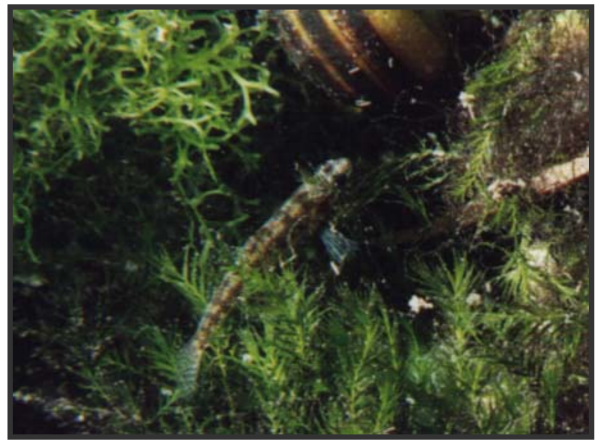
Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem

FINAL 2005 ANNUAL REPORT



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EXECUTIVE SUMMARY

This annual summary report presents a synopsis of methodology used and an account of sampling activities, including sample conditions, locations and raw data obtained during two quarterly sampling events (Comprehensive Monitoring Effort), conducted on the Comal Springs/River ecosystem in 2005. There were no low-flow critical periods or high-flow events triggered in 2005. The data are reported here in an annual report format similar to previous reports; we have not been able to acquire the necessary range of data from all flow levels (specifically low-flow) to conduct stringent data reduction techniques or statistical applications. These techniques will be applied once the appropriate data have been gathered to allow for a complete assessment of variable flow dynamics and will be included in a final report to the Edwards Aquifer Authority.

Flows were consistently higher than that of the average historical record for the entire year in the Comal River. Significant rainfall events in 2004 have maintained aquifer levels and yielded higher than normal (compared to historical data) flows in 2005. Flows peaked in March and continued to decline thereafter into the summer when the lowest flows were recorded for the year. Discharge measurements in the Old Channel and Spring Runs revealed that 17 – 18% of the total discharge passed down the Old Channel during each sampling period in 2005. It was also revealed that 30 – 35% of the total discharge originated in Spring Runs 1 and 3, while 1.5 – 2.5% was generated from Spring Run 2. If flows continue to decline in 2006, there may be critical period conditions that initiate low-flow sampling efforts in the Comal River. Overall water quality was assessed quarterly throughout the entire Comal River during 2000-2002, and data from those years can be found in those reports (BIO-WEST 2002a, 2003). Thermistor data, collected from 2000-2005, have revealed a high degree of thermal uniformity throughout the Comal River ecosystem, despite the wide-ranging flow conditions experienced during the study. In many places the temperature remains nearly constant due to nearby spring inputs, while other locations (typically further away from spring influences) are more substantially influenced by atmospheric conditions. As has been observed in past years, the range of water temperatures in several sites is around 1ºC or less, except for a few acute peaks/troughs. Overall, the Heidelberg site in the Upper Spring Run reach, the deep portions of Landa Lake, and the Spring Runs maintained a narrow range of temperatures in 2005. Most sites did not exceed the water quality standards value of 26.67°C during 2005, except for Blieder's Creek, which is not directly influenced by spring inputs and showed a much larger temperature range than other sites. As with other components of this study, more data are needed to determine the potential impacts of high air temperatures and low flows during an extended period of reduced recharge.

For the fountain darter (*Etheostoma fonticola*), habitat use is largely influenced by aquatic vegetation, and assessments of habitat availability were conducted by mapping this vegetation during each sampling event. Overall, vegetation coverage increased at all sites following the high-flows of 2004. 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 Upper Spring Run reach remained relatively stable, but did increase substantially in bryophyte (*Amblystegium* and *Riccia*) coverage (an important fountain darter habitat component). Fragmentation of important (to fountain darters) vegetation types in the Landa Lake reach led to decreases in *Ludwigia* and *Hygrophila* coverage in 2005. Filamentous algae were still rare in the Old Channel reach, which is unfortunate because it typically contains high densities of fountain darters. Prior to 2003, filamentous algae dominated this reach and provided the highest quality habitat, but since then *Hygrophila* and *Ludwigia* have been the dominant vegetation in this reach. The New Channel

reach underwent drastic changes in vegetation coverage from 2004. By spring 2005, *Ludwigia* was completely eradicated (and has yet to re-establish) and *Hygrophila* only covered about 3% of what it did in fall 2004. This substantial decrease in vegetation coverage was the result of several high-flow events in the Comal River in 2004. The New Channel is highly susceptible to these scouring events because of its lack of complexity. 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 using aquatic vegetation to stratify random sample locations. Filamentous algae and bryophytes continue to support the highest numbers of darters in all vegetation types sampled. Although fountain darter densities are slightly less in bryophytes than in filamentous algae, bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake Reaches, and thus make up a significant portion of the available habitat. Filamentous algae and bryophytes have high densities of fountain darters because they provide cover at the substrate level and high numbers of amphipods (an important food item). *Cabomba* and *Ludwigia* are also important vegetation types for darters, but are far less abundant than exotic plants like *Hygrophila* that maintain lower darter densities. Overall, the size-class distribution for fountain darters collected during the study period is typical of a healthy fish population with a peak near 30 millimeters (mm) total length (TL). Size-class distributions of fountain darters in the Old Channel Reach and Upper Spring Run Reach demonstrated that larger fountain darters were more abundant in fall, and smaller darters were more numerous in spring. Although presence of small fountain darters has been noted in all sample seasons suggesting year-round reproduction, increased abundance of small darters in spring samples (from 2005 as well as previous years) suggests a peak in reproduction in early spring.

In 2005, an effort was made to establish a rapid method for assessing changes in fountain darter population abundance between sample efforts, especially during critical periods (high- and low-flow events). Objectives of this portion of the study were to assess the viability of an alternative dip netting method designed to gather presence/absence data at multiple sites within each reach and thereby increase the number of data points that may be collected, reduce the time necessary to collect data at all sites, and reduce habitat disturbance. Fountain darters were present in 71% of sites on day 1 and in 67% of sites on day 2, showing little variation among randomly selected sites. Small amounts of variation within and among sites suggest that this method is repeatable and can be used to detect trends in the fountain darter population. Although it provides no inference into the magnitude of the change, a statistically significant reduction can be observed which may be used to initiate a management response. If correlated with seasonal drop netting data this method could serve as a surrogate technique to provide continuous monitoring, especially during critical low flow periods.

Although not a fish, one exotic species which has had considerable impact on the vegetation community in the Comal Springs/River ecosystem in the past is the giant ramshorn snail (*Marisa cornuarietis*). Densities of these organisms have declined in recent years, and were at their lowest in 2005. However, since these snails can have severe impacts at high densities, their populations will continue to be monitored especially if a critical low-flow period occurs in 2006. In 2005, new methods were tested to help better identify changes in gill parasite drift densities during the year. These new methods will be implemented in 2006 to better understand drift rates of the parasite and infection rates of fountain darters.

All SCUBA/snorkel surveys revealed the presence of Comal Springs salamanders (*Eurycea sp.*) along the Landa Lake bottom and in each sampled Spring Run, except for the Spring Run at Spring Island in fall 2005. No large (> 3 cm) rocks were found in this area in the fall and may have contributed to the lack of salamander observations. 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.

A total of 144 hours of sample time occurred among the three drift net sites at Comal Springs in 2005 and 11 species were captured. Among species of concern, an average of 14.5 *Stygobromus pecki* (Peck's cave amphipod, many small *Stygobromus* were unidentifiable to species), 3.2 *Heterelmis comalensis* (Comal Springs riffle beetle), and 1.3 *Stygoparnus comalensis* (Comal Springs dryopid beetle) were retrieved during each 24-hour period at all sites. *S. pecki* was found in all three locations. On average the highest number were found in the western shoreline habitat than the other two locations, but in October more were collected in Spring Run 3. *H. comalensis* and *S. comalensis* were found in Spring Runs 1 and 3 but not in the western shoreline habitat of Landa Lake, where a single *S. comalensis* was collected in 2003 and no *H. comalensis* have been found. During 2005, most species had a higher abundance at all sites during the October sample compared to the April sample. Because discharge was approximately the same during each sample (with no recent "spikes" in the hydrograph) it is not likely that these differences are related to discharge, but may represent natural or seasonal variability in these populations. A total of 1,009 Comal Springs riffle beetles were collected in all 2005 samples and the number of larvae (n=227) was far fewer than the number of adults (n=782). Despite the physical differences between sites, there were minimal differences in abundance among sites in 2005. The relatively even distribution of Comal Springs riffle beetles among the three sample areas reveals that the beetles are not just confined to the spring runs, but occur in large numbers in a variety of habitats. The sampling methodology used in 2004-2005 has revealed a much larger population of Comal Springs riffle beetle along the western shoreline of Landa Lake than previous sampling had suggested. Although there were similar numbers of beetles sampled among sites, there were differences within sites. In many cases samples within a site that were geographically close had large differences in total abundance of beetles sampled. These data suggest that the species has precise requirements (e.g., physical characteristics or food source) that govern its distribution.

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 periods of low-flow (particularly from an extended period of low-flow) are essential to fully evaluate the biological risks associated with future critical periods (high or low-flow). Fortunately, the Authority's Variable Flow monitoring program includes an extensive data-collection protocol for periods of critical flow that should address this current limitation. In the interim, efforts to evaluate response mechanisms of the threatened and endangered species to low-flow conditions either via laboratory investigation (as conducted in previous years under this program) or via field (*in situ*) experiments as proposed with the "intensive management areas" concept would provide valuable information for management decisions. Data collected during natural low-flow conditions (Critical Period sampling) is essential to verify any interim results and gather additional information to further evaluate low-flows.

Though the comprehensive portion of the study has been reduced to two annual samples (plus a limited summer effort), it is still adequate to maintain a continuous record of conditions. That is vital knowledge since antecedent conditions influence community-level response to reduced discharge conditions. Sampling only during a low-flow event will not provide the necessary context to adequately assess changes that occur during such conditions.

METHODS

In 2005, only two (reduced from three) comprehensive sampling efforts were conducted with a sampling protocol that was slightly modified relative to 2000 – 2004. The new monitoring program was discussed among BIO-WEST, Inc. (BIO-WEST), the Edwards Aquifer Authority (Authority), and the U.S. Fish and Wildlife Service (USFWS). Modifications to summer sampling included only conducting the dip net sampling for fountain darters (*Etheostoma fonticola*), while the spring and fall sampling periods remained the same. This maintained the continuity of the sampling plan through the summer while reducing the disturbance to the system from multiple sampling activities. The changes described above were intended to maximize the efficiency of this project. The resulting schedule included the following:

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

Aquatic Vegetation Mapping

Fountain Darter Sampling
- Drop Nets
- Dip Nets
- Visual Observations
- Gill Parasite Evaluation

Salamander Observations

Macroinvertebrate Sampling
- Drift Nets
- Comal Springs riffle beetle surveys

Springflow

Total discharge data for the Comal River were acquired from United States Geological Survey (USGS) water resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2006). The discharge data for the Comal ecosystem was taken from USGS gage 08169000 from the Comal River at New Braunfels. This site represents the cumulative discharge of the springs that form this river system.

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

High-Flow Sampling

There were no high-flow events in 2005, but there was a significant flood event in November 2004. During this event, flows in the Comal River reached 6,860 cubic feet/second (cfs) on November 22, 2004. Normally this would trigger a full sampling event, but BIO-WEST had performed four high-flow Critical Period in previous years (August and November 2001, July 2002, and June 2004). Because of these recent data collection efforts on high-flow impacts, 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.

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. On November 22nd, 2004 flows reached 6,860 cfs.

Water Quality

The objectives of the water quality analysis are: delineating and tracking water chemistry throughout the ecosystem; monitoring controlling variables (i.e., flow, temperature) with respect to the biology of each ecosystem; monitoring any alterations in water chemistry that may be attributed to anthropogenic activities; and evaluating consistency with historical water quality information. The water quality component of this study was reduced in 2003, but the two components necessary for maintenance of long-term baseline data, temperature loggers (thermistors) and fixed station photography, were included through 2005. In addition, conventional physico-chemical parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen, water depth at sampling point, and observations of local conditions) were taken at the surface and near the bottom in all drop-net sampling sites using a Hydrolab Quanta. When conditions trigger low-flow sample events in the future, the full range of water quality sampling parameters will be employed, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

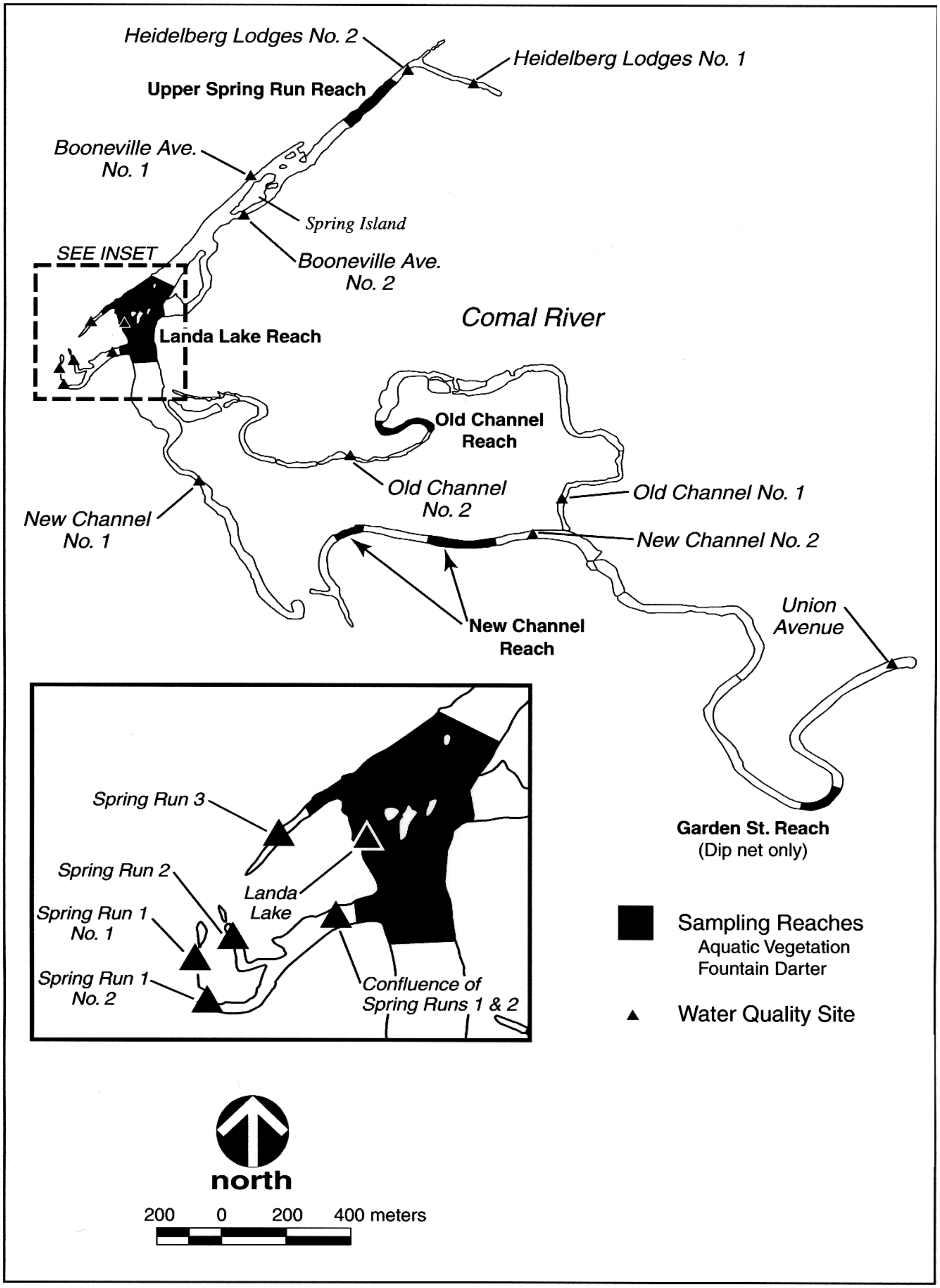

Figure 1. Comal River water quality and biological sampling areas.

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

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

Aquatic Vegetation Mapping

In 2003, one adjustment was made to the vegetation mapping efforts; the upper section of the New Channel was not mapped. The site was dropped from the sample protocol because this additional 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 sample for fountain darters and the one *Hygrophila* site sampled that had been sampled quarterly was typically similar in species composition to samples in the lower section.

Mapping was conducted using a Trimble Pro-XH global positioning system (GPS) unit with real-time differential correction capable of submeter accuracy. The Pro XH receiver was linked to a Trimble Recon Windows CE device with TerraSync software that displays field data as it is gathered and improves efficiency and accuracy. The GPS unit was placed in a 10-meter (m) Perception Swifty kayak with the GPS antenna mounted on the bow. The aquatic vegetation was identified and mapped by gathering coordinates while maneuvering the kayak around the perimeter of each vegetation type at the water's surface. Vegetation stands that measured between 0.5 and 1.0 m in diameter were mapped by recording a single point. Vegetation stands less than 0.5 m in diameter were not mapped.

Hygrophila in the Old Channel Reach

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium*; primarily in the upper Spring Run and Landa Lake) were included in all 2005 sampling events. Difficulties with mapping these vegetation types (patchiness, bryophytes are easily obscured by filamentous algae, etc.) precluded them from early samples; however, these vegetation types are clearly important fountain darter habitat and were included in all sample events beginning in the summer of 2001.

Fountain Darter Sampling

Drop Nets

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

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

Drop Net Data Analysis

The fisheries data collected with drop nets were analyzed in several ways. Calculations of fountain darter density in the various vegetation types during 2000-2005 provide valuable data on species/habitat relationships. These average density values were also used with aquatic vegetation mapping data on total coverage of each vegetation type by sampling effort to create estimates of the population abundance in each reach (fountain darter density within a vegetation type x total coverage of that vegetation type in the given reach). Because there were generally only two drop net samples in each vegetation type within each reach, density estimates between sampling efforts had great variation and population estimates based on those densities would be greatly influenced by this variation. Part of the variation would be due to changes in environmental conditions (discharge, temperature, etc.) that had occurred since the last sample, but part would be due to natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints) it is impossible to tell how much of the variation is attributed to each source within a given sampling effort. Using the average density of fountain darters across all samples for a given vegetation type does not account for changes in density across samples (differences associated with changes in environmental conditions), but the increased sample size substantially reduces the high natural variability. This type of comparison between samples, where density values are held constant across all samples, is based entirely upon changes in vegetation composition and abundance between sampling efforts. Because these abundance estimates use the same density values across sites and seasons, and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Dip Nets

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

To balance the effort expended across samples, a predetermined time constraint was used for each reach (Upper Spring Run - 0.5 hour, Spring Island area - 0.5 hour, Landa Lake - 1.0 hour, New Channel - 1.0 hour, Old Channel - 1.0 hour, Garden Street - 1.0 hour). The areas of fountain darter collection were marked on a base map of the reach. Though information relating the number of fountain darters by vegetation type was not gathered by this method (as in the drop net sampling), it did permit a more thorough exploration of various habitats within the reach. Also, spending a comparable length of time sampling the entirety of each reach allowed comparisons between data gathered during each sampling event.

Dip Net Data Analysis

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

Dip Net Techniques Evaluation

In 2005 an effort was made to establish a rapid method for assessing changes in fountain darter population abundance between sample efforts, especially during critical periods (high- and low-flow events). While drop netting provides quantitative data of fountain darter populations, it is somewhat labor-intensive and destructive to vegetation that is valuable habitat especially in low-flow periods. Dip netting, as it is currently used, provides information on the relative abundance of fountain darters between samples. However, sample sites are selected in high quality channel edge habitat and are not distributed among available vegetation types. In addition, this method yields one data point (a single timed survey) for a given reach. Therefore, this method does not result in data that may be used to determine a statistical difference among samples, or account for possible habitat shifts and clumping under low-flow conditions. Objectives of this portion of the study were to assess the viability of an alternative dip netting method designed to gather presence/absence data at multiple sites within each reach and thereby increase the number of data points that may be collected, reduce the time necessary to collect data at all sites, and reduce habitat disturbance. Although presence/absence data provides no means of calculating fountain darter abundance, repeated sampling does provide a quick and less labor intensive way to monitor trends in the fountain darter population.

Fifty sites were distributed among the 4 sample reaches based on total area, diversity of vegetation, previous fountain darter abundance estimates, and overall biological importance of each reach (Table 1). Sites were randomly selected from a grid overlain on the most recent vegetation map of that reach. After each dip, presence or absence of fountain darters was noted and the entire contents of the net were placed into a plastic tub with river water to avoid recapturing organisms. After all dips were completed at a site, all organisms were released, and times of day and GPS coordinates were recorded. Data were collected for three consecutive days using this sampling method to assess variability both within and among randomly selected sites. To examine variability among randomly selected sites, data were compared between day 1 and day 2 when a different set of randomly selected points were used. On day 3, sites sampled on day 1 were re-sampled to assess variability within sites.

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

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

Visual Observations

Visual surveys were conducted using SCUBA in Landa Lake to verify the continued fountain darter and Comal Springs salamander (*Eurycea sp.*) use of habitat in deeper portions of the lake. The locations of these time-constrained surveys were deeper than drop net or dip net methods for the darters would allow. Observations were conducted in the early afternoon for each effort.

An additional component to these surveys was a grid (0.6 m x 13.0 m) added in summer 2001, and subsequent sampling. The grid was used to quantify the number of fountain darters using these deeper habitats. To sample the area, all fountain darters within the grid were counted. Time constraints limited the sampling to just one grid. A much more labor-intensive effort would have been required to develop an estimate of the true population size in the sample area, but the data were useful in providing an indication of the relative abundance of the fountain darters that are found in areas similar to those sampled. This method also allowed some insight into trends in population dynamics that may occur over time.

Gill Parasite Evaluation

The objectives of 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. In 2005, new methodologies were tested (Lozano 2005) based on Cantu (2003) to determine more efficient ways of studying parasite populations in the Comal River. The main objective of the Lozano (2005) study was to determine the requisite sample number to accurately reflect the number of parasites drifting through a cross-section in the Comal River.

To answer this, one site in the Comal River was chosen as a representative cross-section of the river. The site was located at the Clubhouse Bridge downstream of Landa Lake because the lake is theorized to contain a large population of the parasite. Samples were taken over two days (one cloudy and one sunny), and collected using filtration of the river water similar to the method described in Cantu (2003). 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) with a pump attached to it was used to collect 45 – 5 liter water samples from the top and bottom of the water column along the cross-section. All 5 liter water samples were fixed with a 0.1% formalin solution after collection and prior to filtration to increase the percentage of parasites recovered from the sample. The new methodologies were tested in order to streamline operations and better represent parasite populations and their effects on fountain darter populations.

Comal Springs Salamander Visual Observations

In addition to the visual observations made in the deeper portions of Landa Lake for fountain darters and Comal Springs salamanders, the BIO-WEST project team performed presence/absence surveys for the Comal Springs salamanders in the spring reaches located at the head of the Comal River during all 2005 sampling events. Surveys were conducted in Spring Run 1, Spring Run 3, and the Spring Island area (Figure 1) and performed by two people in each spring reach. Each survey began at the downstream-most edge of the sampling area and involved turning over rocks located on the substrate surface within the Spring Run while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. The Comal Springs salamander locations were noted, along with time and water depth. In order to maintain consistency between samples, all surveys were initiated in the morning and terminated by early afternoon.

Salamander survey in Spring Run 1

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

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 (*Heterelmis comalensis)*, Comal Springs dryopid beetle (*Stygoparnus comalensis*), and Peck's cave amphipod (*Stygobromus pecki*). At the outset of this project, drift nets were used in the middle of Spring Runs 1-3 to explore the movement of organisms downstream of the spring openings since drifting downstream is a primary means of dispersal by benthic invertebrates (Smock 1996). The focus was to determine whether a single species might be used to serve as an indicator for measuring community response to changes in springflow. That portion of the study, completed in 2002 (BIO-WEST 2003) yielded drift rates, densities, and patterns of selected aquatic invertebrates in the Spring Runs. In 2003, there was a shift of focus to the spring openings in order to evaluate the frequency with which the three primarily spring-adapted (troglobitic) endangered species are expelled from the aquifer. In 2003-2005, there were also sampling efforts targeted at the Comal Springs riffle beetle distribution and relative abundance among the three areas identified during a 2001 survey (BIO-WEST 2002b) as having the largest concentrations of individuals and under various discharge conditions within each site.

In 2005, drift nets were placed in spring openings during the spring and fall comprehensive sampling efforts. Drift nets were placed over the openings of Comal Spring Runs 1 and 3 and a moderate-sized spring upwelling along the western shoreline of Landa Lake. The nets were anchored into the substrate directly over the spring opening using rods, with the net face perpendicular to the direction of flow of water. The nets had a 0.45-m by 0.30-m rectangular opening and mesh size of 350 micrometers (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 of these three species were identified and returned to their spring of origin. Voucher specimens included fewer than the 20 living specimens (identifiable in the field) of each species. All other invertebrates were preserved in 70% ethanol for later identification. Water quality measurements (temperature, pH, conductivity, dissolved oxygen, and current velocity) were taken at each drift net site using a Hydrolab multiprobe and DataSonde (model 2) and a Marsh McBirny portable water current meter (model 201D).

In addition to drift nets placed over spring openings, surveys of the endangered Comal Springs riffle beetle, were conducted in the two comprehensive sampling efforts in 2005 (April and November). These samples were conducted in three disjunct areas of Landa Lake on the Comal River, in locations that were previously identified (BIO-WEST 2002b) to have the highest densities of Comal Springs riffle beetles. The three sites included Spring Run 3, the western shoreline of the lake, and upstream of Spring Island. 2005 samples were conducted using the same methodology as in 2004 (see BIO-WEST 2005 for a description of earlier sampling technique). Bed sheets (50% cotton, 50% polyester) were cut into 15-cm x 15-cm squares. At each of the three study sites, 10 springs found in potential habitat were selected and sampled using this method. Depth (ft), current velocity (m/s), and landmark distance measurements were taken at each spring. Each square had the corners folded inward and placed in the spring with rocks loosely stacked over top to keep it in place. Approximately four weeks later, squares were 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 beetle genera (*Microcylloepus* and *Stenelmis*), Comal Springs dryopid beetles, Peck's cave amphipods, and blind isopods (*Lirceolus* near pilus).

Exotics / Predation Study

This sampling component was not included in 2005 but will be included in future low-flow sampling efforts.

OBSERVATIONS

The BIO-WEST project team conducted the 2005 sampling events as shown in Table 2.

Table 2. Components of 2005 sampling events.

EVENT	DATES	EVENT	DATES
Spring Sampling		**Fall Sampling**	
Vegetation Mapping	Apr 15 - 21	Vegetation Mapping	Oct 3 - 5
Fountain Darter Sampling	Apr 20 - 22	Fountain Darter Sampling	Oct 12 - 14
Comal Salamander Observations	Apr 28	Comal Salamander Observations	Oct 13
Macroinvertebrate Sampling	Apr 26 – 28	Macroinvertebrate Sampling	Nov 2 - 4
Summer Sampling			
Fountain Darter Sampling	July 26		

Springflow

The flooding that occurred in November of 2004 contributed to increased daily springflow in spring 2005. Mean daily springflow values peaked in March above 480 cfs and remained above 400 cfs into June (Figure 2). Lack of significant rainfall through the summer resulted in lower flows that remained constant near 370 cfs through the fall. The lowest flow recorded in 2005 occurred in July at 349 cfs (Table 3). Flows in 2005 were very similar to those of 2003 with few peaks or substantial declines in the hydrograph. The monthly averages in 2005 ranged from 70 to 175 cfs above normal compared to that of the historical record (Figure 3).

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

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

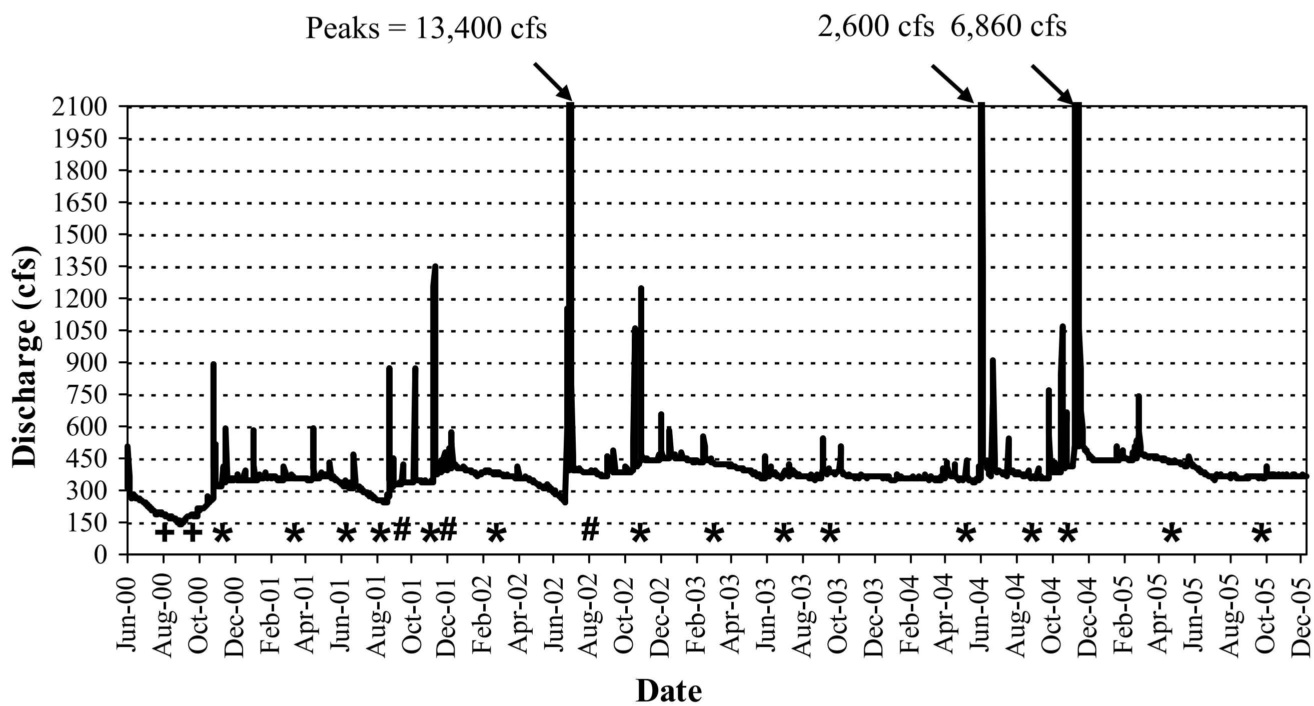

Figure 2. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (*), low (+), and high-flow (#) sampling events are indicated.

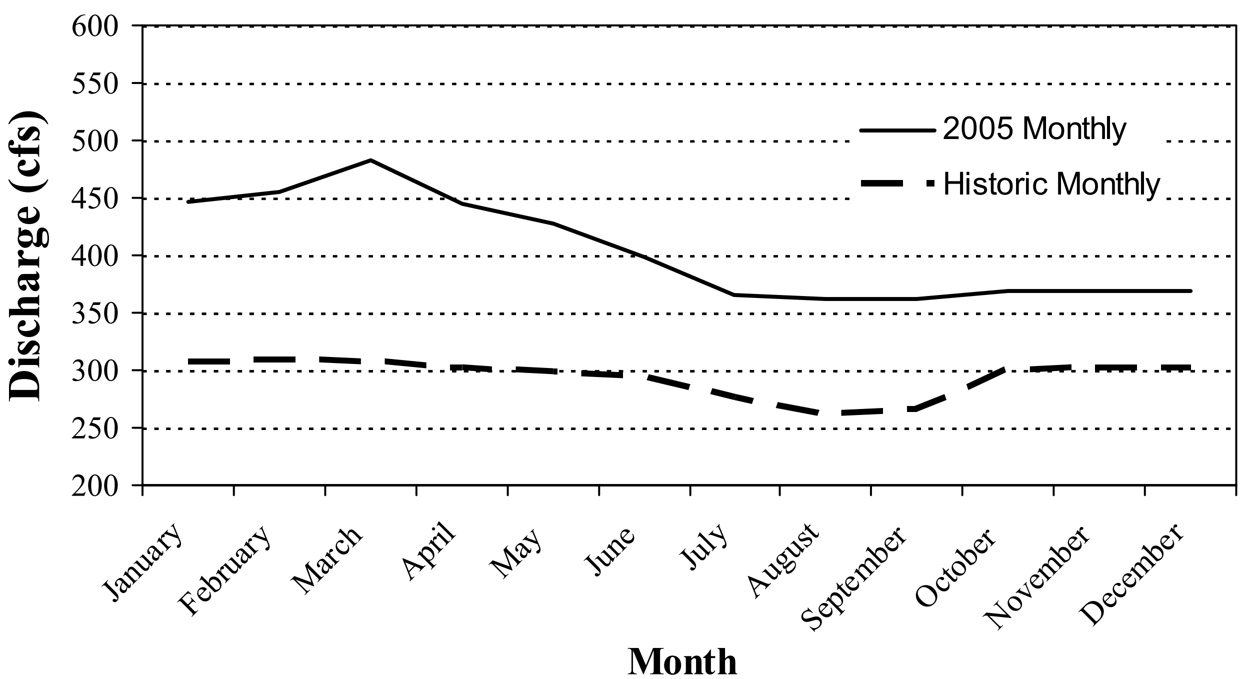

Figure 3. Mean monthly discharge in the Comal River during the 1934-2005 period of record.

Table 4 shows the discharge measured in each of the Spring Runs (including one upstream and one downstream site in Spring Run 3) and the Old Channel. Table 5 shows the proportion that each spring contributed to the total Comal River discharge and the proportion of total flow that traveled down the Old Channel during each sample effort. Overall, discharge at each location was lower in fall 2005 due to the lack of any significant rain events through the latter half of the year. Proportions of discharge were stable or increased slightly from 2004 to 2005, and then decreased substantially by fall 2005. As in 2004, Spring Runs 1 and 3 made up a large proportion of total discharge (27.6%) in the spring of 2005, but the lack of rain contributed to a decrease of more than 5% by fall 2005 (22.1%). Spring Run 2 decreased to a two year low of 1.4% of the total discharge in the river.

Table 4. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, and 3 and Old Channel reach during each sample effort in 2004 - 2005.

	Discharge (cfs)				
Location	**Spring 2004**	**Summer 2004**	**Fall 2004**	**Spring 2005**	**Fall 2005**
Total Discharge Comal River (USGS)	375	377	411	446	368
Spring Run 1	43.3	50.1	50.1	64.2	37.7
Spring Run 2	6.2	8.8	7.2	10.5	5.0
Spring Run 3 (upstream)	36.8	41.8	42.4	45.6	33.8
Spring Run 3 (downstream)	49.5	52.5	54.7	58.8	43.9
Old Channel	91.8	83.8	76.5	77.3	65.4

Table 5. Proportion of total discharge in the Comal River (USGS data) that each Spring Run contributed and proportion that traveled down the Old Channel during each sample effort in 2004 - 2005.

	Proportion of Total Discharge				
Location	**Spring 2004**	**Summer 2004**	**Fall 2004**	**Spring 2005**	**Fall 2005**
Spring Run 1	11.5%	13.3%	12.2%	14.4%	10.2%
Spring Run 2	1.7%	2.3%	1.8%	2.4%	1.4%
Spring Run 3 (upstream)	9.8%	11.1%	10.3%	10.2%	9.2%
Spring Run 3 (downstream)	13.2%	13.9%	13.3%	13.2%	11.9%
Old Channel	24.5%	22.2%	18.6%	17.3%	17.8%

Water Quality

A representative graph of thermistor data for the Comal Springs/River ecosystem is presented in Figure 4; additional graphs can be found in Appendix B. The continuously sampled water temperature data has provided a good view of the conditions experienced by fountain darters and other species throughout the Comal Springs ecosystem in 2000-2005. In many places the temperature remains nearly constant due to nearby spring inputs, while other locations (typically further away from spring influences) are more substantially influenced by atmospheric conditions. At times, it appears that precipitation can have acute impacts (typically very cold rainfall) in some locations, but these are generally short-lived and the overall relationship at these sites is more directly associated with air temperature (also, air temperatures strongly influence precipitation temperatures).

As has been observed in past years, the range of water temperatures in several sites is around 1ºC or less, except for a few acute peaks/troughs (Spring Run 1, Figure 4). The perceived increase in the spring of 2004 at Spring Run 1 was likely due to thermistor failure than an increase in water temperature. Other thermistor failures occurred at Landa Lake and Spring Run 2 (Appendix B). While the Landa Lake thermistor returned to normal, the Spring Run 2 thermistor subsequently failed (as indicated by gradual decreases in the graph). As a result, all thermistors were replaced in 2005 due to high incidence of faulty readings

Overall, the Heidelberg site in the Upper Spring Run Reach (Appendix B), the deep portions of Landa Lake, and the Spring Runs (Figure 4) maintained a narrow range of temperatures that did not exceed 26ºC in 2005. The Spring Island area has two completely different conditions on either side (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 fluctuates 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 required to reach the Spring Island area allowed the water temperatures in the western channel to reflect more of a seasonal trend with the coldest recordings in the winter months. Similarly, Blieder’s Creek (upstream of the Spring Runs and Landa Lake) followed a seasonal trend because of its lack of spring inputs. The greatest fluctuation in water temperature throughout the Comal River system occurred in Blieder’s Creek, where the temperature increased to nearly 28ºC in 2002, 2003, and 2005 (possibly also in 2001, but the thermistor did not function correctly during that period). While the wide fluctuations in temperature found in Blieder’s Creek do not appear suitable to provide habitat for fountain darters during a portion of the year, the effects of these water conditions are highly localized. The lower end of Blieder’s Creek is typically a stagnant pool that does not flow into the Comal River and temperatures observed in the Upper Spring Run Reach (immediately downstream of Blieder’s Creek) were within a much more narrow temperature range (Heidelberg site; Appendix B).

Downstream of Landa Lake, temperature variations were greater – most values were between 20ºC and 26ºC (Appendix B). Except for two spikes in the Old Channel reach in which the thermistor must have been temporarily exposed, these sites did not exceed the water quality standards value of 26.67°C during 2000-2005. The temperature at which fountain darters are believed to have reduced fecundity is 27ºC (Bonner et al. 1998) and the lethal limit for the fountain darter is 34.8°C (Brandt et al. 1993).

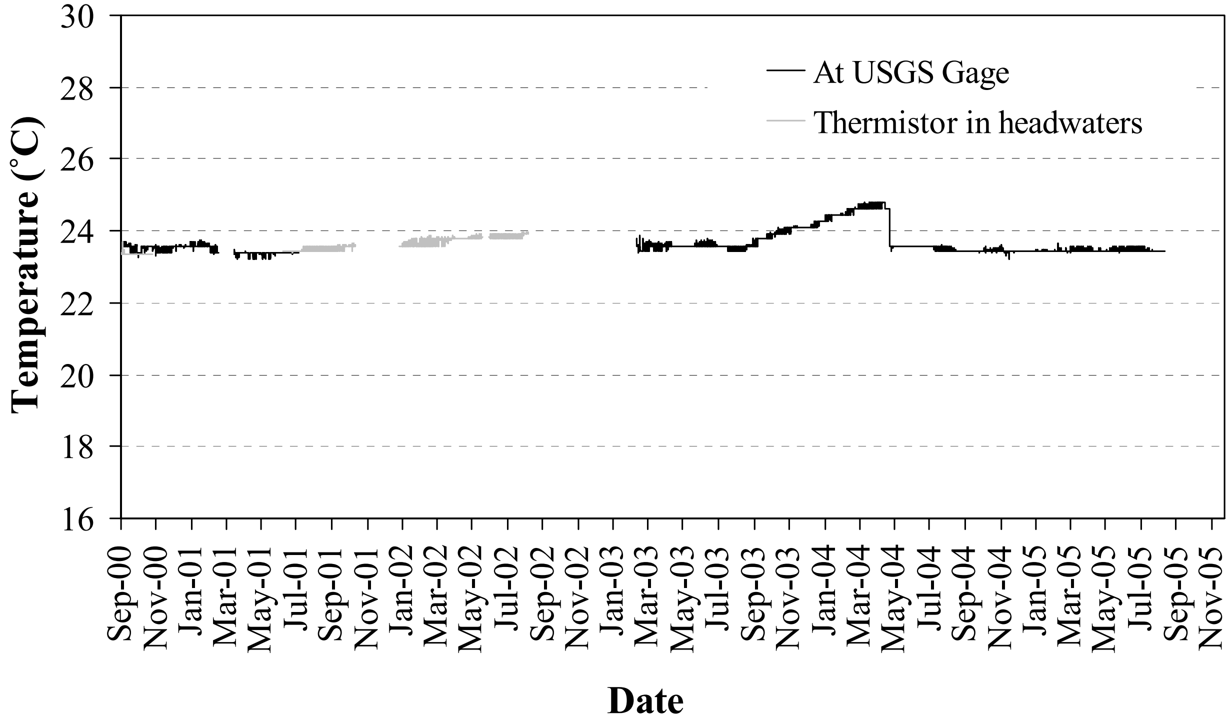

Figure 4. Thermistor data from Spring Run 1.

As with other components of this study, more data are needed to determine the potential impacts of high air temperatures and low flows during an extended period of reduced recharge.

Aquatic Vegetation Mapping

Maps of the aquatic vegetation observed during each sample effort can be found in the Appendix A map pockets. The maps are organized by individual reach with successive sampling trips ordered chronologically. It is difficult to make sweeping generalizations about seasonal and other trip-to-trip characteristics, since most changes occurred in fine detail; however, some of the more interesting observations are described below.

Upper Spring Run Reach

Although the Upper Spring Run Reach is relatively stable compared to other portions of the Comal River, there was an overall trend of increasing aquatic vegetation coverage in 2005. There was a substantial increase in coverage from the fall of 2004. A possible explanation is that the flood in November 2004 provided a scouring effect that stimulated growth of most types of vegetation and the relatively constant discharge in 2005 (though higher than normal) has not provided the kind of flushing flow conditions that stimulate growth.

Cabomba coverage remained relatively constant from 2004 through 2005. *Ludwigia* also changed little in 2005, but more than doubled from the fall of 2004 to the spring of 2005 with new patches appearing in the middle part of the reach.

Hygrophila increased substantially from fall 2004 to the spring of 2005 (an increase of 126.8 m^2) likely due to the flood. This represents an increasing trend of growth for *Hygrophila* since a low of 291.4 m^2 in the fall of 2003. Though *Hygrophila* coverage did decrease slightly in 2005 (by 19.6 m^2) it appears that overall this plant is successfully reseeding in this reach. This is exemplified by new patches appearing in the middle and upper sections of the reach. Although an exotic species, this vegetation type has become important for fountain darters because of the total coverage it provides in the Comal River. The similar (native) species, *Ludwigia*, supports a greater density of fountain darters, but occurs in much lower amounts.

Sagittaria coverage decreased from fall 2004 to spring 2005, but increased by fall 2005 to its greatest area in three years (494.2 m^2). Most of the growth occurred along the north side of the channel in the lower part of the reach in areas that previously had no vegetation. *Sagittaria* supports a low density of fountain darters; thus, the fluctuations in total coverage of that habitat type are less important to the fountain darter population.

Bryophytes continued to increase from fall 2004 through fall 2005 with most of the new coverage appearing in the upper part of the reach. The flood of November 2004 clearly had a positive impact on bryophyte growth throughout the reach. This is one of the most important vegetation types in this reach and the Comal River for supporting populations of fountain darters. Fountain darters are able to occupy this habitat type in much higher densities than other vegetation types because it provides substantial food and cover. However there appears to be a lag time of several months between vegetation increase and darter exploitation of the new habitat. If the bryophytes continue to remain in such high coverage in this reach, fountain darters should respond by increasing population numbers.

Landa Lake Reach

Landa Lake is a large and complex reach encompassing many different habitat types. There are several vegetation types within the reach, but it is clearly dominated by a pure stand of *Vallisneria*. This stand continued an increasing trend from 2004 through 2005 filling in some bare patches in deeper, open water areas. A large bare patch opened around the lookout dock on the southwestern side of the lake where *Vallisneria* previously dominated. This plant species has flourished since the giant ramshorn snail population exploded and denuded the lake of vegetation in the late 1990s and appears largely resistant to scouring flows that impact the other species.

Sagittaria and *Cabomba* increased from 2004 and throughout 2005. For *Sagittaria*, most of the growth occurred in the northwestern part of the reach, while *Cabomba* replaced bare patches in the northeastern section of the lake. *Nuphar* coverage changed little between 2004 and 2005, but decreased by fall 2005 when it was replaced in some areas by *Vallisneria*. Green algae appeared in abundance in Landa Lake in 2005. It started growing in open water near a *Nuphar* patch in the middle of the lake, where it tripled in size over the course of 2005. It is unclear whether the scouring flood of November 2004 was a direct cause of the algae growth (as it may have been displaced from upstream).

Though the flood of late 2004 appeared to initially stimulate plant growth through much of the Landa Lake reach (especially bryophytes), vegetation coverage decreased substantially over 2005. While

Hygrophila increased from 2004, it decreased substantially over 2005 becoming much patchier over its range. The large patch present just north of the easternmost island in the reach had almost completely disappeared by the fall 2005 sampling period. In addition the largest patch between the three islands has shrunk and bare patches within the *Hygrophila* have become more prevalent. Bryophytes have also begun to mix in with much of the *Hygrophila* south of the easternmost island. This patchiness in summer and fall was also seen in 2003 when there was a lack of any scouring flows. In addition, rented paddleboats provide the only access to the lake for the general public and the shallow areas of *Hygrophila* and *Ludwigia* (see below) may be influenced by their use on the lake.

Ludwigia followed a similar trend as *Hygrophila* decreasing from 2004 to 2005 to its lowest coverage in fall 2005. Like *Hygrophila*, it formally occupied larger patches that have since been broken up into smaller distinct plants. As a result, *Ludwigia* coverage by fall 2005 (35.8 m^2) was half of what it was in spring 2005 (72.5 m^2), and it decreased nearly 2/3 from fall 2004 (98.1 m^2). As with *Hygrophila*, the *Ludwigia* occurs in relatively shallow areas that may be susceptible to mechanical disturbance from paddleboats.

The bryophytes are the most important of the vegetation types sampled in Landa Lake because they support the greatest densities of fountain darters in that reach (of the vegetation types sampled). It appears that the flood in November 2004 may have stimulated bryophyte growth in the open areas of the northern section of the lake. The total coverage of bryophytes nearly quadrupled from fall 2004 (735.0 m^2) to spring 2005 (2,800.9 m^2). It was then reduced by approximately half by fall 2005 (1,055.4 m^2), which corresponds with a period of steadily decreasing flows throughout the summer.

The exact cause of the increased abundance of the bryophytes remains unclear but the increase has coincided with the higher flows. It was hypothesized that higher CO_2 levels resulting from higher flows stimulated the growth, and the lab study showed also that growth of the two bryophytes (*Riccia* and *Amblystegium*) was related to CO_2 concentration. It is possible that increased nutrients or mechanical effects of increased flow across the vegetation surface may be the cause, but more research would be needed to examine this issue further. The peak of bryophyte coverage during the study occurred in the spring of 2003 at 4,190 m^2. During this study, the bryophyte coverage was almost exclusively *Riccia* up until the winter of 2002, but *Amblystegium* became much more common in the upper one-third of the reach in all subsequent samples.

Old Channel Reach

Until 2003, the Old Channel Reach maintained the most stable aquatic vegetation community with a structure (culvert) that regulates flow through this section. The culvert was adjusted at some point in late 2002 or early 2003 and as a result, discharge measurements have become much more variable.

Vegetation communities changed substantially from 2004 to 2005, and overall coverage increased over 2005. *Ceratopteris* (an aquarium species) was reduced drastically in spring 2005 (21.4 m^2); the species increased by fall 2005 (34.3 m^2), but its coverage was substantially less in 2004 (189.0 m^2). This reduction was likely due to the flooding in late 2004. *Ludwigia* followed a similar pattern, but became much less patchy by late 2005 (194.0 m^2) compared to earlier in the year (109.4 m^2). It appears that filamentous algae was also negatively affected by the flood in 2004 because there wasn't any detected in the spring 2005 sample. Two small stands were observed in fall 2005 (3.1 m^2), but that was less than half of what it was the previous year (8.6 m^2). As a result, only one stand of algae was sampled for fountain darters in the fall 2005 sample (see drop results below).

Hygrophila coverage continued to increase from 2004 through 2005. Coverage more than doubled from fall 2004 (647.7 m^2) to fall 2005 (1,325.6 m^2), and may be a result of new growth. The biggest increase took place at the large bend where the river is deepest. This deep area was not mapped in 2004 because the bottom could not be seen clearly, but stable flows in 2005 permitted mapping. In addition, beginning in spring 2005, the *Hygrophila* patch appeared to grow higher in the water column, and by fall 2005, it could be distinguished across much of the deeper area. It is unclear whether turbidity levels changed enabling the *Hygrophila* to receive more sunlight and grow, or if the water level decreased. With *Hygrophila* dominating more of this reach, fountain darter populations may decrease because this vegetation type provides lower quality habitat than the vegetation it replaced (filamentous algae and *Ludwigia*). Continued monitoring will be important to determine whether filamentous algae will return in areas that have not been covered with *Hygrophila* and *Ludwigia* and to determine how much area will be covered with these two plant types.

New Channel Reach

The New Channel Reach underwent drastic changes in vegetation coverage from 2004. By spring 2005, *Ludwigia* was completely gone (and has yet to re-establish) and *Hygrophila* only covered about 3% (18.1 m^2) of its total coverage in fall 2004 (619.6 m^2). The summer 2004 flood event had a greater influence in reducing vegetation in this reach then the floods of 2001 and 2002, and the flood in November 2004 almost completely eradicated all vegetation in the New Channel. This reach receives the runoff from the dry Comal River, which can send a substantial surge of flow during storm events. In the latter half of 2005 vegetation began to re-establish in the New Channel Reach. *Hygrophila* rebounded well along the wall on the north side of the river (219.8 m^2), and algae began to establish along the south side (123.3 m^2). The vegetation in this reach will continue to be affected by significant rain events in the future.

These data show that the vegetation community is ever changing in each of the study reaches. Even during periods of relatively constant discharge conditions, it is important to monitor current conditions to estimate the presence of suitable habitat for fountain darters. The true impacts of a major shift in discharge (high- or low-flow) cannot be determined without baseline data for conditions leading up to such an event.

Fountain Darter Sampling

Drop Nets

A total of 496 drop net samples were conducted during 2000-2005 in the Comal Springs/River ecosystem. Twenty-two of these samples were conducted in spring 2005 and 22 were conducted in fall 2005. The number of drop net sites and vegetation types sampled per reach is presented in Table 6. Drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sample event, and data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. There were some changes over the course of the study including a shift from sampling two bare substrate sites during each sampling event in the Upper Spring Run and

Landa Lake in 2000-2001 to sampling two bryophytes sites in those reaches beginning in the summer of 2001. In 2004, there was a change in the sample design for the Old Channel Reach in response to the dramatic shift from a vegetation community dominated by filamentous algae and *Ceratopteris* to one dominated by *Hygrophila* and *Ludwigia.* Also, the 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 2005, no samples were taken in the New Channel Reach due to all vegetated areas being too deep to sample.

Table 6. Drop net sites and vegetation types sampled per reach in 2005.

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes [a] (2)	Bryophytes [a] (2)	None [b]	*Ludwigia* (2)
Sagittaria (2)	*Hygrophila* (2)		*Hygrophila* (2) [c]
Hygrophila (2)	*Cabomba* (2)		Filamentous Algae (2) [d]
	Vallisneria (2)		
	Ludwigia (2)		
Total (6)	**Total (10)**	**Total (0)**	**Total (6)**

[a] Switched from Open to Bryophytes, summer 2001.
[b] Areas with vegetation were too deep to sample in spring and fall 2005.
[c] Three *Hygrophila* sites were sampled in the fall 2005 sample to make up for one filamentous algae site.
[d] Only one filamentous algae site was sampled in the fall 2005 sample due to limited coverage.

The numerous vegetation types found in the Comal Springs/River ecosystem provide a great diversity of habitats to fountain darters covering a wide range of suitability. During 2000-2005, densities of fountain darters in the various vegetation types ranged from 3.7 per 1.0 m^2 in *Ceratopteris* to 28.7 per 1.0 m^2 in filamentous algae (Figure 5). The bryophytes also contained high numbers of fountain darters ($\approx 25/m^2$), followed by *Ludwigia* ($12.8/m^2$), and *Cabomba* ($11.4/m^2$). Although filamentous algae contain the most fountain darters per unit area, it is rather uncommon and occurs mainly in a few small patches in the Old Channel Reach. Coverage of filamentous algae has decreased in the Old Channel Reach in recent years, and this area is now dominated by *Hygrophila* and *Ludwigia* (see aquatic vegetation mapping section). Although fountain darter densities are slightly less in bryophytes than in filamentous algae, bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake Reaches, and thus make up a significant portion of the available habitat. Filamentous algae and bryophytes have high densities of fountain darters because they provide cover at the substrate level and high numbers of amphipods (an important food item). However, they are also the most easily impacted by scouring during high-flows. Although darter densities are relatively high in *Ludwigia* suggesting that it is high quality habitat, it is also susceptible to scouring and has shown large fluctuations in coverage during the study period. *Hygrophila*, an exotic species similar to *Ludwigia*, is abundant within all four reaches, however, it provides substantially lower quality habitat. *Cabomba*, which grows in relatively stable locations with some seasonal variation, provides similar fountain darter habitat suitability (based on density) as *Ludwigia. Sagittaria, Vallisneria,* and *Ceratopteris* exhibited relatively poor habitat suitability. Of these, densities in *Sagittaria* were the highest mostly as a result of association with *Riccia*. *Sagittaria* is on the low end of habitat suitability for fountain darters, except when *Riccia* is abundant and settles on and within this vegetation type resulting in a sharp increase in fountain darter densities. Although *Ceratopteris* was sampled with drop nets in previous years, in 2005 its coverage was drastically reduced and it was not sampled. The wide range in suitability and relative importance of the various aquatic vegetation types makes the composition and abundance of aquatic vegetation a critical factor in monitoring the fountain darter population.

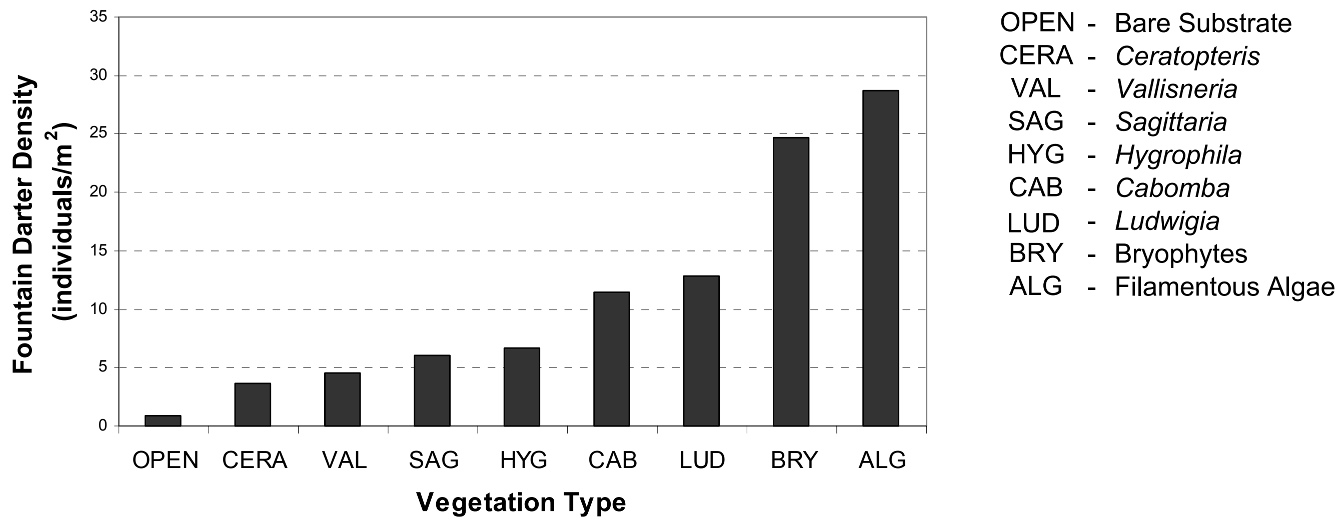

Figure 5. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem (2000-2005).

Estimates of fountain darter population abundance in all reaches (Figure 6) 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 2000-2005 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. Estimates of population abundance were highest in the summer of 2003 when the coverage of bryophytes peaked in Landa Lake (Figure 6). Although population estimates for spring and fall 2005 did not approach the highest estimates observed in summer 2003, they fell within the range of variation observed during the study period and showed a considerable increase from estimates observed after high flows in 2004. 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. All four high-flow Critical Period samples during the study period showed a decrease in the population estimate relative to the previous sample, although there was an increase in the subsequent sample each time. This is most likely related to scouring of important vegetation types resulting in fountain darters becoming more scattered at high flows.

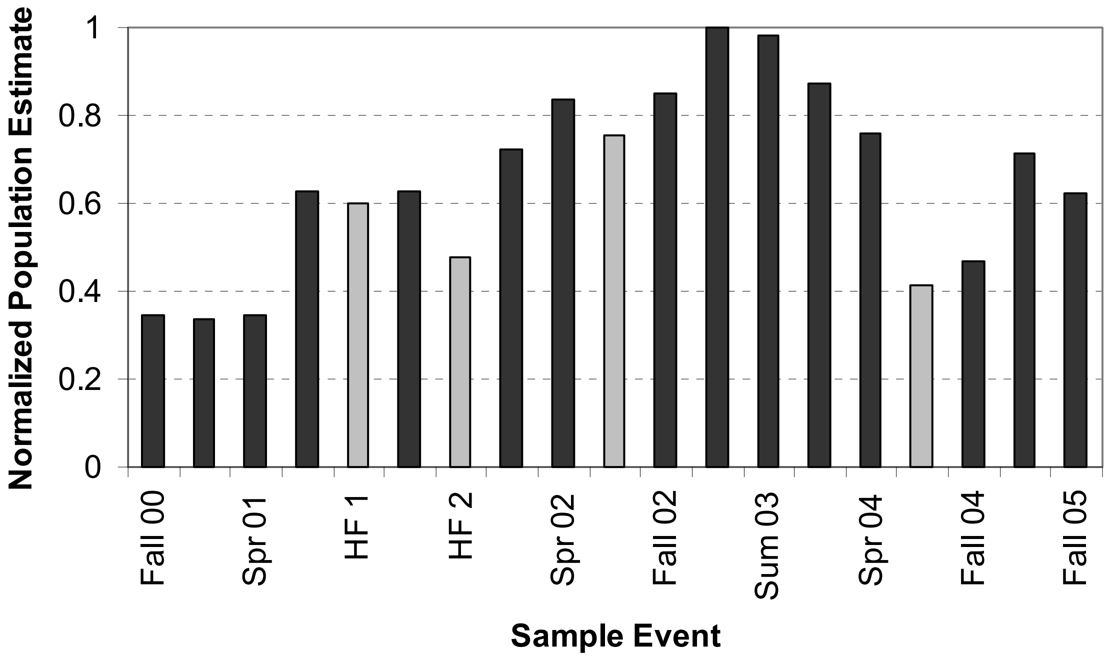

Figure 6. Population estimates of fountain darters in all four sample reaches combined (2000-2005); values are normalized to the maximum sample. Light-colored bars represent high-flow Critical Period sampling events.

Drop netting efforts in 2005 resulted in collection of 833 fountain darters in the Comal River/Springs ecosystem. The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem in 2005 is presented in Figure 7 (all data collected in previous years is presented in Appendix B). In 2005, the greatest number of individuals occurred in the range of 24-34 mm. Data from 2003 and 2004 showed a similar trend. Overall, the size-class distribution for fountain darters collected during the study period is a statistically normal distribution that is typical of a healthy fish population with a peak around 26 mm (Appendix B).

Size-class distributions of fountain darters in the Old Channel Reach and Upper Spring Run Reach demonstrate substantial differences in size structure between spring and fall collections (Figure 8). Larger fountain darters (>25 mm) were more abundant in fall 2005, while smaller darters (<25 mm) dominated spring samples. Although presence of small fountain darters has been noted in all sample seasons suggesting year-round reproduction (see dip net results), increased abundance of small darters in spring samples (from 2005 as well as previous years) suggests a peak in reproduction in early spring. Higher abundance of fountain darters in the Upper Spring Run Reach compared to the Old Channel Reach is most likely a result of differing vegetation communities in these sections. Bryophytes are abundant in the Upper Spring Run Reach, while the Old Channel Reach is dominated by less suitable *Hygrophila*.

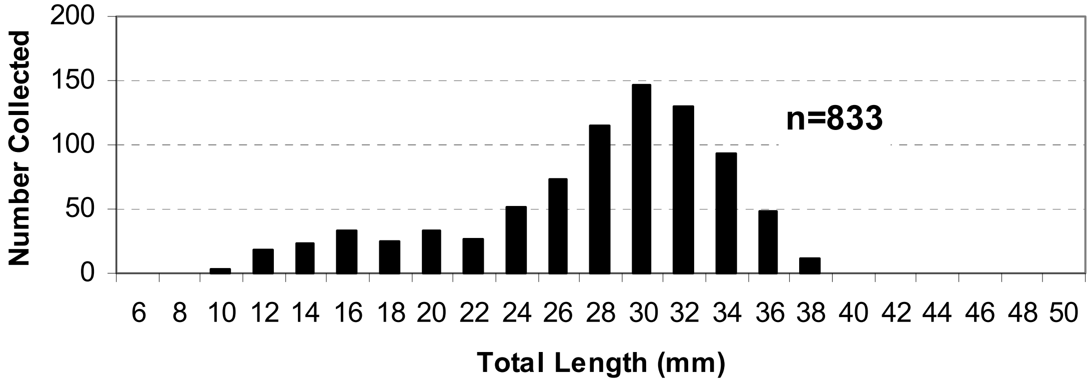

Figure 7. Fountain darter size-class distribution among all drop-net sampling events in the Comal River in 2005.

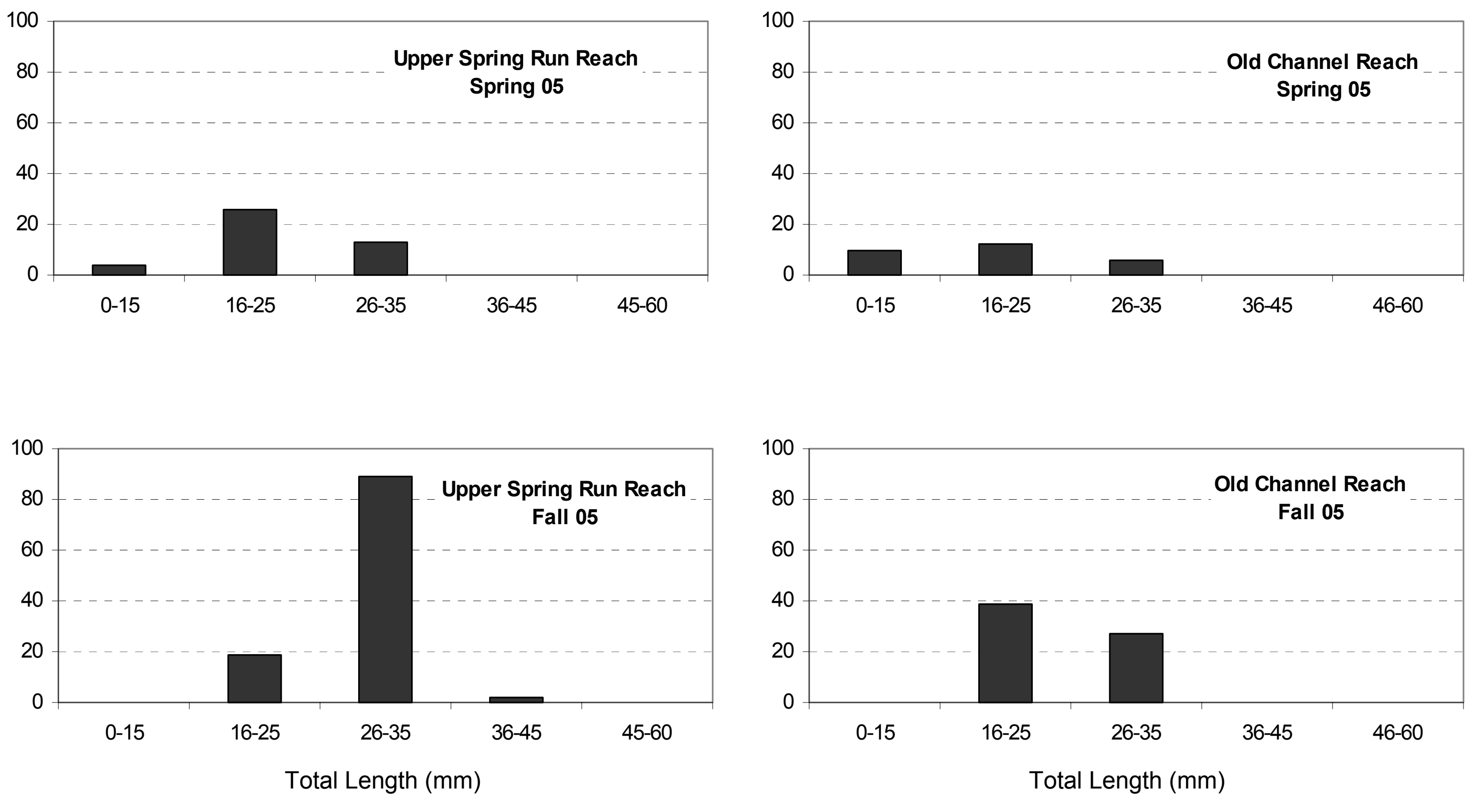

Figure 8. Size-class distributions of fountain darters collected in spring and fall 2005 in the Old Channel and Upper Spring Run Reaches.

In addition to fountain darters, 62,978 specimens representing 22 other fish taxa have been collected by drop netting from the Comal Springs/River ecosystem during the study period; of these, seven are considered exotic or introduced (Table 7). Although several of these species are potential predators of fountain darters, previous data collected during this study suggested that predation by both native and introduced predators is minimal during normal flow conditions. The impact of predation is to be further evaluated under low-flow conditions. Other potential impacts of exotic fish species include effects of herbivorous species such as the suckermouth catfish on algae and vegetation communities that serve as fountain darter habitat.

Although not a fish, one exotic species which has had considerable impact on the vegetation community in the Comal Springs/River ecosystem in the past is the giant ramshorn snail. In the early 1990s, giant ramshorn snails became very dense and caused substantial destruction to the vegetation community in the Comal River. Ramshorn snail densities averaged across all sites and years show that the snails are most common in *Ludwigia*, *Vallisneria*, and *Hygrophila*, and are rare in vegetation types where fountain darter densities are highest (filamentous algae, bryophytes) (Figure 9). Densities of giant ramshorn snails have declined throughout the study period to their lowest numbers in 2005 (Figure 10). However, because this exotic species can have considerable impacts at higher densities, close monitoring of their populations will be continued.

Table 7. Fish taxa and the number of each collected during 2005 drop-net sampling.

			NUMBER COLLECTED	
COMMON NAME	SCIENTIFIC NAME	STATUS	2005	2001-2005
Rock bass	*Ambloplites rupestris*	Introduced	0	18
Black bullhead	*Ameiurus melas*	Native	0	1
Yellow bullhead	*Ameiurus natalis*	Native	15	73
Mexican tetra	*Astyanax mexicanus*	Introduced	21	242
Central stoneroller	*Campostoma anomalum*	Native	0	1
Rio Grande cichlid	*Cichlasoma cyanoguttatum*	Introduced	36	306
Roundnose minnow	*Dionda episcopa*	Native	23	257
Fountain darter	*Etheostoma fonticola*	Native	821	8727
Greenthroat darter	*Etheostoma lepidum*	Native	31	43
Gambusia	*Gambusia sp.*	Native	10106	57321
Suckermouth catfish	*Hypostomus plecostomus*	Exotic	3	57
Redbreast sunfish	*Lepomis auritus*	Introduced	4	116
Green sunfish	*Lepomis cyanellus*	Native	0	10
Warmouth	*Lepomis gulosus*	Native	0	24
Bluegill	*Lepomis macrochirus*	Native	0	30
Longear sunfish	*Lepomis megalotis*	Native	1	36
Spotted sunfish	*Lepomis punctatus*	Native	119	850
Sunfish	*Lepomis sp.*	Native/Introduced	38	585
Spotted bass	*Micropterus punctulatus*	Native	0	1
Largemouth bass	*Micropterus salmoides*	Native	12	76
Texas shiner	*Notropis amabilis*	Native	1	29
Sailfin molly	*Poecilia latipinna*	Introduced	320	2886
Tilapia	*Tilapia sp.*	Exotic	7	16

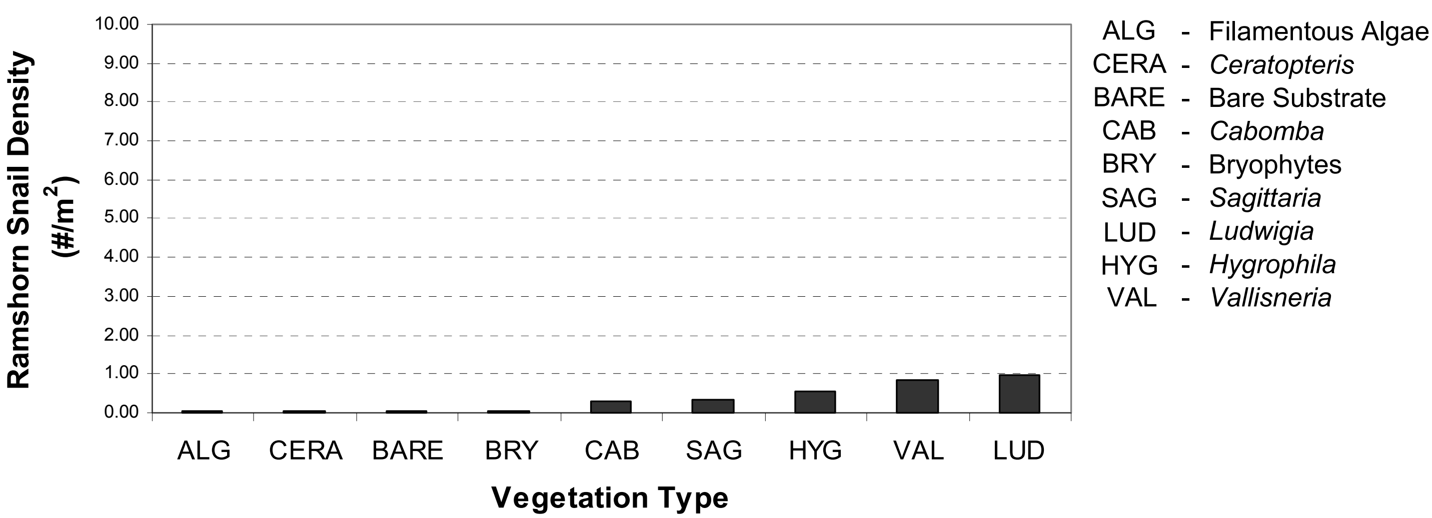

Figure 9. Density of giant ramshorn snails by vegetation type (averaged across all sites).

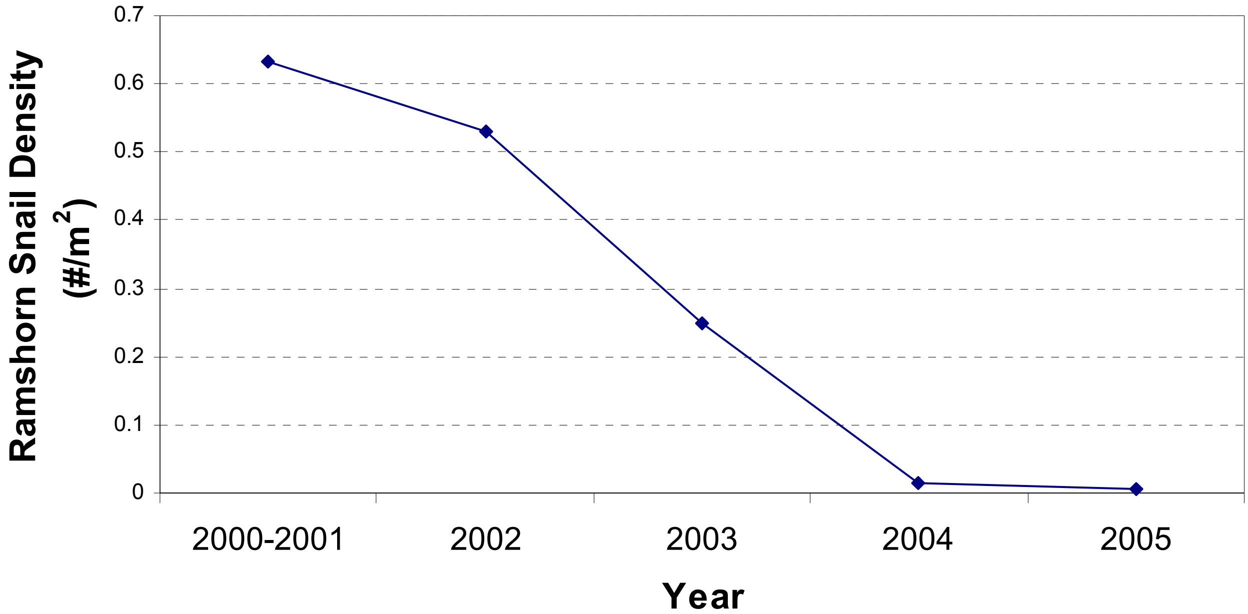

Figure 10. Density of giant ramshorn snails in each year across all vegetation types.

Dip Nets

Data gathered using dip nets are graphically represented in Figure 11 for the Old Channel Reach, and in Appendix B for all other reaches. The boundaries for each section of the dip net collection efforts are depicted in Figure 12. High water and turbid conditions prevented the collection activities from the New Channel and Garden Street sites on a few occasions.

In 2001-2004, the highest numbers of small fountain darters (5-15 mm TL) were usually observed in dip net samples during the spring of each year in all sample reaches (Appendix B). This trend was consistent in 2005 data, suggesting a peak in reproduction in early spring. However, throughout the sample period small fountain darters have been captured in summer, winter, and fall sample periods as well. In 2005, four out of seven sample reaches contained small fountain darters during the summer sample. The Old Channel Reach, in particular, has contained darters of the smallest size group (<15 mm TL) in every sample taken during the study period. This indicates that there is some reproduction occurring year-round, although perhaps on a limited basis and only in certain areas.

In general, large fountain darters (>25 mm TL) are most abundant in summer and fall dip net samples, and are usually in low abundance in spring samples. In the Old Channel Reach, 10% of fountain darters collected in spring 2005 were greater than 25 mm TL, while approximately 46% of those collected in fall 2005 were greater than 25 mm TL. Size class distributions of fountain darters from dip netting correlate well with those of drop netting with small fountain darters most abundant in the spring, and larger darters dominating fall samples.

Variability in the total number of fountain darters collected by dip netting makes any inference into population trends difficult with this method. However, there was a substantial increase in the number of darters collected from the Upper Spring Run Reach in 2003. This increase is associated with an increase in bryophytes in this reach at approximately the same time.

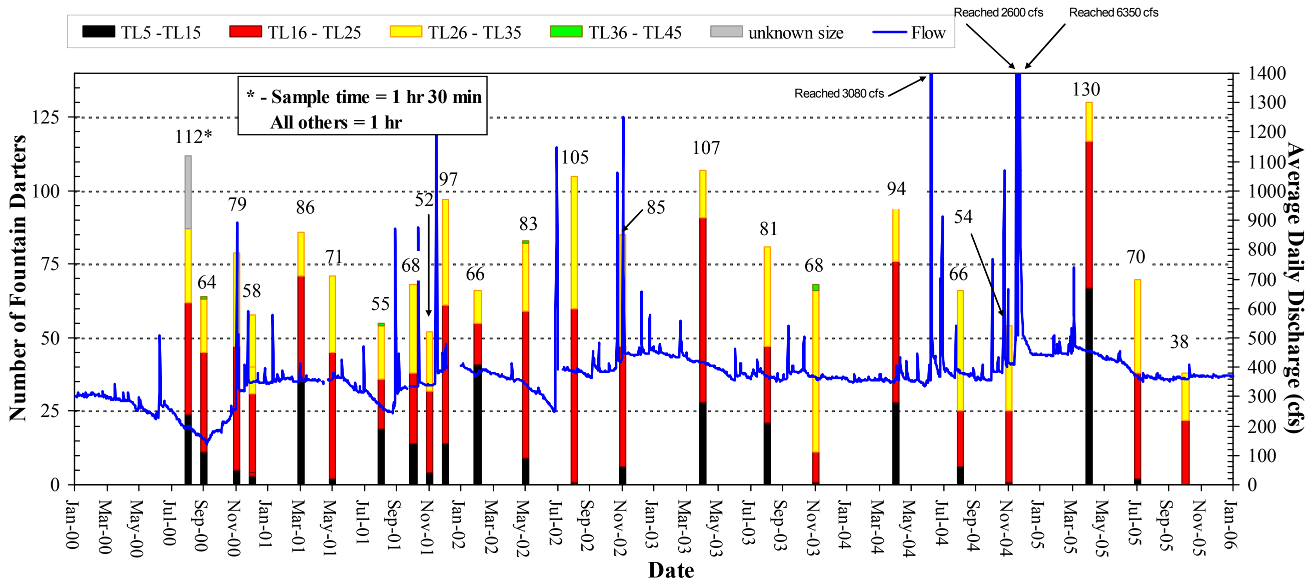

Figure 11. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

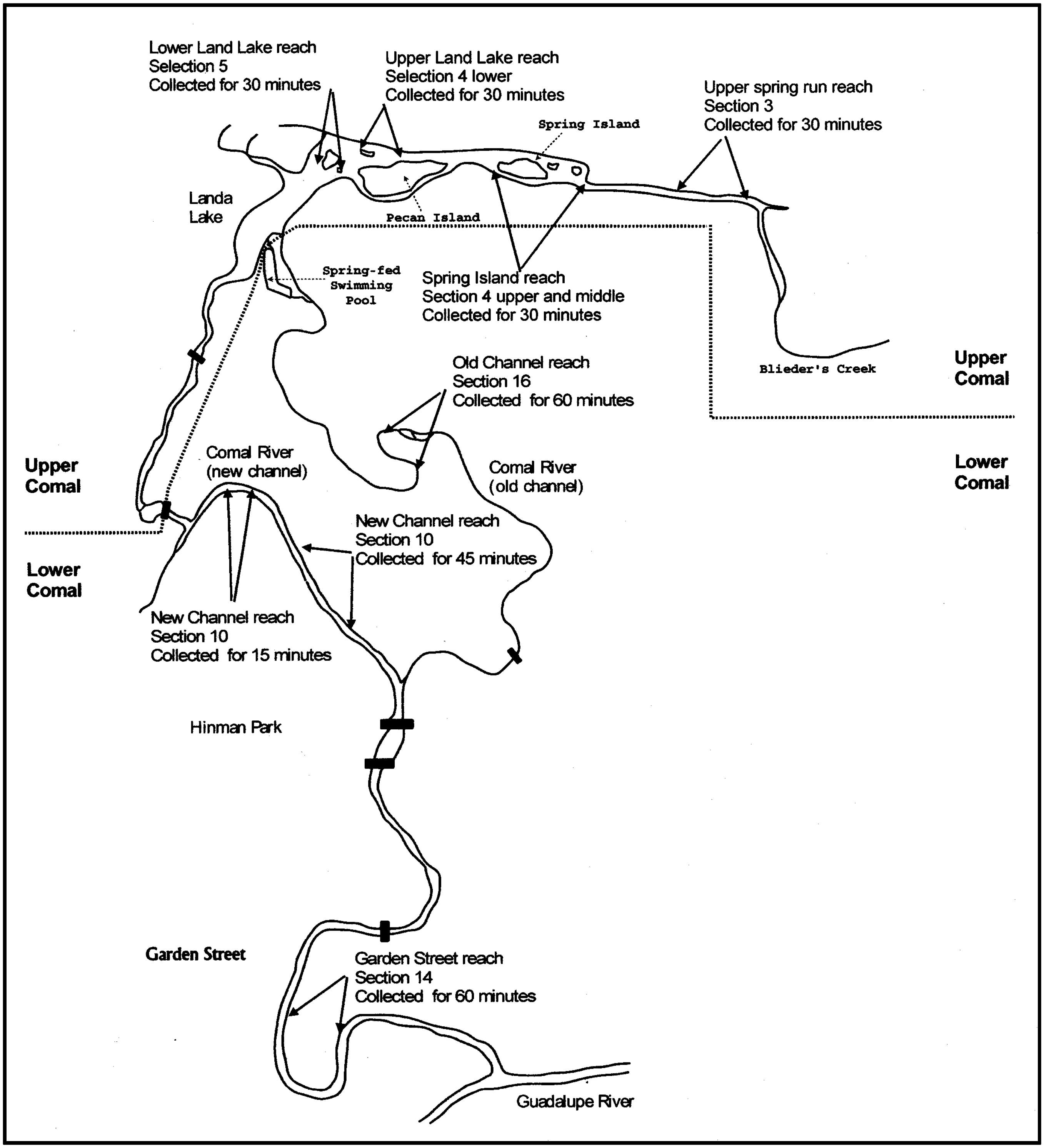

Figure 12. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

Dip Net Techniques Evaluation

The distribution of sites among reaches and vegetation types can be seen in Table 8. Twenty-two sites were established in Landa Lake which is the largest and most diverse reach, and fifteen sites were established in the Old Channel reach due to high abundance of fountain darters in previous surveys. The Upper Spring Run and the New Channel reach each have lower habitat diversity and provide marginal fountain darter habitat, thus only 6 sites were established in each reach.

Table 8. Dip net techniques evaluation sites and vegetation types sampled per reach in 2005.

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes (3)	Bryophytes (8)	*Hygrophila* (6)	*Hygrophila* (8)
Hygrophila (3)	*Hygrophila* (8)		*Ludwigia* (6)
	Cabomba (3)		Filamentous Algae (1)
	Ludwigia (3)		
Total (6)	**Total (22)**	**Total (6)**	**Total (15)**

Across all vegetation types, fountain darters were present at 71% of sites and in 44% of all dips. In sites where fountain darters were observed, presence was noted on the first two dips in the majority of cases (89%), and rarely (3%) was presence noted initially on the fourth dip suggesting that four dips are adequate for sampling.

Fountain darters were most common in filamentous algae and bryophytes, where they were found in 100% and 94% of sites sampled, respectively (Figure 13). Darters were slightly less common in the other vegetation types, occurring in 51-56% of *Cabomba*, *Ludwigia*, and *Hygrophila* sites.

The percentage of sites containing fountain darters as well as the percentage of dips containing fountain darters were consistent from day 1 through day 3 suggesting that this method does effectively capture variation from day to day both within and among randomly selected sites (Figure 14). Fountain darters were present in 71% of sites on day 1 and in 67% of a different set of randomly selected sites on day 2, showing little variation among sites. When the original sites were re-sampled on day 3, fountain darters were present at 73% of sites demonstrating little variation within sites. Small amounts of variation within and among sites suggest that this method is repeatable and can be used to detect trends in the fountain darter population. With a sample size of 50 sites a change of 19% is needed to detect statistically significant differences ($\alpha = 0.05$). Although it provides no inference into the magnitude of the change, if correlated with seasonal drop netting data, this method could serve as a surrogate technique to provide continuous monitoring, especially during critical low-flow periods.

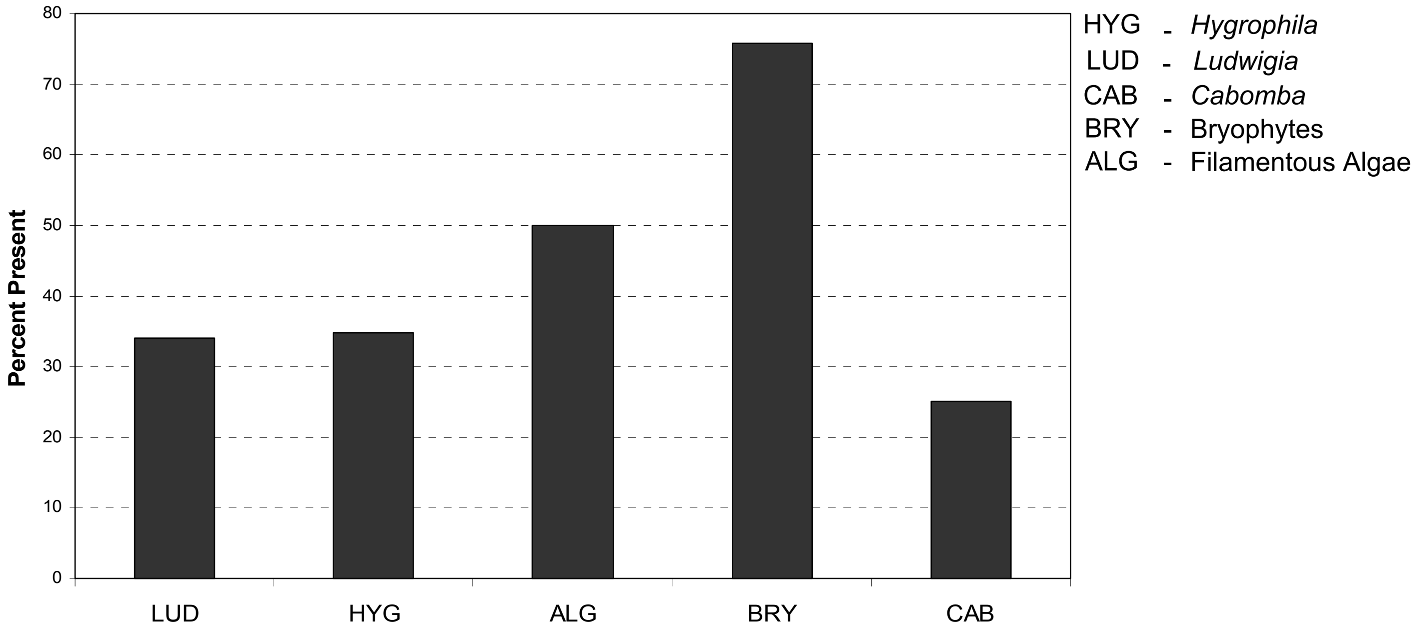

Figure 13. Percentage of sites in which fountain darters were present for each of the five vegetation types sampled by dip netting in the Comal River.

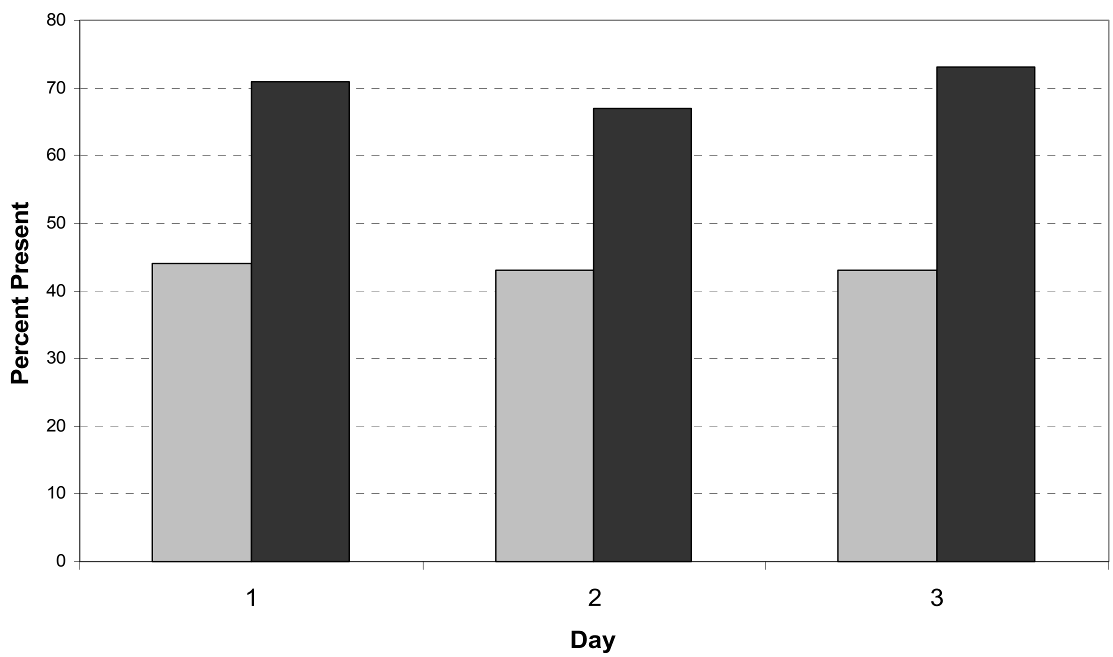

Figure 14. Percentage of sites (dark bars) and percentage of dips (light bars) in which fountain darters were present on each day.

Visual Observations

Fountain darters were observed in the deepest portions of Landa Lake (depths greater than 2 m) during each sampling event, including all low-flow and high-flow events to date. Fountain darters were observed throughout the reach in bare areas and surrounding vegetation, with the greatest concentrations occurring near areas with dense coverage of *Riccia*. Throughout the study period, observations of fountain darters in the sample area have remained consistently high. The quantitative sampling results are limited to a single grid per sampling event; therefore an accurate estimate of the true population size within the sample area is not possible. A much more labor-intensive effort would be required to provide such an estimate. These data simply provide an indication of the relative abundance of the fountain darters that are found in areas similar to that sampled and allow some insight into trends that may be occurring over time. Table 9 shows the number of fountain darters observed in the 7.8 m^2 grid per sampling event.

Table 9. The number of fountain darters observed in Landa Lake per grid/sampling event.

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

These results suggest there is some fluctuation in the use of the deepest areas of Landa Lake by fountain darters. The presence of *Riccia* appears to have a strong influence on the fountain darter. Substantially fewer individuals were found during periods when the percent coverage of *Riccia* was 50% or lower. The highest number was found when *Riccia* coverage was greater than 80%. It appears that there may be also some seasonality controlling populations since the highest numbers were observed during the fall of each year. Little change in the number of fountain darters was observed from fall 2004 to spring 2005 despite a 20% increase in *Riccia* coverage. Despite only two sampling dates this year, the number of fountain darters increased in the fall of 2005 with little change in *Riccia* coverage (5% increase), which again points to seasonality as a driving factor in these populations. Overall, our observations continue to reinforce the hypotheses 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.

Gill Parasite Evaluation

The findings of the 2005 activities related to parasite evaluation efforts are described in more detail in Lozano's thesis (2005; University of Texas at San Antonio) entitled: "The Effects of Turbulence and Snail Populations on the Abundance of *Centrocestus formosanus* Cercaria in the Comal River, Comal County, TX." However, a brief summary of the study is provided here.

From the preliminary efforts it was determined that there were significantly more parasites present in the water column on sunny days compared to cloudy days ($P < 0.01$). There was no difference in parasite abundance vertically within the water column, but as current velocity increased parasites were significantly more abundant ($P < 0.01$). As in Cantu (2003), time of day was an important factor in parasite abundance with the most present between 0930 and 1230 (since depth and horizontal distance across the channel did not significantly affect parasite numbers, it was determined that five samples at each cross section would be sufficient to characterize parasite abundance). However, because this effort was only conducted at one site that may not accurately reflect other sites in the Comal River, this part of the experiment will be repeated at all six sites early in 2006. These findings will direct future sampling protocol for this study.

Monthly samplings of cerceria concentrations in 2006 will take place on sunny days between the hours of 0930 and 1230 at two sites in the Comal River. Using discharge measurements at each cross-section and drift rate, we can generate an estimate of total number of parasites drifting through the cross-section during the sample period. In addition, ten small (20–25 mm TL) fountain darters will be collected to determine parasite infestation during quarterly samples. Gill arches will be removed and examined to determine the number of parasites infecting each fish.

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. 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.

Comal Springs Salamander Visual Observations

All SCUBA/snorkel surveys revealed the presence of Comal Springs salamanders along the lake bottom and in each sampled Spring Run, except for the Spring Run at Spring Island in fall 2005. This is the first time zero salamanders have been observed at any site since the inception of this study. Comal Springs salamanders were observed around portions of the springs, under rocks at depths of up to 2.4 m. No Comal Springs salamanders were observed in any areas with excessive sediment. The total number of Comal Springs salamanders observed at each survey site during each sampling event is presented in Table 10.

The total number of salamanders observed in 2005 (41 and 45), were the lowest since 2001. Much of this decrease occurred at the Spring Island sites, though it is unclear why the changes were substantial at these reaches. Lack of larger rocks (> 5cm) at the Spring Run site at Spring Island may have contributed to this decline as users of the park at this site appear to have removed many of the large rocks salamanders use as cover. As a result, salamanders may have moved the short distance to the east outfall of Spring Island, which may explain why observations increased at this site from spring to fall 2005. 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.

Table 10. Total number of Comal Springs salamanders observed at each survey site during each sample period.

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
August 2000	9	13	11	1	34
September 2000	5	14	6	5	30
Fall 2000	8	4	4	2	18
Winter 2001	16	9	8	1	34
Spring 2001	20	7	17	6	55
Summer 2001	23	15	4	4	46
High-flow 1 2001	31	12	1	6	50
Fall 2001	11	8	13	7	39
High-flow 2 2001	18	2	6	5	31
Winter 2002	18	9	7	3	53
Spring 2002	10	15	6	5	62
High Flow 2002	18	7	3	16	67
Fall 2002	20	10	8	9	47
Spring 2003	20	21	6	13	60
Summer 2003	25	10	3	13	51
Fall 2003	31	10	3	19	63
Spring 2004	36	14	7	12	69
Summer 2004	27	14	4	14	59
Fall 2004	20	2	2	35	59
Spring 2005	18	10	2	11	41
Fall 2005	22	7	0	16	45
Average	19.3	10.1	5.8	9.7	44.3

The high flow event in November 2004 may have contributed to the decrease in salamander observations in 2005. As flows decreased over 2005 (and no substantial rain events occurred), total numbers of salamander observations increased. Observations decreased at Spring Run 3 from spring to fall 2005, but there were no observable changes in habitat to explain this phenomenon.

Macroinvertebrate Sampling

In 2005, 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.

Drift Net sampling

A total of 144 hours of sample time occurred among the three drift net sites at Comal Springs in 2005 and 11 species were captured (Table 11). Table 12 displays the physico-chemical data collected at these sites. Total discharge in the Comal River was approximately 447 cfs during the April sample and 365 cfs during the October sample. There was only one minor "spike" in the hydrograph in 2005 (average daily discharge of 739 cfs), which occurred on March 5, prior to the April sample. However, this was a short-lived event and conditions returned to approximately the same discharge within a few days and should not have had much influence on the sample taken six weeks after this event. There have not been any opportunities with drift net sampling during this study to evaluate low flow conditions. However, there has been variation in flow conditions among sample efforts; the summer and fall samples in 2004 occurred shortly after high flow events.

Despite variable conditions, there have been common observations among samples. Species of the genus *Stygobromus* and *Lirceolus* were the most abundant at all sites during each sampling event (Figure 15) suggesting that they should be considered in any attempts to establish a springflow biological response relationship. *Stygobromus pecki* (Peck's cave amphipod) was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long, which suggests that smaller individuals may be more susceptible to expulsion from the aquifer. Those individuals that were too small to identify to species were recorded as *Stygobromus* sp. and most likely consisted of both *S. russelli* and *S. pecki*.

Among species of concern, an average of 14.5 *Stygobromus pecki* (many small *Stygobromus* were unidentifiable to species), 3.2 *Heterelmis comalensis*, and 1.3 *Stygoparnus comalensis* were retrieved during each 24-hour period at all sites. Although the average number of *S. pecki* was lower in 2005 relative to 2004, the beetle numbers (*H. comalensis* and *S. comalensis*) and combined *Stygobromus spp*. numbers were progressively higher than in 2003 and 2004. As in 2004, *Stygobromus pecki* was found in all three locations. On average more were found in the western shoreline habitat than the other two locations, but in October more were collected in Spring Run 3 than the other two locations. As in 2004, *H. comalensis* and *S. comalensis* were found in Spring Runs 1 and 3 but not in the western shoreline habitat of Landa Lake, where a single *S. comalensis* was collected in 2003 and no *H. comalensis* have been found. As discussed in the methods section, the Landa Lake shoreline location where the drift net is placed does not correspond to the same location where riffle beetles are sampled with cotton squares. Despite the relatively large size of this spring opening and substantial discharge the extensive survey of *H. comalensis* distribution in 2001 (BIO-WEST 2002b) did not identify any individuals in this habitat nor have any drift net samples taken there since. This site has lower current velocities than the two Spring Run sites, so high velocity is not a limiting factor. It just appears that the population has a patchy distribution within the lake and does not occur in this area at this time.

Table 11. Total numbers of troglobitic and endangered species collected in drift nets during April and October, 2005. Federally endangered species are designated with (E). A = adult beetles. L = larvae.

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	48	48	48	144
Crustaceans				
Amphipoda				
Gammaridea				
Crangonyctidae				
Stygobromus pecki (E)	14	21	52	87
Stygobromus russelli		2		2
Stygobromus spp.	153	235	346	734
All *Stygobromus*	167	258	398	823
Hadziidae				
Mexiweckelia hardeni	4	18	0	22
Sebidae				
Seborgia relicta		13	1	14
Artisiidae (=Bogidiellidae)				
Artesia subterranea	1			1
Isopoda				
Asellidae				
Lirceolus (2spp.)	42	49	11	102
Cirolanidae				
Cirolanides texensis	1			1
Insects				
Coleoptera				
Dytiscidae				
Comaldessus stygius		13 A		13
Haideoporus texanus		2 (1L, 1A)		2
Dryopidae				
Stygoparnus comalensis	5 L	3 L		8
Elmidae				
Heterelmis comalensis	8 (6L, 2A)	11(4 L, 7A)		19

Table 12. Results of water quality measurements conducted in 2005 during drift net sampling efforts at Comal Springs.

	Spring Run 1		Spring Run 3		West Shore Upwelling	
Date	April	Oct	April	Oct	April	Oct
Temperature (°C)	22.9	22.9	23.1	23.1	23.5	23.6
Conductivity (mS)	0.547	0.537	0.541	0.545	0.545	0.553
pH	7.0	7.1	7.0	7.1	7.1	7.0
Dissolved Oxygen (mg/L)	5.8	5.8	5.6	5.7	5.7	5.6
Current Velocity (m/s)	0.9	0.8	0.6	0.4	0.5	0.3

During 2005, most species had a higher abundance at all sites during the October sample compared to the April sample. The exceptions are the combined *Stygobromus* sp. along the western shoreline, *Stygoparnus comalensis* in Spring Run 3 and *Mexiweckelia hardeni* in all localities which decreased in number between April and October. Because discharge was approximately the same during each sample (with no strong influence of recent "spikes" in the hydrograph) it is not likely that these differences are related to discharge, but may represent natural or seasonal variability in these populations.

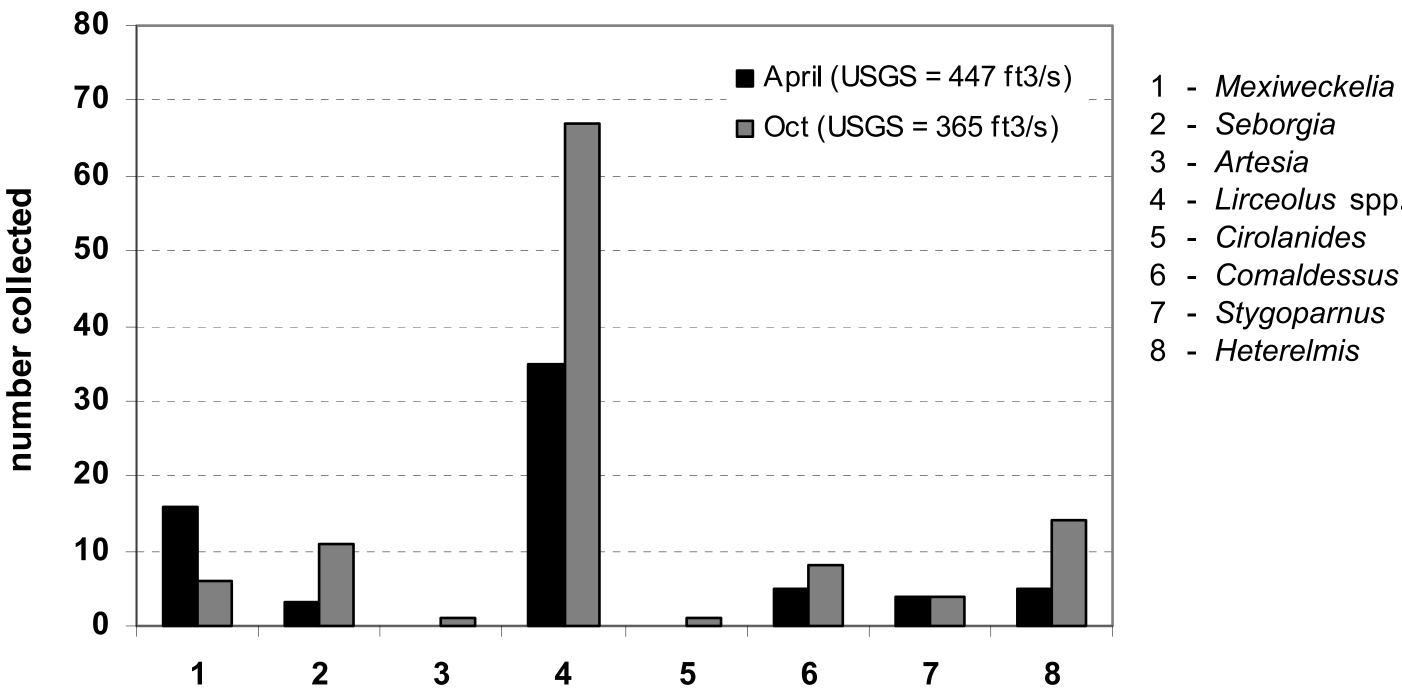

Figure 15. Drift net sampling results for each species (except *Stygobromus* sp.) combined across all sample sites.

One of the most interesting findings of the three years of drift sampling has been the observations of many specimens of an undescribed genus of mite (only in Spring Run 1). The closest known relatives occur in springs and seeps in India and Romania.

Comal Springs Riffle Beetle

The Comal Springs riffle beetle sampling provides basic information on the population dynamics and distribution among sample sites. A total of 1,009 Comal Springs riffle beetles were collected in all 2005 samples (1,186 individuals were observed in 3 samples during 2004). As in 2004, the number of larvae (n=227) was far fewer than the number of adults (n=782). This 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 continue to be sampled in all sampling efforts, 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.

Each of the three sample sites has very different physical characteristics. The Spring Run 3 site has lateral spring flow from the shoreline into the fast moving primary current of the Spring Run, the western shoreline site has lateral spring flow directly into Landa Lake and the Spring Island site has upwelling flow from the bottom of Landa Lake. The Spring Island site had the lowest velocity 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. Despite the physical differences between sites, there were minimal differences in abundance among sites in 2005 (as in 2004). All three sites had similar numbers of observations in 2005 (n=306-381) and the range among sites within each season was very small. The relatively even distribution of Comal Springs riffle beetles among the three sample areas 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 2004-2005 has 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.

Although there were similar numbers of beetles sampled among sites, there were differences within sites. The beetles (both larvae and adults) tended to be patchily distributed with wide ranges of abundance among samples within a site and season. 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 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 was most pronounced in Spring Run 3. In the western shoreline habitat and near Spring Island, the beetles appeared to congregate in different areas during each of the 2005 samples suggesting there may have been higher rates of movement in these habitats. More movement relative to 2004 that may indicate a change in habitat suitability or other conditions that causes individuals to seek new habitat. Because movement appears to be more pronounced in two of the three sites, there may be some differences in habitat conditions; however, this has not influenced the relative abundance of the species among sites.

This valuable information on microhabitat use and distribution within the three sample sites can benefit management strategies that focus on maintaining suitable habitat conditions for Comal Springs riffle beetles in specific areas during a period of low recharge.

Exotics / Predation Study

Because there were no low-flow events in 2005, no samples were made for the exotics / predation component of this study.

Summary

This study remains the most comprehensive biological evaluation that has ever been conducted on the Comal River ecosystem. Overall, flora and fauna flourished in 2005 even though flows declined over much of the year. 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. With rainfall events scarce in 2005, we will closely monitor flows in these systems to assess possible critical low-flow periods in 2006. 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).

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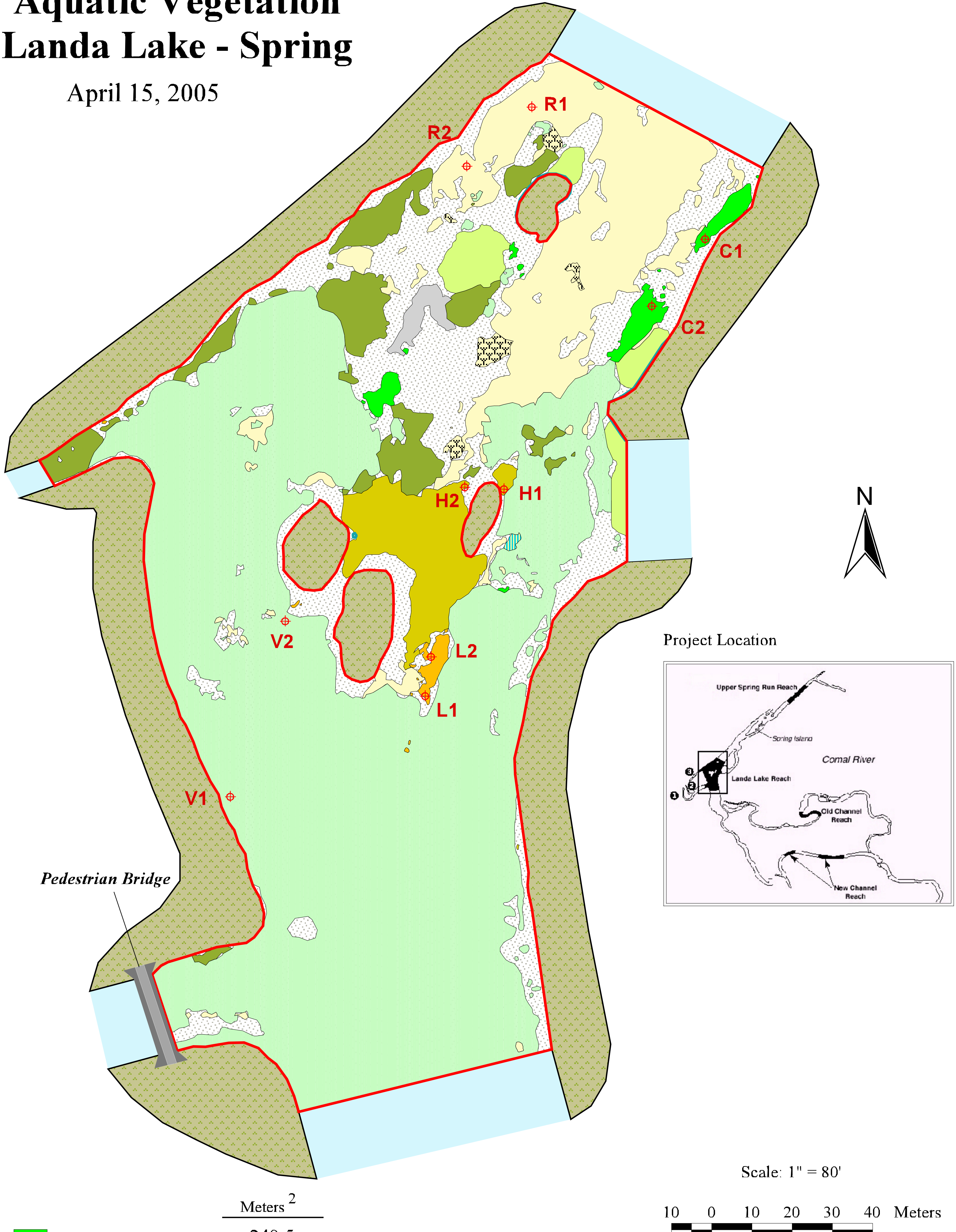
APPENDIX A:
AQUATIC VEGETATION MAPS

Landa Lake Reach

Comal River
Aquatic Vegetation
Landa Lake - Spring

April 15, 2005



	Meters^2
Cabomba	249.5
Hygrophila	846.3
Ludwigia	72.5
Nuphar	460.1
Bryophytes	2,800.9
Sagittaria	1,114.5
Vallisneria	12,822.5
Algae	110.8
Bryophytes / Vallisneria	106.8
Bryophytes < 50%	11.4

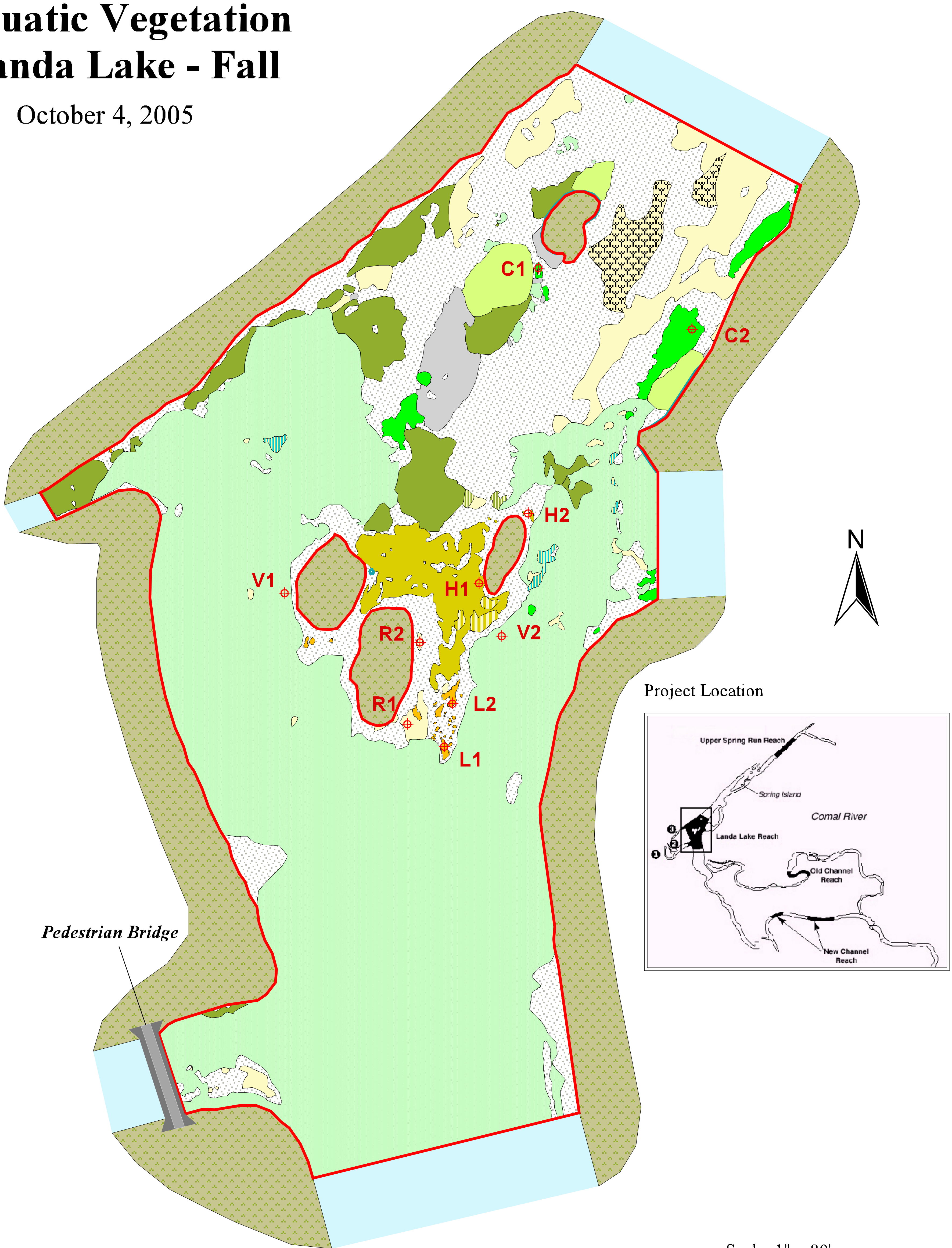
Shore and Islands
River
Study Area (22,551.2 m^2)
Bare Substrate
Colocasia
Drop Net Sample Sites

BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Landa Lake - Fall

October 4, 2005



Project Location

Scale: 1" = 80'

10 0 10 20 30 40 Meters

	Meters ^{2}
Cabomba	300.2
Hygrophila	558.9
Ludwigia	35.8
Nuphar	360.2
Bryophytes	1,055.4
Sagittaria	1,166.3
Vallisneria	13,015.8
Algae	337.2
Bryophytes < *50%*	312.3
Bryophytes / Sagittaria	17.0
Bryophytes / Vallisneria	41.6
Bryophytes / Hygrophila	39.5

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

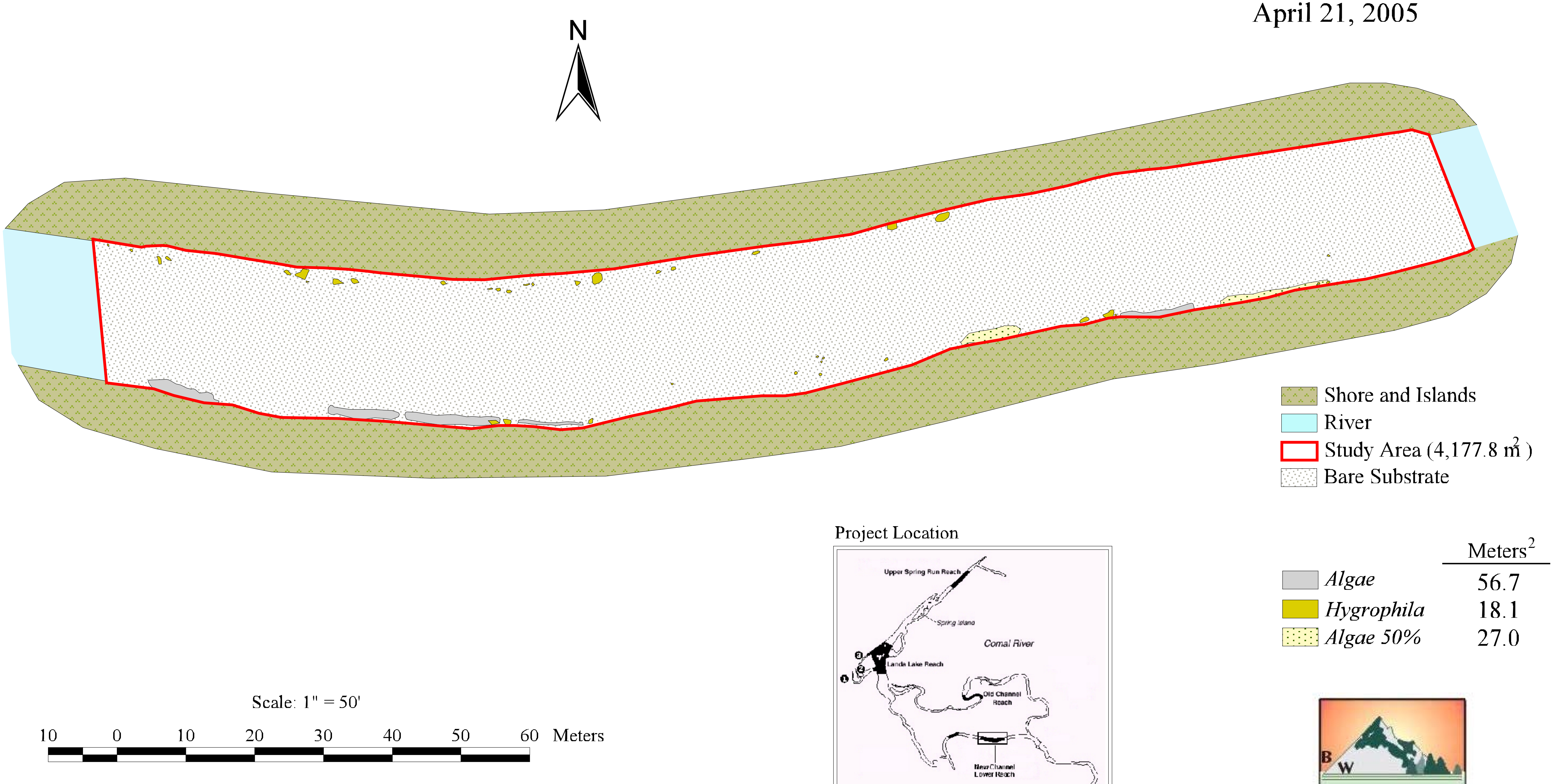
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

New Channel Reach

Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring

April 21, 2005

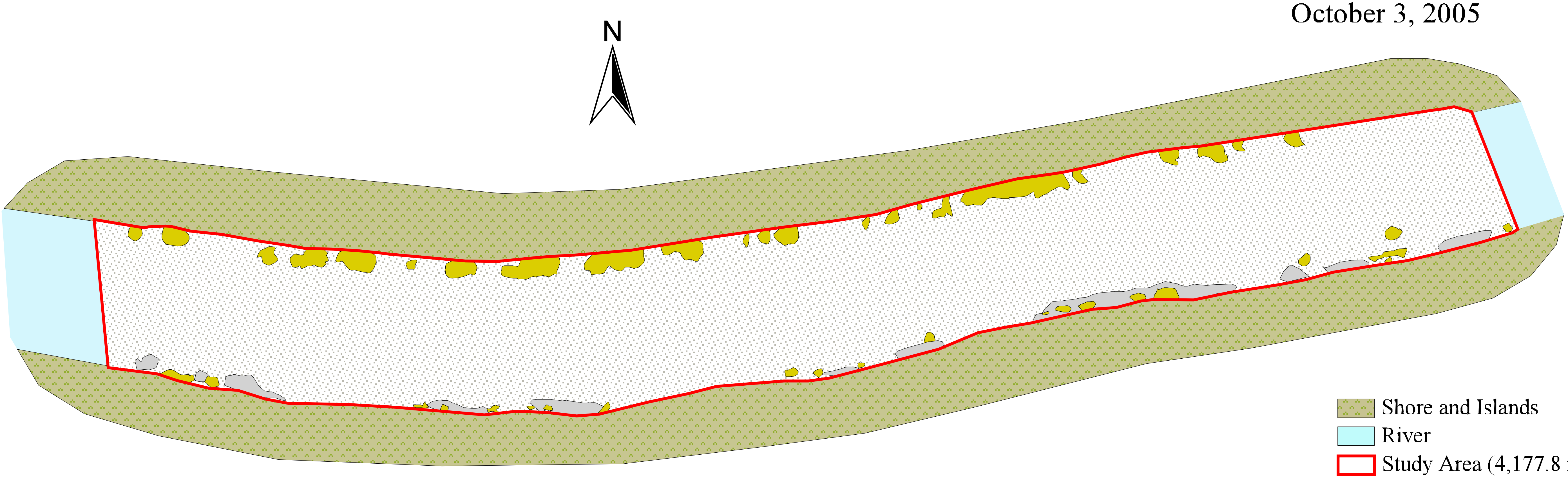


BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
New Channel Lower Reach
Fall

October 3, 2005



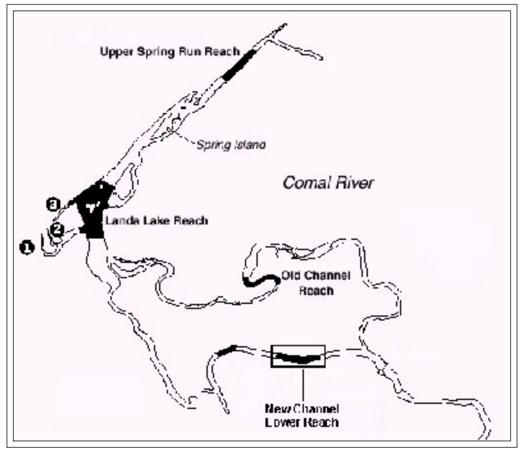
Shore and Islands
River
Study Area (4,177.8 m^2)
Bare Substrate

	Meters^2
Algae	123.3
Hygrophila	219.8

Scale: 1" = 50'

10 0 10 20 30 40 50 60 Meters

Project Location



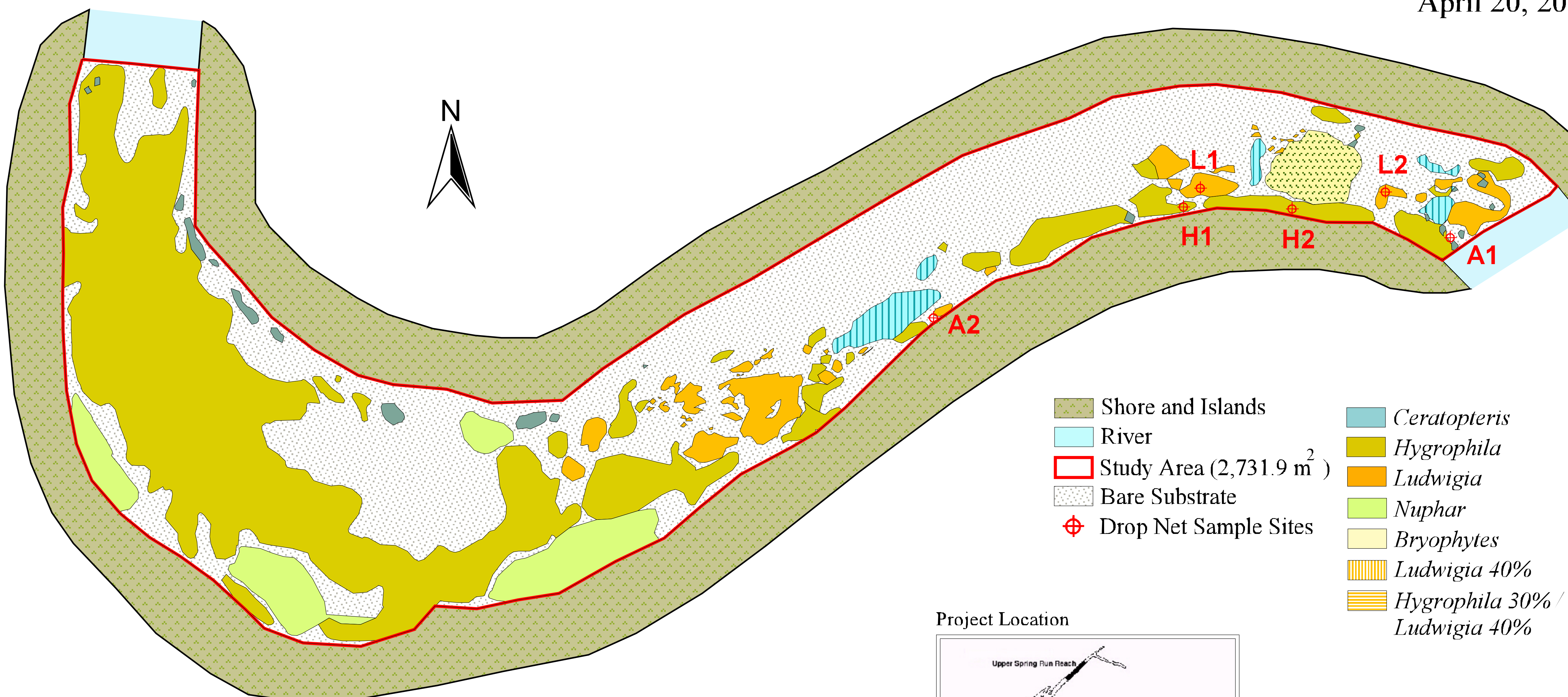
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Old Channel Reach

Comal River
Aquatic Vegetation
Old Channel Reach - Spring

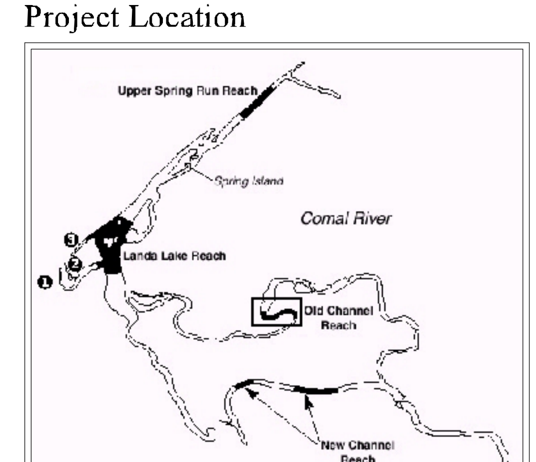
April 20, 2005



- Shore and Islands
- River
- Study Area (2,731.9 m^2)
- Bare Substrate
- Drop Net Sample Sites

	Meters^2
Ceratopteris	21.4
Hygrophila	953.2
Ludwigia	109.4
Nuphar	157.7
Bryophytes	1.0
Ludwigia 40%	46.0
Hygrophila 30% / Ludwigia 40%	44.0

Project Location



Scale: 1" = 35'

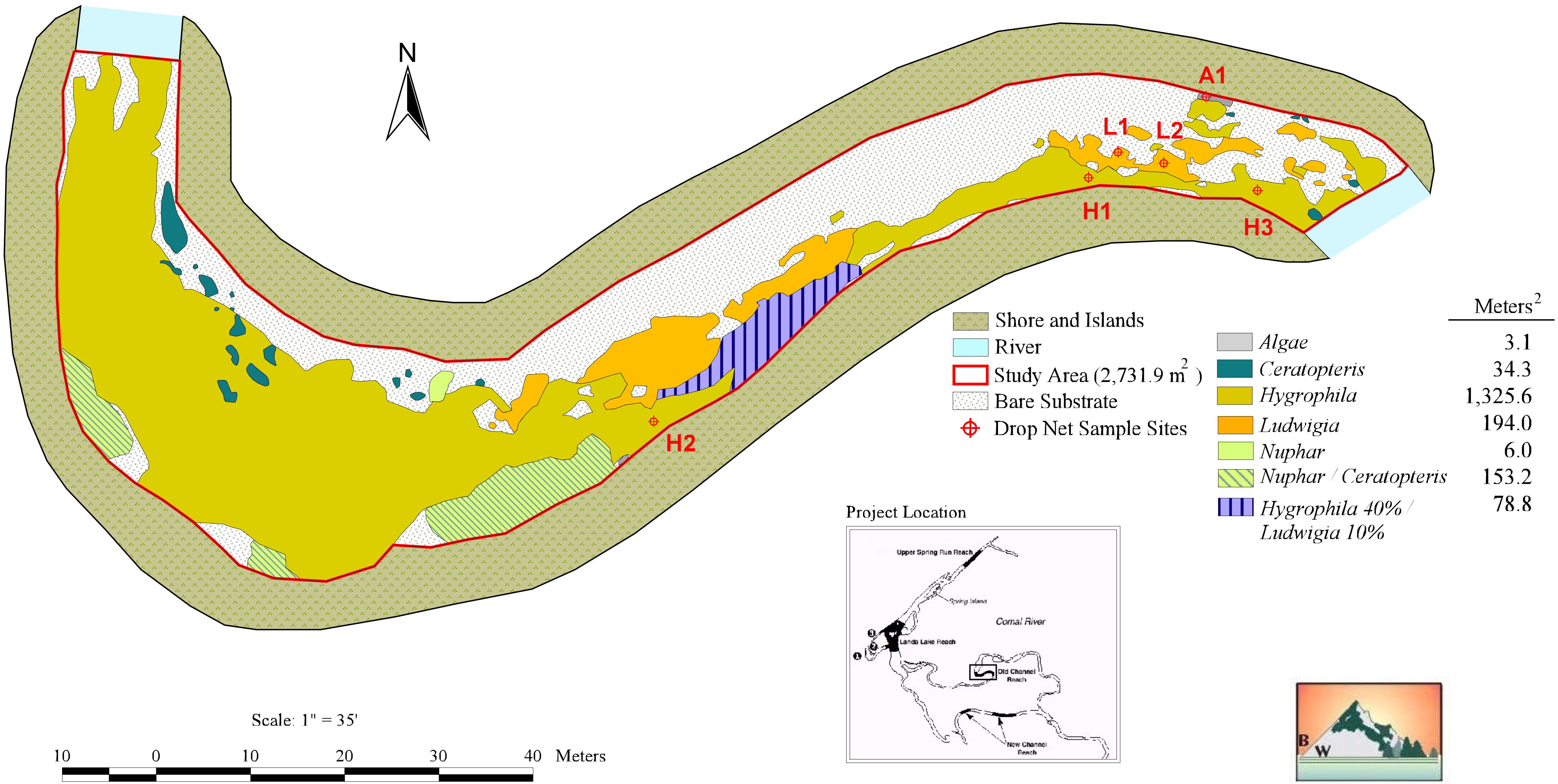
10 0 10 20 30 40 Meters

BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Old Channel Reach - Fall

October 5, 2005



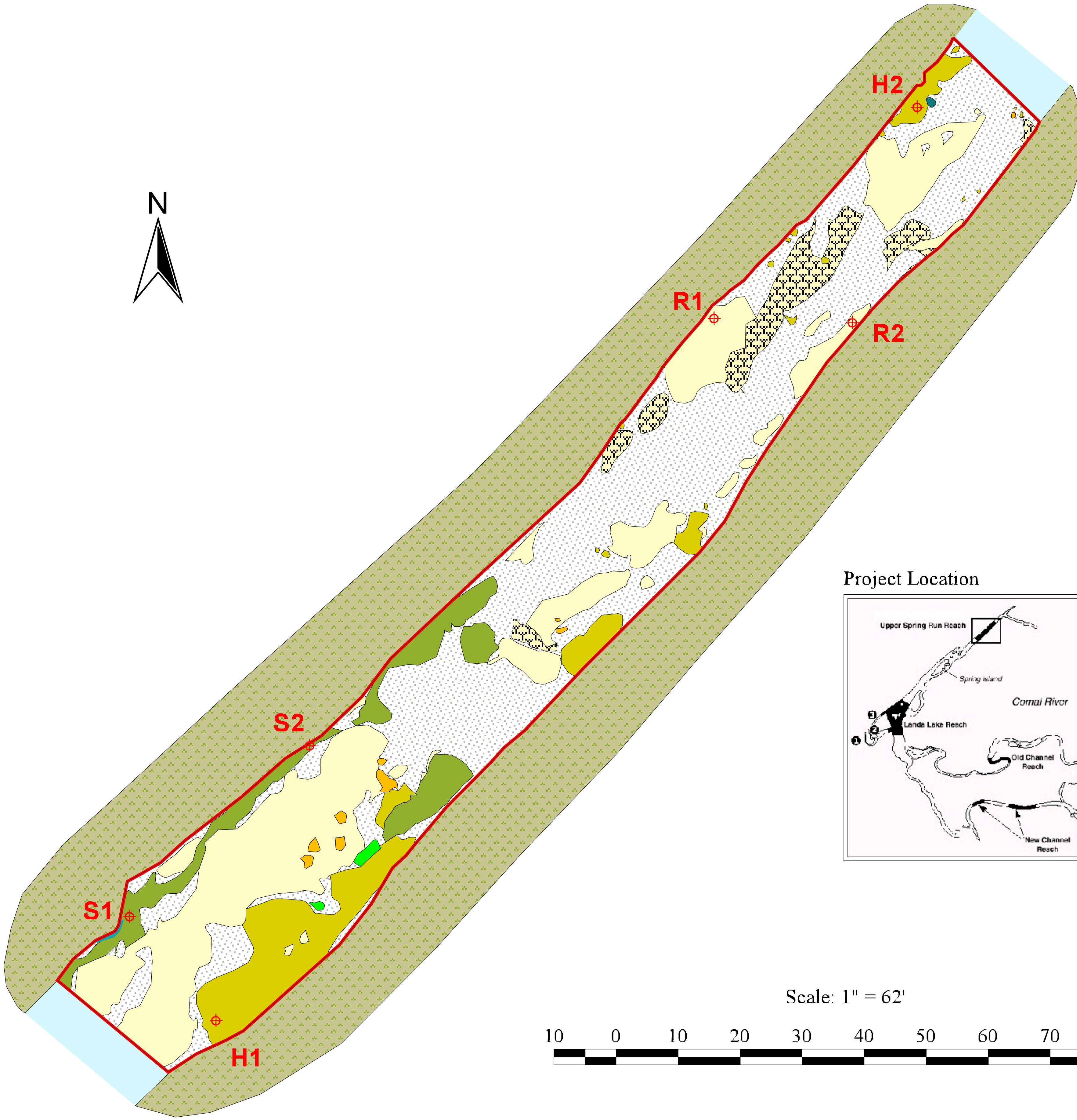
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Upper Spring Run Reach

Comal River
Aquatic Vegetation
Upper Spring Channel Reach
Spring

April 15, 2005



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

Project Location

	Meters^2
Cabomba	10.4
Hygrophila	487.8
Limnophila	1.8
Ludwigia	22.3
Bryophytes	1,385.7
Sagittaria	342.8
Bryophytes < *50%*	250.4

Scale: 1" = 62'

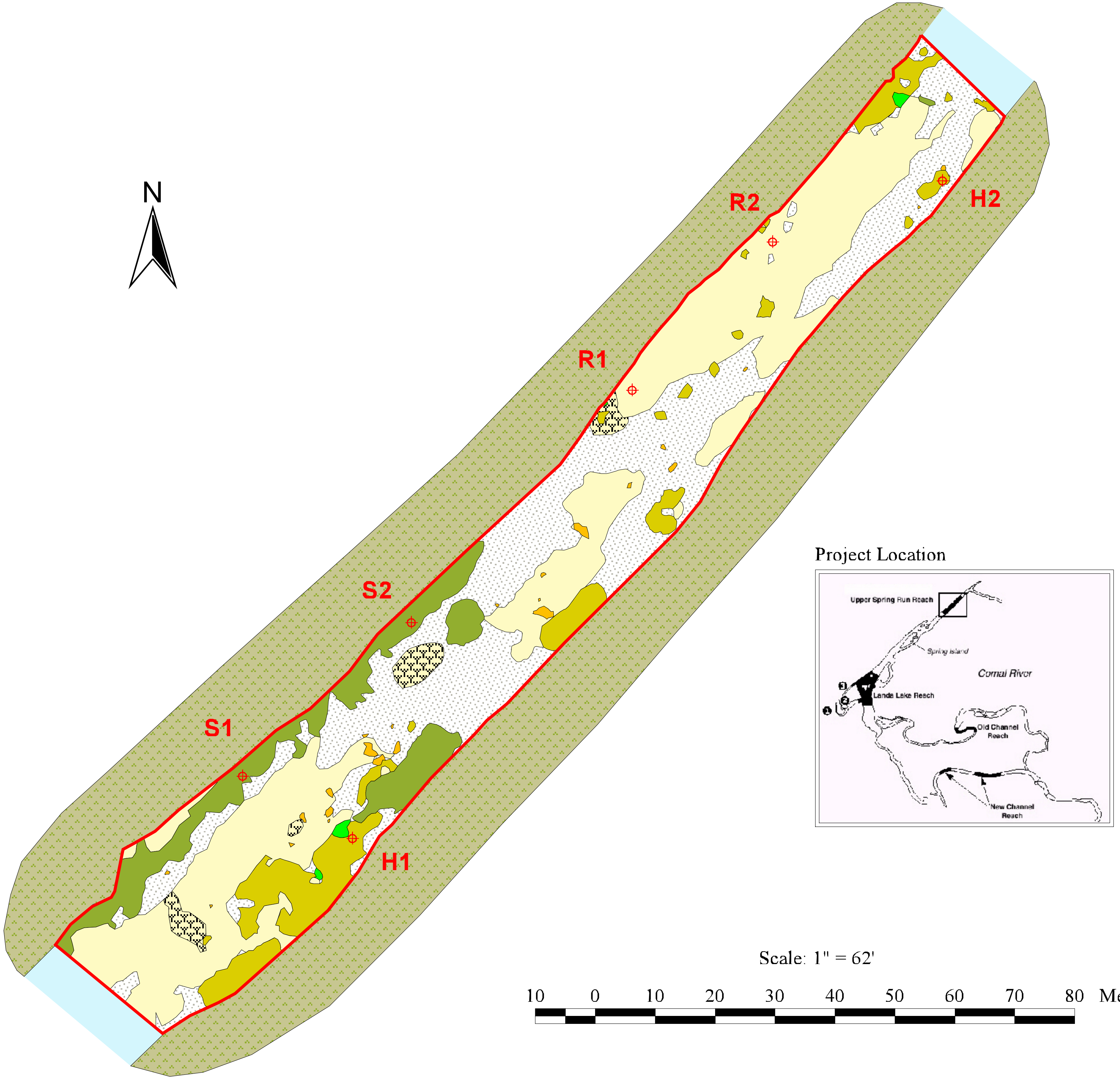
10 0 10 20 30 40 50 60 70 80 Meters

BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

Comal River
Aquatic Vegetation
Upper Spring Channel Reach
Fall

October 3, 2005



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

	Meters^2
Cabomba	12.4
Hygrophila	468.2
Ludwigia	24.4
Bryophytes	1,915.2
Sagittaria	494.2
Bryophytes < *50%*	104.0

Scale: 1" = 62'

10 0 10 20 30 40 50 60 70 80 Meters

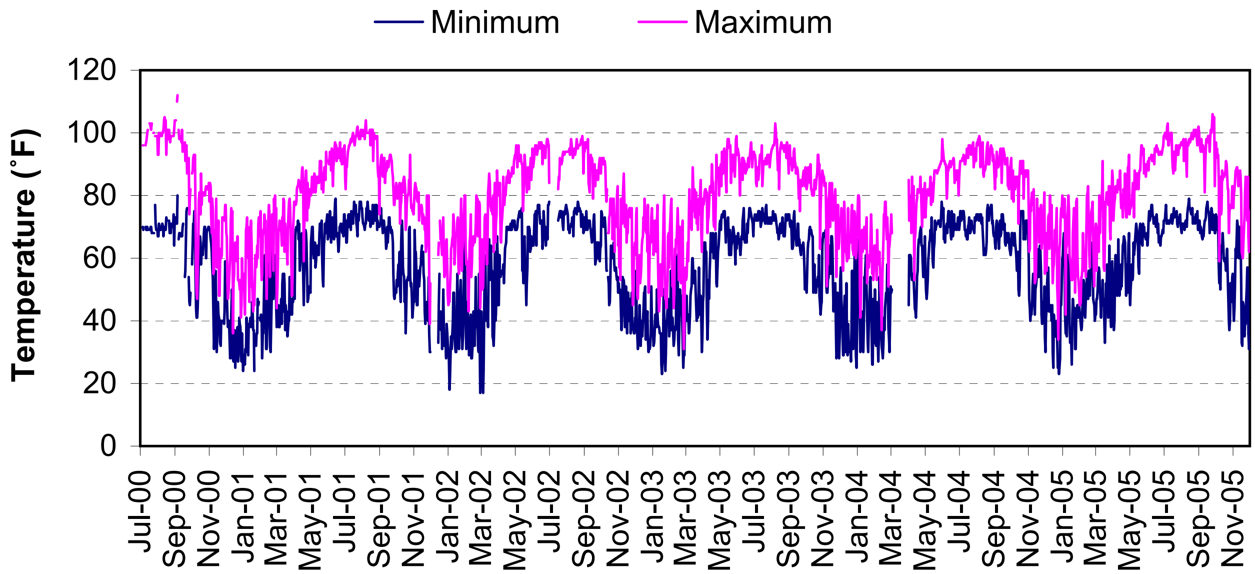
BIO-WEST, Inc.

EDWARDS AQUIFER AUTHORITY

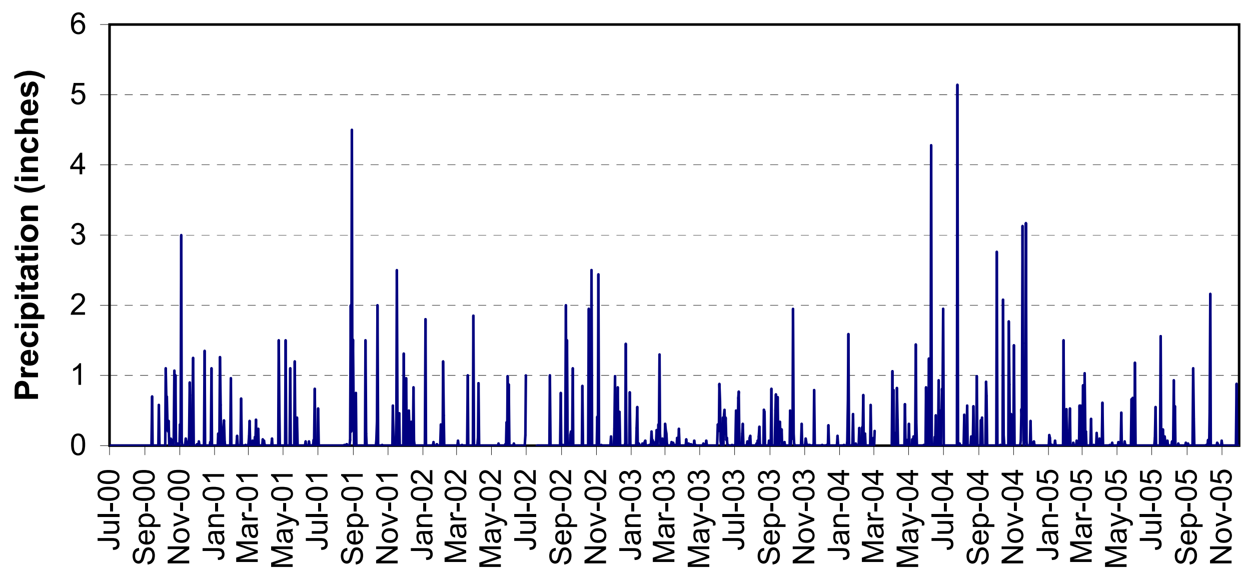
APPENDIX B:
DATA AND GRAPHS

Water Quality Data
and
Thermistor Graphs

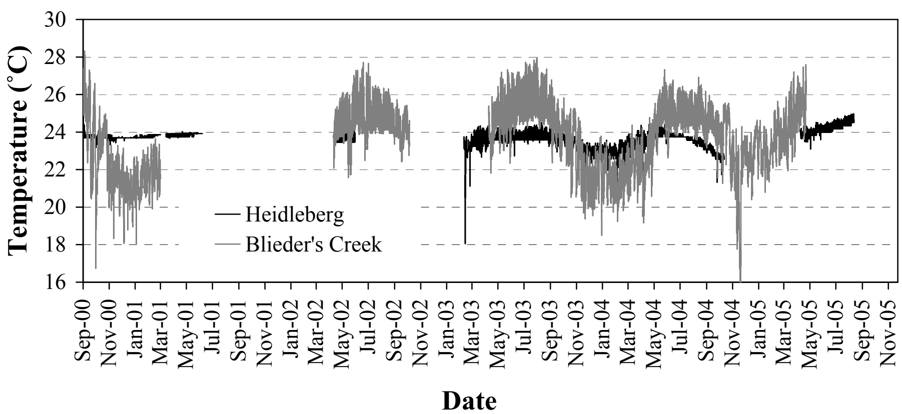
Daily Air Temperature Data for New Braunfels, Texas



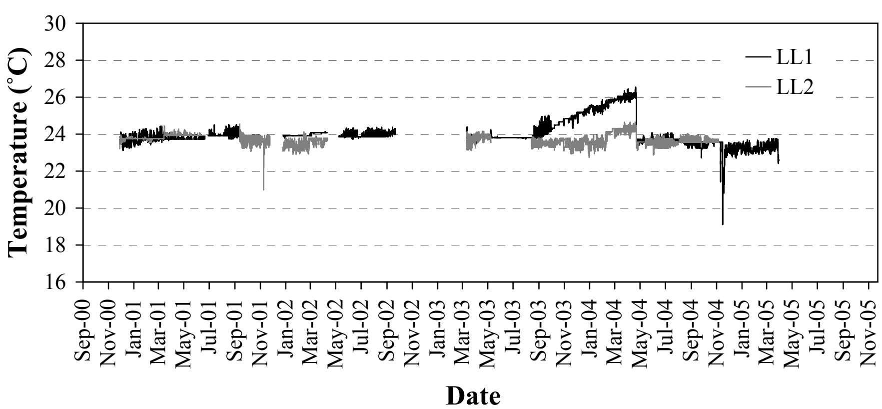
Daily Precipitation Data for New Braunfels, Texas



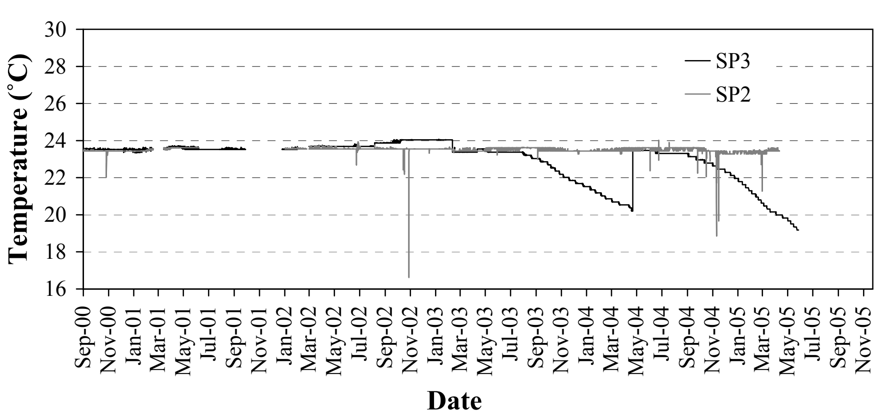
Thermistor Data: Comal Headwaters

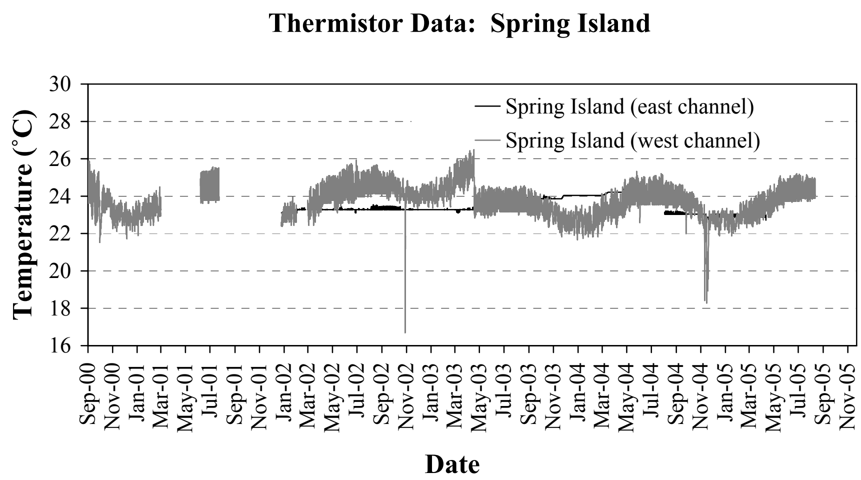


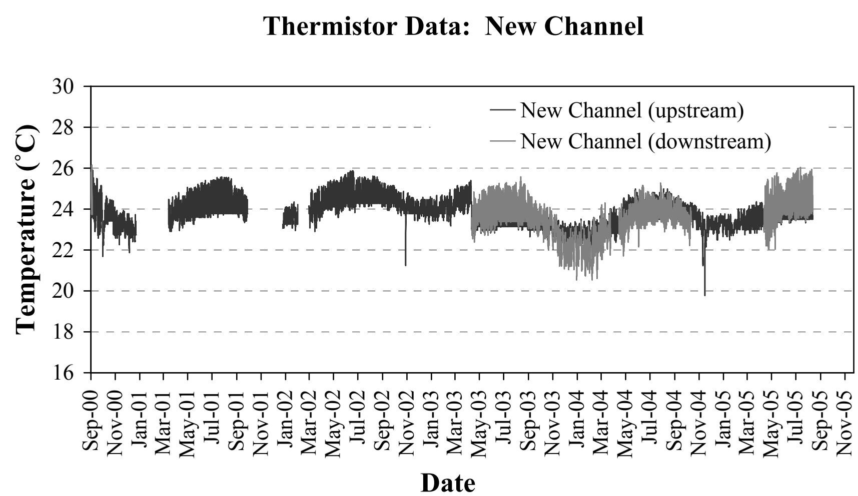
Thermistor Data: Landa Lake Bottom

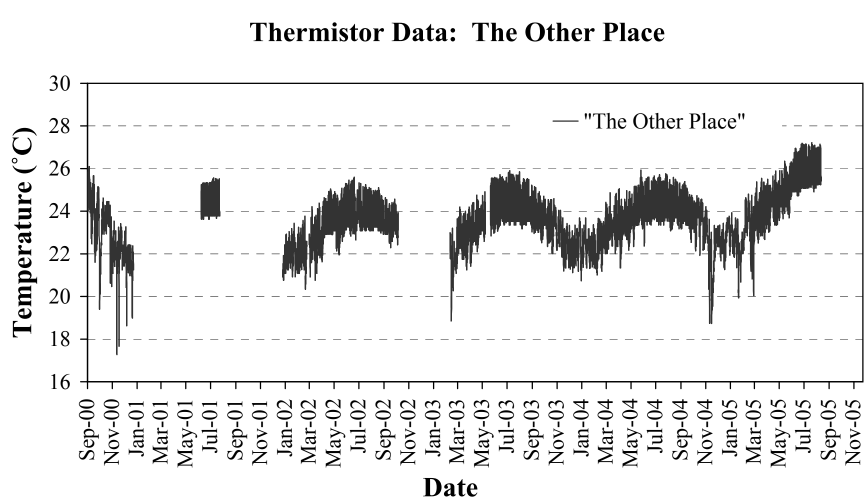


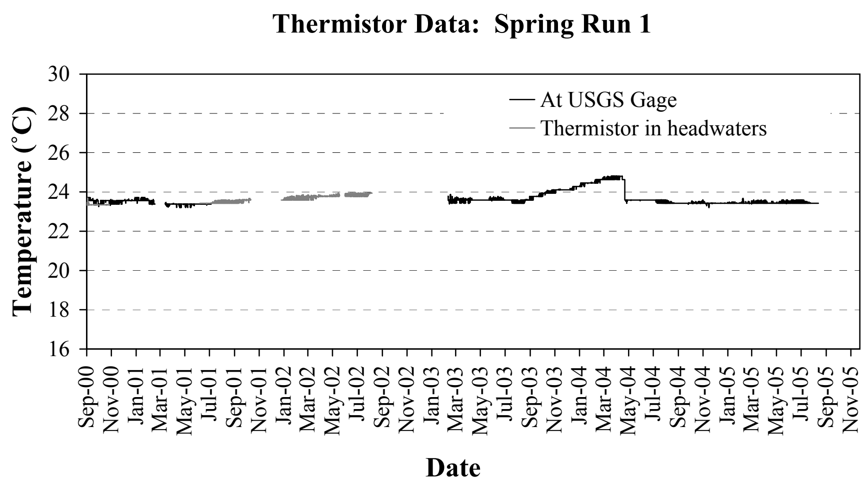
Thermistor Data: Spring Runs 2 & 3




Thermistor Data: Spring Island
Spring Island (east channel)
Spring Island (west channel)
Temperature (°C)
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
Jan-05
Mar-05
May-05
Jul-05
Sep-05
Nov-05
Date


Thermistor Data: New Channel
New Channel (upstream)
New Channel (downstream)
Temperature (°C)
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
Jan-05
Mar-05
May-05
Jul-05
Sep-05
Nov-05
Date


Thermistor Data: The Other Place
"The Other Place"
Temperature (°C)
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
Jan-05
Mar-05
May-05
Jul-05
Sep-05
Nov-05
Date


Thermistor Data: Spring Run 1
At USGS Gage
Thermistor in headwaters
Temperature (°C)
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
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Jan-04
Mar-04
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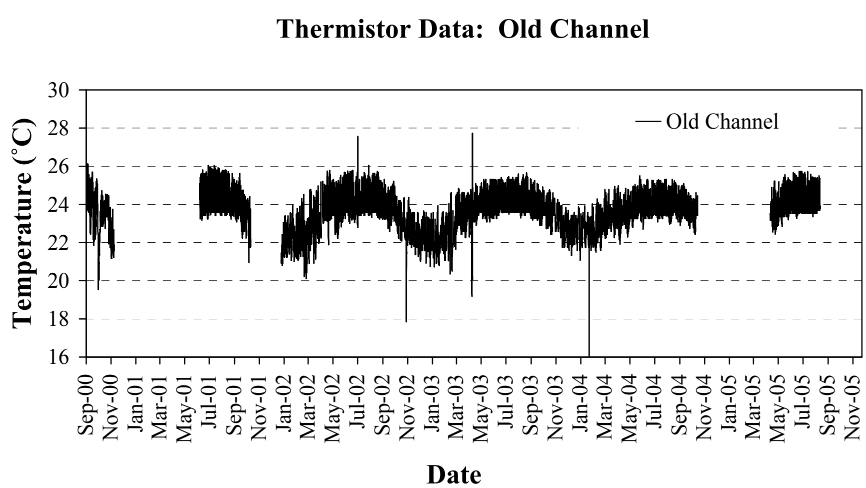
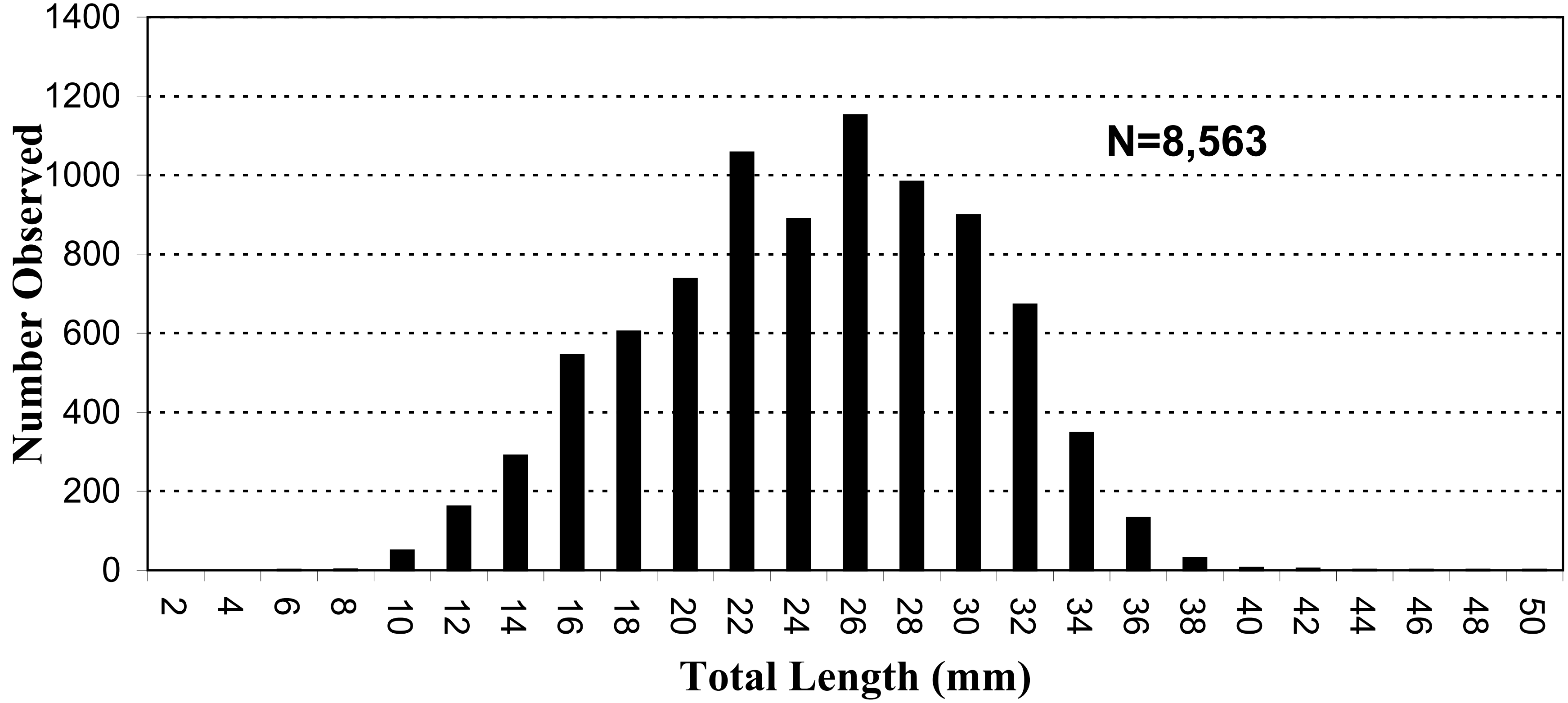
May-05
Jul-05
Sep-05
Nov-05
Date


Thermistor Data: Old Channel
Old Channel
Temperature (°C)
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
Jan-05
Mar-05
May-05
Jul-05
Sep-05
Nov-05
Date

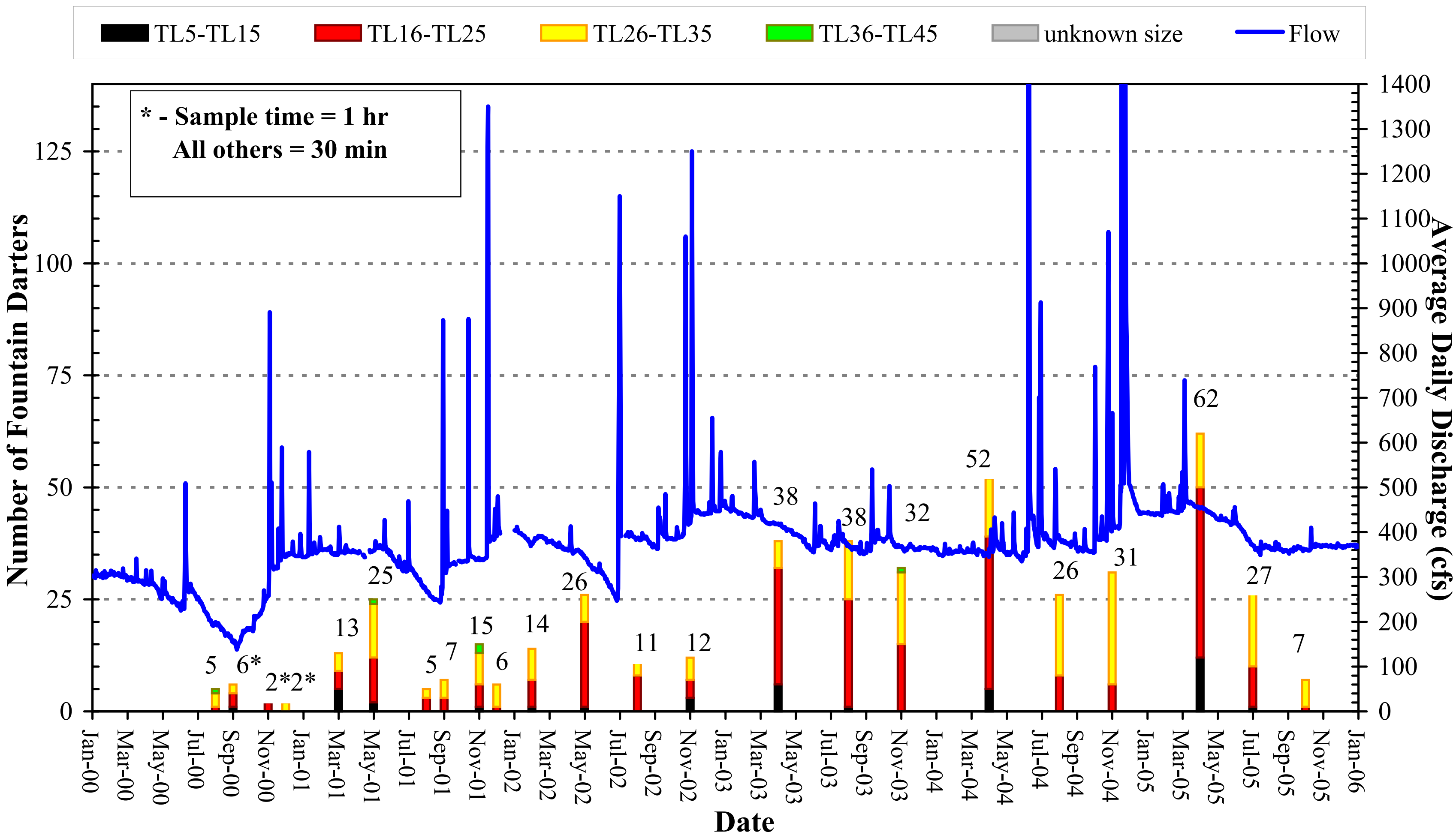
Drop Net Graph

Drop Net Results 2000-2005 in the Comal River

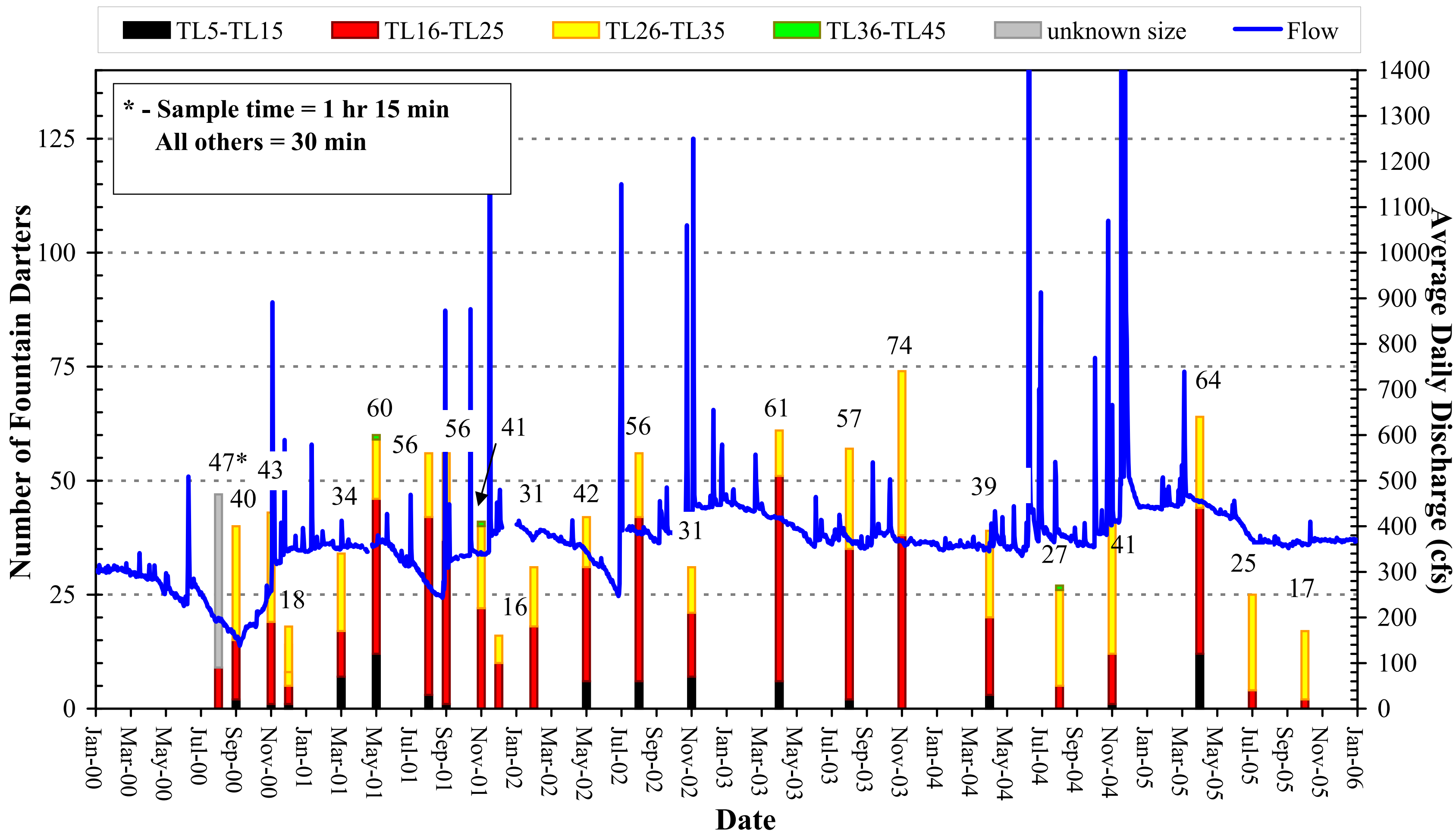


Dip Net Graphs

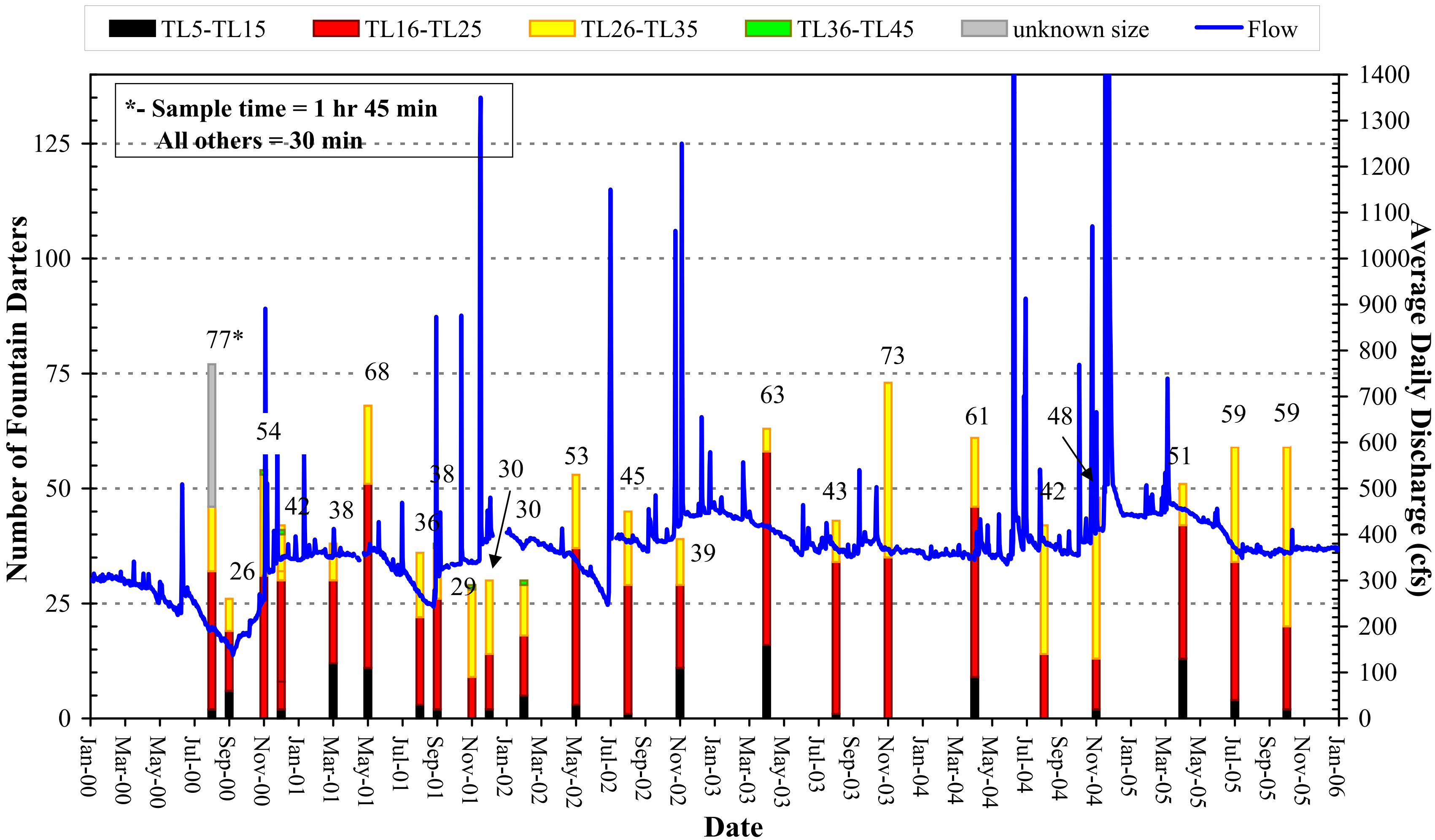
Fountain Darters Collected from the Upper Spring Run Reach (Section 3) Dip Net Results - Comal River



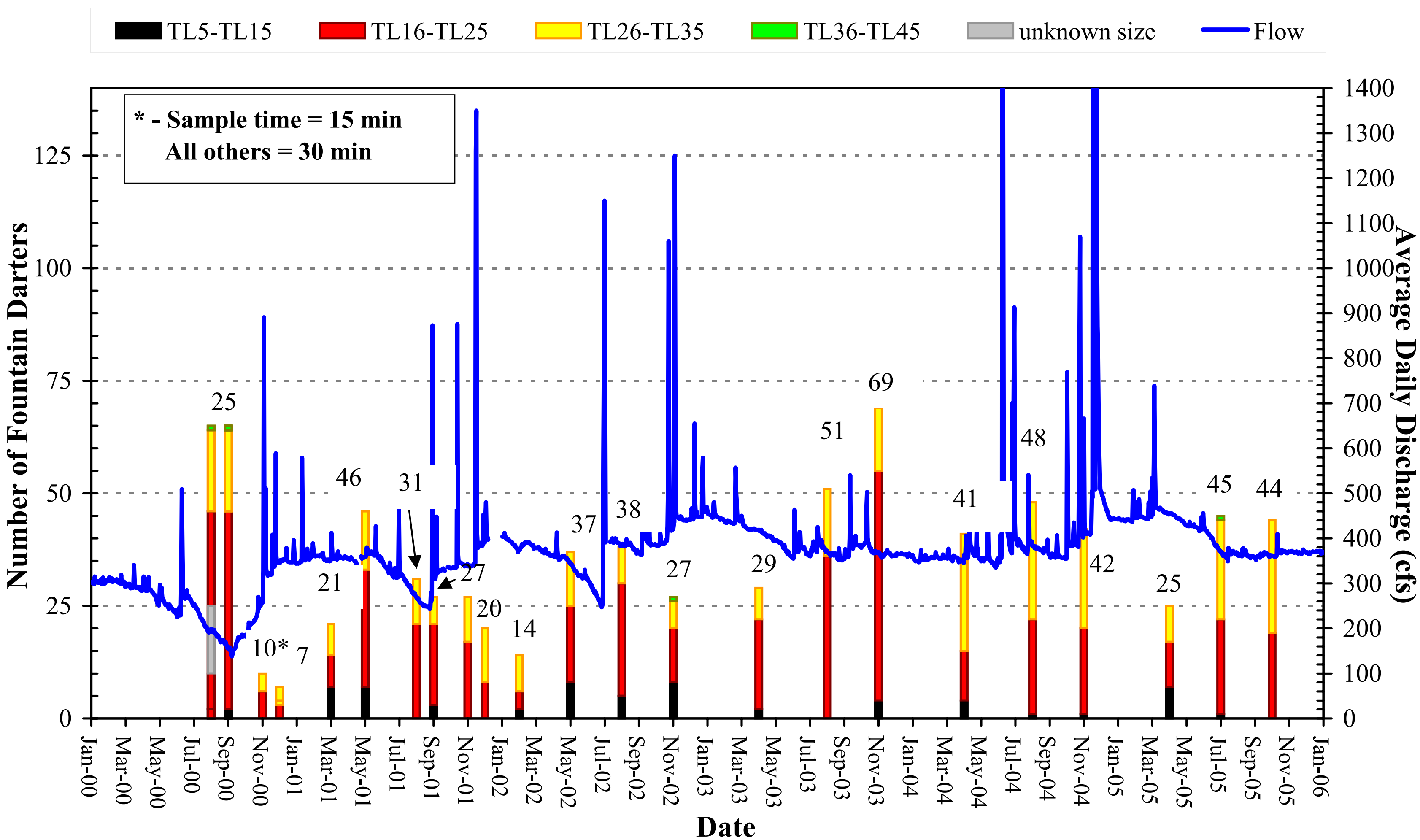
Fountain Darters Collected from the Landa Lake Reach (Section 4L) Dip Net Results - Comal River

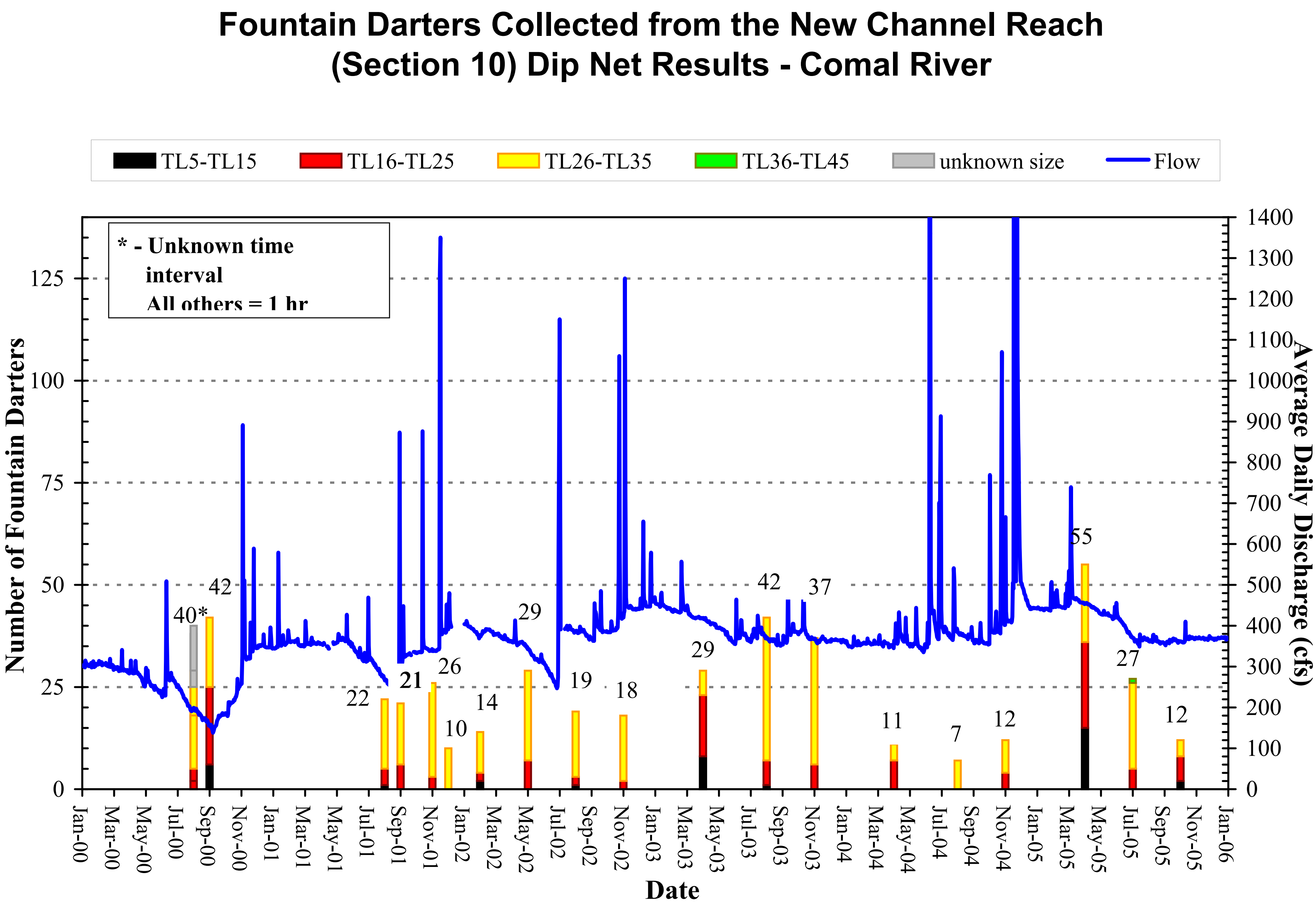


Fountain Darters Collected from the Spring Island Area (Section 4U-M) Dip Net Results - Comal River



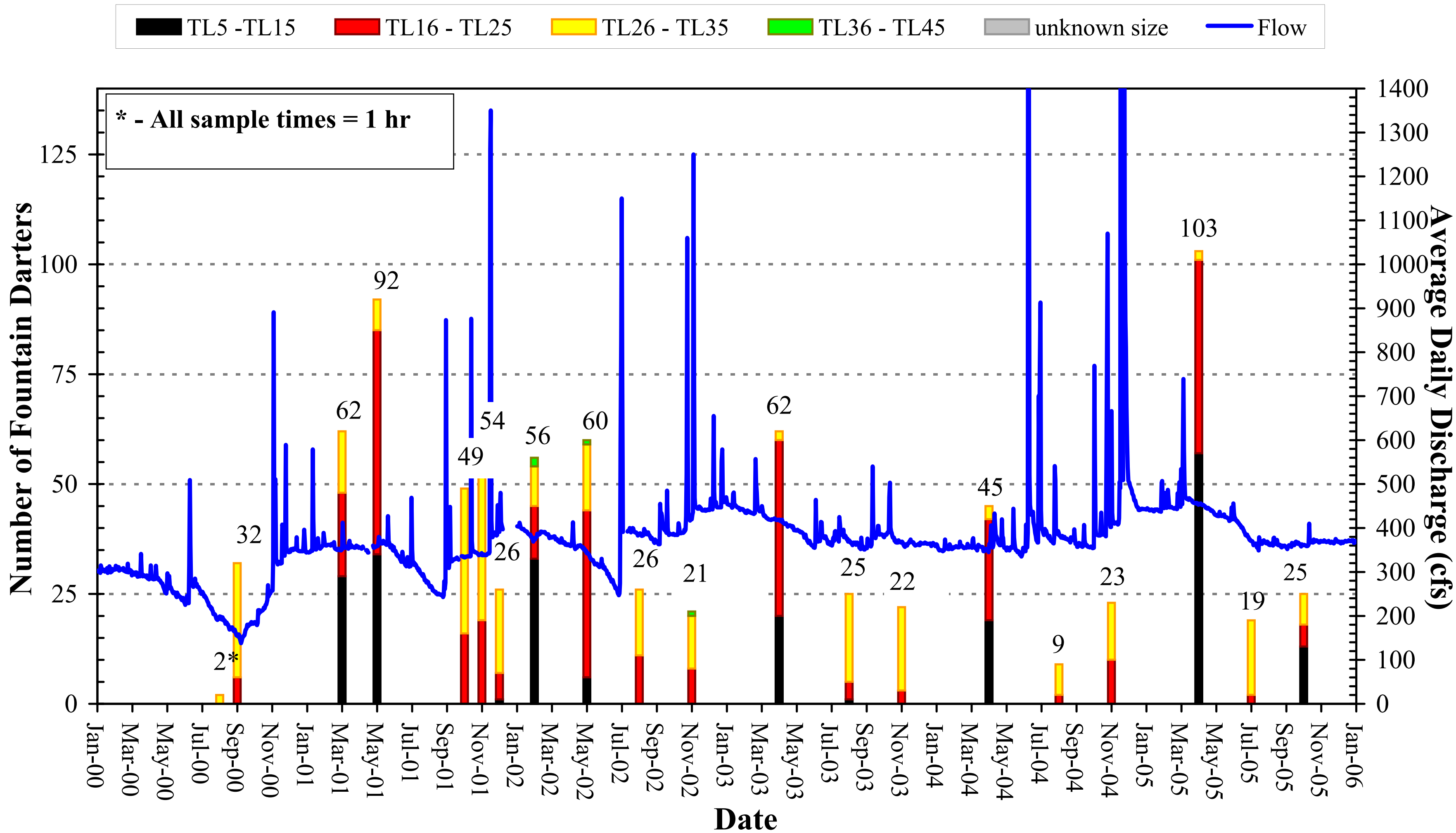
Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River





Fountain Darters Collected from the New Channel Reach
(Section 10) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Unknown time interval
All others = 1 hr
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
40*
42
22
21
26
10
14
29
19
18
29
42
37
11
7
12
55
27
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
Mar-05
May-05
Jul-05
Sep-05
Nov-05
Jan-06

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



APPENDIX C:
DROP NET RAW DATA

(not available online)

APPENDIX D:
LOZANO (2005) THESIS

UNIVERSITY OF TEXAS AT SAN ANTONIO
San Antonio, Texas

The Effects of Turbulence and Snail Populations on the Abundance of *Centrocestus formosanus* Cercaria in the Comal River, Comal County, TX

A Thesis Submitted in Partial Fulfillment of the Requirements for the Bachelor of Science Degree in Biology with Tier 2 Honors in the Honors College

Carlos Lozano

Department of Biology
May 2005

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Dr. Janis K. Bush, Thesis Reader

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Dr. Jyotsna Sharma, Thesis Reader

____.
Dr. Richard Diem, Dean of the Honors College

RECEIVED BY THE HONORS COLLEGE:

May 2005

Abstract

The digenetic exotic trematode from Asia known as *Centrocestus formosanus* has become established in the Comal River, Comal County, TX. The parasites presence in the river threatens the populations of the endangered fountain darter (*Etheostoma fonticola*). The parasite's utilization of three hosts (birds, snails, and fish) to complete its life cycle (Figure 1) complicates monitoring parasite numbers in a stream. Theron (1979) and Prentice (1984) have developed a practical and effective filtration technique for studying the free-living stage of the parasite called cercaria. In this study that filtration technique was modified to evaluate cross-sectional areas within the Comal River. Parasite recruitment from snail populations and the effect of turbulence on their prevalence was observed. It was found that snail populations contribute to the amount of parasites present in the water however, not significantly. It was also found that turbulence decreases the amount of parasites in the water.

Table of Contents

Acknowledgements

I want to thank Tom Brandt and Valentin Cantu of the San Marcos National Fish Hatchery and Technology Center for all the help and support I received during this study. Tom Brandt, my advisor, provided guidance that was important in the completion of the project. Valentin Cantu provided guidance and companionship during my training for this project. I would also like to thank my thesis readers Jyotsna Sharma and Janis Bush who provided me with helpful support and ideas for the project. I also thank Ken Saunders, of the Texas Parks and Wildlife Department, for his assistance and training in taking discharge measurements. I'd like to also thank Eric Hintze from Texas A&M who analyzed the data from my preliminary study. Finally I want to thank the Honors College and especially Dr. Ann Eisenberg who provided me the opportunity to perform this project. Financial support for this study was provided by the Edwards Aquifer Authority through Bio West Inc.

Introduction

The study of trematode population dynamics is important to many different fields of study. Parasites, especially exotic ones, can have a devastating effect on aquatic communities as well as on the sanitary conditions of human water resources. Improvement of the methodology for sampling parasites is a vital aspect for understanding their distributions and behavior.

The presence of an exotic heterophyid trematode in the Comal River was detected in the fall of 1996. That year, a drought threatened the populations of an endangered fish, the fountain darter (*Etheostoma fonticola*). Refugia for the fountain darter were created at the San Marcos National Fish Hatchery and Technology Center and the Uvalde National Fish Hatchery during an earlier drought. Several of the fish that were collected from the Comal River in 1996 for refugium purposes exhibited inflamed gills that extended beyond the fish's opercula. The fish were found to contain a heavy infection of an exotic trematode known as *Centrocestus formosanus* (Mitchell *et al*., 2000). This species of trematode uses birds and mammals as the definitive host and snails and fish as the first and second intermediate hosts, respectively. The snail host was found to be *Melanoides tuberculatus* in the San Marcos and Comal rivers. In both rivers, *C. formosanus* is thought to mainly uses birds as definite hosts (Mitchell *et al*., 2000).

Centrocestus formosanus was more than likely introduced into the U.S. via its snail host *M. tuberculatus*. The native range of this snail spans from Northern Africa to Southern Asia. The snails, along with parasites, were both introduced into the U.S. as hitchhikers in aquarium fish and have since established their populations throughout the southern states from Florida to Texas (Benson 2004).

The existence of *C. formosanus* in the Comal River threatens the survival of the fountain darter, which is found in only one other river, the San Marcos River, in the world. The parasite's second intermediate stage, cercaria, can have a number of negative effects on a fish's health. Once released from its snail host cercaria are present throughout the water column where they can target fish. When it locates a fish the parasite embeds itself into the fish's gills and searches for an area of high oxygen concentration. It then encases its body by forming a cyst around itself. In response to the infection, the fish's gill tissue cells have an inflammatory response further enlarging the cyst. In a study done on heavily infected carp in Mexico, parasites were found to be closely associated with blood vessels. In some cases parasites were found embedded near the cartilaginous base of the primary lamellae of the gill arches (Velez-Hernandez *et al*., 1998).

There can be a number of factors that affect the dynamics of cercarial and miracidial prevalence and distribution. Several projects have been done to study what effects those factors may have on the parasite populations of a stream. Among those factors, water velocity and turbulence have been studied closely. The ability of cercaria and miracidia, a trematode's first intermediate phase, to move through the water column and locate their hosts can be affected by running water. In a study of *Schistosoma mansoni* miracidia it was found that the infection rate on *Biomphalaria glabrata* was severely affected by running water. In that study, infection rates were studied in running and standing water. It was found that in standing water and in running water with a velocity of 13·11 cm/sec and above no infections occurred. It was also found however, that *Schistosoma mansoni* miracidia could infect *Biomphalaria glabrata* at long distances downstream from their release into a stream system aided by water current below a velocity of 13·11 cm/sec. In running water miracidia were able to infect snail populations up to 97 m downstream from their release (Upatham 1972).

Various methods have been used to enumerate cercariae since the early 1900s. Among these methods, different animals that serve as hosts have been used to study cercaria. They include: birds, snails, fish, and mice. In one study mice were exposed to an infected stream in floating cages. The mice were then dissected to count the number of adult parasites. The infection rates were calculated to determine effects on infection due to cercarial concentrations (Upatham 1973). Other methods that have been used are continuous centrifugation and filtration of water samples. In continuous centrifugation, water samples are spun to retrieve a pellet of solids, which can then be examined for parasites. The filtration method involves direct filtration of water samples and examination of filters to count parasite. In a study comparing the efficiency of the two techniques, it was found that the filtration technique was most efficient in determining cercarial presence in water (Sandt, 1973).

Most of the methods that have been used in the past present several difficulties. Using animal hosts to study parasites requires either capturing or raising the animal. This can be time consuming as well as expensive. The continuous filtration technique proved to be very time consuming. It was also difficult to examine the pellet of solids for parasites due to the high amount of sediment contained in the pellet (Sandt 1973). The filtration method has been found to be the most favorable method for studying cercaria in water system. It is generally low cost, and produces efficient results. In this study the filtration technique was used to identify areas of cercarial recruitment particularly from snail populations. It is expected that snail populations contribute to the amount of parasites present in a stream system. Areas where parasite numbers decrease were also observed. Turbulence is expected to decrease the amount of parasites flowing in a river system. The least amount of sampling required to attain an adequate representation of cercarial numbers in the Comal River was also be determined to simplify the filtration procedure.

Methods

Study area .-- The study area for this project begins 1,100 m downstream from Landa Lake and covers 1,500 m of the Comal River's old channel (Figure 2). Discharge from Landa Lake through the study area has been measured to be 1.2 m^3/s. The study area consists of a series of shallow runs that are interrupted by pools and riffles. Vegetation is prevalent throughout the river and may have an effect on both water current and parasite numbers. The stretch also includes two low water vehicle crossings, two golf cart bridges, and a catwalk, that each create a disturbance in the water current. High turbulence is experienced downstream at each bridge site because each has a base of elevated concrete that transects the flow of water. Landa Lake is known to have a high population of *M. tuberculatus* and is a high contributor of cercaria to the old channel. Eleven colonies of *M. tuberculatus* are also present within the study area, which can also contribute to the amount of parasite load in the study area.

Preliminary Study: % Recovery of Pump. – In order to collect parasites from the environment, samples of water were be pumped from the river and filtered using a filtration device used in a previous study by Cantu (2003) which was a modification of the procedures, designed by Theron (1979) and Prentice (1984) (Figure 3). The filter consists of two pre-filter screens, the first having a mesh size of 220 microns, the second has a mesh size of 86 microns and a removable monofilament filter that has a mesh size of 30 microns. The removable filter was used to collect the parasites. After the water was filtered, the nylon filters were removed from the device and treated with 10mL of Rose Bengal stain and 5mL of 10% formalin in Petri dishes. In this study, instead of using a bucket to retrieve water from the stream, as in the procedure in Cantu (2003), a pump was be used to collect water from various locations within a cross-section of the water column. Because of the sticky nature of cercaria, especially when treated with formalin, using a pump can generate possible losses due to attachment to the tubing and pump. In order to test if the loss of parasites in our procedure was significant, a percent recovery test was done to determine if there are any losses of cercaria associated with attachment to the tubing and pump.

This test was set up in the laboratory using buckets and well water. Cercariae were recovered from a stock of infected snails collected from the Comal River. A known number of approximately one hundred parasites (100-108) were used for each of the five trials. In each trial cercariae were added to a 10-L bucket containing 5 L of well water. The bucket was swirled in order to prevent cercariae from attaching to the sides of the bucket. That water was pumped into a second, empty, 10-L bucket. The water in this bucket was then treated with 10 mL of 10% formalin. The purpose of using formalin is to kill the parasites; live parasites tend to wiggle through the device's filters generating more losses. The formalin-treated water from the second bucket was then poured through the filtration apparatus and the filter (filter1) was removed from the device and treated for storage. The filtrate was collected in a 6-L Erlenmeyer flask and re-poured through the filtration apparatus and a new clean filter (filter 2). Between each filtration the filtration apparatus was washed to remove any possible parasites that might have attached to its surfaces. A third bucket with 5 L of clean well water, without cercaria, was treated with 10% formalin and pumped through a third filter (filter3). The procedure was repeated five times and the filters were examined and were counted.

The average recovery rate of parasites from filter1 was 48.4% ($\sigma = \pm 6.83$) suggesting a 50% loss in the procedures. The recovery rate for filter 2 was found to be 3.4% ($\sigma = \pm 1.36$) revealing that some cercaria can bypass the nylon filters. Filter 3 yielded a recovery rate of 2.2% ($\sigma = \pm 0.89$). This proved that by pumping a sample through the tubes and water pump, cercaria attached to their surfaces. During the field collections samples will only be collected during a time slot limiting the amount of samples collected in a day. Due to the limitation of time and because the results of the percent recovery test yielded minimal losses from cercaria going through filters and attachment to the pump and tubing, those losses will be ignored. The significant loss of parasites (50%) was unknown but may have been due to attachment to the buckets or unfiltered, smaller parasites.

Preliminary Study: Determination of Samples Needed for Adequate Representation. -- In order to determine the least number of samples needed for an adequate representation of the number of parasites that are flowing through a cross-sectional area of the river, a preliminary study was conducted at a selected site within the study area. The Clubhouse Road bridge site was selected for this preliminary study because it is the most upstream location in the study area, it exhibits uniform flow and depth, and it was expected that this site would yield the most cercaria because it is closest to the discharge from Landa Lake. During this preliminary study, a large number of samples were taken. To determine the minimum number of samples that needed to be collected to accurately determine the number of cercaria per liter of water contained within a cross-section of the river.

The distribution of samples taken within each cross-section was determined by modifying a standard procedure used for measuring discharge. A measuring tape was stretched across the cross-section of river and anchored at each end a couple of feet from the edge of the bank. The distance between the edges of each bank was divided into 25 vertical sections. At each of the vertical sections, flow readings were recorded at 60% of depth from the surface if the depth was less than 2.5 ft or at 20% and 80% of depth from the surface it the depth was 2.6 ft or greater. The width of the vertical sections varied depending on the flow readings, if an area that experienced a higher readings was found then more vertical section were taken in that area by reducing the horizontal separation of the vertical sections. In sections where the flow readings were lower, the separations of the vertical sections were longer. At the site flow readings were recorded and discharge was calculated.

The flow meter rod, used to position the flow meter in the water column, was also used to position the pump for collecting samples, at the desired depth. The pump was attached to the flow meter rod, using a small bungee cord, parallel to the flow meter but behind the flow meter sensor, so that it remained downstream, keeping it from altering the readings. In each vertical section where flow readings were taken, cercaria samples were also taken at the same depths where the flow readings were obtained.

Additional samples were taken at each fifth of the total distance between each bank. At each of these vertical sections, samples were pumped at 0.3 ft (top) from the surface and 0.3 ft (bottom) from the bottom. This was done because the presence of vegetation in the river forms a buffer from the water current and may accumulate parasites.

The samples were collected on two consecutive days. The first day was cloudy and cool, and the second day was sunny and warmer. A total of 45 samples were collected at the site and the results were analyzed by Eric Hintze, a statistician at Texas A&M University. Horizontal distance from the bank and the number of cercaria were compared as well as, number of cercaria and depth, number of cercaria and flow reading values, and because the study was done on two separate days, a sunny day was compared with a cloudy day.

The number of cercaria collected on the sunny day was much greater than the number of cercaria collected on the cloudy day. The difference was highly significant with a p-value well under 0.05 ($p < 0.000001$). The number of cercaria did not show a significant difference between each of the depths (0.3 ft from surface, 20% of depth from surface, 60% of depth from surface, 80% of depth from surface, and 0.3 ft from bottom) were water samples were collected (p = 0.45091). Because no current velocity values were taken at 0.3 ft from the surface or 0.3 ft from the bottom, these values were not incorporated into the data for calculating a difference between current velocity and cercaria collected. Only values for the sunny day were used to compare these variables because a higher number of cercaria was collected on that day. The sunny day was found to have a significantly increasing number of cercaria as current velocity increased (significance level = 0.00378). A significant negative slope was found for cercaria and time of day (significance level = 0.00001). Cercarial numbers were highest around 9:30, the time sampling began, than the numbers of cercaria collected as time progressed. This relationship conflicted with comparing the horizontal distance from the bank with cercarial concentration. A significant negative trend of number of parasites was found as the distance from the bank increased. Because the samples taken for each horizontal distance were also taken at progressing time, the difference in horizontal cercarial numbers was most likely due to the difference in time as proved before in Cantu (2003).

After reviewing the data cross-sectional sampling was planned. It was decided that for each cross-section, only five evenly spaced samples would need to be taken to accurately represent the entire cercarial flow through a cross-section of river. This decision was made because depth did not have an apparent significance in the number of cercaria collected. Also since horizontal distance was assumed to not have an influence on cercarial numbers, each sample taken at any point within a cross-section would have an equal representation of the total. For the main study's sample collection, special interest in time was taken. Five samples were taken at each cross-section to compare numbers.

Sampling Strategy. – Before major sampling began, cross-sectional areas were selected throughout the entire 1,500-m study area. A map (Figure 4) was developed to record areas that had ideal depth and were free from obstructions. The presence of snails was also recorded on the map. The entire study area was examined for snail populations by scooping sediment from the edges along each bank. Only areas that contained snails that were > 1 inch in length were recorded on the map because only snails that size produce cercaria. Bridges were noted on the map as well because each produced turbulence.

After the map was produced from the observations, a simple sampling strategy was planned. In order to compare changes of cercarial prevalence due to certain factors, those factors being turbulence and presence of snails, two snail inhabiting sites with the highest number of large snails were selected to be sampled. Of the five turbulence areas that were found, including the bridge sites, the two that were selected were the two which had the most uniform depth. At each of the four sites, two cross-sections were sampled, one a few meters upstream and the other a few meters downstream from the snails or turbulence.

The eight sample sites were labeled (1-8) on the map beginning with the upstream-most site (Figure 4). Sampling began on March 16, 2005. This day was cloudy with some precipitation. The first cross-sections that were sampled were cross-section 5, upstream of a snail population, and cross-section 6, downstream from that snail population, were sampled beginning at 9:30am and ending at 11:40am. Only two more cross-sections were sampled that day due to the time restriction. Because the sampling days were selected for weather conditions, two cloudy days and two sunny days, on each day one snail population site and one turbulent site were sampled. After the first two sites were sampled, sampling continued downstream to the Elizabeth St. Bridge turbulence site. Cross-sections 7 and 8 were sampled beginning at 12:00pm and finishing at 1:35pm. The following day was clear and sunny and cross-sections 1 and 2, a snail population upstream from the cross-sections 5 and 6, were sampled from 11:00am to 11:30am. Earlier that day, cross-sections 3 and 4, the turbulent site upstream from the first, were sampled from 9:35am to 8:55am.

Apart from the eight cross-sections at either the snail population or turbulent sites, a cross-section upstream from all eight was sampled on two separate days, one sunny and one cloudy. For this site discharge measurements were also taken.

Cercariometry. – Samples were collected in a time slot between 8:30am and 1:30pm on each of the four days. For each sample, 5 L of water were pumped from the river into a 10-L bucket. To each sample, 5 mL of 10% formalin was added and the sample was poured through the filtration apparatus. After the water samples were filtered through the apparatus, the monofilament filters were removed, placed into Petri dishes, and stained with 5 mL of Rose Bengal staining dye and 10 mL of 10% formalin was added to preserve the cercaria. This process required two workers, one in the water and one on the bank. As before, the pump used to take samples was tied to the flow meter rod using zip ties. The total distance across a cross-section, bank to bank, was measured and partitioned into five equal distances. At every fifth of the total distance, a 5-L sample of water and a flow reading, at 50% of the total depth from the surface, were taken. The pump required a battery that was tugged along inside a plastic container on a diving float.

The 10-L bucket with the water sample was relayed back and forth to the bank for filtration.

Cross-sections 1, 2, 5, and 6 were too deep to wade across, so a kayak was used to take samples across them. In order to do this a rope was tied across the river in order to secure the kayak in place. Again the battery for the pump was towed across on a diving float that was attached to the kayak. The water samples were relayed from the bank to the kayak on the diving float using a simple pulley system.

The filters were analyzed in the laboratory two days after preparation. At least two days were required in order for the dye to adequately stain the parasites. Using Petri dishes with grid paper the filters were submerged in tap water and viewed under a microscope. The numbers of total parasites moving through each cross-section were compared to find increases or decreases of cercaria.

Results

For each sample site (1, 2, 3, 4) the total number of parasites recovered for the upstream cross-sections was compared to the total number of parasites recovered from the downstream cross-sections. A paired sample T-Test was done for the two cross-sections (1-8) of each of the four sites (Figure 5). Discharge going through the study area, taken from the Clubhouse Rd site on the sunny day was 66.82 ft/sec and the discharge on the cloudy day was 81.03 ft/sec.

Comparison of the total number of parasites collected at cross-sections one and two, a snail site (site 1), revealed a slightly higher number of parasites in the downstream cross-section than the upstream cross-section. This difference however, was not significant (significance = 0.840). The same was true for the second snail site (site 3). The increase of parasites collected in the downstream cross-section from the parasites collected in the upstream cross-section at site 3 was higher than the increase of parasites from site 1. However, this difference was not significant as well (significance = 0.206).

The turbulence site (site 2) resulted in a decrease of parasites collected in the downstream cross-section from the upstream cross-section. This difference was found to be significant (significance = 0.017). The second turbulence site (site 4) also yielded a lower number of parasites in the downstream cross-section from the upstream cross-section. This difference was not significant (significance = 0.052).

A regression was made for the number of parasites per sample versus current velocity. This regression was done separately for the two types of day, sunny and cloudy. On the cloudy day a negative relationship was found for parasite numbers as current velocity increased but was not significant (significance = 0.478). On the sunny day however, a positive relationship was experienced but was not significant as well (significance = 0.385).

Discussion

As expected, the presence of a snail population seems to slightly contribute to recruitment of cercariae in the river. Despite the differences not being significant, it is important to note that an increase was found for both the snail population sites that were sampled. Also as expected, the turbulence sites decreased the amount of parasites present in the river. Like for the snail populations, this difference was not significant but it is equally important to note the difference.

One factor that may have affected the results would be the ever-changing discharge of this spring fed river system. Even though the results for current velocity and number of parasites collected per sample did not show a definitive trend in the study, discharge might influence the numbers. Another factor that may have contributed to the outcome of the results is time. Because a negative trend in parasite numbers was found as time progressed from 8:00 am in the preliminary study, there is little doubt that time played a role in the outcome of the results. Since several cross-sections were taken at different times in a single day, the diel behavior that has been observed in previous studies could have influenced the number of parasites collected (Cantu 2003). In the future, if possible, all selected sites that are to be compared should be sampled at the same time.

The presence of a snail population does not necessarily indicate an area of parasite transmission. Snails that are most likely to be infected with *C. formosanus* were found to be between the sizes of 17mm and 38mm shell lengths (Mitchell *et al*., 2000). When the sample sites were selected for this study only the presence and abundance of snails were recorded and not the shell length, snails that were in the size range that are most likely to be infected may have been present in some sites and not in others. Future studies must take snail size into account. It may also be possible to locate transmission foci by using the filtration technique.

In future studies it would be very helpful to perform heavy sampling at sites that experience different current velocities and, various riverbed morphologies to better understand the cross-sectional ecology of *C. formosanus*. By doing so, differences of the cross-sections can be taken into account when analyzing and comparing sites like in this study.

It would also be helpful to do another, very well planned, diel behavior study of *C. formosanus*. It would be important to try to find if it would be possible to sample at times later than 1:00 pm. The current time slot of 8:00 am to 1:00 pm really limits the amount of sampling that can be done in one day. In a previous study by Cantu 2003 the diel pattern that was found was based on a single set of water samples that was taken near the bank (Cantu 2003). By using the techniques that were pioneered in this study it is now possible to find a more definitive pattern based on several samples.

The results of this study and the results from previous studies in the Comal River are important to the management of this river system. The information learned from this project will help to plan new studies that will increase the understanding of the ecology of *C. formosanus*.

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Appendices

Figure 1: Map of study area labeled with snail populations and bridges.

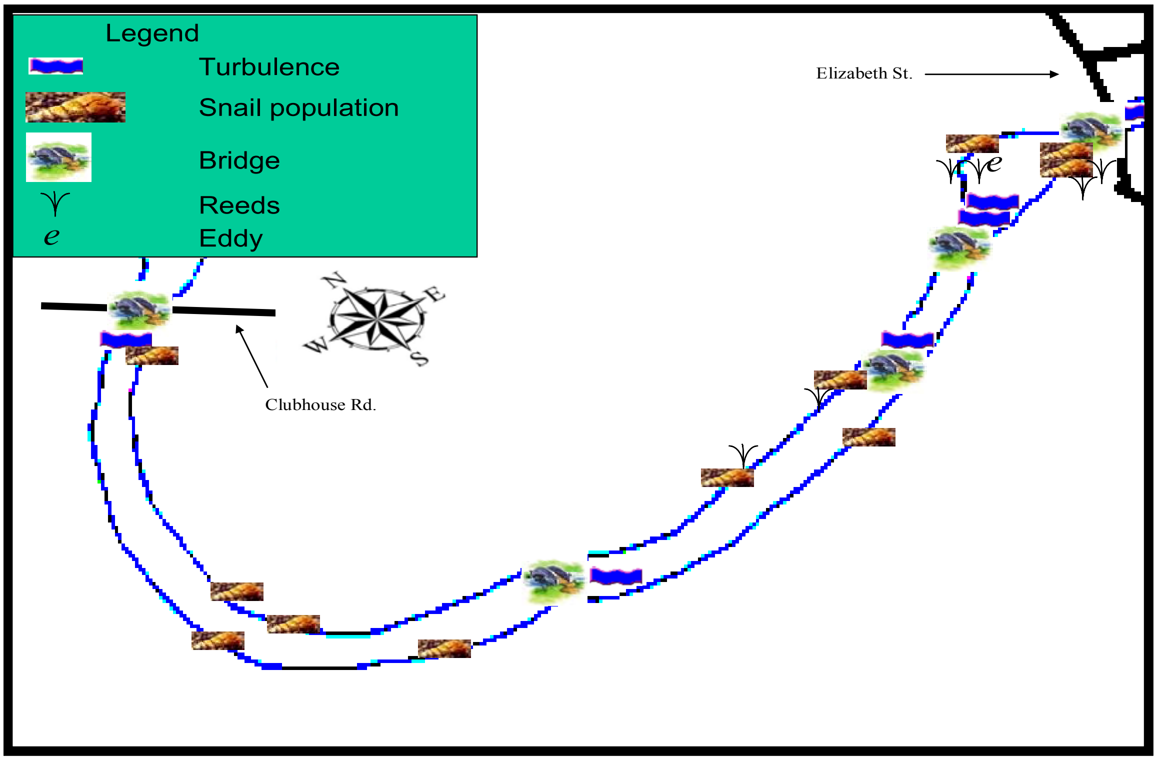

Figure 2: Filtration apparatus used in the study.

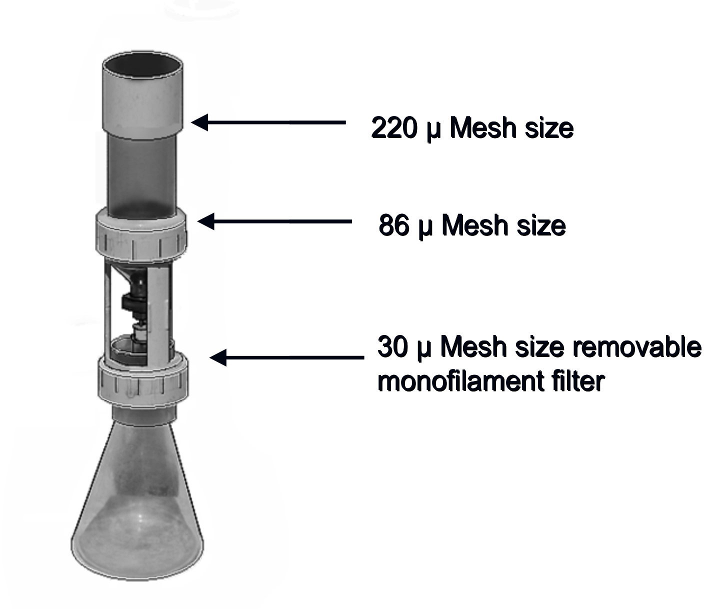

Figure 3: Map of sampling strategy. Each cross-section sampled is labeled (X#).

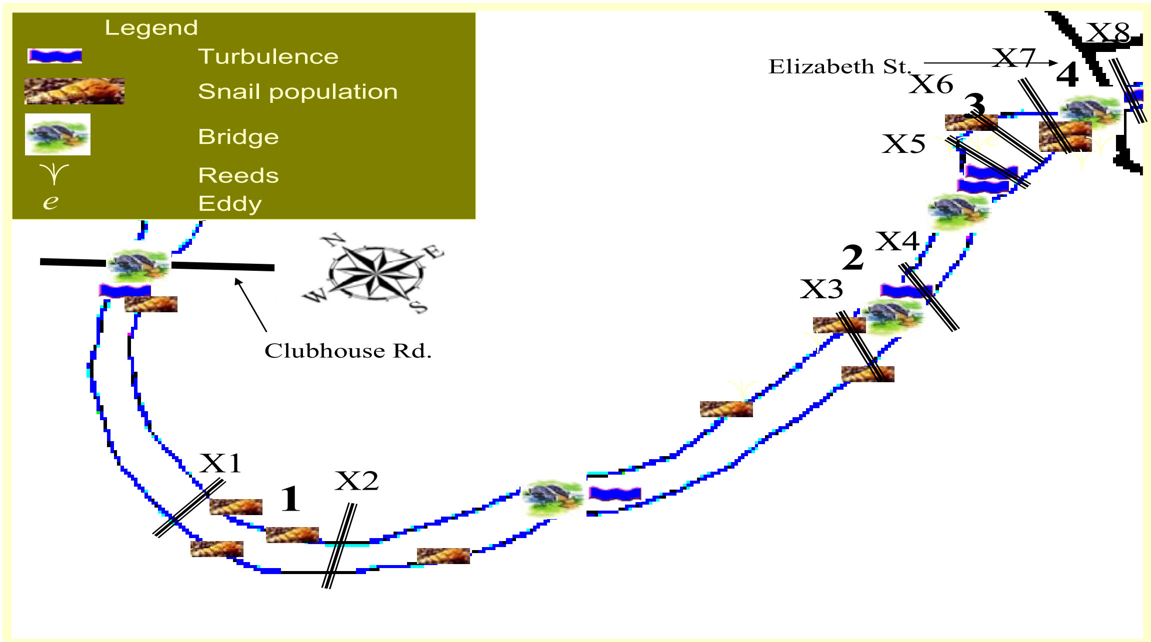

Figure 4: Box plot diagrams comparing parasites per liter between the eight cross-sections that were sampled.