 0.2 mg/l respectively (BIO-WEST 2003, Appendix B).

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

Nitrate values also exceeded the water quality standards screening level of 1.0 mg/l in most cases, whereas, ammonium values were well below the screening level of 1.0 mg/l (Table 6). Nitrate values in the Comal River were fairly constant throughout the river and throughout the year (ranging from 1.3 to 2.5 mg/l), except at two sites during the August 2000 sampling event, (the Other Place and Island Park) where nitrate values reported were near 6.0 mg/l. In contrast, ammonium concentrations varied throughout the sampling period and among sites, and they were well below the screening level. The TN values for the Comal River were strongly influenced by the high nitrate concentrations. Because the median concentration of nitrate in the Edwards Aquifer ranges from 1.4 to 1.7 mg/l (Bush et al. 1998), it is spring flow and not anthropogenic inputs to the immediate surface waters that affected this parameter.

Hydrolab measurements of water quality at each drop net sample site generated a large dataset across a range of sites in the Comal Springs/River ecosystem. There were 474 sites in the Comal Springs/River ecosystem during 2000-2004 and the vast majority of data suggested superior water quality conditions. Point measurements of dissolved oxygen fell below the water quality standard of 5.0 milligrams per liter (mg/l) several times during 2000-2004. These observations occurred only in Landa Lake and (the majority) in the Upper Spring Run. Most of these observations (20 out of 26) occurred during 2002. The lowest value was 4.2mg/l and values below 4.5mg/l occurred only 7 times in 2000-2004. Dissolved oxygen concentrations tended to be very similar among reaches with average readings of 7.2 to 7.5mg/l between 2000-2004. Water temperature measurements at drop net sites were also similar among reaches and specific conductivity and pH measurements remained within very narrow ranges at all drop net sample locations.

Water temperature data collected continuously with thermistors in multiple sites throughout 2000-2004 are presented in select locations in Figures 5 and 6 and in all sample locations in Appendix B. Over the course of the project, the range of water temperatures in sites associated with springs (Spring Runs, east side of Spring Island, deep portion of Landa Lake) remained less than 1ºC, except for a few acute peaks/troughs. (Several "spikes" in the graphs appear to be a result of a temporary exposure of a thermistor, for instance there were two occasions when the thermistor in the Old Channel spiked to 28ºC and above.) In locations that did not have direct influence of springs (west side of Spring Island, Old and New Channels, "Other Place" resort) there were wider daily fluctuations in temperature of approximately 2ºC and seasonal variation of 4-6ºC between 20-26ºC (Appendix B). The Heidleberg site located in the Upper Spring Run, which is not immediately adjacent to spring openings, was intermediate between these groups with daily fluctuations between 1-2ºC and seasonal fluctuations between 3-4ºC. The greatest fluctuation in temperature (between 18-28ºC) occurred in Blieders Creek where there is little influence of spring inputs.

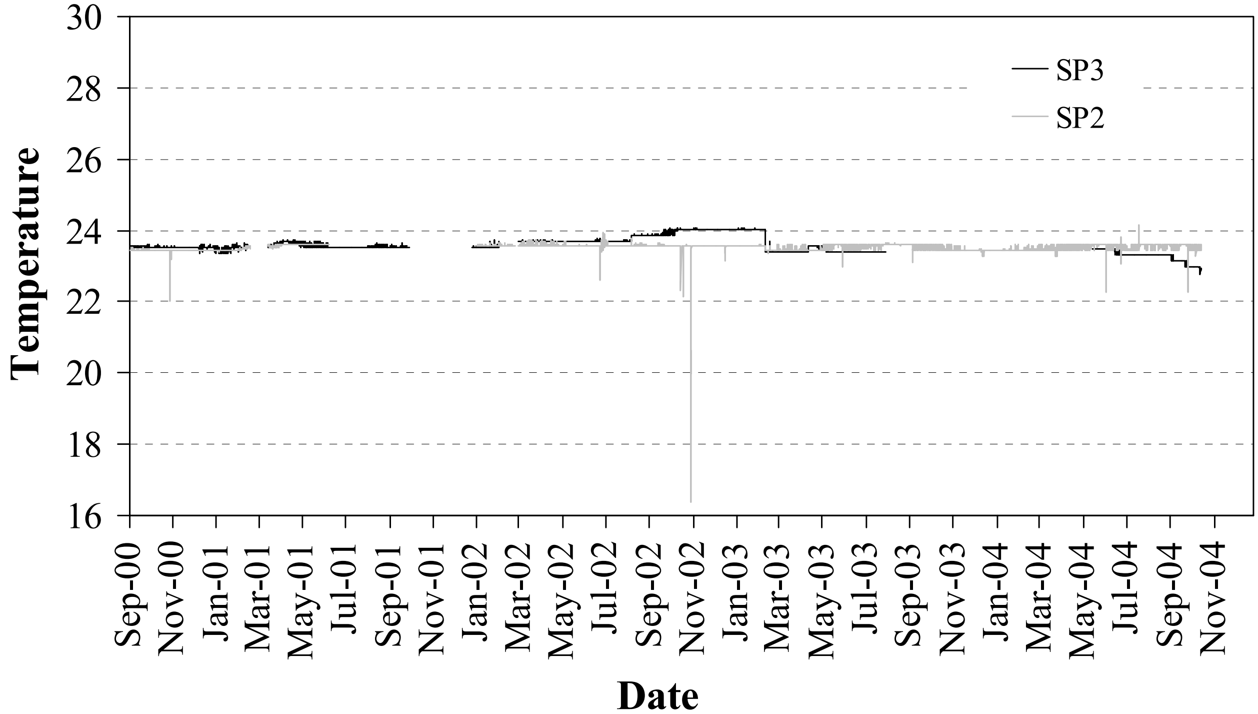


Figure 5. Thermistor data from Spring Runs 2 and 3.

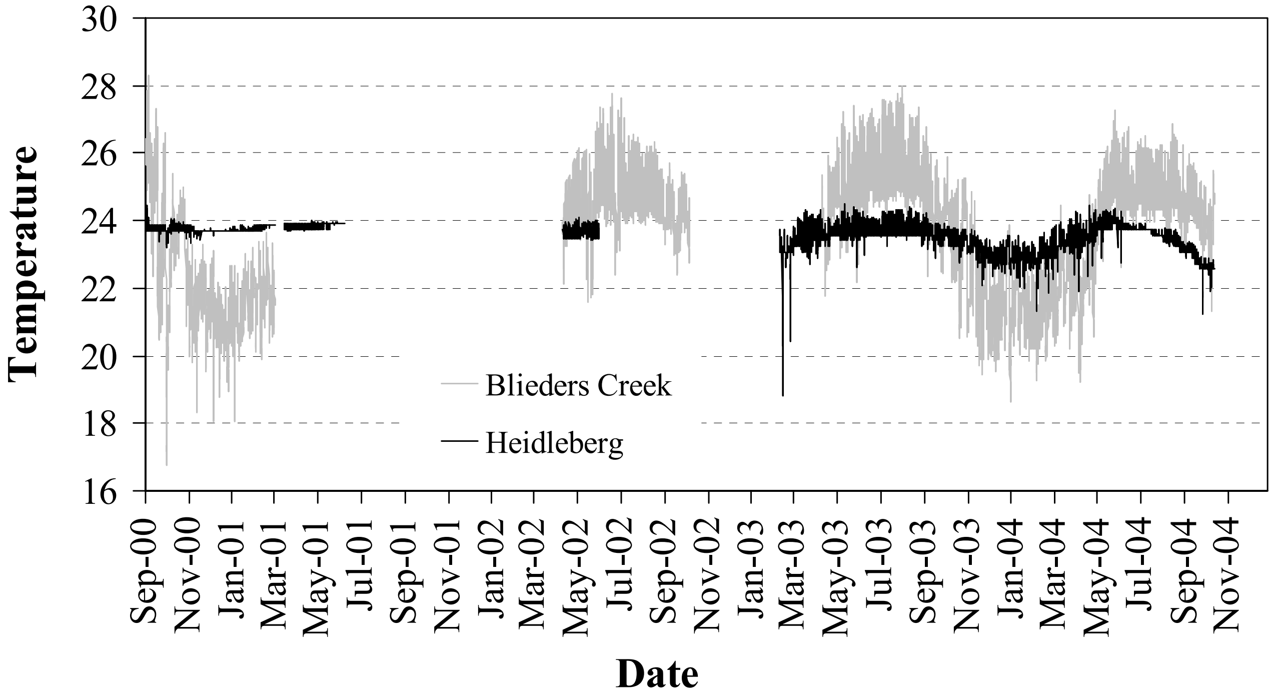


Figure 6. Thermistor data from Blieders Creek and Heidleberg Lodge area (Upper Spring Run).

Sites with wider temperature ranges are more likely to exceed critical water temperature thresholds. The TCEQ critical threshold for water quality in the Comal River is 26.7°C, which is similar to the temperature at which fountain darters are believed to have reduced fecundity (27ºC; Bonner et al. 1998); the lethal limit for the fountain darter is 34.8°C (Brandt et al. 1993). Most sites did not exceed 26ºC at any time in 2000-2004 (Appendix B). Sites that were closer to spring inputs were less likely to approach this threshold. For example, the Spring Island area has two distinctly different conditions on the east and west sides and thus the data collected from the two sites differed dramatically (Appendix B). The deeper channel that runs to the west of the island receives little direct input from the spring in the center of the island and fluctuated in temperature to a much greater extent than the area just downstream of the spring inputs of the island (on the eastern side). Most of the flow in the deeper western channel comes from spring inputs further upstream. The residence time between the headwaters and the Spring Island area allowed the water to reach 25ºC or more during the hottest months. The data for the Spring Island west channel site show that temperatures exceeded 26ºC in March 2003, but there was some "drift" in the thermistor resulting in inaccurate readings. Once the thermistor was replaced, readings were much lower than 26ºC. As mentioned above there were two "spikes" in the continuous record of water temperature in the Old Channel when water temperature exceeded 27ºC in July 2002 and April 2003, but in both instances daily high temperature values before and after the day with the spike were near 25ºC. The one site that legitimately reached 26ºC and above was in Blieder's Creek, which is not directly influenced by spring inputs.

Blieders Creek also registered much lower water temperatures during the winter than areas more directly influenced by springflow, but water temperature reached the lowest values in the furthest downstream sites. Minimum temperatures occasionally dropped below 18°C downstream of Landa Lake but usually remained above 20ºC (Appendix B). While the wide fluctuations in temperature found in Blieders 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 Blieders Creek is typically a stagnant pool that does not flow into the Comal River; temperatures observed in the Heidelberg site just downstream, but within the influence of Comal Springs were within a much more narrow temperature range (Appendix B).

Data on air temperature and precipitation during the project are also found in Appendix B. It appears that precipitation had acute impacts in some locations (typically very cold rainfall resulted in rapid drop in water temperature), but these effects were generally short-lived. Although spring inputs have the greatest affect on water temperature in the Comal Springs/River ecosystem, air temperature affects sites that are further away from spring openings.

Aquatic Vegetation Mapping

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

Upper Spring Run Reach

The Upper Spring Run Reach remained relatively stable throughout the study compared to other portions of the Comal River. The reach is characterized by *Hygrophila* sp. and *Sagittaria* sp. near each shoreline and small patches of *Ludwigia* sp. in the middle of the reach. These macrophytes fluctuated in abundance over the study period depending largely upon flushing flows, but even after scouring, each vegetation type tended to return within the same areas it had previously occupied. This reach also contains both bryophytes, *Riccia* sp. and *Ablystegium* sp., which had more variability in their distributions than the other vegetation types. Bryophytes were not very abundant in this reach during the early part of the study (they were first mapped in summer 2001) but increased dramatically in total coverage after the larger flushing flow events. The largest increase in the bryophyte coverage occurred after the summer 2002 flood. The bryophytes expanded to cover most of the previously bare substrate throughout this reach through 2003. However, between the fall of 2003 and the spring of 2004 the total coverage declined dramatically back down to levels that were similar to pre-2002 flood levels and remained low through 2004. The flushing flows in the summer of 2004 had a similar effect to the 2002 flood event with some scouring evident immediately after the flood, but it is unknown whether the re-growth observed in the fall 2004 sample will continue into 2005 as occurred after the 2002 flood.

Landa Lake Reach

Landa Lake is a complex reach containing many different aquatic vegetation types, but the largest proportion is a pure stand of *Vallisneria* sp. That stand did not change much in total coverage from 2000-2004 and averaged approximately 55% of the total area within the reach during this time. There were some areas where the *Vallisneria* expanded into previously-bare substrate and other areas that were thinned and filled in with *Riccia*; however, the *Vallisneria* in this reach was very stable overall under the flow conditions experienced during this study. Two other relatively stable vegetation types were *Hygrophila* and *Sagittaria*, which each occupied between 2-5% of the total area on average during the study. The *Hygrophila* grows in a large patch in the shallow area between the three islands in the middle of Landa Lake while the *Sagittaria* grows along shoreline areas, primarily the Northwest shoreline of the lake. At times, the *Hygrophila* patch was dense during the study, but at other times (not always associated with flushing flows) the stand was patchier with “holes” of bare substrate or *Riccia*. The *Sagittaria* patch was very consistent with some increase in total coverage over the course of the project. Large portions of the *Sagittaria* and *Vallisneria* stands were also covered with *Riccia* during the course of the study.

Similar in physical appearance to *Hygrophila* the *Ludwigia* was less common in Landa Lake and grew in only one location within Landa Lake. This macrophyte was highly susceptible to flushing flows but quickly re-grew after being scoured. *Cabomba* sp. was also a relatively minor component of the vegetation community in total coverage. This vegetation type was fairly resistant to major impacts from flushing flows due to its tendency to be along the shoreline in depositional areas and away from the primary flow, but it did fluctuate seasonally more than any other plant. The *Cabomba* did not grow well in the winter and spring and tended to be very sparse during these times, but it grew much more densely in the summer and fall.

As in the Upper Spring Run, the bryophytes were the most variable vegetation type in the Landa Lake Reach during the study (Figure 7). In this reach the *Riccia* was more common than the *Amblystegium* with the former dispersed throughout the lake and the latter restricted to the upper areas on otherwise bare substrate (generally areas with gravel and small cobble). In Landa Lake, the *Riccia* tended to fill-in patches of bare substrate within other vegetation types and also to settle on top of other vegetation types (notably *Sagittaria* and *Vallisneria*). Over previously bare substrate, this plant type fluctuated in a similar fashion to that observed in the Upper Spring Run and increased most dramatically following flood events. The total coverage of bryophytes doubled (from 1,940 m^2 to 3,985 m^2) in the six months between the fall 2001 high-flow event and the spring of 2002. The bryophytes remained high through most of 2002-2003, but began to decline in fall 2003 and throughout 2004.

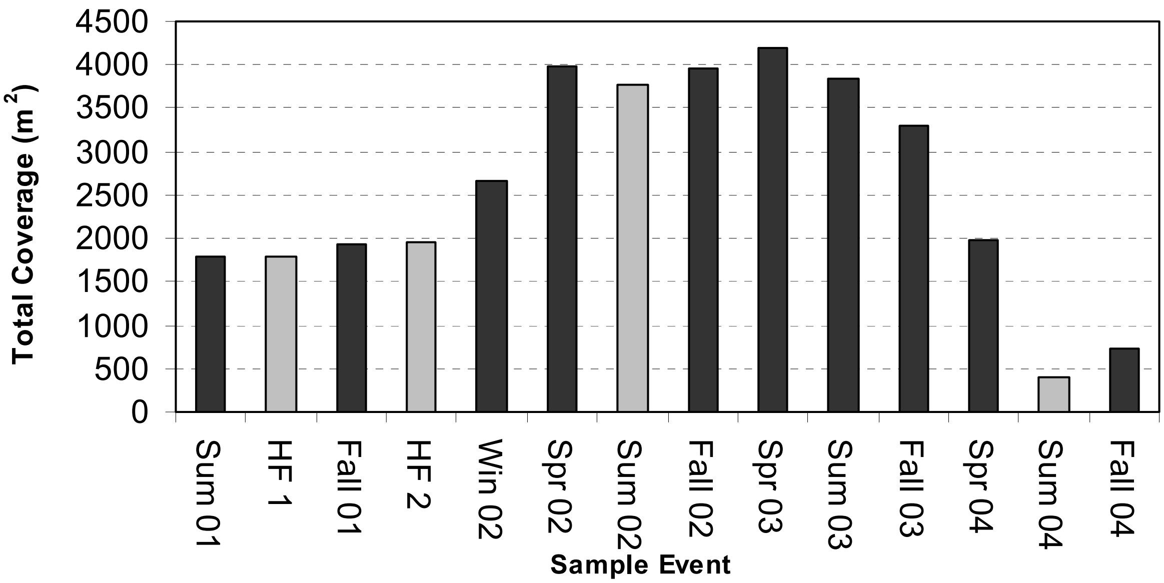


Figure 7. Total coverage of bryophytes in Landa Lake Reach (2000-2004). Light-colored bars represent high-flow Critical Period sampling events.

Old Channel Reach

Because flow in this reach is regulated by two culverts that allow flow into the channel from Landa Lake (one directly from the lake and another through the Landa Park swimming pool), this reach usually has the most stable flow conditions among the study reaches. However, flooding can have drastic impacts on the small channel; there was a dramatic influence observed following the summer 2002 flood. Prior to that time (2000-2002) filamentous algae dominated the reach; the only other common vegetation type was the aquarium species of the genus *Ceratopteris*. After the summer 2002 flood (beginning in the fall of 2002), the filamentous algae virtually disappeared and remained very low throughout 2003. During this time, flows were higher than normal through this reach because the culverts were opened wider (to reduce the lake level). During this period, small patches of *Hygrophila* started to become established by the fall 2002 sample and some *Ludwigia* appeared by the spring 2003 sample (neither were present in the reach prior to this point). Both species grew at about the same rate, but since it started to establish a few months before the *Ludwigia* appeared, the *Hygrophila* was slightly more abundant. In 2004, filamentous algae re-established in deeper areas within the reach, however, *Hygrophila*, and to a lesser extent *Ludwigia*, have also expanded into much of the deeper portion of this reach.

New Channel Reach

Hygrophila dominated the New Channel Reach during this study, with a moderate amount of *Cabomba* and very small amount of *Ludwigia* (observed only in 2003) also contributing to the total available habitat types. The total coverage of *Hygrophila* did not fluctuate much between 2000-2003. There were some minor decreases in total coverage due to scouring after floods during that time period, but the bare patches are quickly re-vegetated. That was not true of the summer of 2004, which had much more dramatic effects on this reach. Virtually all of the vegetation was scoured out of this reach during that event, which apparently had a more rapid flushing flow than the summer 2002 event despite a lower total discharge. The *Cabomba* in this reach appears to be well positioned to avoid scouring by flooding in this reach, as almost all patches remained after flooding in 2002 and most had increased in size (although virtually all vegetation, including *Cabomba* were removed in the summer 2004 event). Only *Hygrophila* is in water that is shallow enough to sample with drop nets within this site.

Fountain Darter Sampling

Drop Net Data

The 2004 drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sampling event; previous drop net site locations were indicated on maps provided with each annual report. The 2004 data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. A total of 714 drop net samples were conducted during 2000-2004 with 474 occurring in the Comal Springs/River ecosystem. The number of drop net sites and vegetation types sampled per reach in 2004 is presented in Table 7. There were some changes over the course of the study including a shift from sampling two bare substrate sites 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. Also, the upper section of the New Channel Reach was removed from the study due to inability to sample the deep areas and the similarity between that site and the lower section. 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* sp. to one dominated by *Hygrophila* and *Ludwigia.*

Table 7. Drop net sites and vegetation types sampled per reach in 2004.

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes [a] (2)	Bryophytes [a] (2)	*Hygrophila* (2) [b]	*Ludwigia* (2)
Sagittaria (2)	*Hygrophila* (2)		*Hygrophila* (2)
Hygrophila (2)	*Cabomba* (2)		Filamentous Algae (2) [c]
	Vallisneria (2)		*Ceratopteris* (2) [d]
	Ludwigia (2)		
Total (6)	**Total (10)**	**Total (2)**	**Total (6)**

[a] Switched from Open to Bryophytes, summer 2001.
[b] Upper section removed starting fall 2002; only two sites in lower section were sampled in 2003-2004.
[c] Only one filamentous algae site was sampled in the Fall 2004 sample due to limited coverage.
[d] *Ceratopteris* was discontinued after the Spring 2004 sample to permit sampling of the more abundant *Hygrophila* and *Ludwigia*.

<u>Importance of aquatic vegetation</u>

One of the primary observations during 2000-2004 was the wide range of suitability (as fountain darter habitat) of the various vegetation types found in both the Comal and San Marcos Rivers (Figure 8). Compared to the San Marcos Springs/River ecosystem, the Comal Springs/River ecosystem had a greater diversity of habitats available to fountain darters during 2000-2004 and there was a wider range of suitability observed in the latter ecosystem (Figure 8). The densities of fountain darters sampled in the Comal Springs/River ecosystem ranged from 3.6 per m^2 in *Ceratopteris* (sampled only in Old Channel) to 27.9 per m^2 in filamentous algae (sampled only in the Old Channel Reach). [The algae was the type similar to that found in the Spring Lake Reach (San Marcos), which differs from the finer algae found in the Landa Lake and Upper Spring Run Reaches (Comal).] Prior to 2004, *Sagittaria* supported the lowest densities of fountain darters at 2.4 per m^2, but *Riccia* occurred in high densities within the *Sagittaria* in 2004 and much higher densities when fountain darters were sampled in 2004. The high sample numbers in *Sagittaria* in 2004 resulted in an average density for all samples (2000-2004) of 5.0 per m^2. This wide range in relative importance of the various aquatic vegetation types as fountain darter habitat makes the composition and abundance of aquatic vegetation a critical factor in monitoring the fountain darter population.

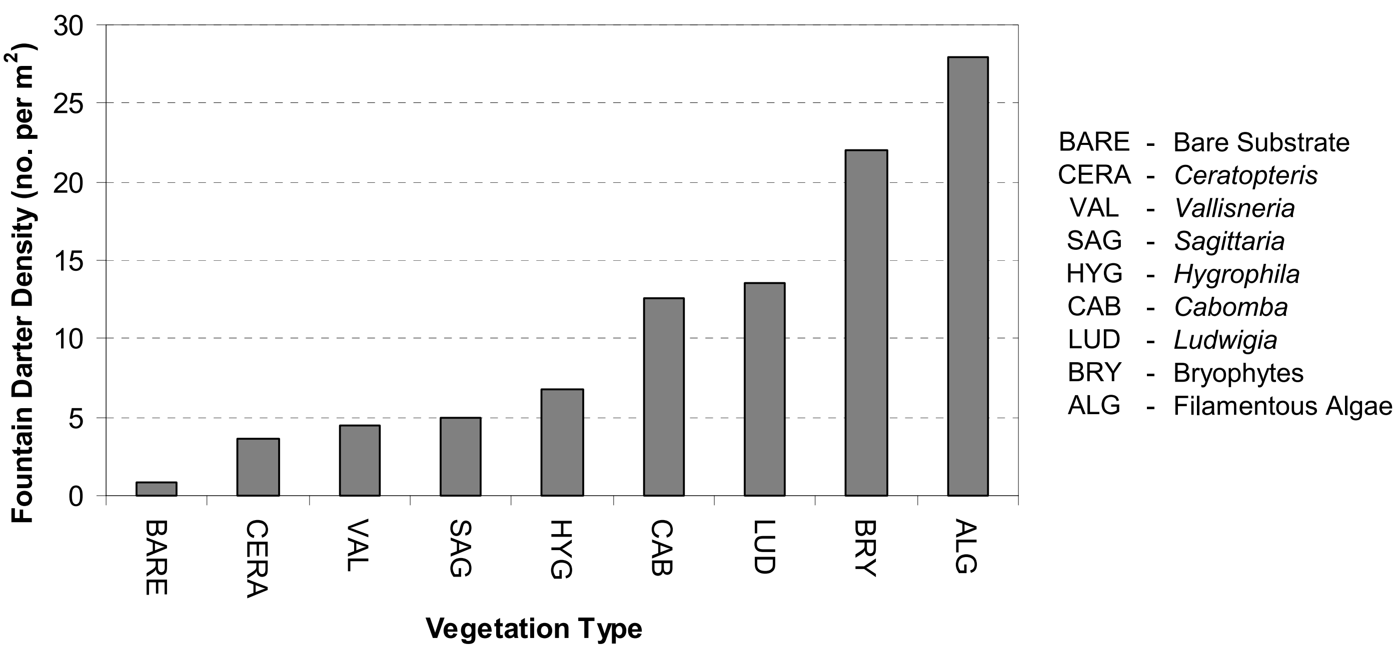


Figure 8. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem (2000-2004).

Although the filamentous algae clearly supported the most fountain darters, it was primarily restricted to one area (the Old Channel Reach) and was severely reduced in total coverage following the 2002 flood (see aquatic vegetation mapping section for more detail). The bryophytes (*Riccia* and *Amblystegium*) also contained high numbers of fountain darters (22.0 per m^2 on average). The bryophytes, as well as filamentous algae, provide cover at the substrate level and high numbers of amphipods, an important food item for fountain darters. Overall, the composition of bryophytes is very important to the fountain darter population since it appears to provide such high quality habitat but also because the bryophytes can be very abundant relative to total available habitat. There was a wide variation in the abundance of bryophytes in Landa Lake and the Upper Spring Run during 2000-2004 that appears to be related to the higher discharge conditions. The bryophytes and filamentous algae, the two highest quality fountain darter habitats, were the most susceptible to scouring during flooding (see aquatic vegetation mapping section for more detail).

In addition to the bryophytes and filamentous algae in the Comal Springs/River ecosystem, *Ludwigia* samples had high fountain darter density (13.6 per m^2) and it appears to provide high quality habitat. *Hygrophila* is similar in structure to *Ludwigia*, but this exotic species, which is relatively abundant in both the San Marcos and Comal Rivers, provides substantially lower-quality habitat than the native *Ludwigia.* *Ludwigia* remains relatively uncommon in Landa Lake and is susceptible to wide fluctuations in total coverage; it tends to be scoured with flushing flows and may be susceptible to mechanical disturbance by boats (described in the vegetation mapping section). The increase of both *Ludwigia* and *Hygrophila* in the Old Channel Reach has resulted in a net loss of fountain darter habitat suitability in that area. *Vallisneria* is very stable but does not support a high density of fountain darters and *Cabomba*, which grows in relatively stable locations with some seasonal variation, provides a similar fountain darter habitat suitability (based on density) as *Ludwigia*. *Sagittaria* is on the low end of habitat suitability for fountain darters, except when *Riccia* is abundant and settles on top of and within this vegetation type. During such times, the density of fountain darters observed in this vegetation type increased to as high as 16.6 per m^2 compared to only 0.9 per m^2 without the *Riccia.* A similar effect was observed in *Vallisneria* and *Ludwigia* in Landa Lake. It thus appears that these vegetation types become much more favorable to fountain darters with *Riccia* interspersed within the stand and that the fountain darter population may increase as a result.

<u>Abundance Estimates</u>

Estimates of fountain darter population abundance (Figure 9) were based on the changes in vegetation composition and abundance and the average density of fountain darters found in each, as described in the methods section. The vegetation type that had the greatest influence on these estimates during 2000-2004 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 in this vegetation type. The estimates of population abundance were highest in the summer of 2003 when the coverage of bryophytes peaked in Landa Lake (Figure 7). (The 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.) In all four high-flow Critical Period samples during 2000-2004, there was a decrease in the population estimate throughout the four sample reaches. However, in the sample following each high-flow Critical Period sample, there was an increase in population estimates.

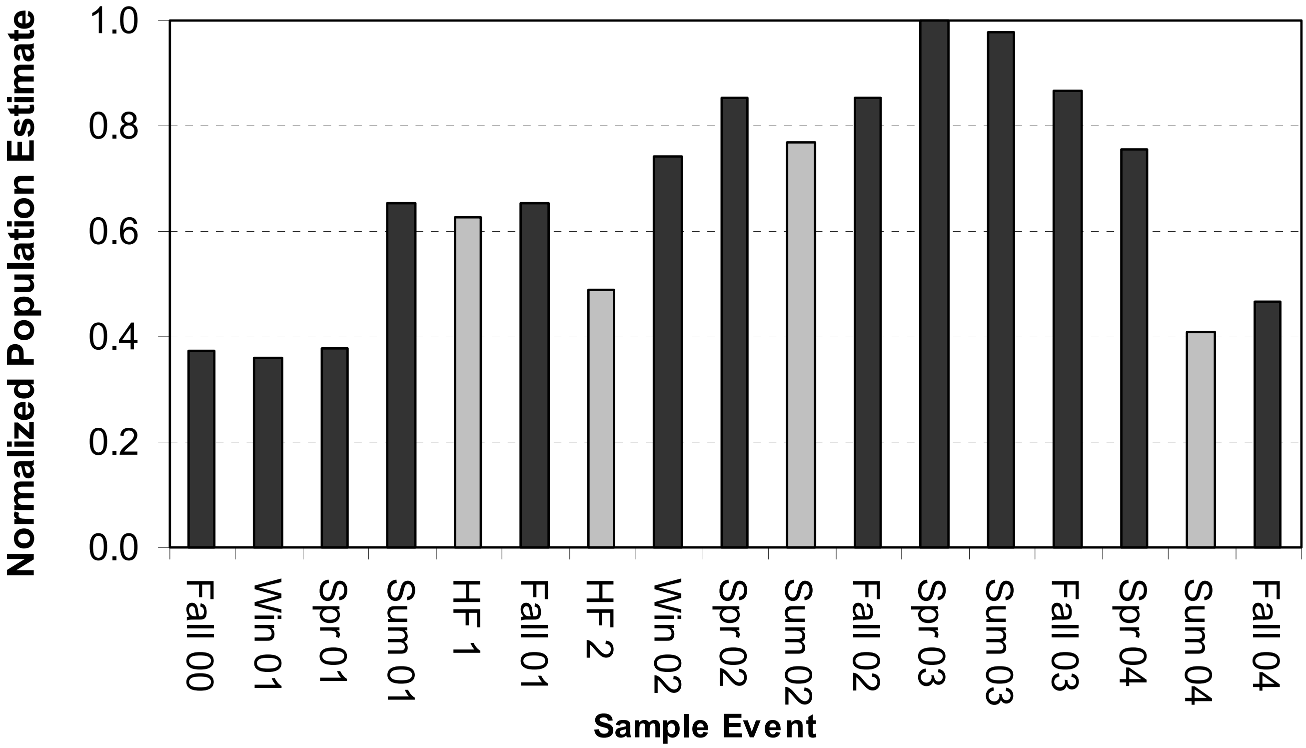


Figure 9. Population estimates of fountain darters in all four sample reaches combined (2000-2004); values are normalized to the maximum sample. Light-colored bars represent high-flow Critical Period sampling events.

Population Modeling

An initial evaluation of a model that incorporated all habitat parameters (Table 8) revealed that there were significant problems with multiple parameters varying together (multicollinearity) which limited the value of a single model to evaluate changes in fountain darter abundance. As an example of multicollinearity, substrate type and water velocity at 15cm above the substrate were highly correlated since lower velocity habitats primarily consisted of silt and sand and higher velocity habitats had much larger substrates. One of the more significant pairs of highly correlated parameters is season and discharge. Fountain darters appeared to have some seasonal variation in density as discussed in previous annual reports, but the high correlation between season and discharge makes it difficult to differentiate which parameter may be the cause of these differences. Additional data at a range of discharges (especially lower flows) may result in a data set that has a greater capability to differentiate effects associated with these two parameters. As a result of the multicollinearity problem, only a few variables could be feasibly incorporated into a model. Therefore, variables had to be evaluated individually or in smaller subsets. In order to evaluate the three variables that are considered to be most critical (total discharge in the Comal River, vegetation, and location/reach) a series of linear regressions were conducted on the relationship between fountain darter density (transformed with square-root equation) and discharge in each combination of vegetation and reach. These regressions allowed us to evaluate whether the range of discharge conditions resulted in a significant change in fountain darter density in each reach x vegetation combination. Additional variables (Table 8) were evaluated independently with similar regression analysis.

Table 8. Parameters collected at drop net locations that were incorporated into initial population model.

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

Individual regressions revealed that many of the relationships are not significantly different from baseline because of the relatively small sample size, but there were some trends that suggest potential relationships that may develop with additional data. Only three reach x vegetation combinations had a significant relationship ($p<0.05$) with discharge using the data collected through 2004 (Figure 10). *Ceratopteris* in the Old Channel Reach had a significant positive slope ($p=0.0274$) indicating that lower total discharge in the Comal River resulted in lower densities within this vegetation type. *Hygrophila* in the Upper Spring Run Reach had a very similar relationship between fountain darter density and discharge. The other significant relationship was in bare substrates in the Old Channel Reach where lower discharge resulted in higher densities of fountain darters. In each case the proportion of variation in density explained by discharge (R^2) is relatively low (0.14 – 0.21) but that is typical of ecological data where multiple variables affect a population simultaneously and random error is often very high. The bryophytes in Landa Lake had a nearly significant ($p=0.065$) relationship with higher densities observed at lower discharge, which indicates a trend in the data that should be explored further as more low-flow data are collected.

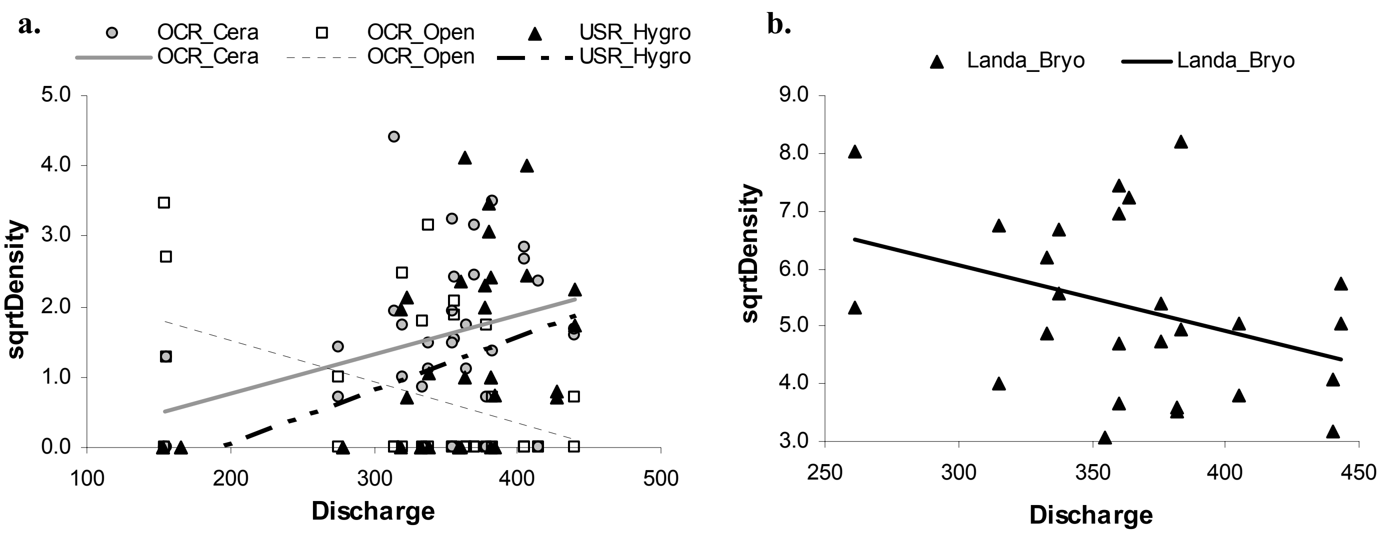


Figure 10. Relationships between discharge and fountain darter density (square-root transformed) were significant ($p<0.05$) for (a) *Ceratopteris* sp. and bare substrate sites in the Old Channel Reach and *Hygrophila* sp. sites in the Upper Spring Run Reach and nearly significant ($p=0.0650$) for (b) bryophytes in Landa Lake. Genus names of the vegetation types are abbreviated: Cera=*Ceratopteris*, Hygro=*Hygrophila*, and Bryo=bryophytes; Open=bare substrates.

Each of the individual variables had significant relationships with fountain darter density; some had significant linear relationships and some had significant quadratic relationships (Figure 11). The significant relationships suggest that there were differences in fountain darter density across the range of each variable in all samples from the Comal Springs/River ecosystem, but does not necessarily mean that each variable has a direct influence on the distribution and abundance of fountain darters. Nonetheless, each of the individual parameters clearly explained very little of the variation in fountain darter densities observed across samples as evidenced by the wide variation and low R^2 values seen in the regression analyses (Table 9).

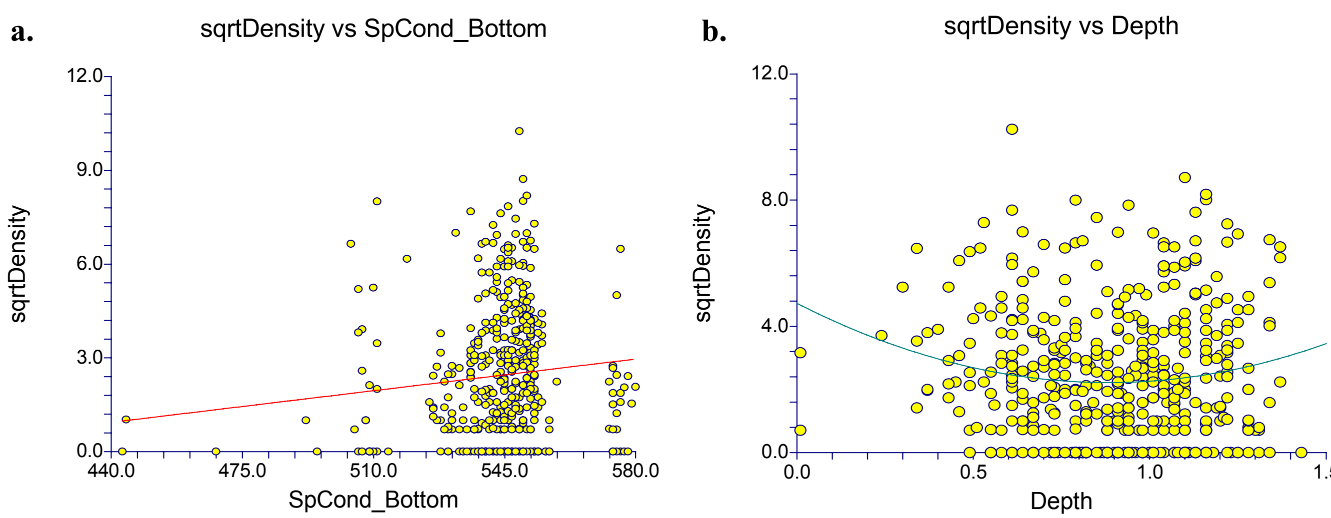


Figure 11. Examples of relationships between individual water quality variables and fountain darter density (square-root transformed) including (a) a significant (p=0.026) linear relationship and (b) a significant (p=0.010) quadratic relationship.

Table 9. Type of relationship (linear or quadratic) between individual variables and fountain darter density during 2000-2004 and R^2 value associated with that relationship.

Parameter	Relationship Type	R^2
Discharge	Linear	0.022
Depth	Quadratic	0.015
Substrate	Quadratic	0.016
Water Velocity at 15cm	Quadratic	0.052
Temperature at bottom	Quadratic	0.023
DO at bottom	Quadratic	0.029
SpCond at bottom	Linear	0.011
pH at bottom	Linear	0.081

Size-class distribution

The size-class distribution for fountain darters collected by drop nets from the Comal Springs/River ecosystem from 2000-2004 is a statistically normal distribution that is typical of a healthy fish population with a peak between 22 mm and 26 mm (Figure 12). There was a slight shift toward larger individuals in 2003 and a more distinct shift in 2004 (Figure 13). In addition to visually comparing size-class distributions, the mean length of fountain darters was evaluated among discharge levels, vegetation types, and reaches in the same way as density with linear regression. Unlike density, mean length could not have zero values, if no darters were captured in a sample it was not included in this analysis. This analysis revealed that there was not a significant relationship between discharge and mean length of fountain darters overall or within any reach (Appendix B).

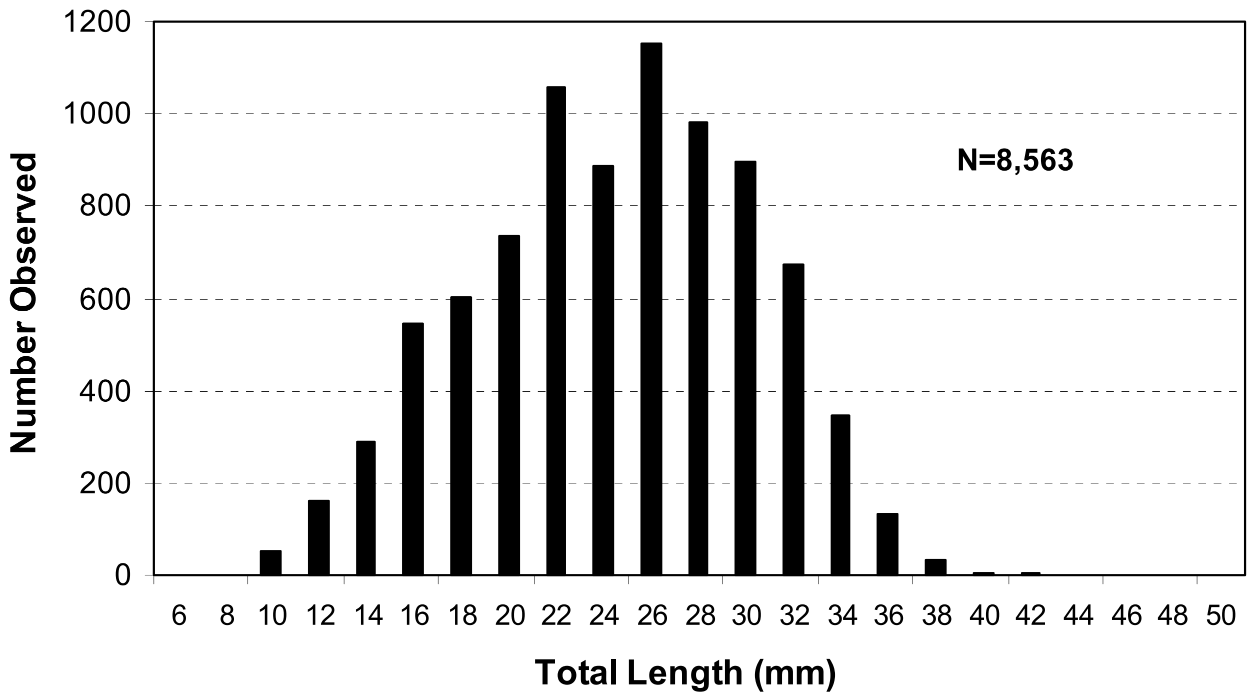


Figure 12. Fountain darter size-class distribution among all drop net sampling events in the Comal River in 2000-2004.

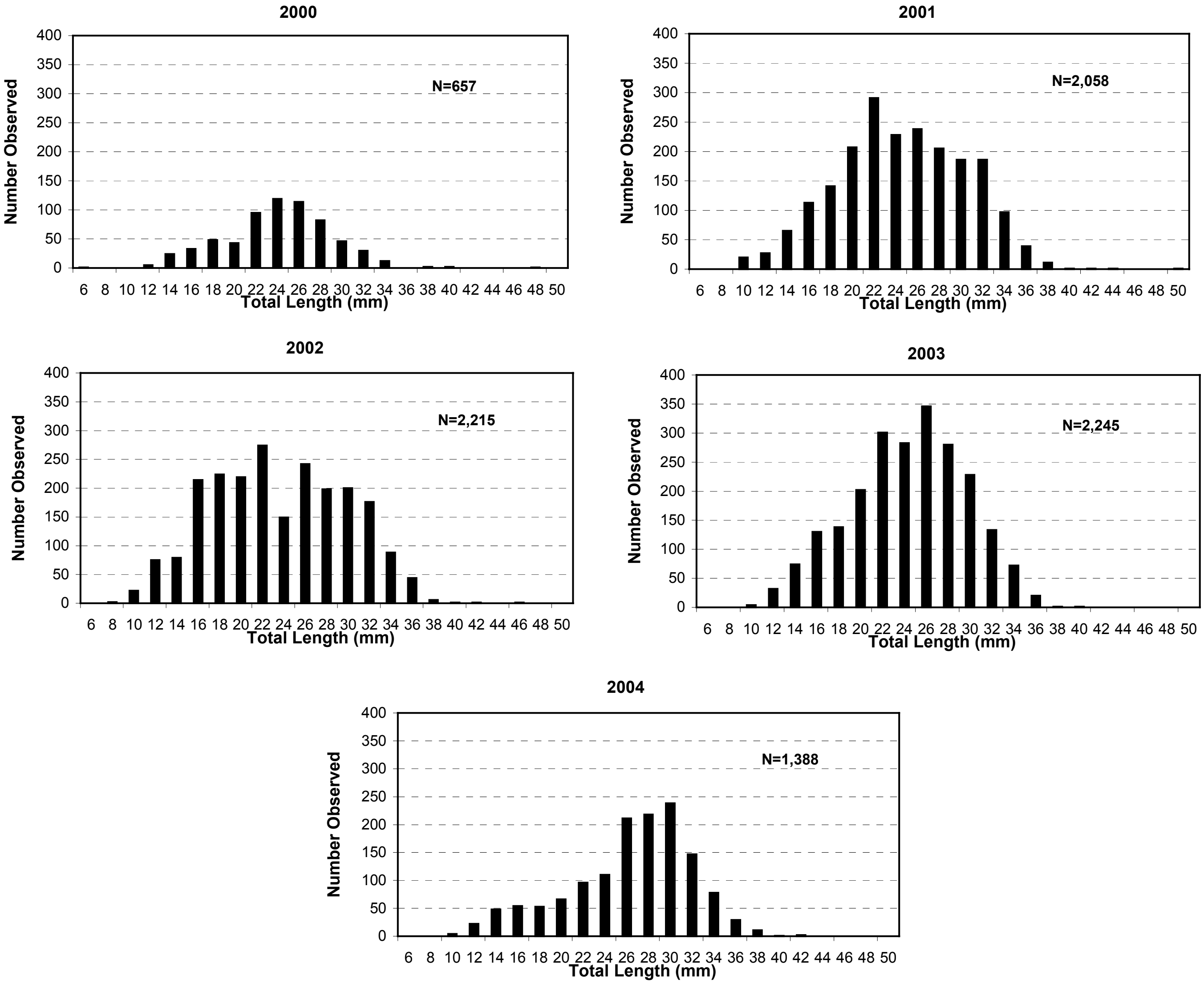


Figure 13. Annual Fountain darter size-class distribution among drop net sampling events by sample year in the Comal River.

Other Species

Including fountain darters, a total of 23 fish species and 60,147 individuals were collected from the Comal ecosystem in 2000-2004; of these, 7 species are considered exotic (introduced) (Table 10). An analysis of community composition (Shannon Weaver diversity index) revealed that among reaches, the Landa Lake reach had substantially lower diversity of species than the other reaches (Figure 14). This is largely due to the abundance of western mosquitofish and sailfin molly in those samples. Examined by reach, there were no discernable patterns in diversity among sample dates (Appendix B).

Among exotic species, the giant ramshorn snail elicits the most concern because of its recent impacts (early 1990s) on aquatic vegetation in the Comal River. Figure 15 shows the densities of live giant ramshorn snails observed in 2000-2004 in the Comal ecosystem by vegetation type. There was some variation in the densities observed during each year, but no strong trends were observed over time. The greatest density was 1.1 snails per m^2 in *Ludwigia*; filamentous algae, *Ceratopteris*, bryophytes and bare substrate all had fewer than 0.1 snails per m^2 sampled.

Table 10. Fish species and the number of each collected in drop net sampling (2000-2004 combined).

COMMON NAME	SCIENTIFIC NAME	STATUS	TOTAL NUMBER
Rock bass	*Ambloplites rupestris*	Introduced	18
Black bullhead	*Ameiurus melas*	Native	1
Yellow bullhead	*Ameiurus natalis*	Native	58
Mexican tetra	*Astyanax mexicanus*	Introduced	221
Central stoneroller	*Campostoma anomalum*	Native	1
Rio Grande perch	*Cichlasoma cyanoguttatum*	Introduced	270
Roundnose minnow	*Dionda episcopa*	Native	234
Fountain darter	*Etheostoma fonticola*	Native	7,906
Greenthroat darter	*Etheostoma lepidum*	Native	12
Gambusia	*Gambusia sp.*	Native/Introduced	47,215
Suckermouth catfish	*Hypostomus plecostomus*	Introduced	54
Redbreast sunfish	*Lepomis auritus*	Introduced	112
Green sunfish	*Lepomis cyanellus*	Native	10
Warmouth	*Lepomis gulosus*	Native	24
Bluegill	*Lepomis macrochirus*	Native	30
Longear sunfish	*Lepomis megalotis*	Native	35
Spotted sunfish	*Lepomis punctatus*	Native	731
Sunfish	*Lepomis sp.*	Native/Introduced	547
Spotted bass	*Micropterus punctulatus*	Native	1
Largemouth bass	*Micropterus salmoides*	Native	64
Texas shiner	*Notropis amabilis*	Native	28
Sailfin molly	*Poecilia latipinna*	Native	2,566
Tilapia	*Tilapia sp.*	Introduced	9

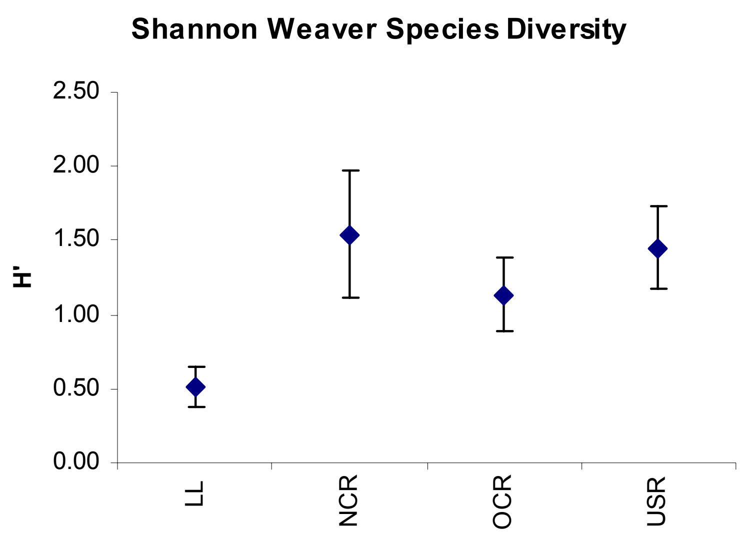


Figure 14. Average value of Index of species diversity (Shannon Weaver) for each sample reach with +/- one standard deviation indicated. LL = Landa lake Reach; NCR = New Channel Reach; OCR = Old Channel Reach; USR = Upper Spring Run Reach.

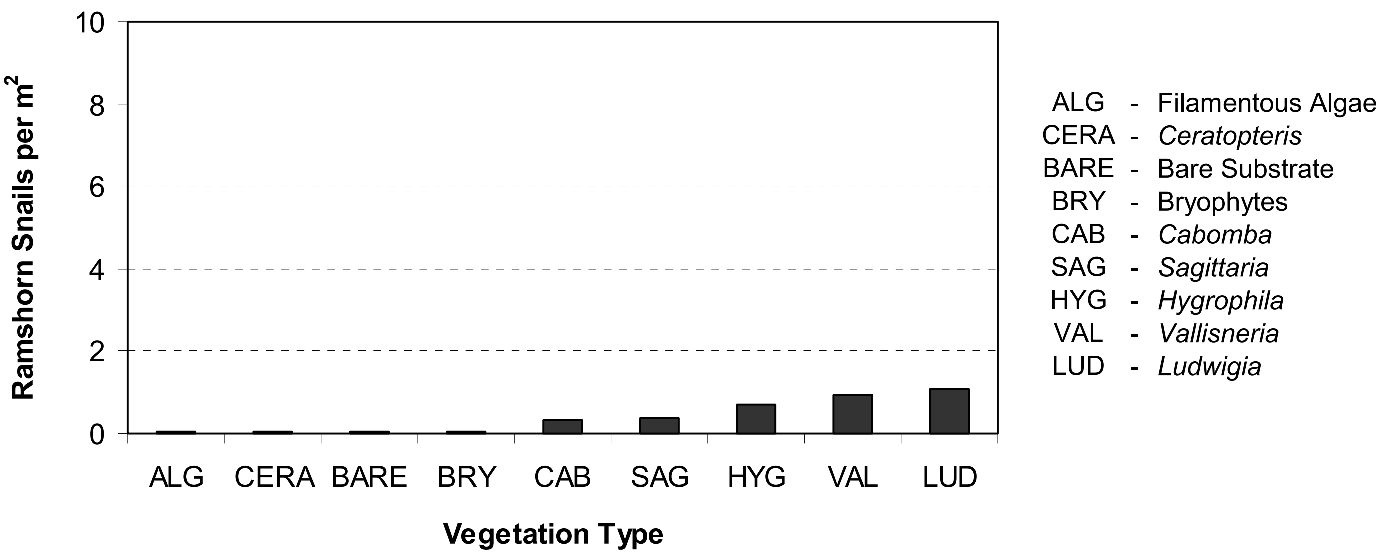


Figure 15. Density of giant ramshorn snail by vegetation type (averaged across all sites).

Dip Net Data

The boundaries for each section of the dip net collection efforts are depicted on Figure 16. Data gathered using dip nets are graphically represented in Figure 17 for the Old Channel Reach, and in Appendix B for all other reaches. High water and turbid conditions prevented the collection activities from the New Channel and Garden Street sites on a few occasions in 2000-2002, but these areas were sampled during each Comprehensive sampling effort in 2003-2004.

In general, the highest numbers of small fountain darters (5-15 mm) were observed in dip net samples during the spring of each year in all sample reaches (Appendix B). In the spring of 2003, all reaches had some fountain darters of the smallest size class, including three that had the greatest number of such observations during the entire study (Upper Spring Run, Spring Island, and New Channel Reaches). This contrasts with 2002 data since several sites in that year had the fewest observations of small fountain darters in the spring. However, the Old Channel, New Channel and Garden Street Reaches have had the highest number of small darters observed during the spring throughout the study.

In the Old Channel Reach (Figure 17) there was clearly a peak in reproduction during each spring, however, there were fountain darters of the smallest size group (5-15 mm) observed in almost every sample in this reach between 2000 and 2004. This indicates that some reproduction occurred in the Old Channel Reach in all seasons during 2000-2004, despite the clear peak in the spring season. The reduction of algae in the summer of 2002 followed by the change in vegetation composition (to *Hygrophila* and *Ludwigia*) in 2003 appears to have had little influence on dip net samples of fountain darters. In addition to continued observations of the highest numbers of small fountain darters in the spring in 2003 and 2004, the greatest numbers overall were observed during the spring, with the total number sampled similar to those observed 2000-2002. There did appear to be fewer small fountain darters overall in 2003 and 2004 compared to earlier samples and also fewer fountain darters of all sizes in the fall.

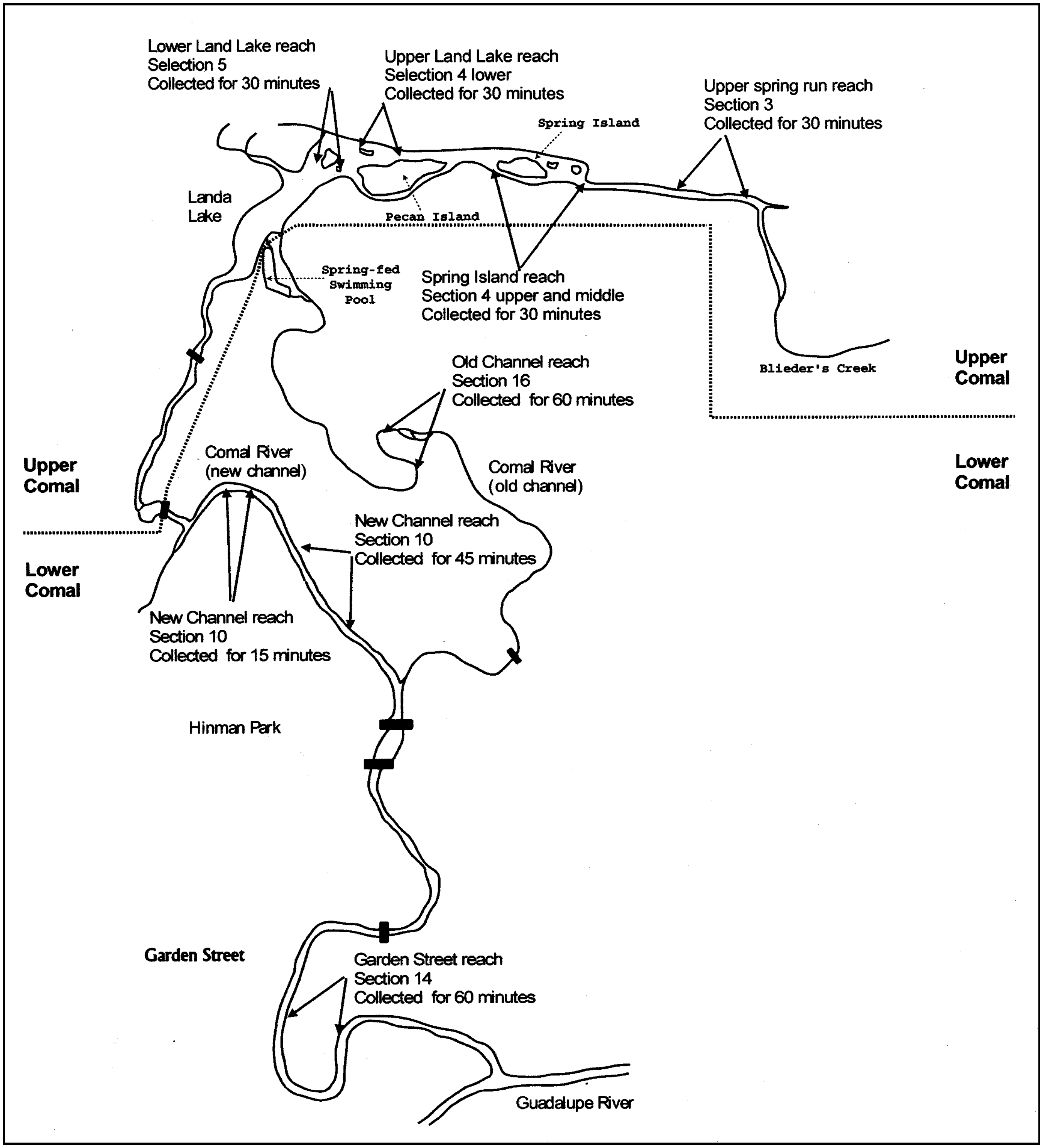


Figure 16. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

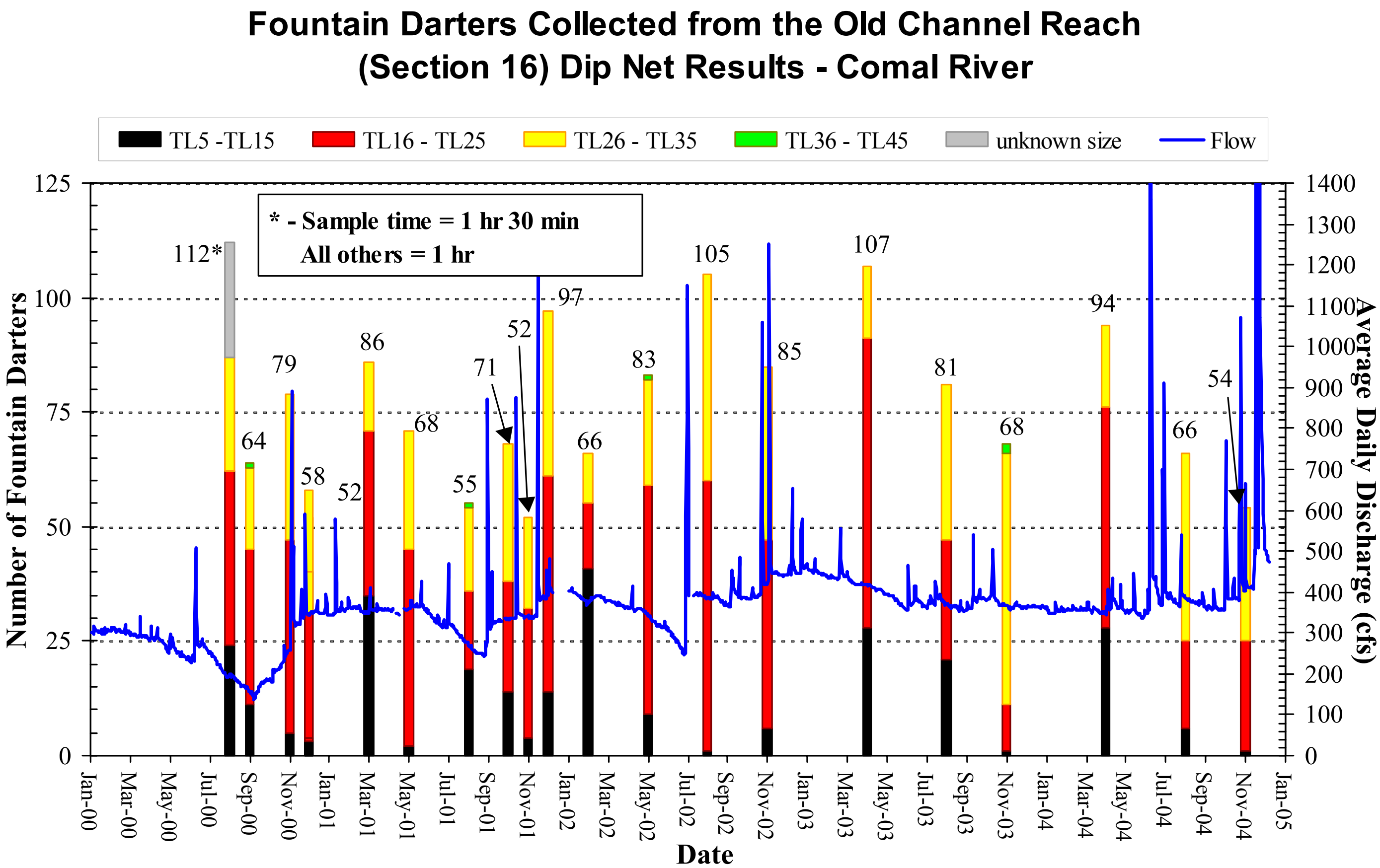


Figure 17. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

In the Upper Spring Run Reach (Appendix B), dip net samples revealed a substantial change in total numbers of fountain darters during 2000-2004. Prior to 2003, half of the samples (7 of 14) resulted in fewer than 10 individuals with the highest sample having 26 individuals. In 2003-2004, the lowest sample size was 26 and the highest 52. There was also little change in the number of small fountain darters (5-15 mm) during this time. The number of small fountain darters observed in the spring of each year in 2003-2004 was similar to the number observed in the spring of 2001 and few small fountain darters were observed in the summer or fall of 2003-2004. This suggests that if there was local reproduction that stimulated this increase, it had to occur in the winter, when dip net samples were not taken. Alternatively, fountain darters may have migrated from downstream to utilize the improved habitat conditions that were observed in this reach in 2003 and 2004 (discussed in aquatic vegetation mapping section).

Visual Observations

Fountain darters were observed in the deepest portions of Landa Lake (depths greater than 2 m) during each sampling event during this study, including all low-flow and high-flow events. Fountain darters were observed throughout the reach in areas of bare substrate and in surrounding vegetation, but the greatest concentrations occurred near areas with dense coverage of *Riccia.* Throughout the study, observations of fountain darters in the sample area remained consistently high. Table 11 shows the number of fountain darters and percentage of *Riccia* observed in the sampling grid used during each sampling event since the Summer 2001 sample and Figure 18 shows the relationship between the prevalence of *Riccia* and the abundance of fountain darters observed.

The results of the grid sampling suggest there was some fluctuation in the use of the deepest areas of Landa Lake by fountain darters during the study period. The number of observations appeared to be strongly influenced by the abundance of *Riccia* where more *Riccia* resulted in higher fountain darter counts. A regression of the two variables revealed a significant relationship and high R^2 value.

Table 11. 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

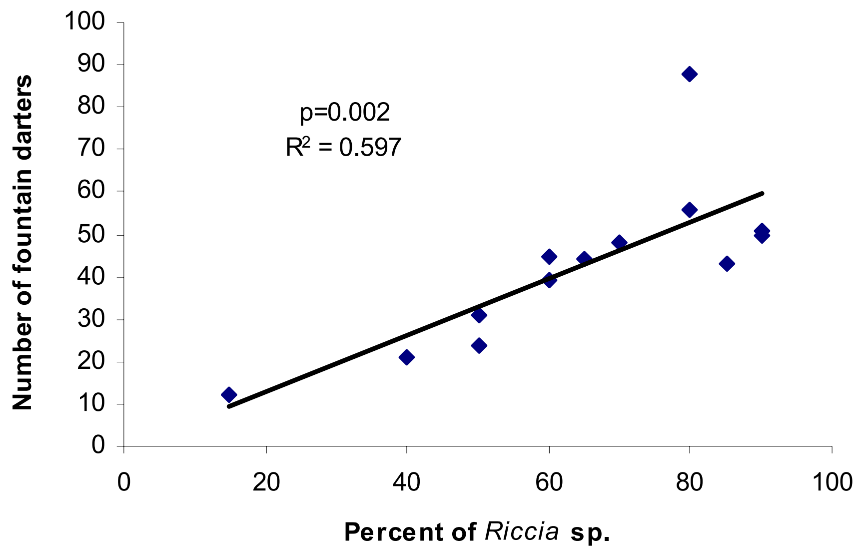


Figure 18. Relationship between the prevalence of *Riccia* sp. and abundance of fountain darters observed in Landa Lake. A regression of the two variables results in a significant relationship with a high R^2 value.

Gill Parasite Evaluation

Similar to the observations during the initial study, there were substantial differences in the number of parasites observed among sample sites (Figure 19). The highest concentrations (10.1±2.1 SE) were consistently detected at Spring Island (SI) in the Comal Springs/River ecosystem whereas three sites (in the New Channel, Upper Spring Run and in Comal River just upstream from the Guadalupe River) generally had less than 1.0 parasite per liter in each sample. Differences in parasite concentrations across seasons were not as distinct (Figure 20), but there tended to be more parasites sampled in the summer.

Even though parasite samples were collected from various habitats (pool, slow and fast runs) with a range of current velocities, there was no significant correlation between current velocity and parasite concentrations (Appendix B); however, the data do suggest a weak correlation. At the two sites with monthly samples, the results were similar with no statistically significant relationships between discharge or current velocity and parasite abundance (Appendix B).

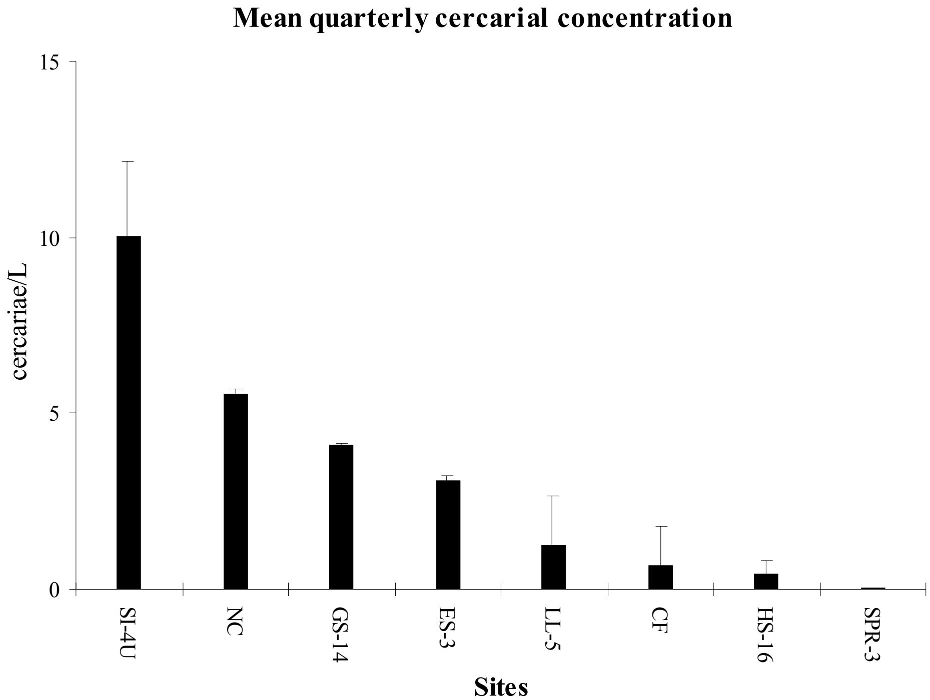


Figure 19. Mean (+SE) abundance of *Centrocestus formosanus* per Liter in each of the eight sample sites in the Comal River.

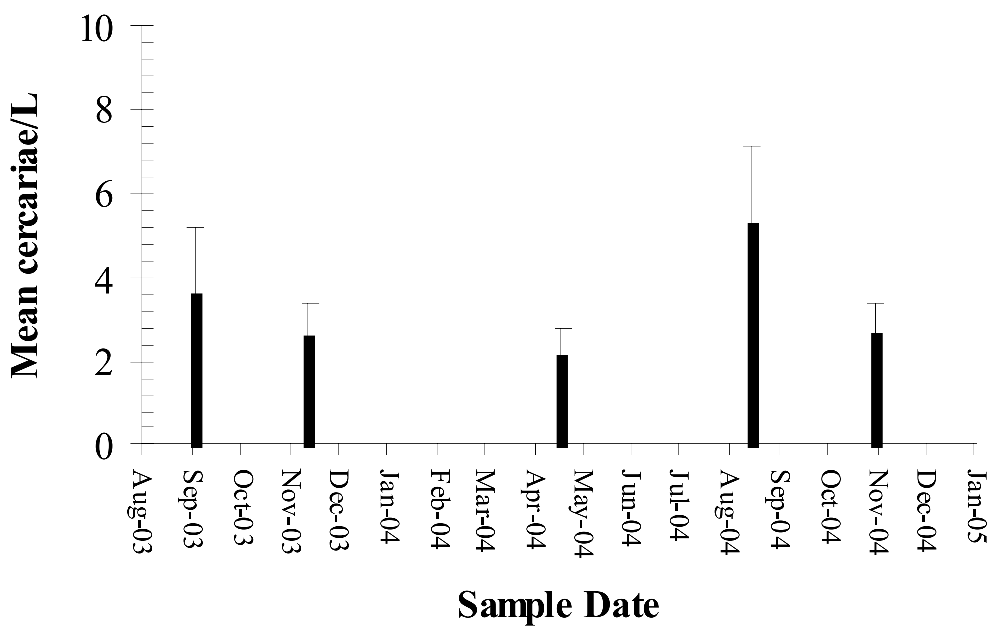


Figure 20. Mean (+SE) abundance of Centrocestus formosanus per Liter in all sample sites in the Comal River by sample date.

Comal Springs Salamander Surveys

All SCUBA/snorkel surveys revealed the presence of Comal Springs salamanders along the lake bottom and in each sampled Spring Run. Comal Springs salamanders were observed around portions of the springs and under rocks at depths of up to 2.4 m. No Comal Springs salamanders were observed in any areas with excessive sediment. Since the inception of the project, Comal Springs salamanders have been observed in each sample location during each sample period. The total number of Comal Springs salamanders observed at each survey site during each sampling event is presented in Table 12.

The greatest cumulative number of Comal Springs salamanders observed for all sites during any one sampling event was 69, which occurred during spring 2004; the fewest (18) occurred during fall 2000. Spring Run 1 had the greatest number of Comal Springs salamanders among all areas sampled during the study to date (19.3 average per effort). This is partially due to the greater amount of area covered and time spent searching in Spring Run 1, but high densities are regularly observed in its headwaters.

High flow events had an influence on salamander habitat in 2000-2004 by covering the substrate with silt in some areas, particularly in Spring Run 3. On each occasion that there was a severe high-flow event, silt was observed in Spring Run 3 and there were substantially fewer observations of salamanders than the previous sample in that site. This was particularly true after the second high-flow event in 2001 and in the fall of 2004 when only 2 salamanders were surveyed in Spring Run 3.

Table 12. 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
Average	19.3	10.3	6.3	9.3	48.8

Macroinvertebrate Sampling

In 2003-2004, 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 in more detail than previous work involving drift netting in the Spring Runs. Data from sampling conducted in 2000-2002 is presented in the 2002 annual report (BIO-WEST 2003) and 2003 data is presented in BIO-WEST (2004a). Data collected in 2004 are presented below.

Drift Net sampling

A total of 215 hours of sample time occurred among the three drift net sites at Comal Springs and 13 species were captured (Table 13). Total discharge in the Comal River was approximately 355 cfs in the May and August samples and over 500 cfs in the October sample and water quality conditions were very similar among samples (Table 14). There were no recent "spikes" in the hydrograph prior to the May sample but several significant spikes occurred prior to the summer sample in August and October in the fall prior. The day before the August sample discharge was approximately 408 cfs and the October sample took place while discharge ranged from 543 cfs to 842 cfs.

Species of the two genera *Stygobromus* and *Lirceolus* were the most abundant at all sites during each sampling event; *Stygobromus pecki* was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long; 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*. There was an observation of one *Stygobromus flagellatus* in Spring Run 1 during the May 2004 sample. This species has previously been sampled only in the Texas State University artesian well, Ezell's Cave, Spring Lake, and Barton Springs.

Among species of concern, an average of 19.2 *Stygobromus pecki* (Peck's cave amphipod; many small *Stygobromus* were unidentifiable to species), 1.6 *Heterelmis comalensis* (Comal Springs riffle beetle), and 1.1 *Stygoparnus comalensis* (Comal Springs dryopid beetle) were retrieved during each 24-hour period at all sites. These numbers were higher than the 10.6, 1.2, and 0.3 averages per 24-hours observed in the respective sites in 2003. As in 2003, *Stygobromus pecki* was found in all three locations, but there were substantially more found in the western shoreline habitat than the other two locations. Also similar to 2003, *H. comalensis* was not found in the upwelling along the western shoreline of Landa Lake. One 2004 observation that differed from 2003 samples was that *S. comalensis* was found in Spring Run 3 in 2004 but not in the western shoreline habitat, the opposite occurred in 2003.

Estimates of drift rate and drift density (Appendix B) were calculated for each family/genus/species that were identified in the three sample efforts. A new method for measuring velocity was incorporated into 2004 samples to improve accuracy and repeatability of measurements. As a result, drift density values in 2004 are presumed to be more accurate than 2003 values and not directly comparable to the earlier estimates.

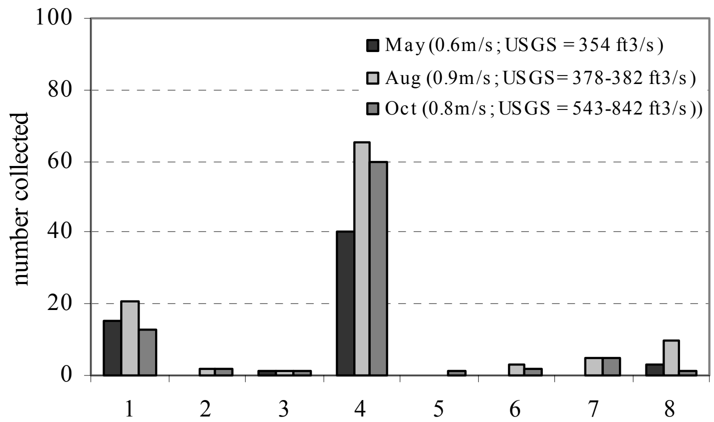
There was no distinct trend observed in abundance for any species across the three sample dates in 2004 (Figures 21 and 22). There were more *Mexiweckelia hardeni*, *Lirceolus* spp., and *Heterelmis comalensis* in August when all sites are combined, but in Spring Run 3, the highest numbers of nearly every species were sampled in October, including substantially higher numbers of *S. pecki*, *Lirceolus* spp., and *Heterelmis comalensis*.

Table 13. Total numbers of invertebrate species collected in drift nets from 1 May to 24 October, 2004 (three sample dates). Federally endangered species are designated with (E). A = adult beetles. L = larvae.

	Run 1	Run 3	Upwelling	Total
Crustaceans				
Amphipoda				
Gammaridea				
Crangonyctidae				
Stygobromus pecki (E)	25	37	110	172
Stygobromus russelli	9	5		14
Stygobromus flagellatus	1			1
Stygobromus spp.	211	170	409	790
Hadziidae				
Mexiweckelia hardeni	26	20	3	49
Sebidae				
Seborgia relicta	2	1	1	4
Artisiidae (=Bogidiellidae)				
Artesia subterranea	2	1		3
Isopoda				
Asellidae				
Lirceolus (2spp.)	101	49	15	165
Cirolanidae				
Cirolanides texensis			1	1
Insects				
Coleoptera				
Dytiscidae				
Comaldessus stygius	1 A	4 A		5
Haideoporus texanus		9 (6L, 3A)		9
Dryopidae				
Stygoparnus comalensis (E)	5 L	5 L		10
Elmidae				
Heterelmis comalensis (E)	8 (6L, 2A)	6		14

Table 14. Results of water quality measurements from drift net sampling locations at Comal Springs.

	Spring Run 1			Spring Run 3			Upwelling		
Date	May	Aug	Oct	May	Aug	Oct	May	Aug	Oct
Temperature (^{o}C)	23.0	23.0	23.0	23.1	23.1	23.1	23.6	23.6	23.6
Conductivity (mS)	0.576	0.575	0.561	0.575	0.574	0.537	0.570	0.572	0.561
pH	7.1	6.8	7.1	7.1	6.8	7.3	7.1	6.9	7.1
Dissolved Oxygen (mg/L)	6.3	6.6	5.7	6.2	6.2	5.5	5.8	6.1	5.4
Velocity (m/s)	0.6	0.9	0.8	0.6	0.6	0.6	0.4	0.3	0.4



1 - *Mexiweckelia*
2 - *Seborgia*
3 - *Artesia*
4 - *Lirceolus* spp.
5 - *Cirolanides*
6 - *Comaldessus*
7 - *Stygoparnus*
8 - *Heterelmis*

Figure 21. Drift net sampling results for each species (except *Stygobromus* spp.) combined across all sample sites.

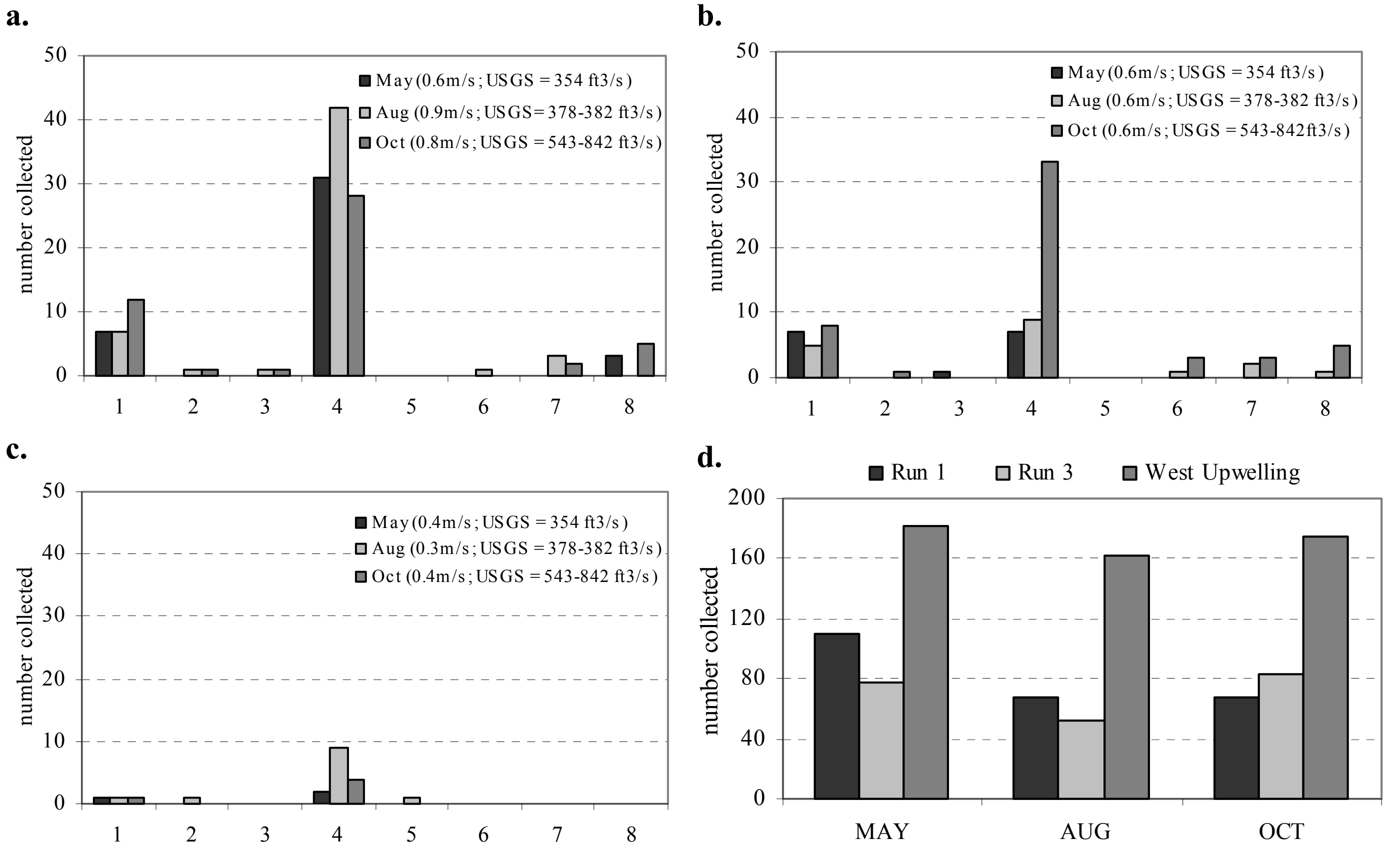


Figure 22. Number of individuals collected in 2004 drift samples by species in (a) Spring Run 1, (b) Spring Run 3, (c) the western shoreline, and (d) *Stygobromus* spp. in all sites. The legend for species is the same as in Figure 21.

Comal Springs Riffle Beetle

There was a moderate amount of variability in the presence/absence of the Comal Springs riffle beetles in individual quadrat (0.36 m^2) samples in 2003, but there were always at least 5 individuals captured in the four quadrat samples combined from each sample site. A total of 276 Comal Springs riffle beetles were collected during all 2003 samples including a maximum of 43 beetles in one quadrat in the Spring Island area during the January 2003 sample. More details on quadrat sampling in 2003 can be found in the 2003 annual report (BIO-WEST 2004a).

Using the new sample method in 2004, a larger number of samples were collected, which allowed more quantitative comparisons. A total of 1,186 Comal Springs riffle beetles were collected in all 2004 samples. There were more than twice as many adults (n=810) as larvae (n=376). The beetles tended to be patchily distributed with wide ranges of abundance among samples within a site and season. Although samples were not conducted in precisely the same area on successive dates, certain areas within each site tended to have higher numbers than other areas.

Sampling with the quadrat in 2003, the data suggested that there were some differences in abundance between sample areas, with the western shoreline producing the fewest individuals; however, 2004 data revealed that the abundance of both adults and larvae was similar among sites (Figure 23). Each of the three sample sites has very different physical characteristics (Figure 24). The Spring Run 3 (R3) site has lateral spring flow from the shoreline into fast moving primary current of the Spring Run, the western shoreline (WS) site has lateral spring flow directly into Landa Lake and the Spring Island (SI) site has upwelling flow from the bottom of Landa Lake. The Spring Island site had the lowest velocity flow, 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. The Spring Island site also had much deeper samples since these occurred in the lake rather than along the shoreline.

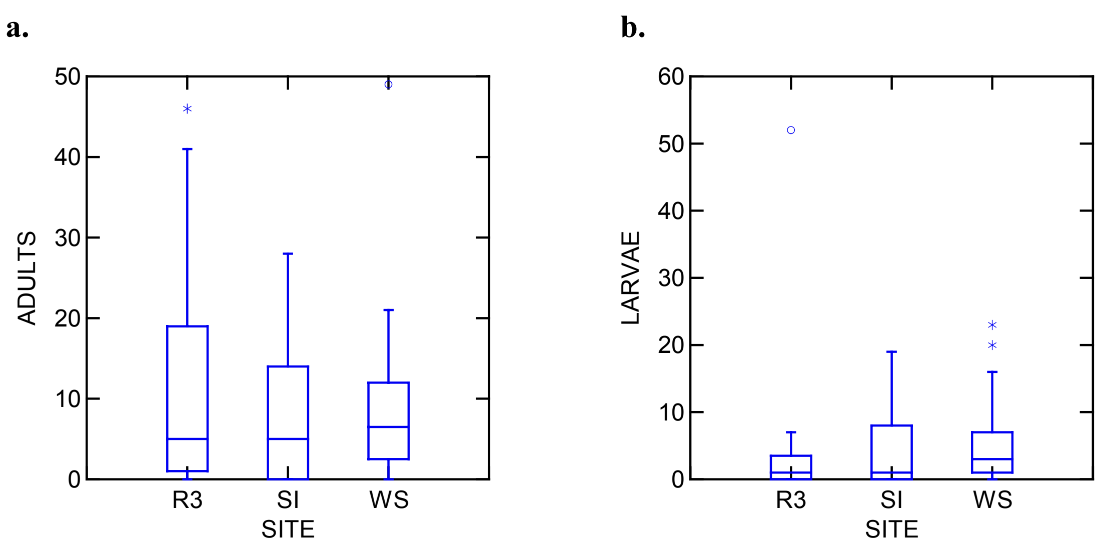


Figure 23. Comal Springs riffle beetle (a) adult and (b) larval abundance in each of the three sample sites in 2004.

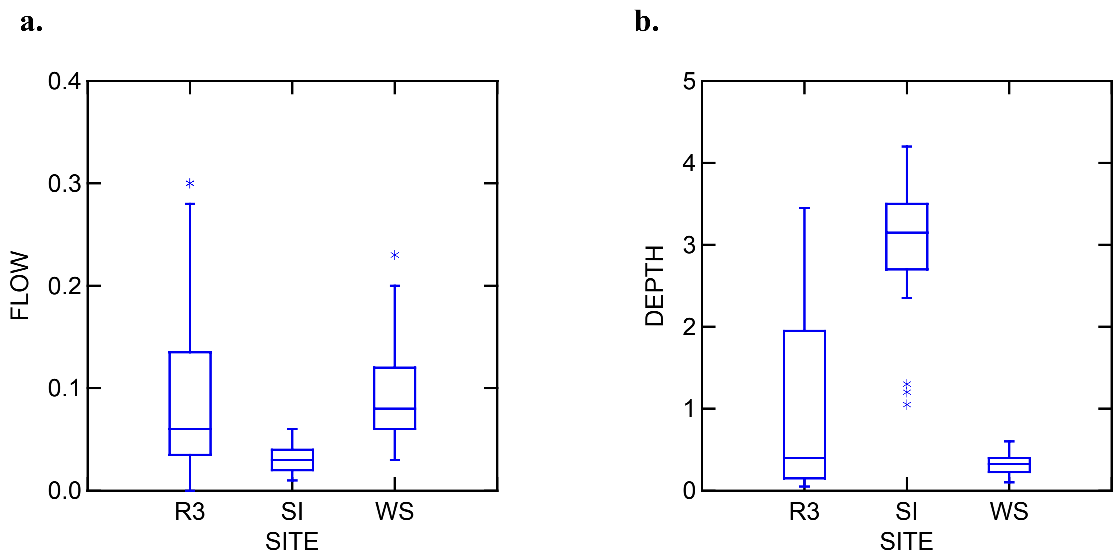


Figure 24. Box plots of (a) depth and (b) flow in each of the three Comal Springs riffle beetle sample sites in 2004.

Despite the physical differences between sites, comparisons of mean abundance of Comal Springs riffle beetles among sites and among seasons (Figure 24) a Kruskal-Wallis one-way Analysis of Variance revealed that there were no significant differences ($p<0.05$) in adult or larval Comal Springs riffle beetle abundance among sites or seasons in 2004 (Table 15, Figures 23 and 25). The near-significant value ($p=0.08$) of differences in larval beetle abundance between sites becomes significant ($p=0.043$) when the outlier value of 52 is removed from the data (Table 15). However, larvae tend to be patchily distributed and a high density such as observed in that sample was not unexpected.

A Spearman correlation test (Table 16) revealed that there were no significant relationships ($p<0.05$) between adult or larval riffle beetle abundance and either depth or flow in 2004, although a near-significant value of $p=0.09$ did occur between larval riffle beetles and flow (Table 16). Removing the extreme outlier from the larval beetle data did not change the significance of the p-values.

Table 15. Kruskal-Wallis one-way ANOVA and two-way ANOVA results for Comal Springs riffle beetle abundance among sites and seasons.

	ADULT Riffle Beetles	LARVAL Riffle Beetles
SITES	p = 0.658	p = 0.081 (p = 0.043 w/out 1 outlier)
SEASON	p = 0.140	p = 0.211 (p = 0.07 w/out 3 outliers)

Table 16. Spearman Correlation values for Comal Springs riffle beetle response to flow and depth.

	ADULT Riffle Beetles	LARVAL Riffle Beetles
FLOW	p = 0.207	p = 0.093 (p = 0.117 w/out outlier)
DEPTH	p = -0.243	p = -0.256

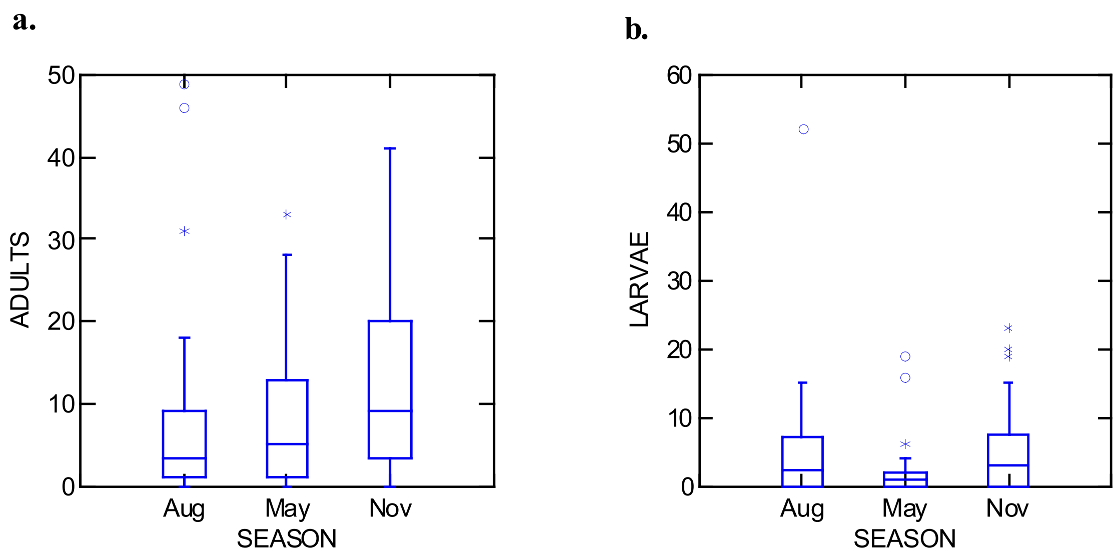


Figure 25. Seasonal abundance of (a) adult and (b) larval Comal Springs riffle beetles in each of the three sample sites.

Exotics/Predation Study

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

DISCUSSION

Springflow

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

The high flows in the Old Channel have progressively decreased in 2003 through 2004 from near 115 cfs in the spring of 2003 to approximately 76 cfs in the fall of 2004. The flows are still substantially higher than the estimated value of 40 cfs given by the USFWS in 2000. The higher flows in 2003 had substantial impacts on the vegetation community in this reach that are unlikely to be reversed, even with a return to lower flows. Maintaining flows in the Old Channel is critical, but higher flows appear to have negative consequences as well. The discharge data collected in the Old Channel and Spring Runs are also providing a valuable record of the relative contribution of the Spring Runs to total discharge and the proportion that flows down the Old and New Channels.

Water Quality

The overall assessment of water quality in 2000-2002 supported observations during other studies that there are no parameters of substantial concern. Since total discharge remained at or above normal during most of that time there was little evidence to suggest that any parameter provided unfavorable conditions for any of the threatened or endangered species in the Comal River. There were some patterns, as expected, in water temperature and dissolved oxygen as water moved away from the spring openings. Temperature tended to increase downstream of springs and dissolved oxygen was lowest near spring openings, however, neither parameter exceeded the thresholds that would cause concern for any species during the study. The continuous record of water temperature collected with thermistors has provided extensive data during 2000-2004 to show that this parameter has remained within the range that allows reproduction of fountain darters (considered to be the most restrictive temperature limitation). Most of the river has remained buffered from wide temperature variations due to spring inputs and only the very upper end of the Comal River in Blieders Creek and the lower reaches have had temperatures above the threshold for a reduction in reproductive capabilities of fountain darters. Water temperature will be one of the most important variables to monitor when low-flow conditions occur and it is important to maintain this continuous record through such conditions.

Total dissolved solids is another parameter that may be important to monitor during low flows since it exceeded the state water quality value in 2001. However, no previous mention of exceedences by the TCEQ suggests that the agency does not consider this water quality parameter a concern. Nitrate values also exceeded the water quality standards screening level in most cases, but the values were within the median concentration of nitrate in the Edwards Aquifer. The standard parameters of specific conductivity and pH remained within very narrow ranges over the course of the study; these variables have little direct effect on population dynamics of fish, salamanders or invertebrates except when a substantial deviation occurs.

The sample site with the most fluctuation in water quality conditions was Blieder's Creek, which has no direct influence from springs and probably does not support habitat for any of the threatened or endangered species. This site is near the headwaters of the Comal River and feeds directly into the Upper Spring Run. Therefore, runoff and flow from Blieders Creek also affects water quality conditions at the Heidelberg site (in the Upper Spring Run) to a greater extent than at the other Comal Springs/River sites. Downstream sites in the Old and New Channel and past the confluence of these also tended to have higher fluctuation in water quality parameters than those near spring openings so it will be important to continue to monitor these during lower flows.

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

Aquatic Vegetation Mapping

Throughout this study it has become clear that vegetation type is one of the most significant factors determining distribution of the fountain darter in the Comal Springs/River ecosystem. In general, aquatic vegetation remained abundant in all reaches under the range of flows experienced in the study. However, there were some differences in the relative value of each reach for fountain darter habitat because of differences in vegetation composition and the response of the vegetation to variable flow conditions. Reaches with greater amounts of high quality habitat (vegetation types that support highest densities of fountain darters) are logically more critical to maintain during periods of stress (e.g., low discharge). The susceptibility of high quality vegetation types to variable flow conditions also influences the suitability of an area for fountain darters. In addition, the Ramshorn snail population, which virtually denuded the Lake in the mid-1990s, is an important consideration. Although the snail does not appear to be a problem anymore, continued monitoring is necessary as there is little understanding about what is currently limiting the snail population and whether another "boom" in the population may occur in the future. If it does become a problem again, it will be important to determine which vegetation types are most vulnerable and which reaches are most susceptible to an impact.

The vegetation composition and susceptibility of that vegetation to variable flow conditions largely determines the relative value of different reaches as fountain darter habitat. Identifying these differences will provide guidance for management goals that focus the greatest effort on maintaining the highest quality habitats. A substantial amount of information has been collected in each of the sample reaches in 2000-2004 despite the limited low-flow data.

<u>Upper Spring Run Reach</u>

Although the two dominant vegetation types, *Sagittaria* and *Hygrophila* are relatively stable and persist through a range of flow conditions, these two vegetation types do not support a high density of fountain darters. *Ludwigia* is a higher quality fountain darter habitat type, but it is very limited in abundance in this reach. If these were the only three vegetation types in the reach, this reach could be classified as poor habitat for fountain darters, but the presence of bryophytes has a strong influence. Alternatively, if the bryophytes were consistently abundant, the reach could be classified as high quality habitat since fountain darter densities are so high in the bryophytes; however, the abundance of the bryophytes in this reach is much more variable than the other vegetation types. In the Upper Spring Run the bryophytes consist primarily of *Amblystegium* with less *Riccia* than in Landa Lake. There was a distinct change in the abundance of bryophytes in this reach after flushing flows in 2000-2004. Although scoured by flushing flows during these events, the bryophytes appear to have a strong positive response to flooding over an extended period as evidenced by the substantial increase in total coverage after the two large floods in 2001 and 2002. The decline of the bryophytes between the fall of 2003 and spring of 2004 is difficult to explain since flows remained higher than normal during this period. It appears that flooding provides some stimulus for increased growth of the bryophytes in the short-term, but the growth can not be maintained over extended periods. Clearly, population dynamics of the fountain darter in the Upper Spring Run Reach is largely dependent on the condition and abundance of bryophytes. It is unclear how the bryophytes will respond to an extended period of low flows, but it will be important to collect this information to fully characterize this reach. With the fountain darter population so dependent on this vegetation type and its wide variability, this reach does not provide the consistent habitat of other areas in the Comal Springs/River ecosystem and should be classified as marginal habitat.

Landa Lake Reach

The stability of the *Vallisneria* stand in Landa Lake, which appears well adapted to resist flushing flows, is good for providing long-term habitat for fountain darters, however this vegetation type does not support high densities compared to other vegetation types. *Sagittaria* is also relatively abundant and stable, but supports very low densities of fountain darters. The presence of *Riccia* within large portions of the *Sagittaria* and *Vallisneria* in Landa Lake presumably (based on drop net sampling in the Upper Spring Run reach) increased the suitability of *Sagittaria* and definitely (based on direct drop net sampling) increased the suitability of *Vallisneria* for fountain darters. *Hygrophila* provides better habitat than *Sagittaria* or *Vallisneria* for fountain darters in the lake, but was also more vulnerable to scour during flooding. *Ludwigia* supported a much higher density of fountain darters than *Hygrophila*, but it had a small proportional distribution relative to the *Hygrophila* and other vegetation types. Although the *Ludwigia* does support a relatively high density of fountain darters compared to most other vegetation types in this reach, it appeared to be in a constant state of flux with its high susceptibility to scouring (and potentially paddle boats since it grows in a shallow area) and rapid re-growth. *Cabomba* is a relatively important component of the vegetation habitat since it supports relatively high densities of fountain darters and because of its stability during flushing flows, but it is also a relatively minor component of the vegetation community in total coverage.

As in the Upper Spring Run, the most important habitat type for fountain darters in the Landa Lake reach, the bryophytes, was also the most variable. In this reach the *Riccia* was more common than the *Amblystegium* with the former dispersed throughout the lake and the latter restricted to the upper areas on otherwise bare substrate (generally areas with gravel and small cobble). In Landa Lake, the *Riccia* tended to fill-in patches of bare substrate within other vegetation types and also to settle on top of other vegetation types (notably *Sagittaria* and *Vallisneria*). This generally increased the densities of fountain darters in these habitat types and made low or moderate quality habitat into high quality habitat in the short-term. Unlike in the Upper Spring Run, the bryophytes in Landa Lake did not decrease dramatically during flood events, but such events appeared to stimulate rapid growth. The total coverage of bryophytes increased dramatically (from 1,791 m^2 to 3,985 m^2) between the fall 2001 high-flow event and the spring of 2002. The bryophytes remained high through most of 2002-2003, but began to decline in fall 2003 and throughout 2004 inexplicably considering flows were high and conditions were presumably favorable for continued growth. There appears to be some sort of boom-bust cycle that occurs with this species that is not seasonal (the increase lasted >1 year) and may have been stimulated primarily by the flushing flows. It is not clear whether a similar cycle of rapid growth and slow decline would have occurred without the flood stimulus or how this cycle may be affected by low-flow conditions.

Although plants that support low densities of fountain darters dominate much of Landa Lake, these plants were very stable during flooding. In addition, there are localized habitat features (e.g., *Ludwigia* and *Cabomba*) that provide high quality habitat. Although the bryophytes are variable in this reach, this critical habitat type was not as susceptible to scouring during flooding as the bryophytes in the Upper Spring Run Reach. Overall, Landa Lake is a very important reach for fountain darters and maintaining habitat there should be a high priority. It will be important to track the influence of low-flow conditions on the high-quality habitat found in Landa Lake through the remainder of this study.

Old Channel Reach

Because the flow is regulated in this reach by two culverts that allow flow into the channel from Landa Lake (one directly from the lake and another through the Landa Park swimming pool), this reach usually provides the most stable conditions for fountain darters (and potentially Comal Springs riffle beetles). However, flooding can have drastic impacts on the small channel and there was a dramatic influence observed following the summer 2002 flood. Prior to that time (2000-2002) filamentous algae, dominated the reach; the only other common vegetation type was the aquarium species of the genus *Ceratopteris*. The filamentous algae in this reach consistently supported a greater density of fountain darters than any other found in the Comal Springs/River ecosystem, as many as 105 per m^2 in the spring of 2002. After the summer 2002 flood (beginning in the fall of 2002), the filamentous algae virtually disappeared with most small patches restricted to areas along the shoreline. Instead of the filamentous algae returning, the majority of the area that had previously been occupied by the algae was covered with *Ludwigia* and *Hygrophila* one year later and remained that way through the final sample of 2004. Although both of these plant types support fountain darters in other locations throughout the ecosystem, they do not support the same densities as the algae. In addition, sampling has revealed that few fountain darters had colonized the new vegetation as of the fall of 2004; densities in *Hygrophila* and *Ludwigia* were 4.2 and 3.4 per m^2, respectively in 2004, compared to 6.2 and 14.9 per m^2 in the rest of the Comal Springs/River ecosystem. Although filamentous algae has re-established in deeper areas within the reach (too deep to sample with drop nets) the shift in habitat conditions appears to be a major impact on fountain darter habitat quality in this reach. It is uncertain whether these changes occurred in other portions of the Old Channel, but despite the changes, this channel should be considered among the highest quality habitat and among the highest priority to maintain. This situation will continue to be monitored through the extension of this project to see whether the fountain darter densities increase in these two plant types in the Old Channel.

New Channel Reach

Hygrophila dominated the New Channel Reach during this study, with a moderate amount of *Cabomba* and very small amount of *Ludwigia* (observed only in 2003) also contributing to the total available habitat types. The total coverage of *Hygrophila* did not fluctuate much in 2000-2003. There were minor decreases in total coverage due to scouring after floods during that time period, but the bare patches were quickly re-vegetated. That was not true in summer 2004, when there were much more dramatic effects in this reach. Virtually all of the vegetation was scoured out of this reach during that event, which apparently had a more rapid flushing flow than the summer 2002 event despite a lower total discharge. *Cabomba* presumably supports higher densities of fountain darters in this reach than *Hygrophila* (as observed in other locations) however all *Cabomba* patches are too deep to sample with drop nets in this reach. The *Cabomba* in this reach appears to be well positioned to avoid scouring by flooding in this reach, as almost all patches remained after flooding in 2002 and most had increased in size (although virtually all patches were removed in the summer 2004 event). Despite the effects of the 2004 flood event, this reach provides highly stable habitat, though lower quality overall than other reaches in the Comal Springs/River ecosystem.

Effects of High-flow conditions

Flushing flows associated with flood conditions had sometimes-dramatic influences on habitat conditions in the Comal Springs/River ecosystem. Generally, flushing flows scoured the vegetation with vulnerable areas and vulnerable vegetation types experiencing the most dramatic changes. The changes were generally short-lived with rapid re-growth, but in some instances the scouring changed the composition of the vegetation community in a reach.

In the Comal Springs/River ecosystem, filamentous algae and bryophytes (*Riccia* and *Amblystegium*) were two types of vegetation that were most susceptible to flushing flows during the project. These are not rooted macrophytes, which presumably makes them highly susceptible to displacement during elevated flow conditions. These are also the two plant types that support the greatest density of fountain darters. In the larger of the two 2001 floods, filamentous algae (primarily found in the Old Channel) was reduced by 79%. During that same flood, the bryophytes were reduced by 57% in the Upper Spring Run. In Landa Lake the bryophytes were largely buffered from scouring during high-flow events and responded to such events with rapid growth resulting in much higher coverage levels than before the flooding. Bryophytes in the Upper Spring Run Reach also responded to flooding with rapid re-growth. The algae returned in similar quantity during 2001, but the flood in summer 2002 resulted in a dramatic shift in the vegetation composition. This susceptibility of the highest quality fountain darter habitat to flooding may have substantial influence on the fountain darter population in the Comal Springs/River ecosystem and it will be particularly important to monitor these two vegetation types during low flows.

In addition to the physical structure of each vegetation type, the impact of flooding on each vegetation type in the Comal Springs/River ecosystem was influenced by spatial distribution among various mesohabitats within each reach and among reaches. *Ludwigia* and *Cabomba* occur in relatively similar abundances in Landa Lake, but *Cabomba* tends to grow in areas that are out of the main current in margin habitat where sediment is deposited. These areas are spared the full impact of a flushing flow event and its location provides the *Cabomba* with some protection from these events. *Ludwigia* is much more exposed to the main current in its distribution (primarily in Landa Lake) and is readily scoured during most flushing flows. Different reaches also had different impacts resulting from flooding. This was most dramatic in the New Channel Reach after the flushing flow in the summer of 2004. A near complete removal of all vegetation in the reach occurred during that event such that the two dominant plant types there, *Hygrophila* and *Cabomba* were reduced by 98% and 99.6%, respectively. This reach receives the runoff from the dry Comal River, which can send a substantial surge of flow during storm events. The Upper Spring Run Reach is similarly influenced by Blieder's Creek. The Old Channel is more buffered from moderate flooding events since flow is controlled by the culvert in Landa Lake, but with substantial runoff conditions, this reach has suffered severe reductions in aquatic vegetation coverage. Much of Landa Lake is also very well buffered from scouring flows.

Despite the severe impacts on the distribution and abundance of certain vegetation types in the Comal Springs/River ecosystem, the vegetation has displayed rapid re-growth following these events. In many cases, the original distribution of a stand of plants expanded shortly after a flood event such that the net result of a flushing flow is an increase in a particular type of aquatic vegetation. This is exemplified by the increase of 28% in *Hygrophila* in the Upper Spring Run between the summer and fall samples after the flood in summer 2002. The total amount of *Hygrophila* after the flood was 992 m^2 compared to a total of 881 m^2 in the spring just before the flood. In most cases, the growth of new vegetation following a flood did not result in a major change in vegetation distribution, with the obvious exception of the alteration of the Old Channel composition after the 2002 flood. In most locations, flooding scoured certain plant types and reduced the amount of available habitat for fountain darters and other species for a short period of time, but the same plant type usually re-grew in each affected area.

Effects of Low-flow conditions

As with other study components a complete assessment of aquatic vegetation impacts resulting from variable flows is not possible due to the lack of low-flow data. It is hypothesized that some plant types will become less abundant and/or provide lower quality habitat under low-flow conditions, but it is unclear at what point conditions may deteriorate or to what extent. A critical component of fountain darter habitat composition, the distribution and abundance of various types of vegetation is a very important component of this study. Additional data at lower flows will improve our understanding of species/habitat relationships and the influence of discharge on these relationships.

Fountain Darter Sampling

Drop Net Data

Importance of aquatic vegetation

In general, the fountain darter population appears to be largely affected by the composition and distribution of suitable habitat (aquatic vegetation) in the Comal Springs/River ecosystem. There is some seasonal variation in certain plants, strong distributional patterns among reaches, and a range of susceptibility of the individual plant types to changes in discharge which all contribute to variation in the fountain darter population abundance over time. The influence of vegetation on fountain darter population dynamics may change at lower flows where other physico-chemical parameters, such as water temperature, may have a greater influence, but additional work is needed to obtain that information.

Because of the dramatic increase in fountain darter density observed in *Vallisneria* in Landa Lake and *Sagittaria* in the Upper Spring Run when *Riccia* was present, it appears that a population estimate based upon vegetation coverage would be greatly affected by the presence/absence of *Riccia* among other vegetation types. Slight changes in the difference in suitability (i.e., density of fountain darters found there) of a given plant type can make a large difference in a population estimate and this should be taken into consideration.

The data collected in this study suggested that fountain darters exploited the favorable changes in habitat conditions in Landa Lake (dramatic increase in *Riccia*) with an expansion of the population, but the trend would presumably be reversed if and when the *Riccia* diminishes.

<u>Abundance Estimates</u>

It is important to clarify the relationships between fountain darter densities and dominant vegetation in the Comal Springs/River ecosystem. As the relationships are improved, long-term monitoring may be shifted to focus on habitat mapping with fewer labor-intensive drop net samples required as verification. The mean densities observed in each vegetation type during this study could be used for long-term monitoring where vegetation coverage is calculated regularly to provide a rough estimate of population size. However, there continues to be large variation in the observed fountain darter densities in each habitat type. Additional drop net sampling is necessary to continue to refine the relationship between fountain darters and each vegetation type, especially in low-flow conditions since the suitability of each vegetation type may change dramatically under those conditions. Using the mapping data during 2000-2004 and the mean densities of fountain darters observed during that time, there were some interesting trends in the data, including a decrease in total population in Landa Lake after each high-flow event. However the trend in Landa Lake was a dramatic increase through early 2003 and rapid decline in population since. This appears largely based on the fluctuations in bryophytes in that reach but will be monitored closely in 2005 and beyond.

<u>Population modeling</u>

This modeling effort will ultimately provide a greater ability to assess changes in the fountain darter population in response to changes in discharge than estimates of population abundance based solely on vegetation. The major shortcoming to the model results is the limited data during low discharge. All of the significant relationships observed between fountain darter density and total discharge in the Comal Springs/River ecosystem were largely influenced by just a few data points from low discharge conditions (approximately 150 cfs). For the Landa Lake bryophytes relationship (nearly significant at $p=0.065$) the lowest discharge was 261 cfs.

The slope of the relationship of bare substrate sites in the Old Channel suggests that the fountain darters will use bare substrate habitat more commonly when discharge decreases. There are a number of possible explanations for why that might occur, for example, fountain darters may be moving between habitats more frequently or water velocity may be more suitable, however, this relationship is based on few data points. In contrast, the slopes of the *Ceratopteris* in the Old Channel Reach and *Hygrophila* in the Upper Spring Run Reach both indicated fewer fountain darters are found in those habitats when discharge decreases. *Ceratopteris* consistently had near zero fountain darters in the lowest discharge conditions. If *Ceratopteris* remains common in the Old Channel with the current change in vegetation composition there (see vegetation mapping section), this relationship should be explored more carefully with more samples at lower discharge. Clearly, these relationships rely very heavily on a few data points during low discharge but may indicate trends that should be examined further.

The near-significant relationship for the Landa Lake bryophytes may become statistically significant with more data, but the trend is counterintuitive to what might be expected. One might expect that darter density would decrease at lower flows in all vegetation types; however, the bryophytes were not sampled during the low-flow Critical Period samples, so the range of discharge conditions for that vegetation type is only 260 cfs to nearly 450 cfs. Data at lower flows may indicate a decrease in density below some discharge level. This data was also confounded by the rapid growth of the bryophytes during the higher flow conditions, which resulted in low densities of fountain darters until the population was able to increase and exploit the increased habitat. This lag time between the change in habitat and change in fountain darter density resulted in low density estimates when the bryophytes were abundant.

One important possibility in all of these discharge relationships (including those that are non-significant) is that fountain darter density may increase or remain the same as discharge decreases and then start to decrease suddenly under very low discharge conditions. Since there is little data at low discharge, that possibility can not be evaluated at this time. More data are needed to fully explore all of these relationships before hypotheses are developed and evaluated.

Although each of the other variables that were evaluated individually indicated significant differences in fountain darter density, there was substantial variability in the density values and very low R^2 values. This suggests that no one variable is particularly influential on fountain darter densities, but that all contribute in some way. We believe that a large part of the reason for these low R^2 values is that during normal-to-high discharge conditions, such as those experienced in 2000-2004, the distribution and abundance of fountain darters is not greatly affected by any of these variables, but largely by the availability of high quality aquatic vegetation. When discharge declines to levels below those experienced during 2000-2004, certain variables such as temperature and dissolved oxygen may become more important in determining the distribution and abundance of the fountain darter in the Comal Springs/River ecosystem. As more low-flow data are collected these relationships, and potentially additional models to incorporate multiple variables, will be explored further. The low-flow data will be critical to improve our understanding of which variables contribute the most to fountain darter distribution and abundance.

<u>Size-class distribution</u>
The size-class distribution for fountain darters collected by drop nets from the Comal Springs/River ecosystem in 2000-2004 is a statistically normal distribution that is typical of a healthy fish population with a peak between 22 mm and 26 mm. This occurred primarily in the Upper Spring Run and Landa Lake Reaches where there appeared to be a boom in the population as a result of increased habitat suitability in 2003 (more *Riccia*). Improved conditions (i.e., a dramatic increase in bryophytes) and limited intra-specific competition in the new habitat may have also contributed to a rapid growth rate in some individuals. Part of the reason may also be that many small fountain darters were sampled in the filamentous algae in the Old Channel and that habitat type was very limited in 2003-2004.

<u>Other Species</u>
There are some natural differences in fish species diversity among reaches in the Comal River as evidenced by the differences in the Shannon Weaver diversity index values. However, there were no discernable patterns of change in diversity at the reach level during 2000-2004. Patterns of community composition will be examined more closely at lower discharge to determine whether species diversity is altered under certain flow conditions.

The exotic giant ramshorn snail has occurred in low numbers throughout 2000-2004 in the Comal Springs/River ecosystem. In the two most preferred fountain darter vegetation types (bryophytes and filamentous algae) there were essentially no giant ramshorn snails present. In the past, the giant ramshorn snails were observed in far greater densities than those reported here. For perspective, the maximum value on the y-axis of Figure 15 is 10 m^2, which was among the lowest concentrations reported in the early 1990s (T. Arsuffi, Texas State University aquatic ecologist, pers. comm.). During that period the greatest giant ramshorn snail density was near 400 snails per m^2, and the vegetative community was literally being devoured (T. Arsuffi, Texas State University aquatic ecologist, pers. comm.). By all indications the densities of giant ramshorn snails observed in the Comal ecosystem during the study period (including the 2000 low-flow events) pose no serious threat to the aquatic vegetative community. However, because of the impact that this exotic species can have substantial impacts at higher densities, close monitoring of this should continue into the foreseeable future.

Dip-net Data

One of the greatest values of the dip net sampling is the ability of this gear to sample small fountain darters, which can be an indicator of recent reproduction. The observation of these small fountain darters during all samples in the Old Channel Reach indicate that some level of reproduction can occur year-round although there was still a distinct peak in the spring. These findings correlate well with the drop net results, in which there were usually fountain darters of the smallest size class in both the spring and summer and primarily large fountain darters (26-35 mm) in the fall. However, other results differed between the two sample methods. The changes in vegetation since the summer of 2002 in the Old Channel Reach had distinct effects on fountain darters sampled with drop nets. Although the drop net samples clearly showed fewer fountain darters using new habitat types, the dip net samples in 2003 and 2004 were very similar to earlier samples. Since the dip net sampling focuses on edge habitat near the shoreline, it shows that some habitat remained unaffected by the change in vegetation composition. This suggests that fountain darters continued to use edge habitat in similar abundance regardless of changes in the main channel. The disparate findings between the two sample methods indicate that both provide valuable information that may not be gathered by relying entirely on one method (in their current forms). Changing the sampling protocol to use only one method (e.g., dip netting) would require some alteration to ensure that all habitat types are sampled within a given stream reach.

The increase in fountain darter abundance in the Upper Spring Run Reach in 2003-2004 has important implications for fountain darter response to changing habitat conditions. The Upper Spring Run is an area of marginal fountain darter habitat that may deteriorate more rapidly than other areas during low discharge. If fountain darters are able to migrate to higher quality habitat conditions, as indicated by the increase in abundance with no evidence of increased local reproduction, then they may be able to migrate downstream to Landa Lake where there would be higher quality habitat during lower flows.

Visual Observations

Overall, the observations in this component of the study have shown that (1) Landa Lake is an integral component to the habitat of species found in the Comal Springs ecosystem, and (2) a sizable portion of the fountain darter population is found there. There is also a clear relationship between the abundance of *Riccia* and fountain darters abundance in the deep portion of the lake as indicated by the regression analysis. In previous reports, we suggested the possibility of seasonal variation in the use of these habitats, but the vegetation composition is clearly the primary factor.

Gill Parasite Evaluation

Parasites had substantial variation in spatial distribution during this study, but observations of temporal variation were less pronounced. The greatest number was found in an area (near Spring Island) where water velocities were low and snail density historically high. The high local snail density probably contributed to the high numbers found in this area, but more studies are needed to evaluate the relationships between snails and abundance of parasites. In addition, migratory green heron, a suspected host of the parasites, also tend to be observed in this location and may influence the high numbers there. The year-round, constant thermal temperatures of the spring-fed river (Brune 1981 and USFWS 1996) may be the primary cause for a weak trend in temporal distribution of the parasites. Although there was a slight peak in the summer samples, these peaks were not as pronounced as in regions that experience drastic seasonal changes in temperatures and prolonged droughts and rainy seasons. Other studies have shown seasonal variation in trematode infections due to temperatures that regulate sporocyst development in snails (Pitchford et al. 1969, Pitchford and Visser 1969, Shiff et al. 1975, Sankurathri and Holmes 1976), droughts that dry up snail habitats, and prolonged rains that scoured away snail populations (Sturrock et al. 1994, Muhoho et al. 1997).

The data from this study do not show a significant relationship between parasite abundance in the water column and water velocity, but there does appear to be a weak trend that will be explored further with additional data. Other field studies of cercariae have shown that the concentration is dependent on water volume and flow (Yousif et al. 1996, and Ouma et al. 1989). One of the shortcomings for using this data set for evaluating questions about flow may be that the current sampling method does not measure the direct effects of water volume moving through a sampling site. When comparing sites of similar current velocities, concentrations probably differ due to the considerable differences in river basin depths and widths. The increases or decreases in river cross-section widths and depths serve to dilute or concentrate the number of parasites moving through a single sampling point in the river, even though they have similar current velocities. This potential relationship between parasite density and water velocity will be evaluated in 2005 and beyond.

Overall, the gill parasite sampling efforts in 2000-2004 have provided valuable data on parasite distribution and abundance in localized areas throughout the Comal Springs/River ecosystem. The results show that *C. formosanus* are present in the water column and infect fountain darters year-round in the Comal River. Information on spatial and temporal variability will provide important data that can be used to focus efforts on reducing the host snails in "hot spots" that may substantially improve conditions for resident fountain darters. Time of year does not appear to be a critical factor that might influence removal efforts, but any efforts would probably be most valuable prior to or during reduced flows when parasite concentrations may be highest. Future monitoring of parasites in the water column in these study sites will provide a continuous dataset for evaluating future trends in parasite concentrations, including information on low-flow conditions. As with other components of the study, more data are needed from low Critical Period flows to fully evaluate the change in parasite effects at lower discharge levels; however, the data being gathered are vital to maintain a record of ongoing conditions to properly assess low-flow conditions when they do occur. Fortunately, the parasite is not prevalent in the San Marcos River, but that could change and information collected in the Comal River could be useful for management in that system as well.

There will be some modifications in 2005 to improve the sampling design and the ability to evaluate parasite distribution and abundance throughout the Comal Springs/River ecosystem. If future sampling efforts continue with this same methodology, an extensive data set would be generated on the trends in parasite abundance and response to fluctuations in discharge, but only in the individual sample areas. A more random sampling design would provide a greater ability to evaluate trends throughout the river, but since parasites tend to be locally abundant, such an approach would require too many samples. Attempting to stratify sampling efforts would also improve the design, but this would require extensive effort to delineate habitats and likely still require too many samples. Thus, instead of random sampling, some samples will focus on cross-sections of the river in several locations to assess fluctuations in parasites drifting through a given area among sample sites and dates. This will hopefully lead to a better understanding of the relationship between discharge and parasite concentration. In addition, some samples will be maintained in the same locations that have had high densities of parasites during the sampling efforts to date. The precise details of number of samples and locations will be developed in early 2005.

Comal Springs Salamander Surveys

Observations of Comal Springs salamanders have varied in number within and between locations during 2000-2004, but individuals have been observed in each sample location during each sample period. There is no distinct pattern of variation in salamander abundance with changes in discharge; however, there was a trend of increasing numbers of salamanders surveyed in the eastern outfall adjacent Spring Island in 2003 and 2004. This increase may be related to increased abundance of *Riccia* and will continue to be monitored with future samples.

Silt appears to affect habitat suitability at the substrate surface since no Comal Springs salamanders were observed in any areas with excessive silt and fewer salamanders were surveyed immediately after sediment was washed into Spring Run 3. The response of the Comal Springs salamander to the influx of sediment into Spring Run 3 following high-flow events may support the preliminary theory that under situations of acute disturbance/habitat modification the species migrates deeper into the substrate and return to the surface when conditions are again suitable. Comal Springs salamanders predictably decreased with increased silt, but did not move laterally in the Spring Run channel since surveys were conducted through the entire channel and lower numbers were observed throughout. The decrease in abundance was only temporary though as individuals returned to the sample area within one or two sample dates to numbers similar to pre-disturbance observations. This pattern was repeated several times during 2000-2004.

It is not clear whether there may be a response by the Comal Springs salamander to low-discharge conditions, since there have been only two occasions to sample at low flow during this study. As with other study components, additional data are needed within that critical flow range.

Macroinvertebrate Sampling

Drift net Sampling

Although the hydrograph in 2004 did not present any opportunities to gather data on low-flow impacts to macroinvertebrates around spring openings, the three sample events presented distinct conditions. The first sample was during higher-than-normal flows, but with no recent flood events. All of the 2003 hydrograph was similar to what occurred prior to this sample. The other two samples were influenced by high-flow conditions; a substantial flood event in early summer and spikes in the hydrograph prior to the August sample and high-flow conditions during the October sample.

Despite the differences in springflow conditions relative to 2003, many of the findings in 2004 were very similar to those in 2003 (see BIO-WEST 2004a for more details on 2003 findings). Species of the two genera *Stygobromus* and *Lirceolus* were again the most abundant at all sites during each sampling event. Since most of the *Stygobromus* were small individuals (only a few millimeters long) it might be possible that juveniles or sub-adults are more susceptible to expulsion from spring openings. As described in the 2003 annual report (BIO-WEST 2004a), the high numbers of *Stygobromus* and *Lirceolus* relative to the other species/groups suggests that any attempts to establish a springflow-biological response relationship should probably focus on these groups. Because the endangered invertebrates, the Comal Springs dryopid beetle and Comal Springs riffle beetle were sampled in low numbers using this sample technique, neither is suitable for use as indicators of springflow changes. However, it is valuable to continue to monitor the presence of each in the three spring openings.

There were some differences in the numbers of the three endangered macroinvertebrates observed in 2003 and 2004. There were a few more Comal Springs dryopid beetles and Comal Springs riffle beetles in 2004 than 2003. All Comal Springs dryopid beetles were larvae, which are thought to be terrestrial, so it is possible that the higher numbers may be a result of higher flows reducing available terrestrial habitat (i.e., air “pockets) in the aquifer forcing the larvae out of spring openings. The higher numbers of Comal Springs riffle beetles are probably not enough to suggest that there might be some difference in population dynamics associated with differences in springflow. There was a much greater difference in Peck’s cave amphipods with substantially more sampled in 2004. This was largely due to an increase in numbers observed in the western shoreline upwelling; there were only 47 Peck’s cave amphipods observed there in all of 2003 and 110 in 2004. Total numbers of *Stygobromus* spp. in this habitat increased from 188 to 409. If the increased numbers had occurred only in the August and October samples, it might suggest that the individuals found around this spring opening are more susceptible to high-discharge events than in the two other sites, but there were similarly high numbers observed in all samples. Additional data collected in 2005 should help explore this trend further.

The only other trends observed in the data related to the higher numbers of *Mexiweckelia hardeni*, *Lirceolus* spp., and *Heterelmis comalensis* in August across all sites, but in Spring Run 3, the highest numbers of nearly every species were sampled in October. Looking only at Spring Run 3 this is a pattern that might be expected given that the highest discharge occurred during the October sample, when many organisms might have been actively dispelled from the spring opening. It is unclear why this pattern was not observed in all three sites. Spring Run 1 and the upwelling on the western shoreline of Landa Lake appeared to be less influenced by these higher flows. It will be important to assess whether there are similar differences in response among sites to low-discharge conditions.

Comal Springs Riffle Beetle

The primary value of the Comal Springs riffle beetle sampling was to provide some basic information on the population dynamics and distribution among sample sites. The new sample method adopted in 2004 provided a larger sample size and a greater ability to examine population dynamics. The new method also improves the ability to evaluate potential differences in population abundance among sites, seasons and with different depths and flows. One of the first observations is the difference in abundance of adults (n=810) and larvae (n=376), which could indicate that larvae do not use the same habitat types as adults or that there may be a bias of this sampling technique where adults are more attracted to the cloth. Larvae were sampled in all 2004 samples and all but the December sample on the western shoreline of Landa Lake in 2003, which suggests either that the larval stage is long enough that there are always some present, or that reproduction occurs at more than one time during the year. Members of the family (Elmidae) are known to require anywhere from 6 months to 3 years to complete the life cycle from egg to adult (Arsuffi 1993), but little is known of the frequency or timing of reproduction. Overall, the beetles (both larvae and adults) tended to be patchily distributed with wide ranges of abundance among samples within a site. In many cases samples within a site that were geographically close had large differences in total abundance of beetles sampled. Although samples were not conducted in precisely the same area on successive sample dates, certain areas within each site tended to have higher numbers than other areas. These data suggest that the species has precise requirements (e.g., physical characteristics or food source) that govern its distribution. This valuable information on microhabitat use and distribution within the three sample sites can benefit management strategies that focus on maintaining suitable habitat conditions in specific areas during a period of low recharge.

The relatively even distribution of Comal Springs riffle beetles among the three sample areas (under 2004 flow conditions) also has implications for management of the species. Although previously believed to occur primarily in the spring runs, it is clear that the Comal Springs riffle beetle occurs in large numbers in other habitats. The sampling methodology used in 2004 has also revealed a much larger population of Comal Springs riffle beetle along the western shoreline of Landa Lake than previous sampling had suggested. Management for the Comal Springs riffle beetle should account for all three populations identified in this study.

The lack of statistically significant seasonal variation in Comal Springs riffle beetle abundance in 2004 is not surprising since spring discharge was high (minimum=335 ft^3/sec), although there were several peaks in the hydrograph. Although not a significant difference, there were greater numbers observed in November in each of the three sites during a time when flows were very high as a result of high recharge. It might be possible that the high flows encouraged individuals that may have been deeper in the substrate to move up to the surface where they are more readily sampled. The lack of a relationship between the species' abundance and water depth is not surprising since the sample areas were all in locations with spring openings and depth is probably inconsequential (though there may be issues with pressure around springs found in deeper parts of Landa Lake). The more significant factor should be water velocity from the spring openings, which may be expected to influence the species abundance and distribution during lower discharge conditions. However, this variable also did not show a relationship during the high discharge conditions in 2004. Additional sampling during a range of flow conditions (particularly during low flow) will improve our understanding of the relationships between discharge, water velocity at spring openings and the abundance and distribution of Comal Springs riffle beetle.

Exotics/Predation Study

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

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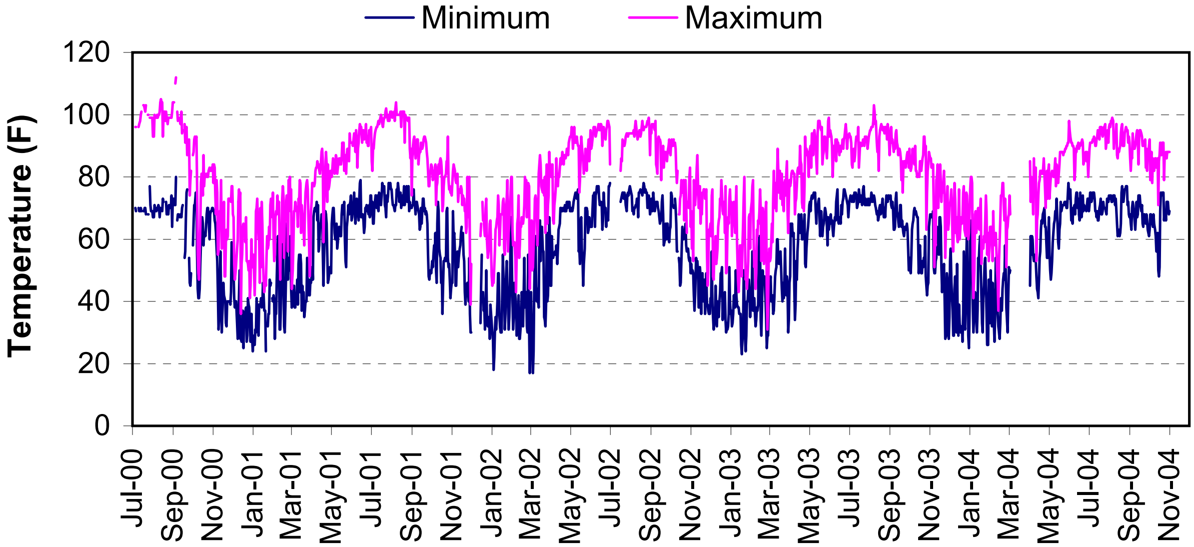
APPENDIX A:
AQUATIC VEGETATION MAPS

(separate file)

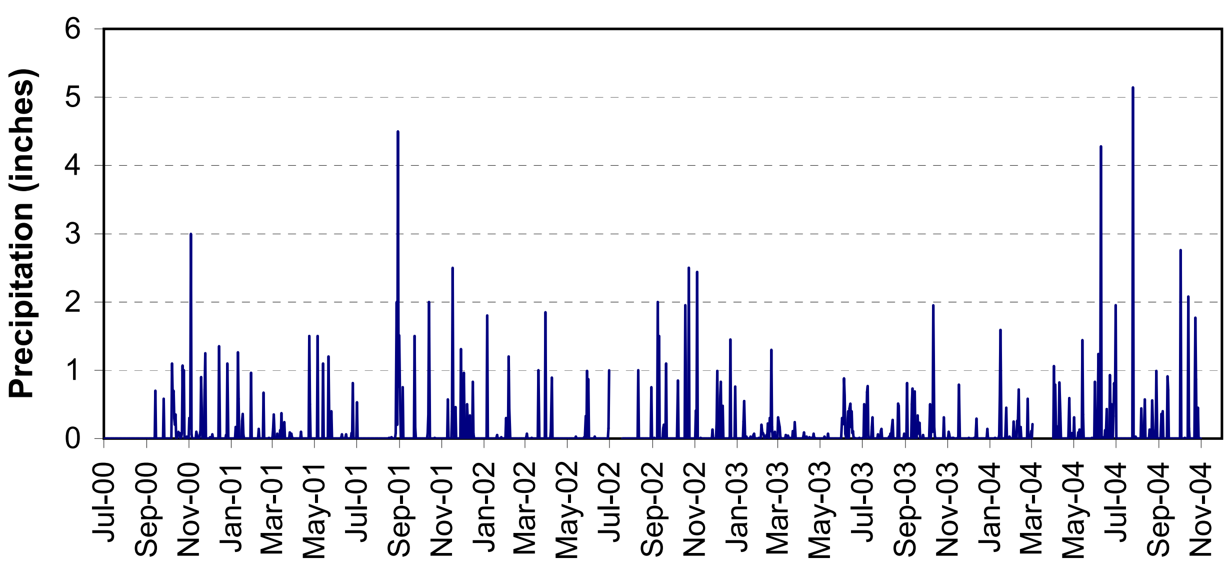
APPENDIX B:
DATA AND GRAPHS

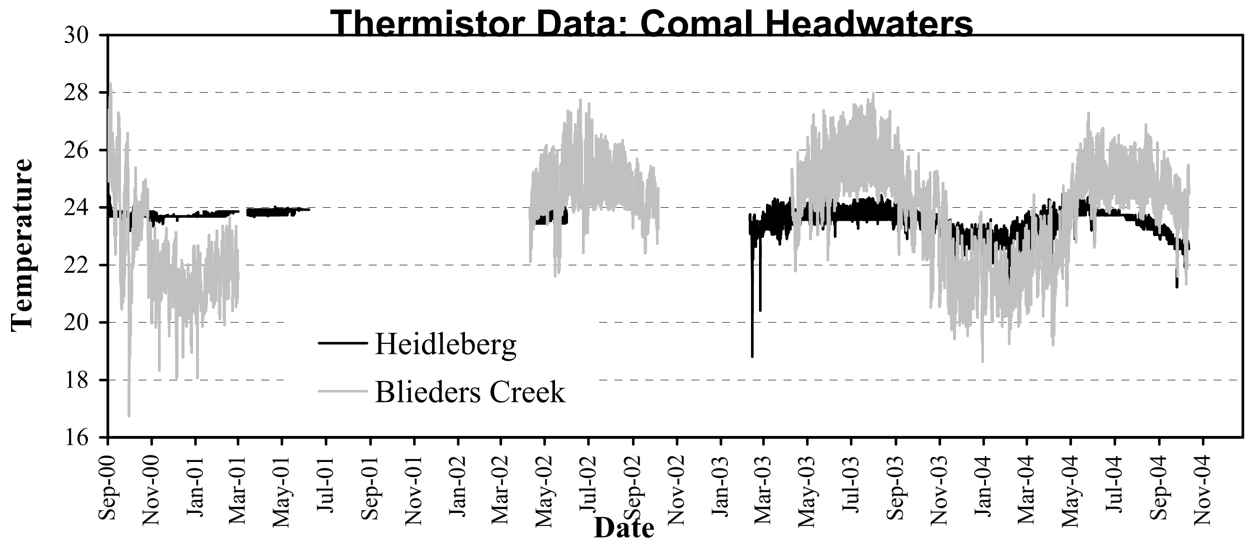
Water Quality Data
and
Thermistor Graphs

Daily Air Temperature Data for New Braunfels, Texas



Daily Precipitation Data for New Braunfels, Texas




Thermistor Data: Comal Headwaters
Temperature
30
28
26
24
22
20
18
16
Heidleberg
Blieders Creek
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
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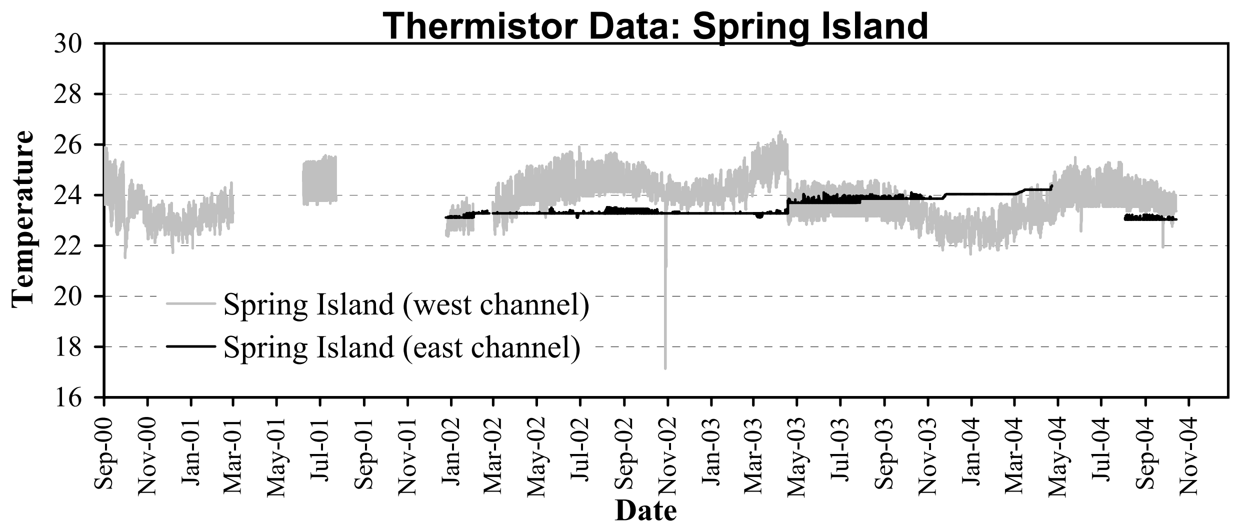
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Date


Thermistor Data: Spring Island
Temperature
30
28
26
24
22
20
18
16
Spring Island (west channel)
Spring Island (east channel)
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
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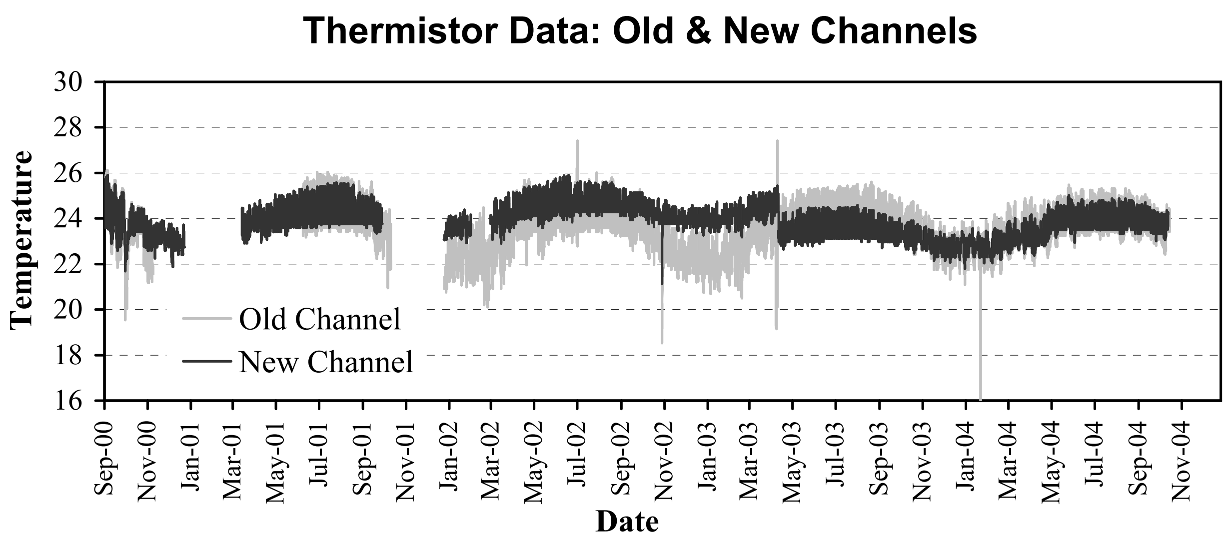
Jul-04
Sep-04
Nov-04
Date


Thermistor Data: Old & New Channels
Temperature
30
28
26
24
22
20
18
16
Old Channel
New Channel
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
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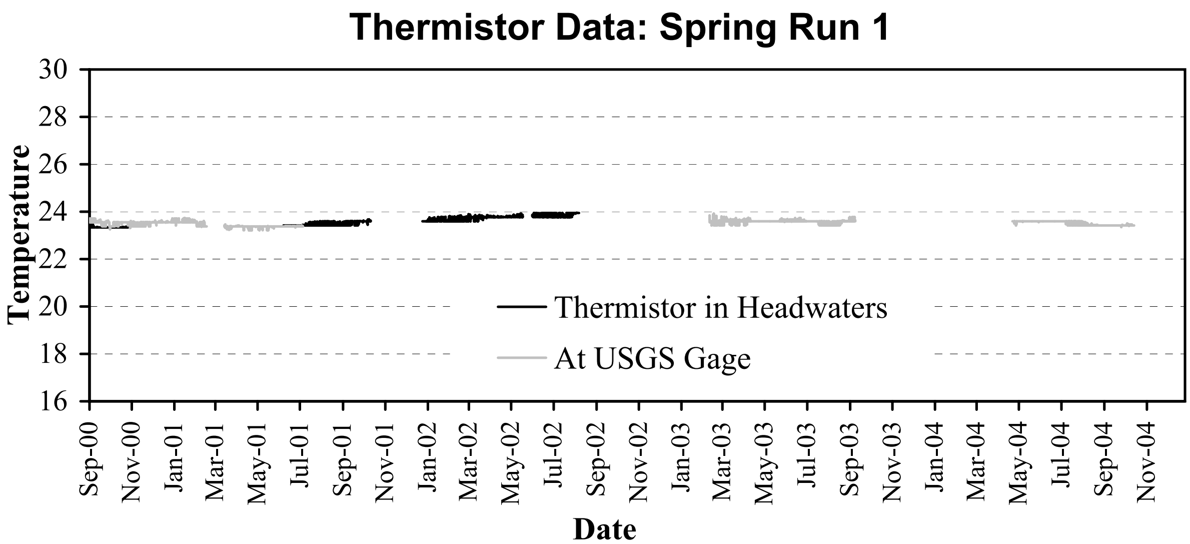
Sep-04
Nov-04
Date


Thermistor Data: Spring Run 1
Temperature
Date
Thermistor in Headwaters
At USGS Gage
30
28
26
24
22
20
18
16
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
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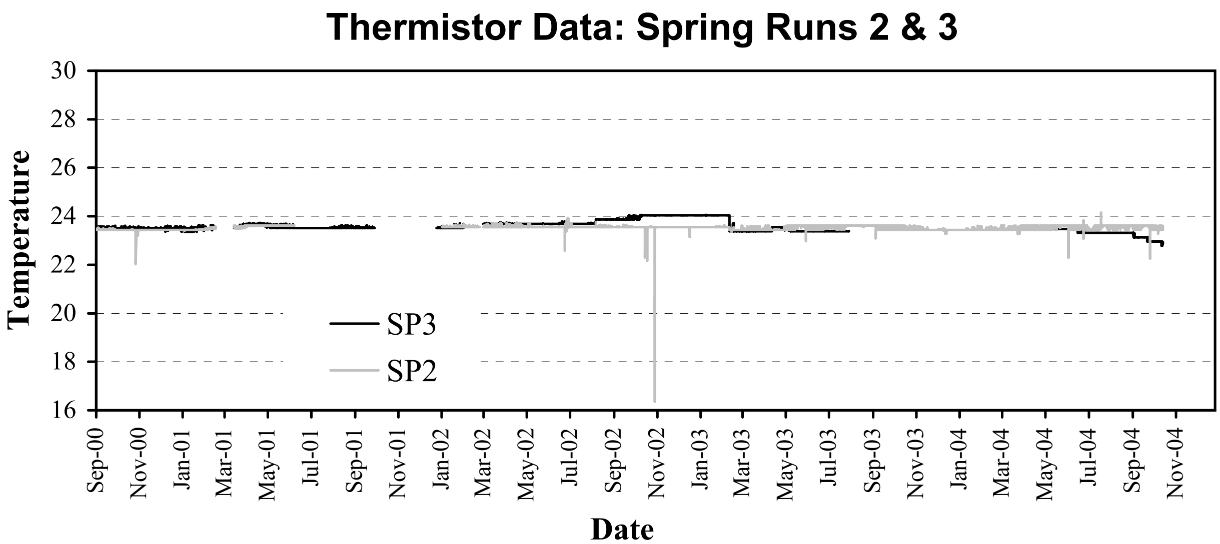
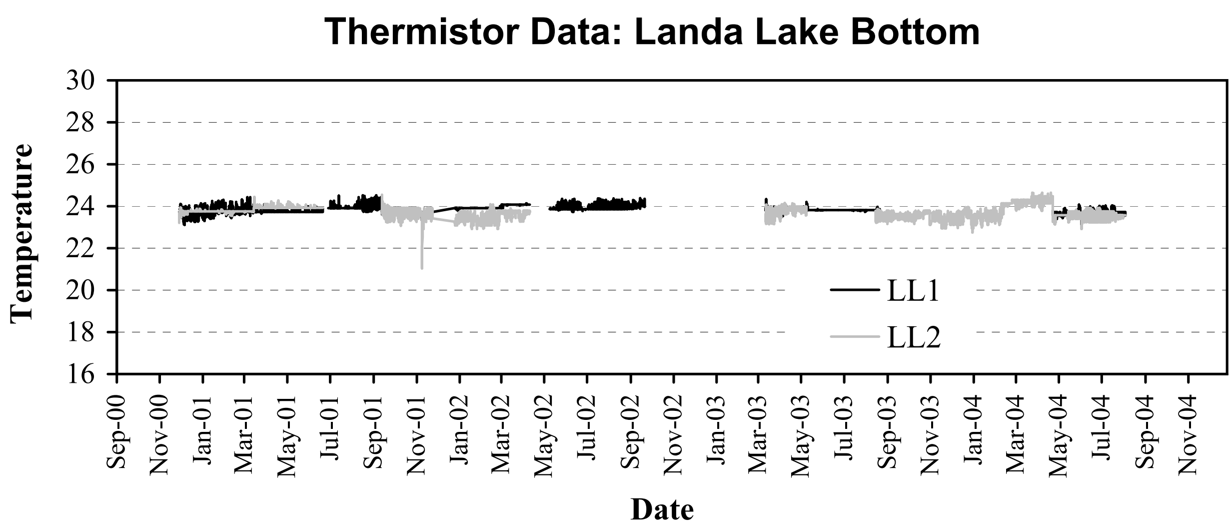
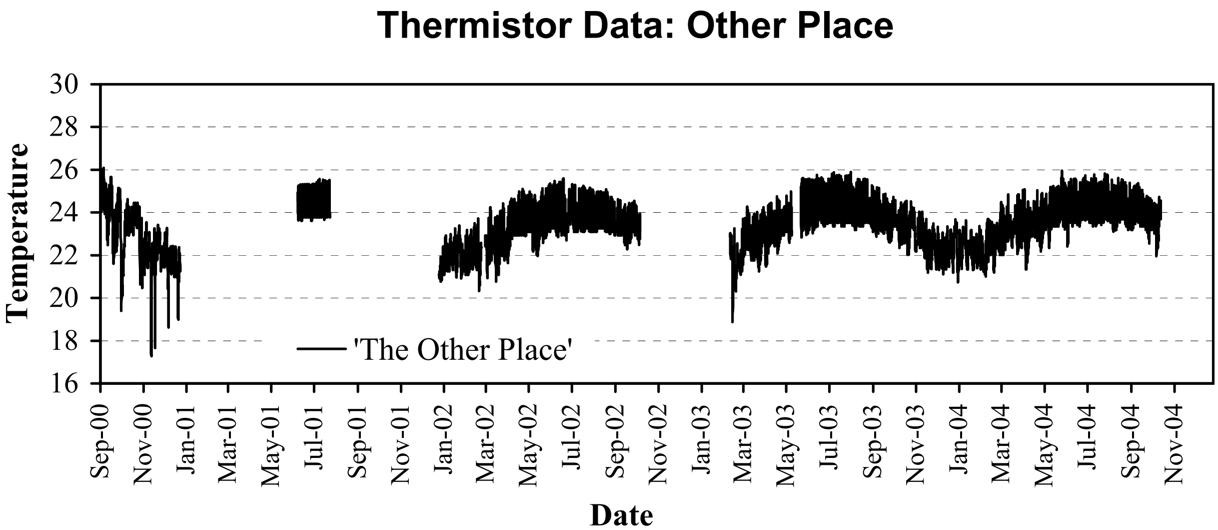
Jul-04
Sep-04
Nov-04


Thermistor Data: Spring Runs 2 & 3
Temperature
Date
SP3
SP2
30
28
26
24
22
20
18
16
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04

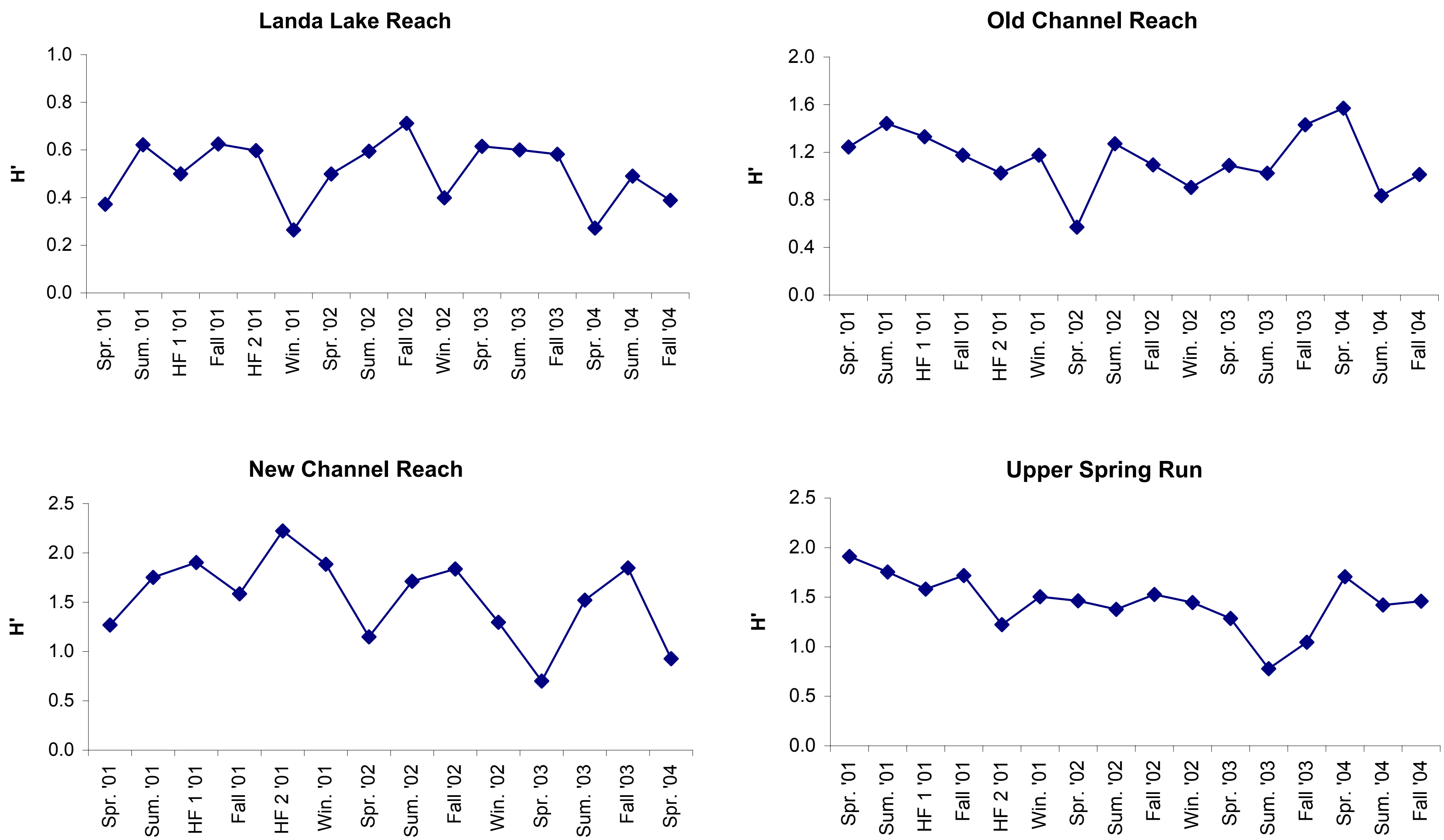

Thermistor Data: Landa Lake Bottom
Temperature
Date
LL1
LL2
30
28
26
24
22
20
18
16
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04



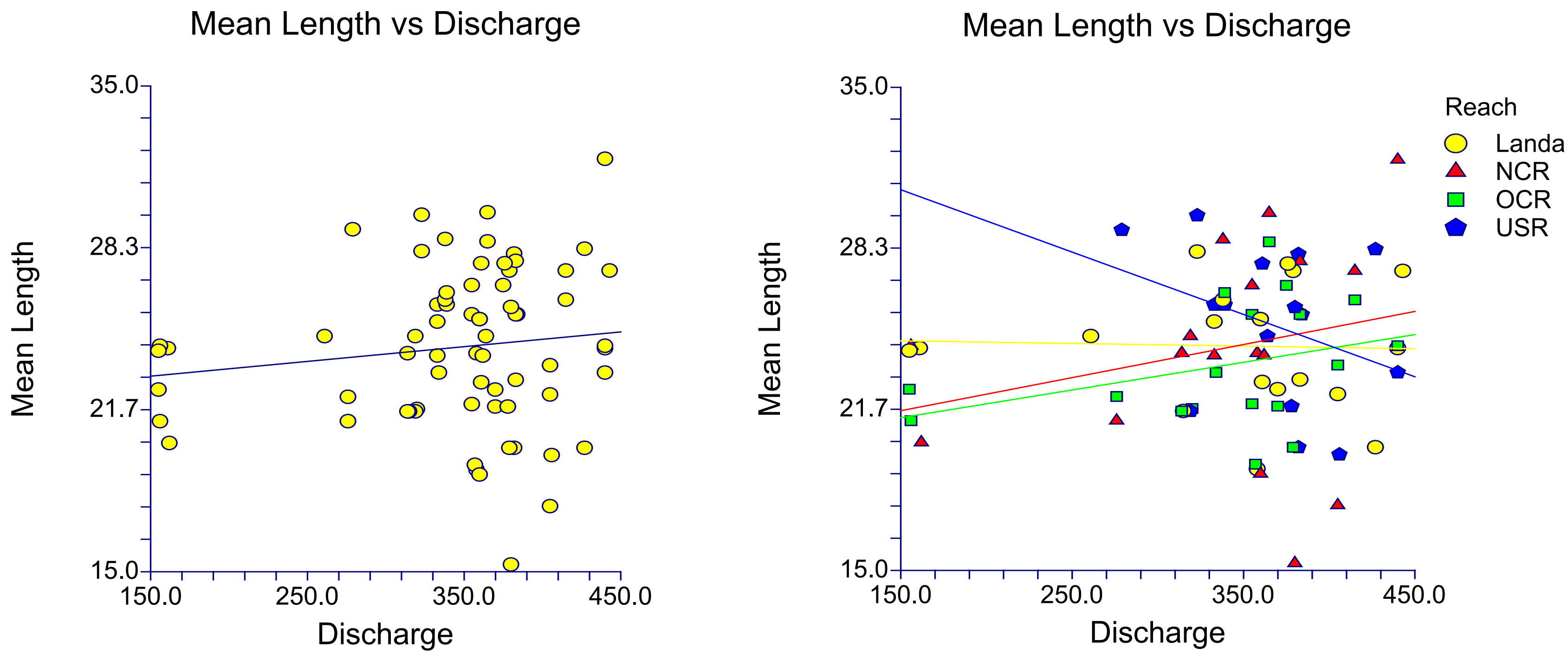
Thermistor Data: Other Place
Temperature
30
28
26
24
22
20
18
16
'The Other Place'
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Date

Drop Net Data:

Species Diversity and Fountain Darter Mean Length



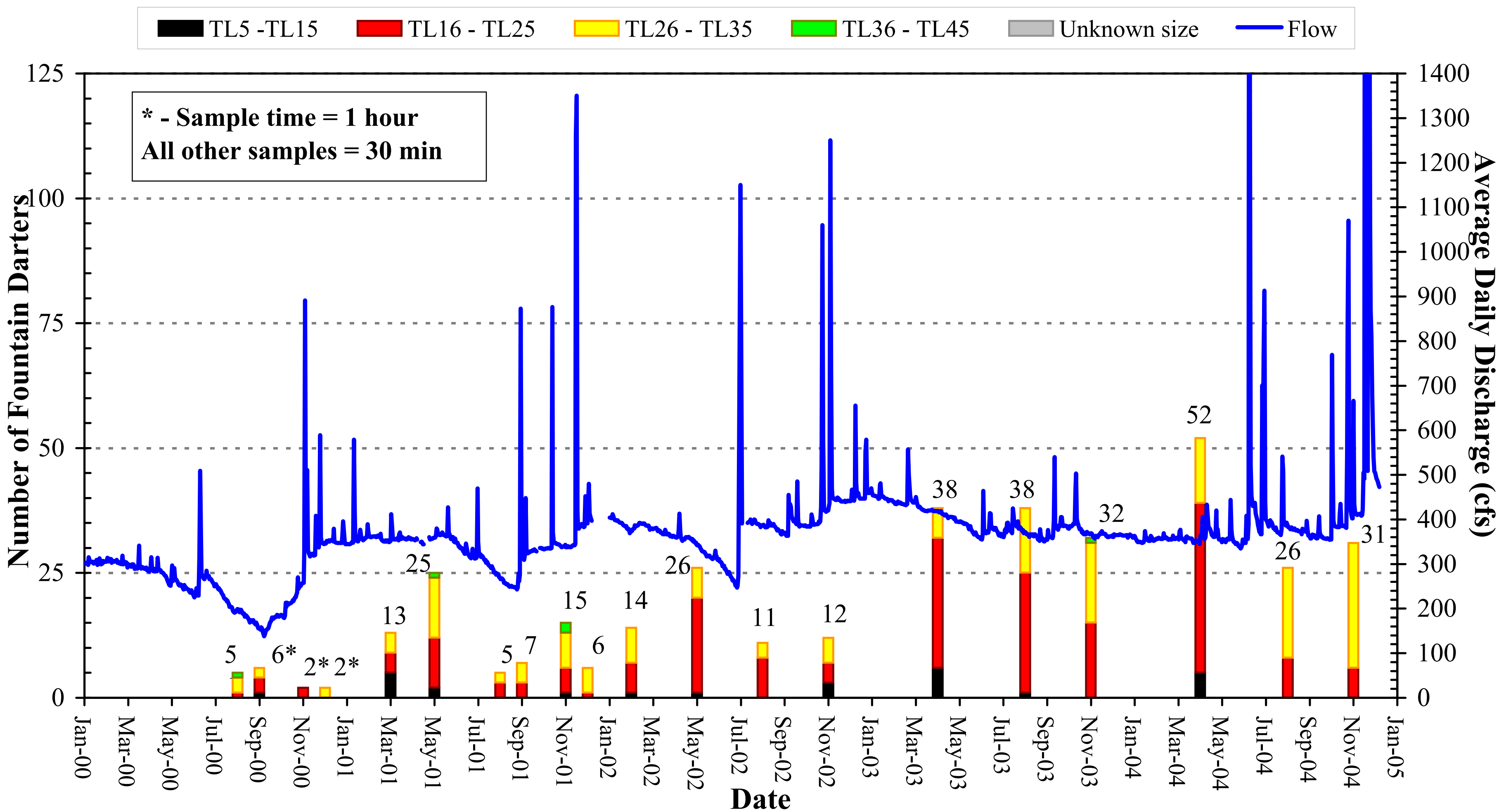
Shannon Weaver diversity index value for each sample date in each sample reach.

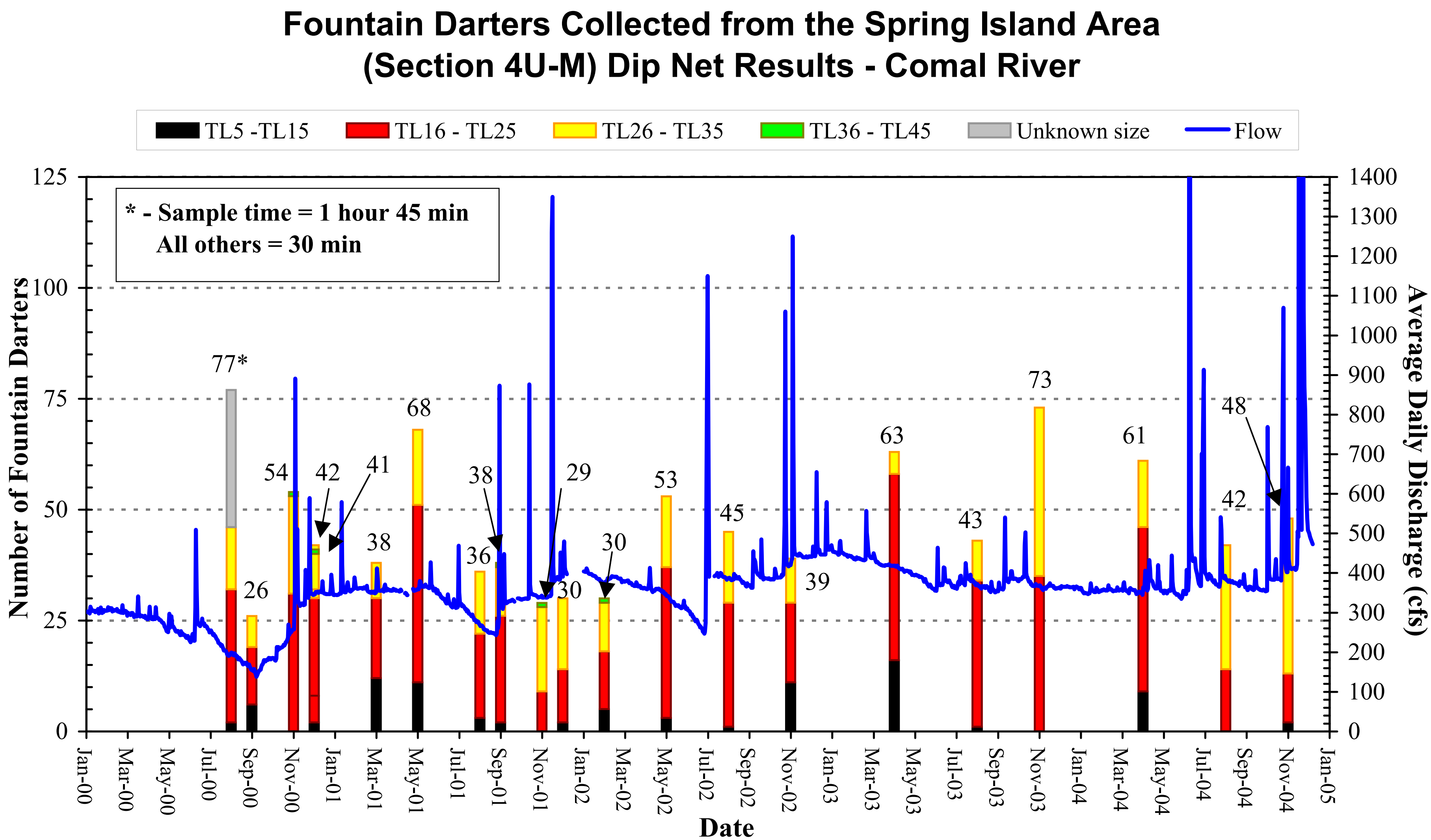


The relationship between mean length of fountain darters and total discharge in the Comal River (2000-2004) was not significant (a) in all reaches combined (p=0.275) or (b) in any reach individually.

Dip Net Graphs

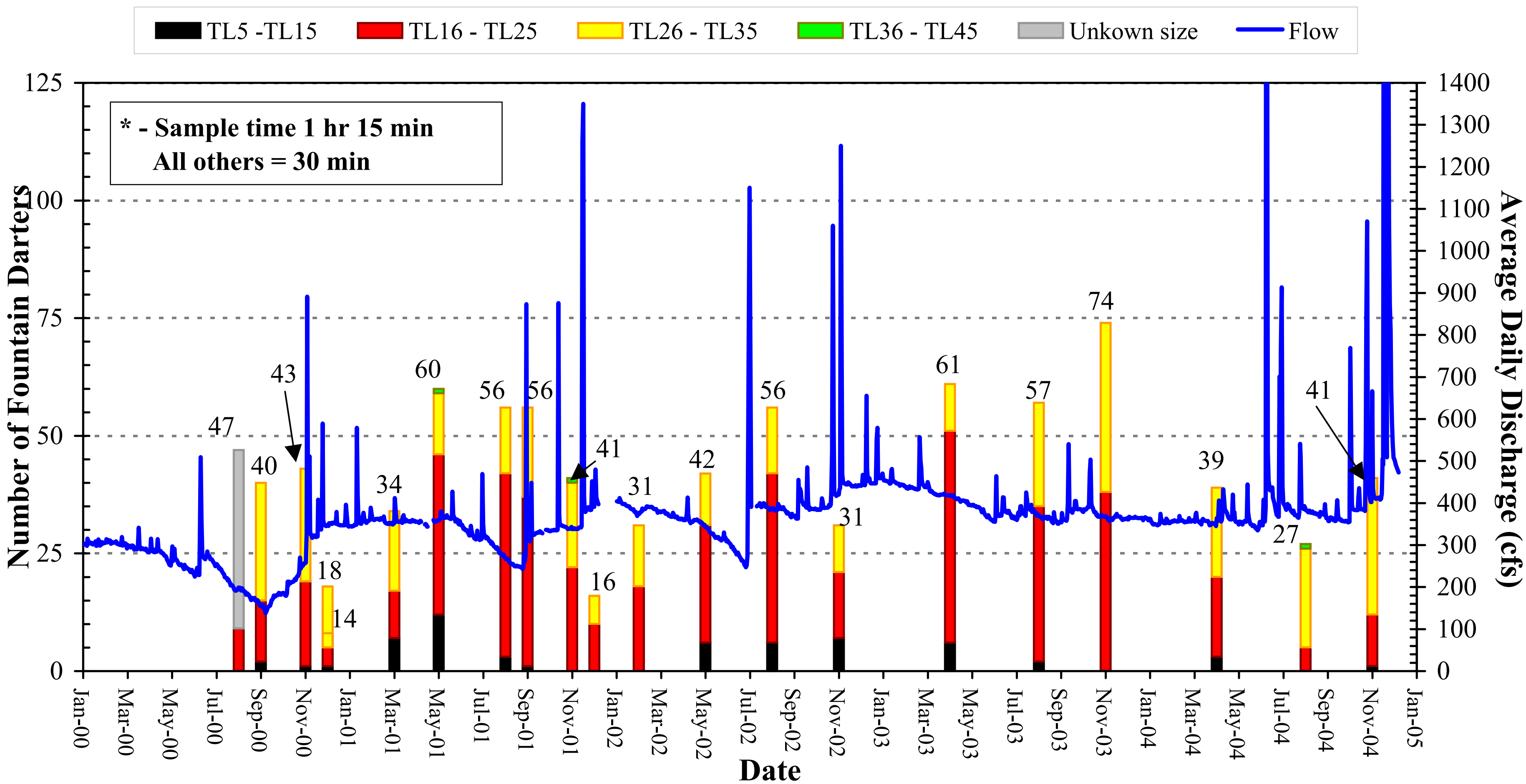
Fountain Darters Collected from the Upper Spring Run Reach (Section 3) Dip Net Results - Comal River

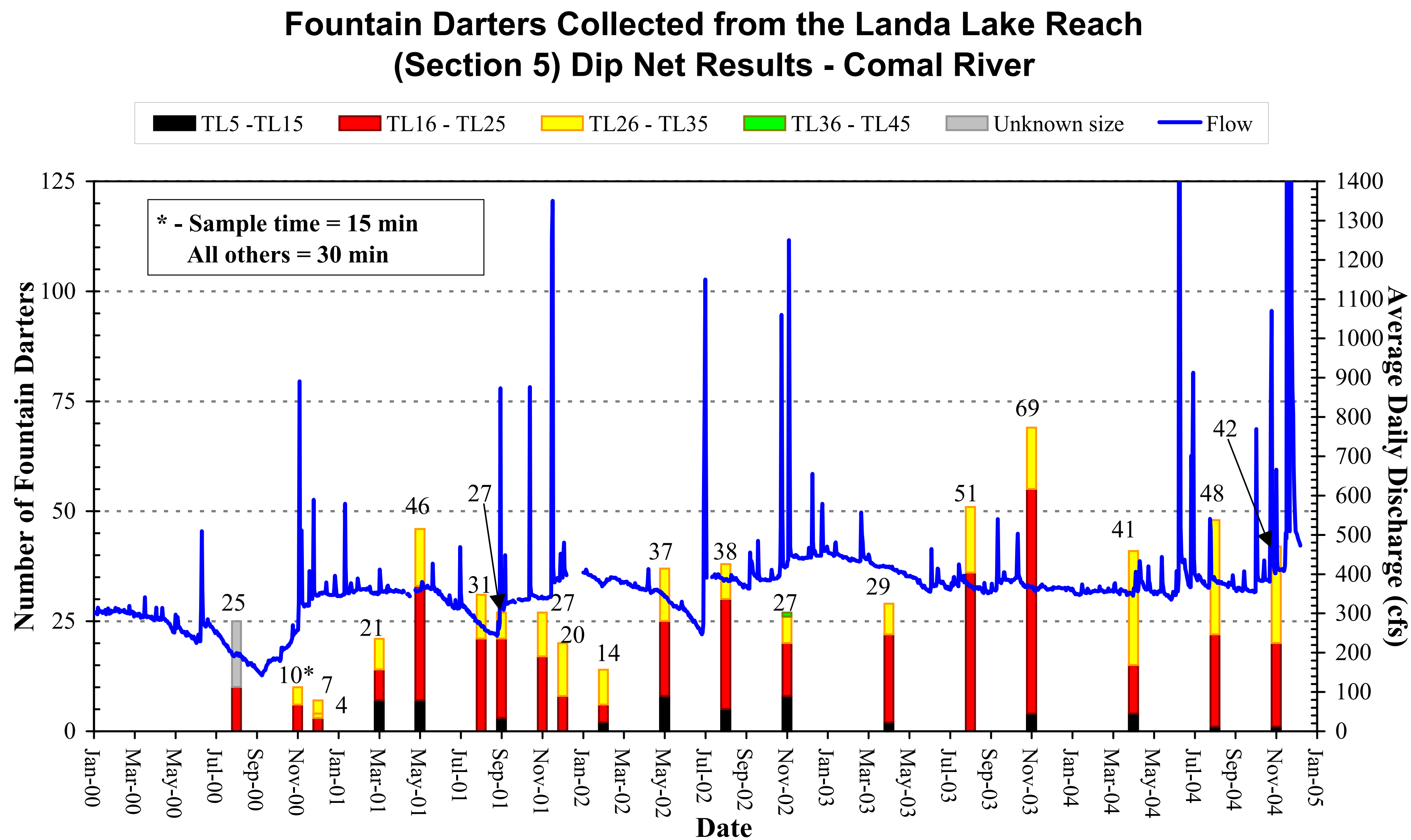




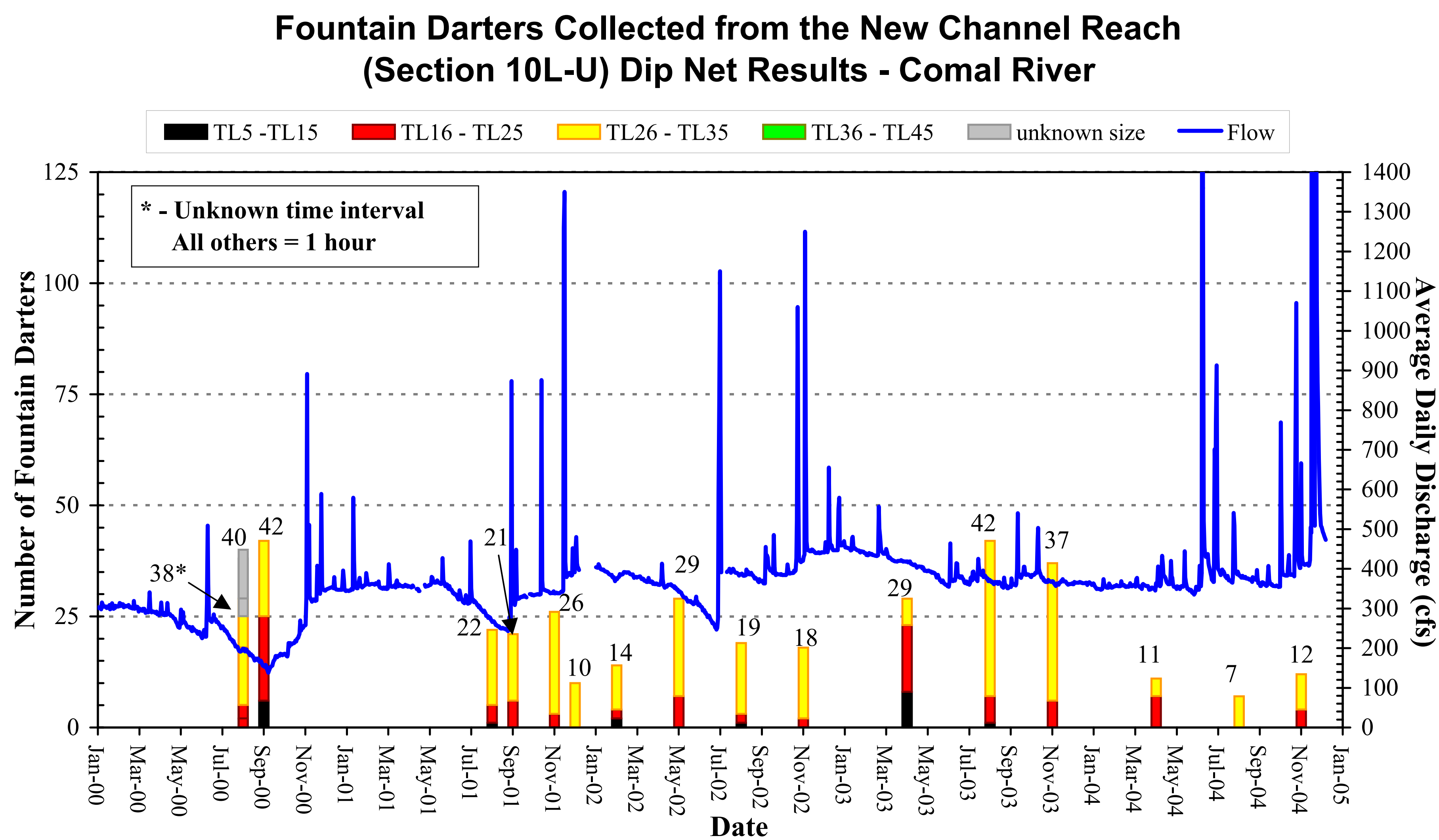
Fountain Darters Collected from the Spring Island Area
(Section 4U-M) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
Unknown size
Flow
* - Sample time = 1 hour 45 min
All others = 30 min
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
77*
26
54
42
41
38
68
36
38
29
30
30
53
45
39
63
43
73
61
42
48
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Jan-05

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

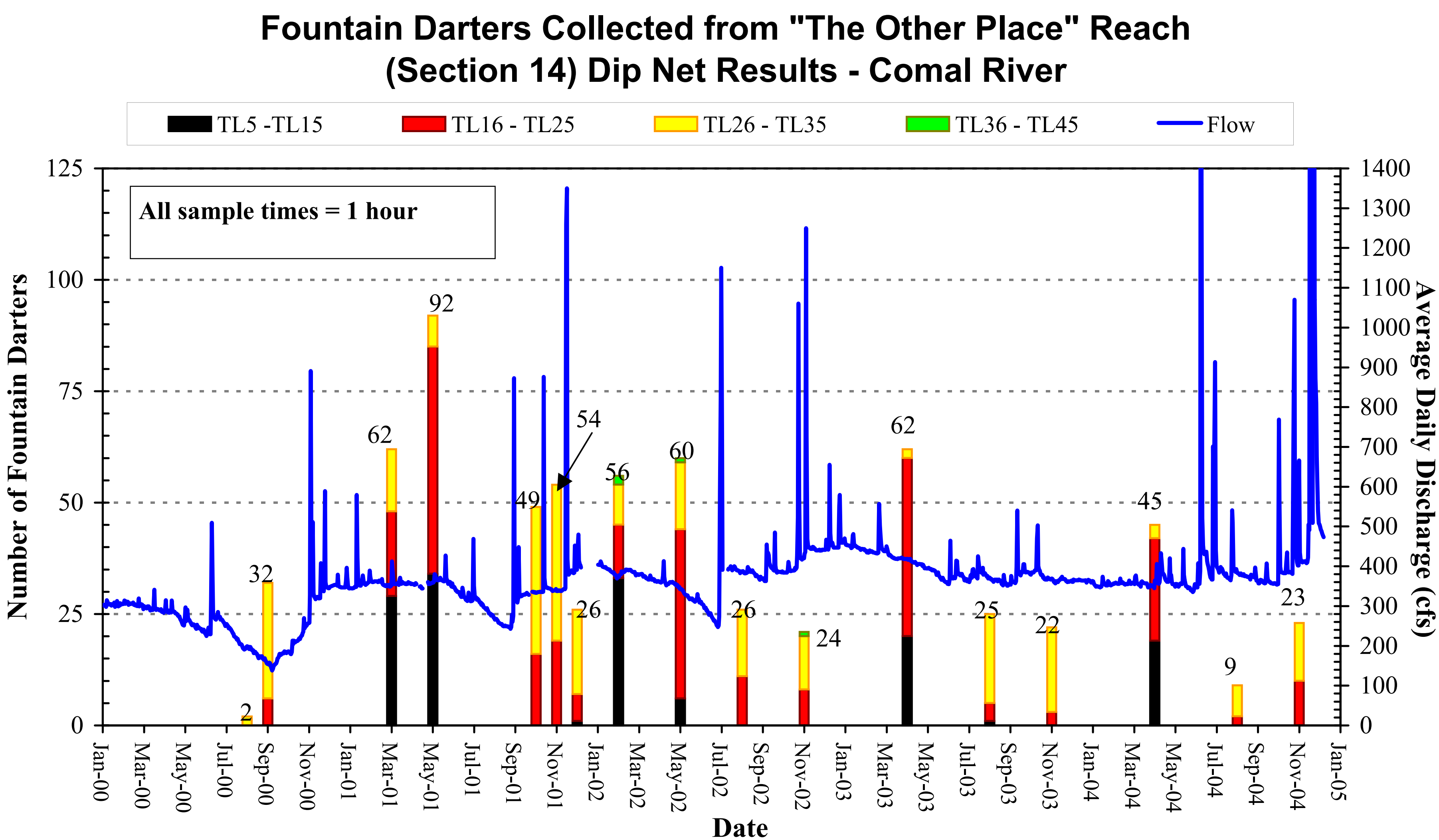




Fountain Darters Collected from the Landa Lake Reach
(Section 5) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
Unknown size
Flow
* - Sample time = 15 min
All others = 30 min
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
125
100
75
50
25
0
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
25
10*
7
4
21
46
27
31
27
20
14
37
38
27
29
51
69
41
48
42
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Jan-05



Fountain Darters Collected from the New Channel Reach
(Section 10L-U) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
unknown size
Flow
* - Unknown time interval
All others = 1 hour
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
38*
40
42
22
21
26
10
14
29
19
18
29
42
37
11
7
12
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Jan-05


Fountain Darters Collected from "The Other Place" Reach
(Section 14) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
Flow
All sample times = 1 hour
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
0
25
50
75
100
125
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
2
32
62
92
49
54
26
56
60
26
24
62
25
22
45
9
23
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
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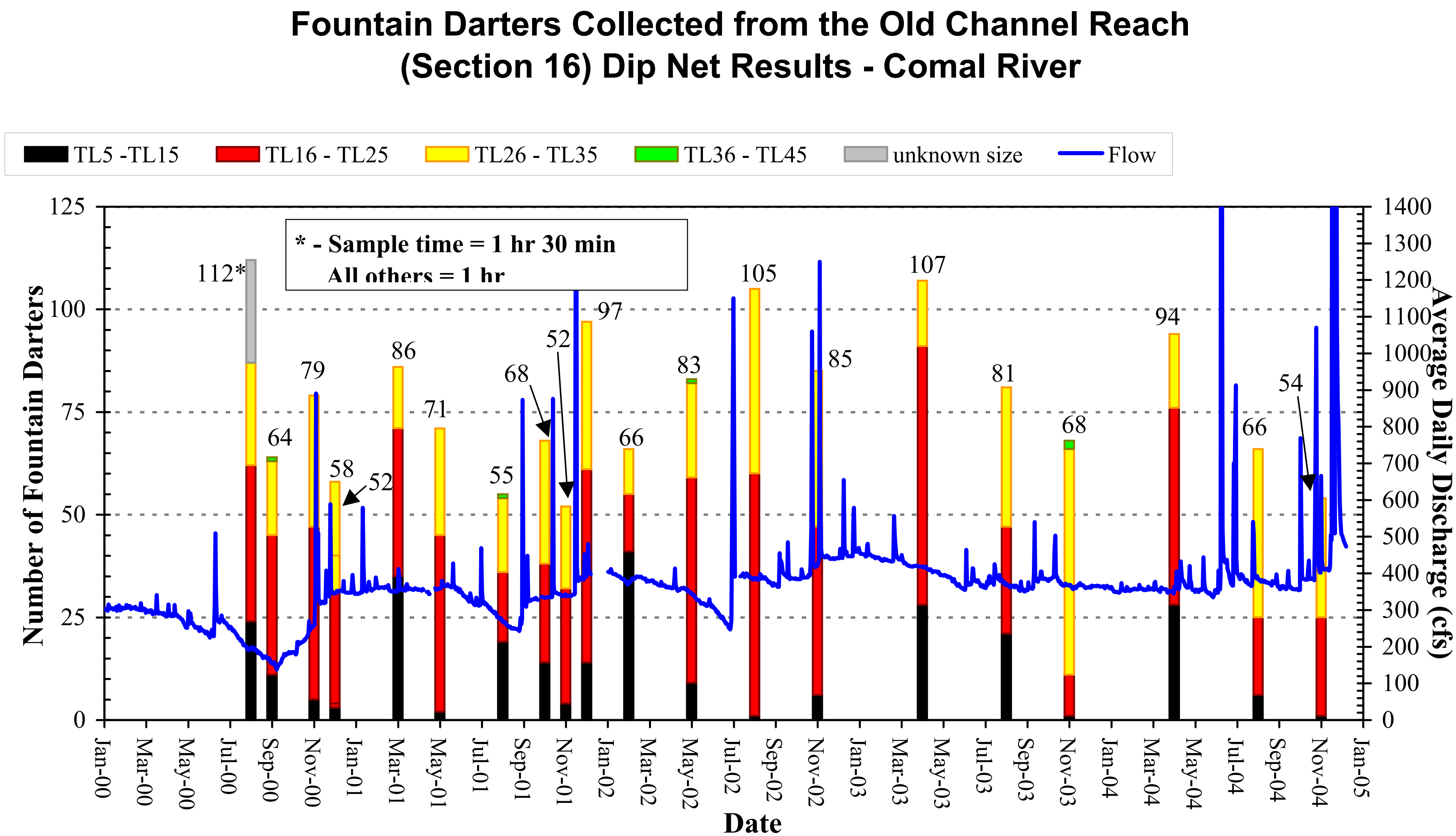
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Jan-05



Fountain Darters Collected from the Old Channel Reach
(Section 16) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
unknown size
Flow
* - Sample time = 1 hr 30 min
All others = 1 hr
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
112*
64
79
58
52
86
71
55
68
52
97
66
83
105
85
107
81
68
94
66
54
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
Nov-02
Jan-03
Mar-03
May-03
Jul-03
Sep-03
Nov-03
Jan-04
Mar-04
May-04
Jul-04
Sep-04
Nov-04
Jan-05

Macroinvertebrate Data:

Drift Rate and Drift Density

Drift rate of each taxa idenitified in 2004 drift net sampling by location and date. Two additional values are presented (bold numbers): the mean drift rate for each taxa during all 2004 samples and the mean drift rate of all organisms captured by date.

	Spring Run 1				Spring Run 3			
	May	Aug	Oct	**Mean**	May	Aug	Oct	**Mean**
Stygobromus pecki	11.5	10	11.5	**8.7**	10.6	8.0	18.0	**12.2**
Stygobromus russelli	2.9	4	2.9	**3.1**	1.9	1.0	2.0	**1.6**
Stygobromus spp.	91.2	54	91.2	**73.4**	61.4	43.0	63.0	**55.9**
All *Stygobromus*	105.6	68	105.6	**85.2**	73.9	52.0	83.0	**69.7**
Mexiweckelia hardeni	6.7	7	6.7	**9.0**	6.7	5.0	8.0	**6.6**
Seborgia relicta	0.0	1	0.0	**0.7**	0.0	0.0	1.0	**0.3**
Artesia subterranea	0.0	1	0.0	**0.7**	1.0	0.0	0.0	**0.3**
Lirceolus (2spp.)	29.8	42	29.8	**35.1**	6.7	9.0	33.0	**16.1**
Cirolanides texensis	0.0	0	0.0	**0.0**	0.0	0.0	0.0	**0.0**
Comaldessus stygius	0.0	1	0.0	**0.3**	0.0	1.0	3.0	**1.3**
Haideoporus texanus	0.0	0	0.0		0.0	3.0	6.0	**3.0**
Stygoparnus comalensis	0.0	3	0.0	**1.7**	0.0	2.0	3.0	**1.6**
Heterelmis comalensis	2.9	0	2.9	**2.8**	0.0	1.0	5.0	**2.0**
All Troglobitic & endangered fauna	**150.7**	**135.0**	**150.7**	**144.3**	**90.2**	**76.0**	**148.0**	**104.5**

	Upwelling - West Shoreline				All Sites Combined			
	May	Aug	Oct	**Mean**	May	Aug	Oct	**Mean**
Stygobromus pecki	35.5	45.0	28.0	**36.2**	19.2	21.0	17.3	**19.2**
Stygobromus russelli	0.0	0.0	0.0	**0.0**	1.6	1.7	1.4	**1.6**
Stygobromus spp.	139.2	117.0	147.0	**134.5**	97.3	71.3	96.0	**88.2**
All *Stygobromus*	174.7	162.0	175.0	**170.6**	118.1	94.0	114.7	**108.9**
Mexiweckelia hardeni	1.0	1.0	1.0	**1.0**	4.8	4.3	7.4	**5.5**
Seborgia relicta	0.0	1.0	0.0	**0.3**	0.0	0.7	0.7	**0.4**
Artesia subterranea	0.0	0.0	0.0	**0.0**	0.3	0.3	0.4	**0.3**
Lirceolus (2spp.)	1.9	9.0	4.0	**4.9**	12.8	20.0	22.9	**18.4**
Cirolanides texensis	0.0	1.0	0.0	**0.3**	0.0	0.3	0.0	**0.1**
Comaldessus stygius	0.0	0.0	0.0	**0.0**	0.0	0.7	1.1	**0.6**
Haideoporus texanus	0.0	0.0	0.0	**0.0**	0.0	1.0	2.1	**1.0**
Stygoparnus comalensis	0.0	0.0	0.0	**0.0**	0.0	1.7	1.8	**1.1**
Heterelmis comalensis	0.0	0.0	0.0	**0.0**	1.0	0.3	3.5	**1.6**
All Troglobitic & endangered fauna	**177.6**	**177.0**	**181.0**	**178.5**	**139.5**	**129.3**	**159.5**	**142.4**

Drift densities of each taxa identified in 2004 drift net sampling by location and date. Two additional values are presented (bold numbers): the mean drift density for each taxa during all 2004 samples and the mean drift density of all organisms captured by date.

	Spring Run 1				Spring Run 3			
	May	Aug	Oct	**Mean**	May	Aug	Oct	**Mean**
Stygobromus pecki	0.16	0.09	0.04	**0.09**	0.14	0.11	0.25	**0.17**
Stygobromus russelli	0.04	0.04	0.02	**0.03**	0.03	0.01	0.03	**0.02**
Stygobromus spp.	1.25	0.49	0.76	**0.79**	0.84	0.59	0.86	**0.76**
All *Stygobromus*	1.44	0.62	0.82	**0.91**	1.01	0.71	1.14	**0.95**
Mexiweckelia hardeni	0.09	0.06	0.15	**0.10**	0.09	0.07	0.11	**0.09**
Seborgia relicta	0.00	0.01	0.01	**0.01**	0.00	0.00	0.01	**0.00**
Artesia subterranea	0.00	0.01	0.01	**0.01**	0.01	0.00	0.00	**0.00**
Lirceolus (2spp.)	0.41	0.38	0.34	**0.38**	0.09	0.12	0.45	**0.22**
Cirolanides texensis	0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**
Comaldessus stygius	0.00	0.01	0.00	**0.00**	0.00	0.01	0.04	**0.02**
Haideoporus texanus	0.00	0.00	0.00	**0.00**	0.00	0.04	0.08	**0.04**
Stygoparnus comalensis	0.00	0.03	0.02	**0.02**	0.00	0.03	0.04	**0.02**
Heterelmis comalensis	0.04	0.00	0.06	**0.03**	0.00	0.01	0.07	**0.03**
All Troglobitic & endangered fauna	**2.06**	**1.23**	**1.51**	**1.55**	**1.23**	**1.04**	**2.02**	**1.43**

	Upwelling - West Shoreline				All Sites Combined			
	May	Aug	Oct	**Mean**	May	Aug	Oct	**Mean**
Stygobromus pecki	0.73	0.92	0.77	**0.81**	0.30	0.27	0.25	**0.27**
Stygobromus russelli	0.00	0.00	0.00	**0.00**	0.02	0.02	0.02	**0.02**
Stygobromus spp.	2.86	2.40	4.02	**3.01**	1.50	0.92	1.39	**1.25**
All *Stygobromus*	3.59	3.32	4.79	**3.82**	1.82	1.22	1.66	**1.55**
Mexiweckelia hardeni	0.02	0.02	0.03	**0.02**	0.07	0.06	0.11	**0.08**
Seborgia relicta	0.00	0.02	0.00	**0.01**	0.00	0.01	0.01	**0.01**
Artesia subterranea	0.00	0.00	0.00	**0.00**	0.00	0.00	0.01	**0.00**
Lirceolus (2spp.)	0.04	0.18	0.11	**0.11**	0.20	0.26	0.33	**0.26**
Cirolanides texensis	0.00	0.02	0.00	**0.01**	0.00	0.00	0.00	**0.00**
Comaldessus stygius	0.00	0.00	0.00	**0.00**	0.00	0.01	0.02	**0.01**
Haideoporus texanus	0.00	0.00	0.00	**0.00**	0.00	0.01	0.03	**0.01**
Stygoparnus comalensis	0.00	0.00	0.00	**0.00**	0.00	0.02	0.03	**0.02**
Heterelmis comalensis	0.00	0.00	0.00	**0.00**	0.01	0.00	0.05	**0.02**
All Troglobitic & endangered fauna	**3.64**	**3.63**	**4.95**	**4.00**	**2.15**	**1.68**	**2.31**	**2.02**

APPENDIX C:
DROP NET RAW DATA

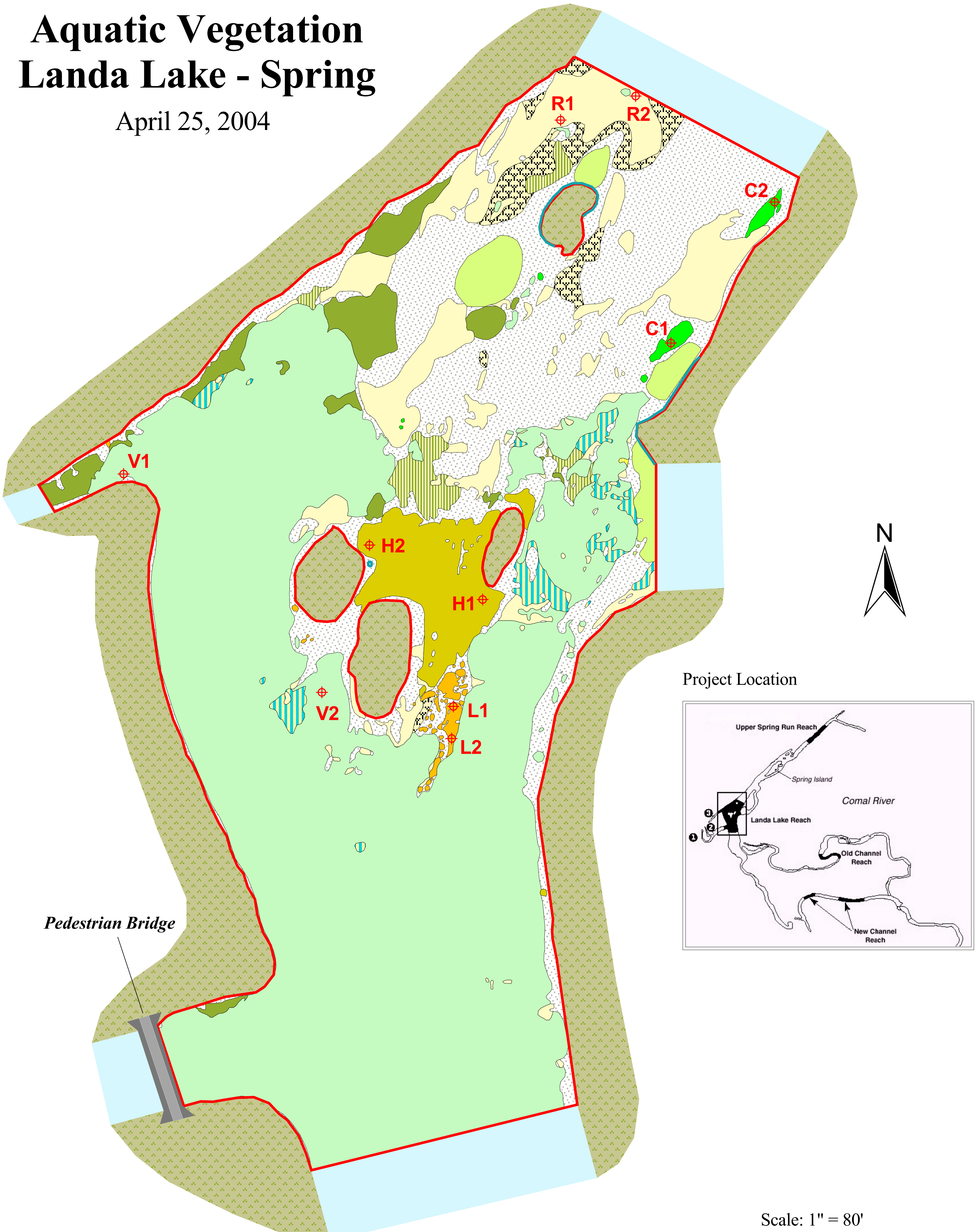
(not available online)

APPENDIX A:
AQUATIC VEGETATION MAPS

Landa Lake Reach

Comal River
Aquatic Vegetation
Landa Lake - Spring

April 25, 2004



Scale: 1" = 80'

10 0 10 20 30 40 Meters

	Meters^2
Cabomba	76.8
Hygrophila	830.0
Ludwigia	99.1
Nuphar	451.7
Bryophytes	1,970.8
Sagittaria	661.6
Vallisneria	12,364.9
Bryophytes < 50%	407.7
Bryophytes / Sagittaria	287.7
Bryophytes / Vallisneria	294.8

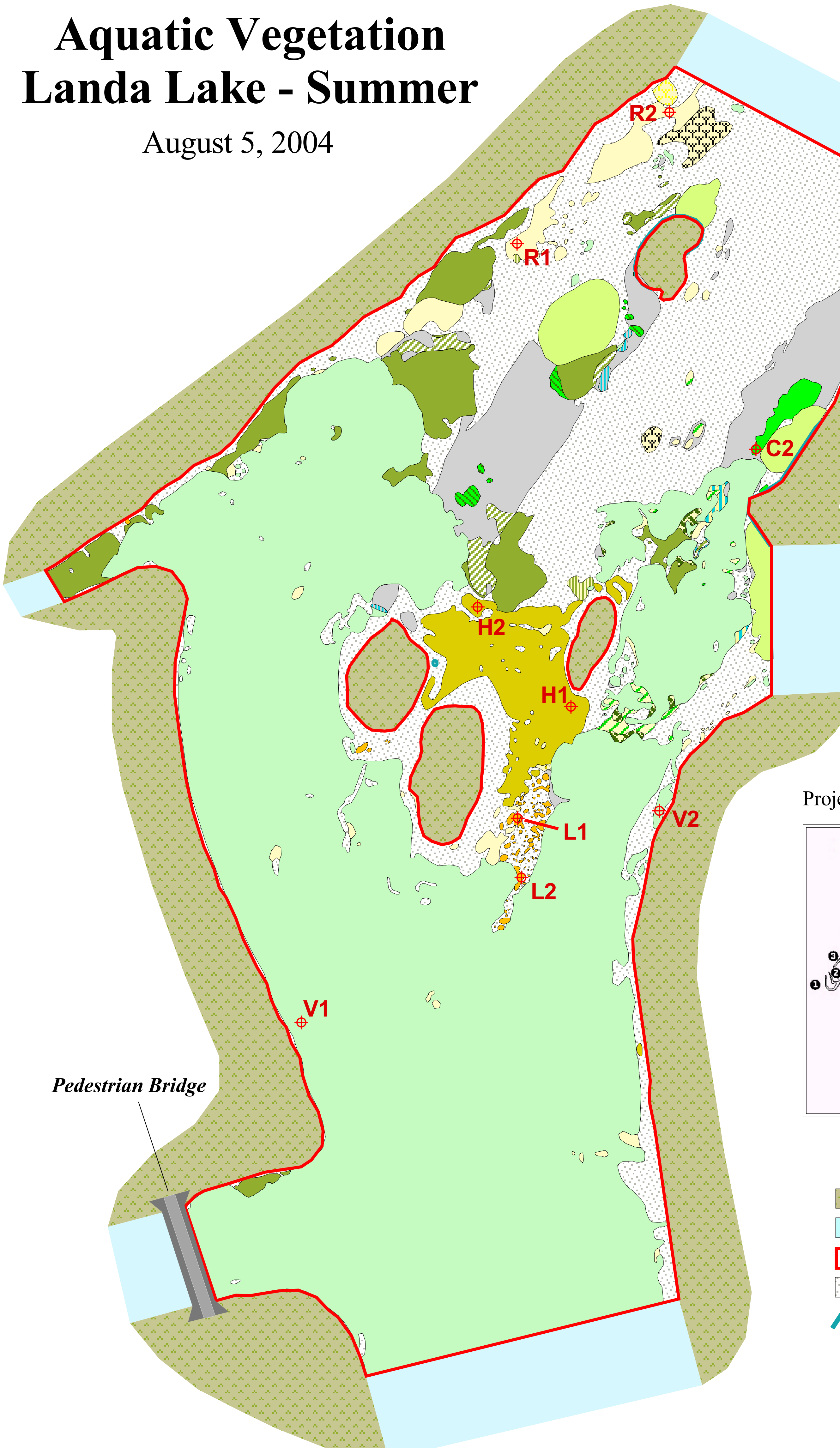
- Shore and Islands
- River
- Study Area (22,551.2 m^2)
- Bare Substrate
- *Colocasia*
- Drop Net Sample Sites

BIO-WEST, Inc.

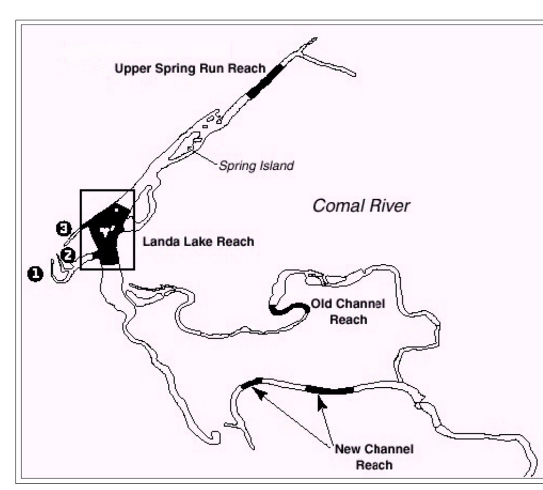
EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Landa Lake - Summer

August 5, 2004



Project Location



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

Scale: 1" = 80'

10 0 10 20 30 40 Meters

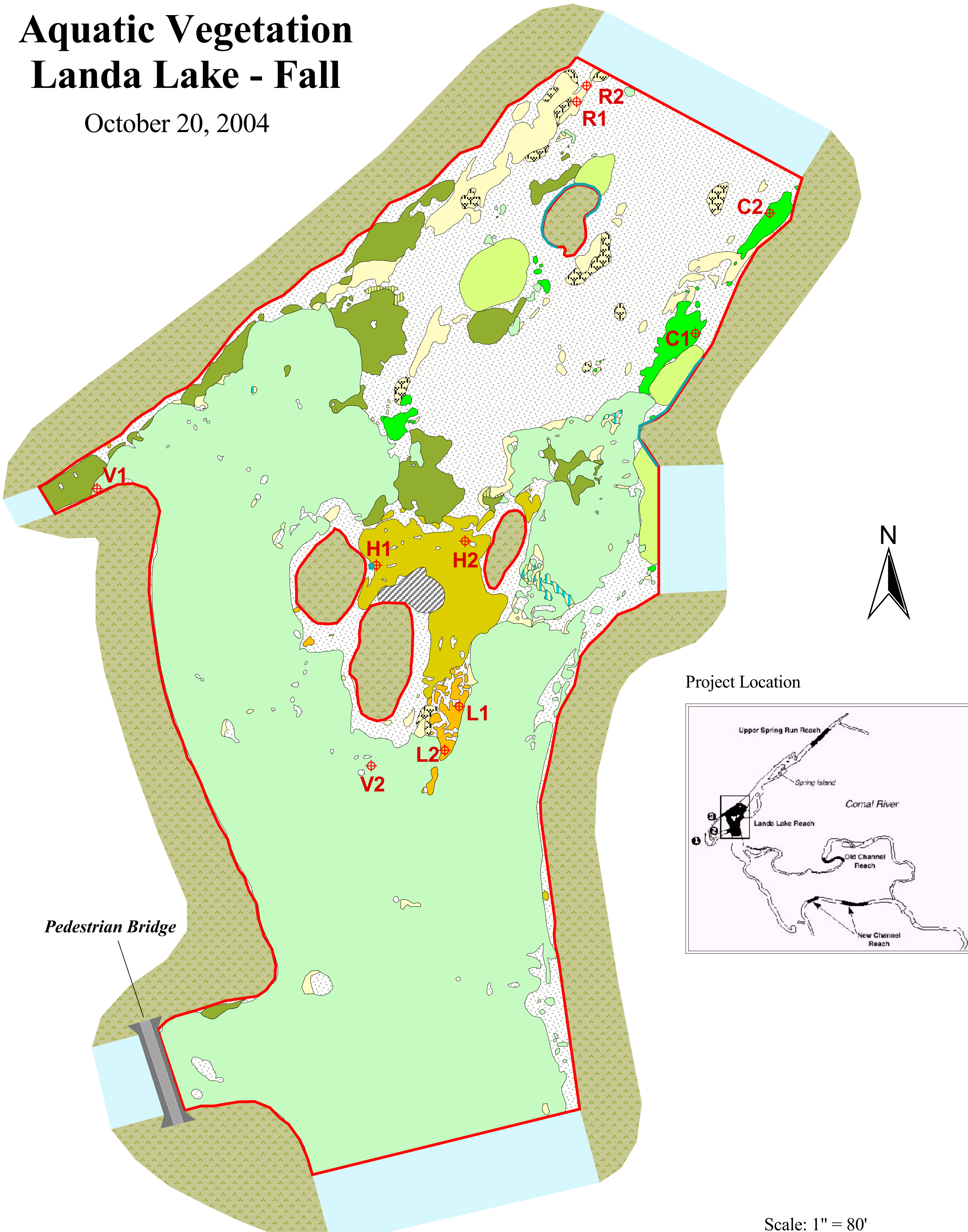
	Meters ^{2}		Meters ^{2}
Cabomba	111.2	*Bryophytes / Vallisneria*	17.6
Hygrophila	683.7	*Bryophytes / Hygrophila*	0.8
Ludwigia	48.8	*Bryophytes / Algae*	59.6
Nuphar	451.0	*Algae / Cabomba*	32.6
Bryophytes	400.1	*Algae / Sagittaria*	119.4
Sagittaria	855.9	*Algae / Vallisneria*	17.8
Vallisneria	12,597.3	*Algae / Vallisneria / Bryophytes*	35.5
Algae	1,267.8		
Bryophytes < 50%	112.5		
Bryophytes / Sagittaria	17.8		

BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Landa Lake - Fall

October 20, 2004



Scale: 1" = 80'

10 0 10 20 30 40 Meters

	Meters ^{2}
Floating vegetation mat	99.4
Cabomba	233.1
Hygrophila	684.8
Ludwigia	98.1
Nuphar	461.2
Bryophytes	735.0
Sagittaria	1,038.0
Vallisneria	12,721.1
Bryophytes < 50%	162.6
Bryophytes / Sagittaria	15.3
Bryophytes / Vallisneria	34.5

Shore and Islands
River
Study Area (22,551.2 m ^{2})
Bare Substrate
Colocasia
Drop Net Sample Sites

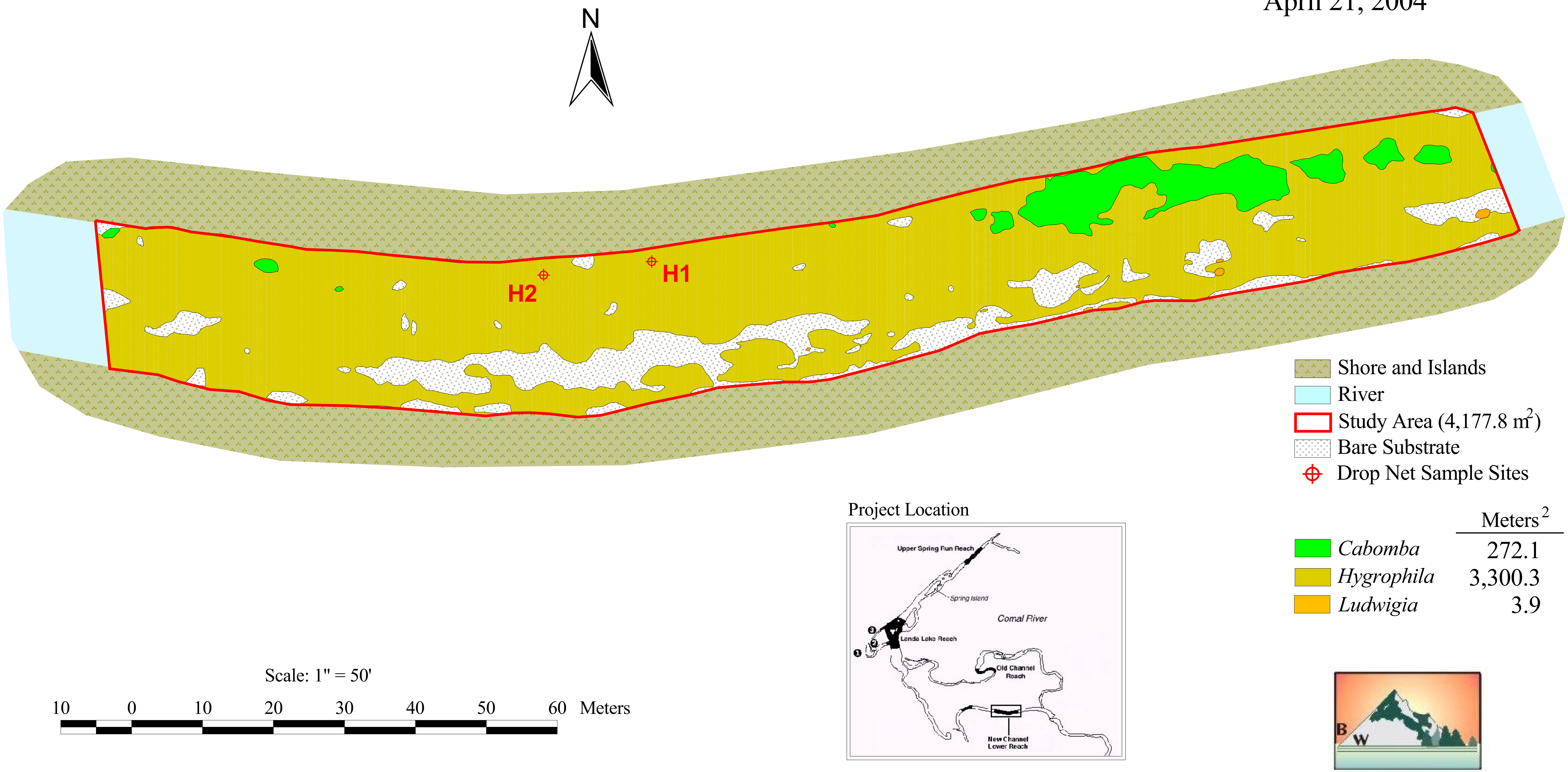
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

New Channel Reach

Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring

April 21, 2004

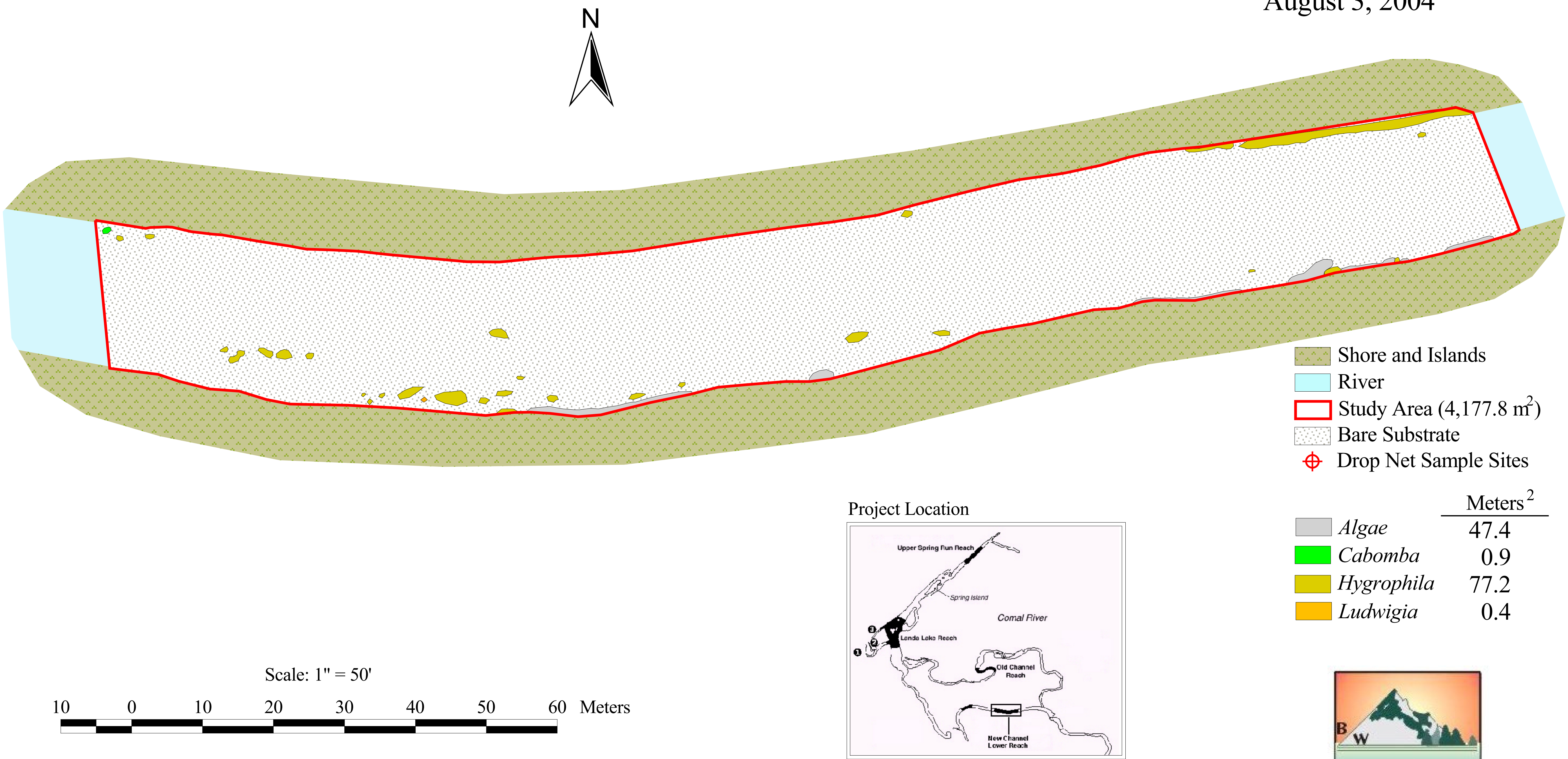


BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
New Channel Lower Reach
Summer

August 3, 2004

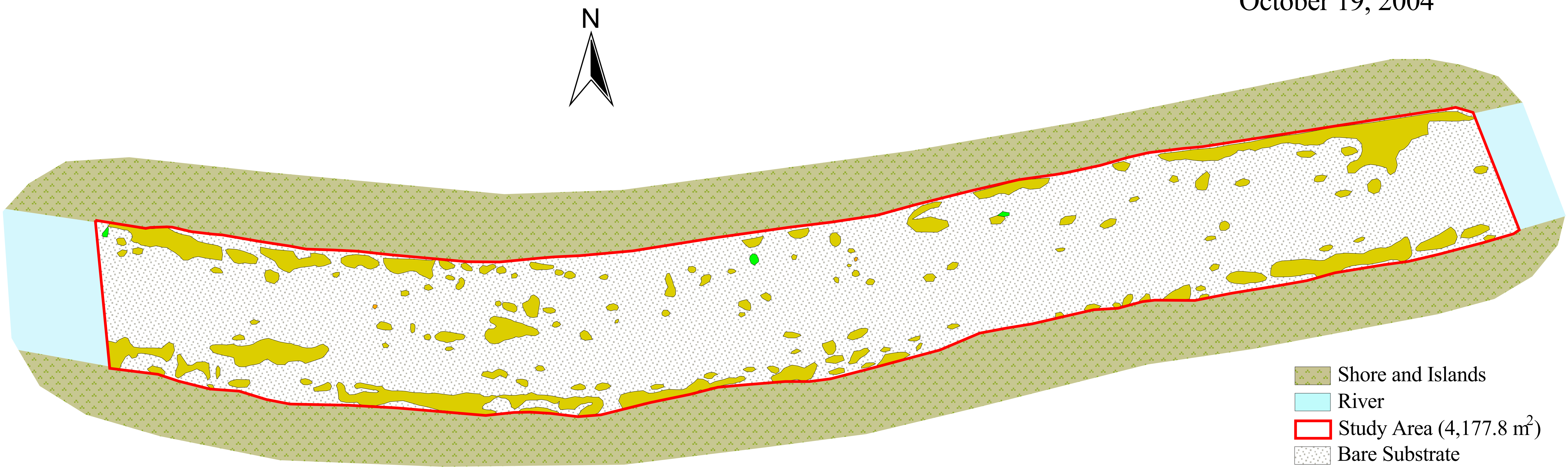


BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
New Channel Lower Reach
Fall

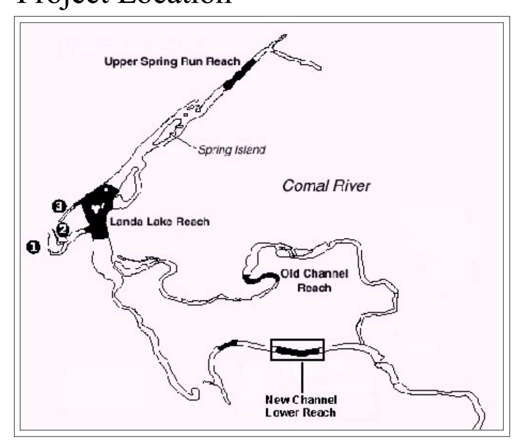
October 19, 2004



Shore and Islands
River
Study Area (4,177.8 m^2)
Bare Substrate
Drop Net Sample Sites

	Meters^2
Cabomba	3.1
Hygrophila	619.6
Ludwigia	0.5

Project Location



Scale: 1" = 50'

10 0 10 20 30 40 50 60 Meters

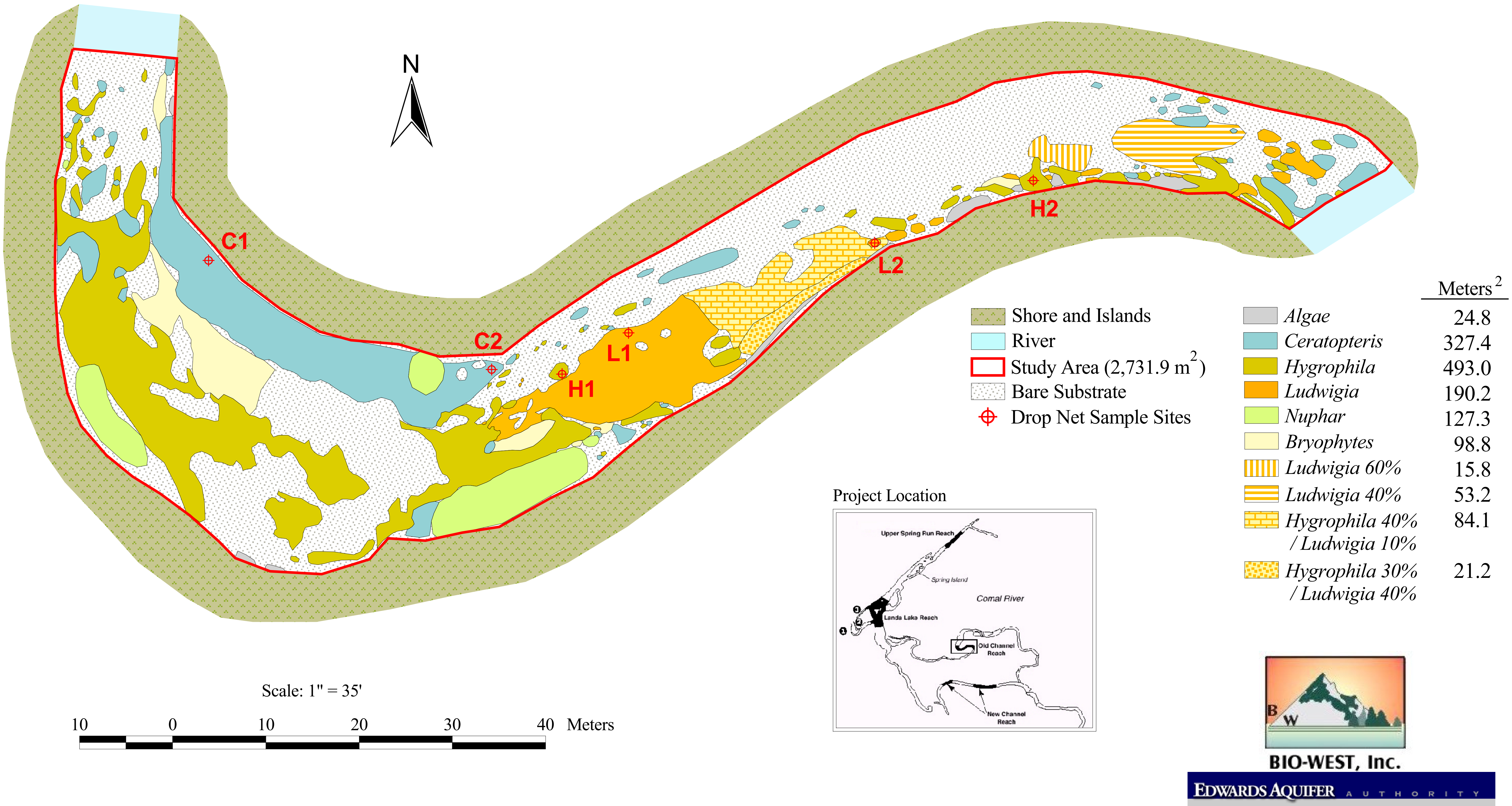
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Old Channel Reach

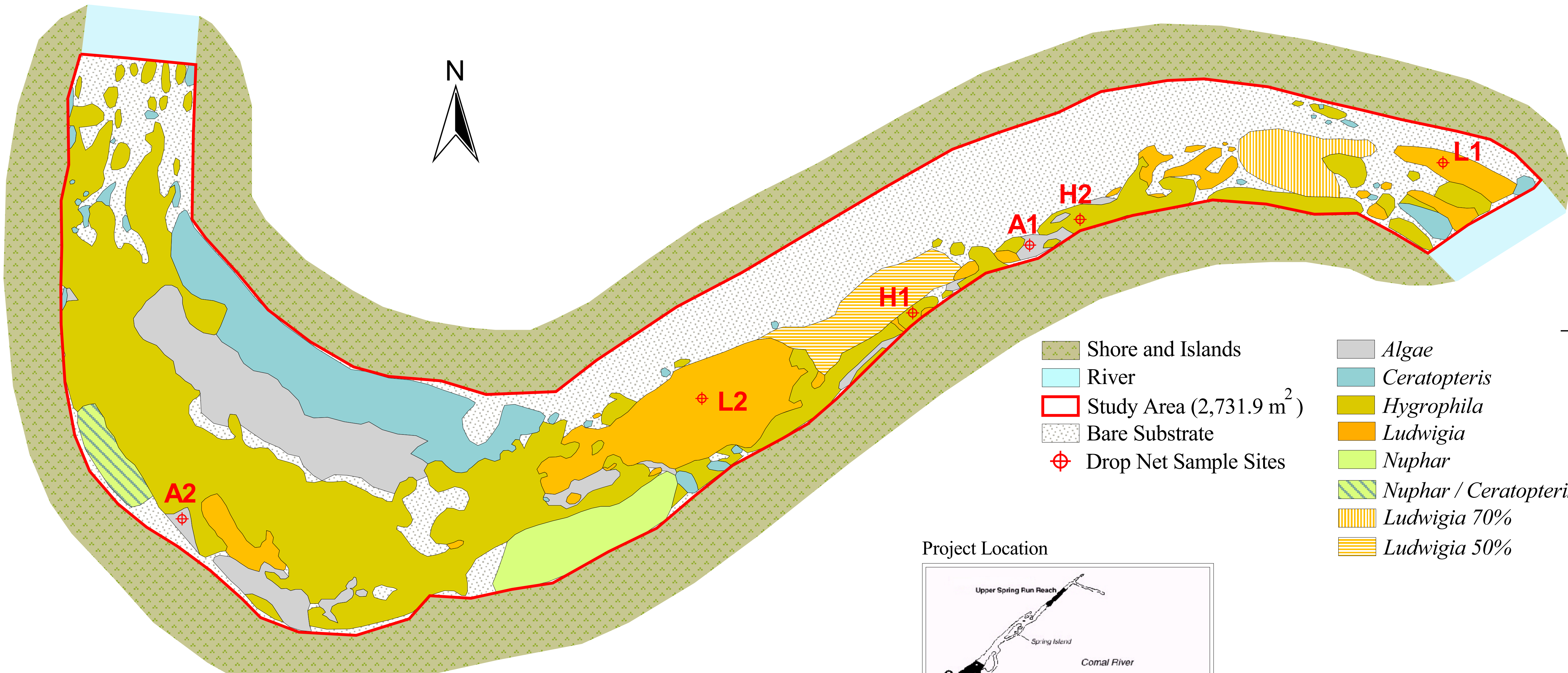
Comal River
Aquatic Vegetation
Old Channel Reach - Spring

April 21, 2004



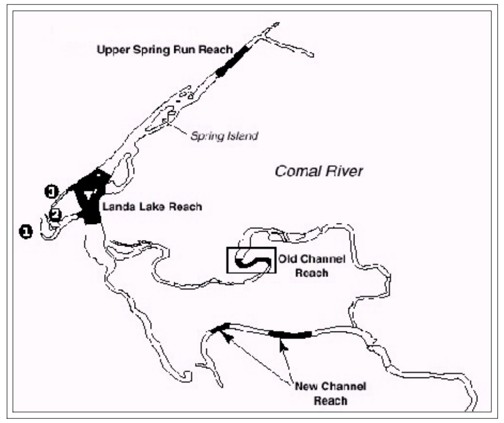
Comal River
Aquatic Vegetation
Old Channel Reach - Summer

August 4, 2004



		Meters^2
Shore and Islands	*Algae*	230.1
River	*Ceratopteris*	233.0
Study Area (2,731.9 m^2)	*Hygrophila*	862.0
Bare Substrate	*Ludwigia*	260.4
Drop Net Sample Sites	*Nuphar*	84.3
	Nuphar / Ceratopteris	30.4
	Ludwigia 70%	53.9
	Ludwigia 50%	74.7

Project Location



Scale: 1" = 35'

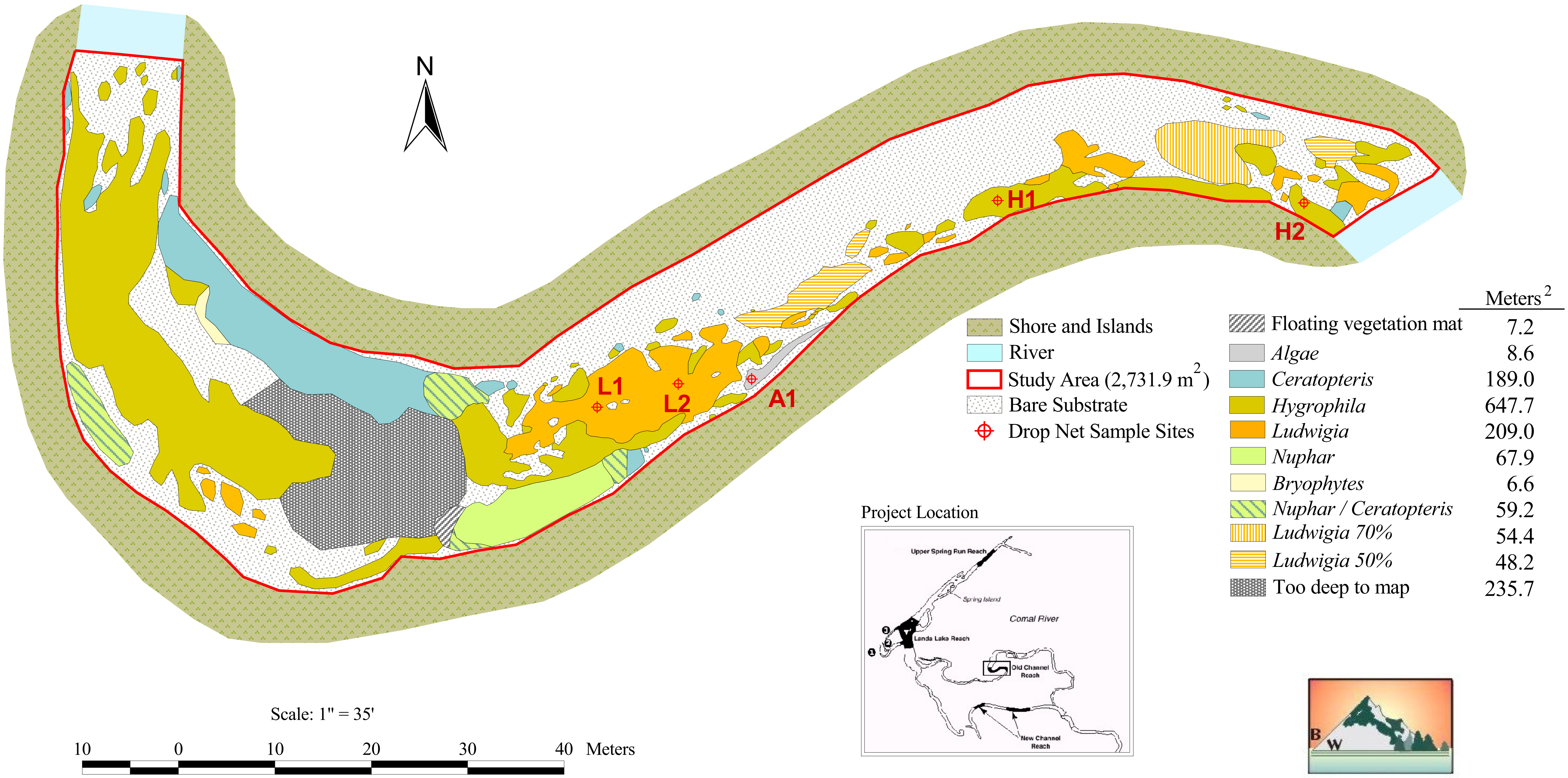
10 0 10 20 30 40 Meters

BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Old Channel Reach - Fall

October 21, 2004

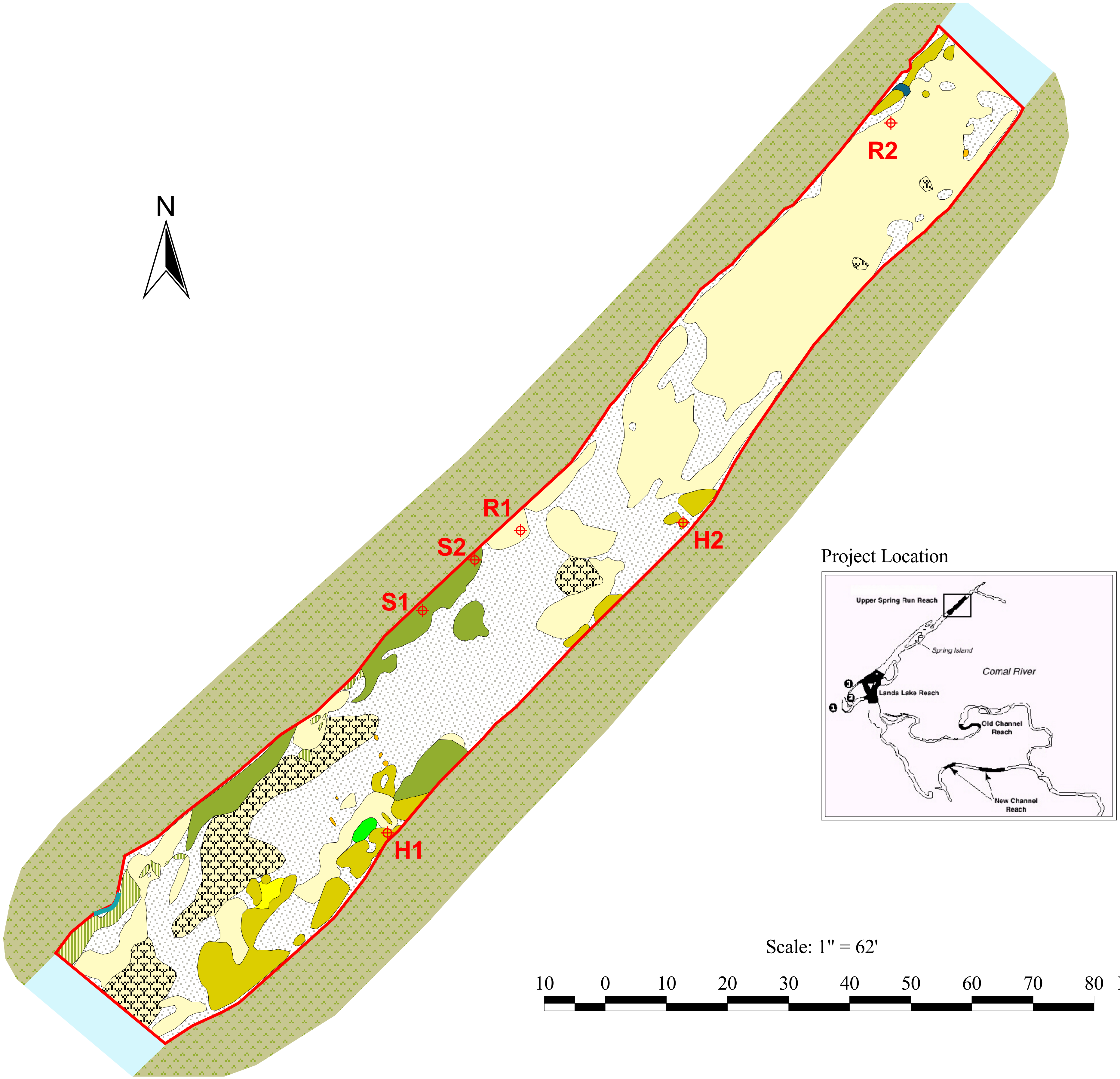


BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Upper Spring Run Reach

Comal River
Aquatic Vegetation
Upper Spring Channel Reach
Spring
April 22, 2004



Shore and Islands	
River	
Study Area (4,723.5 m^2)	
Bare Substrate	
Colocasia	
Drop Net Sample Sites	

	Meters ^{2}
Cabomba	9.2
Hygrophila	293.9
Limnophila	3.2
Ludwigia	3.3
Bryophytes	1,859.3
Sagittaria	252.5
Bryophytes < 50%	517.0
Bryophytes / Sagittaria	63.7

Scale: 1" = 62'

10 0 10 20 30 40 50 60 70 80 Meters

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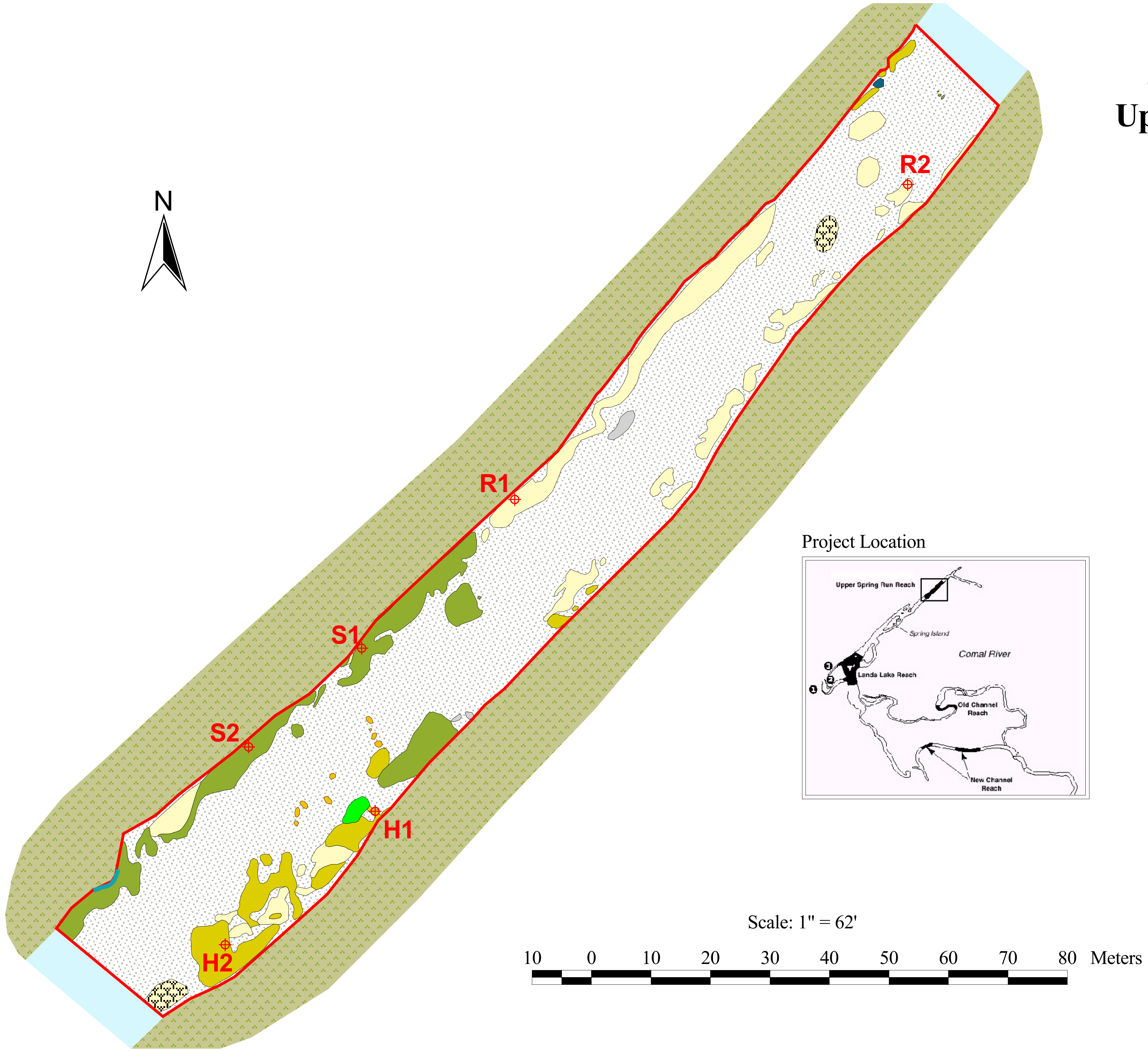
EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Upper Spring Channel Reach
Summer
August 3, 2004



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

	Meters^2
Algae	12.3
Cabomba	12.3
Hygrophila	223.9
Limnophila	2.1
Ludwigia	7.3
Bryophytes	481.4
Sagittaria	370.8
Bryophytes < 50%	42.6

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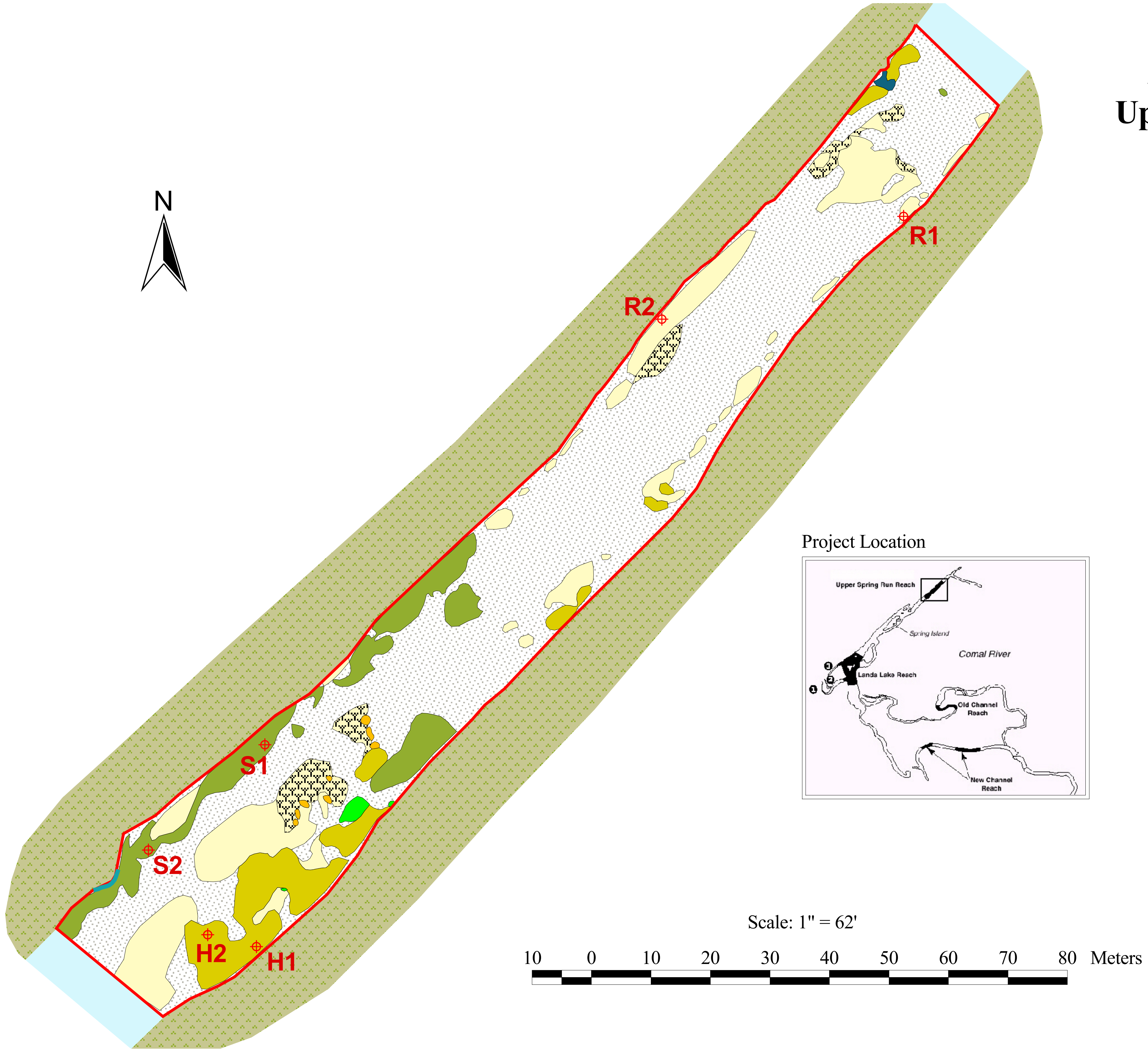
EDWARDS AQUIFER AUTHORITY

Comal River Aquatic Vegetation

Upper Spring Channel Reach Fall

October 19, 2004



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

	Meters^2
Cabomba	14.1
Hygrophila	361.0
Limnophila	6.2
Ludwigia	10.9
Bryophytes	712.0
Sagittaria	399.8
Bryophytes < 50%	161.0

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EDWARDS AQUIFER AUTHORITY