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

FINAL 2003 ANNUAL REPORT

Prepared for:

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

Prepared by:

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

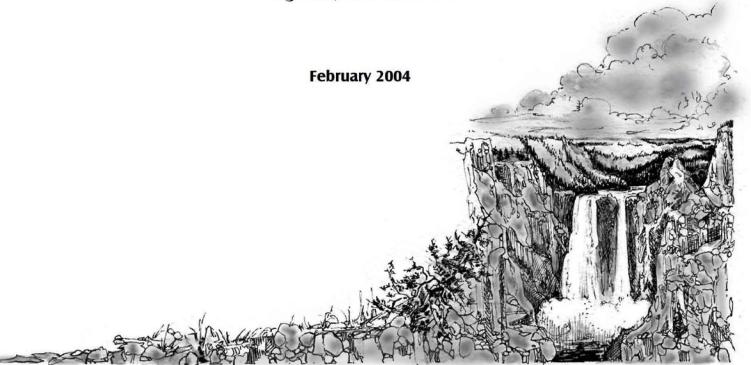


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EXECUTIVE SUMMARY

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 three quarterly sampling events (Comprehensive Monitoring Effort), conducted on the Comal Springs/River ecosystem in 2003. There were no low-flow critical periods or high-flow events triggered in 2003. 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.

Although low flows in late summer 2000 initiated the Variable Flow Study, flows since that time have been largely above average levels. Significant rainfall events in late fall 2001 and during summer 2002 yielded near record high-flow conditions that have maintained the aquifer levels and higher flows in the Comal River throughout 2003. Typical summertime conditions occurred in summer 2001 and 2002, but flows never declined to trigger levels. In 2003, flows remained high through the summer and did not follow the typical pattern of decline. Monthly mean flows in 2003 remained at least 70cfs higher than the monthly means from the period of record. Discharge measurements in the Old Channel and Spring Runs revealed that approximately 25-28% of the total discharge passed down the Old Channel each time it was measured in 2003. These measurements also revealed that Spring Runs 1 and 3 (combined) contributed approximately 25% of the total discharge in the Comal River while Spring Run 2 contributed <2%.

Baseline data have continued to show that the Comal River is an ecosystem with high water quality according to the chemical and physical variables that were measured. Thermistor data have revealed a high degree of thermal uniformity throughout the Comal River ecosystem, despite the wide-ranging conditions experienced during the study. As has been observed in past years, the range of water temperatures in several sites is around 1°C or less, except for a few acute peaks/troughs. 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 that did not exceed 26°C in 2000-2003. Downstream of Landa Lake, temperature variations were greater; most observations were between 20°C and 26°C. Except for two spikes in the Old Channel reach in which the thermistor must have been temporarily exposed, the sites downstream of Landa Lake did not exceed the water quality standards value of 26.67°C during 2000-2003. The greatest fluctuation in water temperature occurred in Blieders Creek (upstream of the Spring Runs and Landa Lake) where the temperature increased to nearly 28°C in both 2002 and 2003. Blieders Creek also registered much lower water temperatures during the winter than areas more directly influenced by springflow, but water temperature reached the lowest values in the furthest downstream sites. Minimum temperatures occasionally dropped below 18°C downstream of Landa Lake but usually remained above 20°C. Although, water temperatures do occasionally exceed the water quality standards value of 26.67°C in the Blieders Creek site, the impacts are isolated and overall, water temperature data do not present any cause for concern in the Comal River. However, low-flow conditions have not been fully examined during this study and an evaluation is still necessary throughout the system. With no low-flow triggers in 2003, other water quality parameters were not monitored.

Aquatic vegetation has remained abundant throughout the study period (2000-present) and has provided suitable habitat for biological communities. After the scouring effects of floods in both 2001 and 2002, many vegetation types responded with rapid re-growth and expanded to a total coverage that was far greater than in previous samples. For the most part, these increases in vegetation coverage were maintained throughout 2003. However, there was some decline in coverage that occurred in the fall of 2003 in many reaches despite the high flows and presumably favorable conditions. This emphasizes the fact that conditions are dynamic even when flows remain relatively constant and habitat should not be predicted on flows alone. In addition, there will always be a need for baseline data of habitat conditions immediately preceding any low-flow period to fully evaluate the changes that occur during the lower flows. The moderate decline that was observed in many vegetation types between the summer and fall of 2003 will need to be monitored into 2004 to evaluate whether this is the beginning of a period of habitat decline (possibly a return to conditions more similar to 2002) or whether it was just natural (seasonal) variation.

One of the more important observations with vegetation composition in 2003 was the dramatic shift in habitat within the Old Channel Reach. Prior to 2003, filamentous algae dominated this reach and provided the highest quality habitat (supported the greatest density) for fountain darters. Beginning in the fall of 2002 *Hygrophila* and later *Ludwigia* appeared in the reach and have grown significantly in 2003 to nearly complete coverage of areas that had previously supported filamentous algae. Although *Ludwigia* is an important (native) habitat type for fountain darters, *Hygrophila* (exotic) is much less favorable and neither species supports the densities found in the filamentous algae. This change has important implications to the fountain darter population in that reach and needs to be monitored closely in 2004. If *Hygrophila* eventually dominates the reach as is typical in other areas where both *Hygrophila* and *Ludwigia* are present, there would be a substantial decline in net habitat quality and ultimately the population abundance of fountain darters that the reach will support.

Fountain darters were collected from each sample reach during each sampling event in 2003. The suitability of the various vegetation types in the Comal River ranges dramatically from supporting densities of 2.5 fountain darters (in *Sagittaria*) to nearly 30 per square meter (in filamentous algae). The bryophytes (*Riccia* and *Amblystegium*) and *Ludwigia* also support high densities of fountain darters (>20 per m²). In 2002, the bryophytes in Landa Lake increased dramatically in areal coverage, and this increase was maintained throughout 2003. In addition, some of the *Riccia* settled down into other vegetation types, most notably *Sagittaria*, *Vallisneria*, and *Hygrophila*, and dramatically increased the number of fountain darters within those vegetation types. The increased densities observed in these vegetation types in 2003 was enough to increase the overall density calculations (from 2000-2003) to much higher numbers than those reported in 2002. The net result of the increase in bryophytes, including the improvement of habitat suitability of other species, is most likely a substantial increase in the fountain darter population. This will be an important situation to monitor in 2004 and beyond to evaluate the impact of an eventual decline of the bryophytes when conditions become less favorable.

The overall size-class distribution for the Comal ecosystem represents a healthy fish assemblage, though some differences in reproduction in individual sample reaches yielded variable size-class distributions by season in some reaches. There were also some differences between 2002 and 2003. In 2002, there was a relatively uniform distribution with similar numbers of individuals observed between 16mm and 32 mm, but in 2003 there was a clear peak of larger individuals (24-34 mm). Distributions by sample reach revealed seasonal variation in the size-classes. In previous years, the greatest number of fountain darter observations occurred in the spring and the fewest in the fall, but that trend was reversed in 2003.

Dip-net data reveal that the Old Channel, New Channel and Garden Street Reaches have had spring peaks in the number of small darters (evidence of reproduction) but that the Old Channel has had year-round reproduction. Few observations of small fountain darters in the fall 2003 sample in the Old Channel may indicate a change in reproductive patterns accompanying the change in vegetation community in that reach.

Two areas of additional concern to fountain darters, the density of giant ramshorn snails (*Marisa cornuarietis*) and evidence of gill parasites on fountain darters, were monitored in 2003. By all indications the densities of giant ramshorn snails observed in the Comal ecosystem during the study period to date (including the 2000 low-flow events) pose no serious threat to the aquatic vegetative community (i.e., fountain darter habitat). However, because of the impact that this exotic species can have under heavier densities, close monitoring of this species should continue. Gill parasites were found on fountain darters in varying densities among different sample areas and appear to be concentrated in areas with slow-moving water and high snail densities. Therefore, these low-velocity areas with abundant snail populations (intermediate host) should be targeted for efforts to reduce parasite numbers. The best means for future monitoring of the gill parasite problem may be to filter water and estimate the density of parasites in the water column.

A total of 216.6 hours of drift net sample time occurred in 2003 among the three sites at Comal Springs and 13 species were captured. Among species of concern, an average of 10.6 Peck's cave amphipod (Stygobromus pecki; many small Stygobromus were unidentifiable to species), 1.2 Comal Springs riffle beetle (Heterelmis comalensis), and 0.3 Comal Springs dryopid beetle (Stygoparnus comalensis) were retrieved during each 24-hour period at all sites. Stygobromus pecki was found in all three locations (fewest in Spring Run 1), but H. comalensis was not found in the upwelling along the western shoreline of Landa Lake and S. comalensis was not found in Spring Run 3 during any sample. Species of the two genera Stygobromus and Lirceolus were the most abundant at all sites during each sample event. Thus, any attempts to establish a springflow-biological response relationship should probably focus on these groups. It is difficult to evaluate the influence of springflow on the abundance of invertebrates found in the net because discharge remained so similar among the three sample events in 2003, however, some interesting patterns emerged.

There was a moderate amount of variability in the presence/absence of the Comal Springs riffle beetles in individual quadrat samples in 2003, but there were always at least 5 individuals captured in the four quadrat samples combined from each sample site. A maximum of 43 beetles was observed in one quadrat in the Spring Island area during the January 2003 sample. Most of the beetles (32) in that sample were found on a small stick (1.5 ft long, 0.2 ft wide) that had settled on top of the spring upwelling, which suggests a preference for this habitat type. In the Spring Run 3 and western shoreline sample locations, individuals were most often found among leaves at the water's edge, particularly where seeps flowed over rocks and in areas where leaves accumulated. Both of these locations have a greater diversity of habitat than the Spring Island area and high numbers were frequently found in samples there. The upwelling areas near Spring Island have provided a wide range of observations and it appears that individuals congregate in particular habitat conditions.

Suitable habitat for the Comal Springs salamander (*Eurycea* sp.) was noted in the Spring Runs and Spring Island area; Comal Springs salamanders were observed in each area during each sampling event. In 2001 and 2002, sediment accumulation in Spring Run 3 following high-flow events did appear to have an acute effect on the number of Comal Springs salamanders present. In 2003, stable flows

reduced the sediment accumulation and more salamanders were observed in the Spring Run 3 sample area. As documented via SCUBA surveys, the Comal Springs salamander population was maintained in the deeper portion of Landa Lake throughout the study period.

As described above, the data in this report are preliminary and, although they have been carefully evaluated to determine trends and observations of particular interest, stringent data reduction techniques and/or statistical applications have not yet been applied to this incomplete data set. More data from low-flow periods (particularly from an extended low-flow period) are essential to fully evaluate the biological risks associated with future critical periods (high or low flow). Although quarterly sampling events do not yield vital low-flow data, this sampling is extremely important to maintain a continuous understanding of current conditions in order to be prepared for a low-flow period and monitor changes that may occur. Sampling only during a low-flow event will not provide the necessary context to adequately assess such changes.

This study remains the most comprehensive biological evaluation that has ever been conducted on the Comal River ecosystem. Variable flow conditions encountered to date have provided an excellent confirmation that the study design is well suited to address the concerns of variable flow and water quality on the biological resources in the Comal and San Marcos River ecosystems. As noted in the 2002 annual report, 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).

METHODS

In 2003, three quarterly sampling efforts were conducted with a sampling protocol that was slightly modified relative to 2000 - 2002. 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) during a meeting in August 2002 and implemented beginning with the Fall 2002 quarterly sample effort. Modification included a reduced evaluation of water quality during quarterly sample efforts to include only thermistors and fixed station photographs; the exotic / predation component was also removed from quarterly sample efforts. Also, macroinvertebrate sampling was modified to include placing drift nets over spring openings and Comal Springs riffle beetle surveys were added. 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

High-Flow Sampling

Unlike in 2001 and 2002, there were no high-flow sampling events in 2003.

Springflow

Total discharge data for the Comal River were acquired from USGS water resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2000). The discharge data for the Comal ecosystem 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.

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 during quarterly sampling events for 2003, but the two components necessary for maintenance of long-term baseline data, thermistors and fixed station photography, were included. In addition, conventional in-situ physico-chemical parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen, water depth at sampling point, and observations of local conditions) were taken at the surface and near the bottom in all drop-net sampling sites using a Hydrolab Quanta. When conditions trigger low-flow sample events in the future, the full range of water quality sampling parameters will be employed, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

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

Fixed station photography allowed for temporal habitat evaluations; these photographs included an upstream, a cross-stream, and a downstream picture taken at each water quality site depicted on Figure 1.

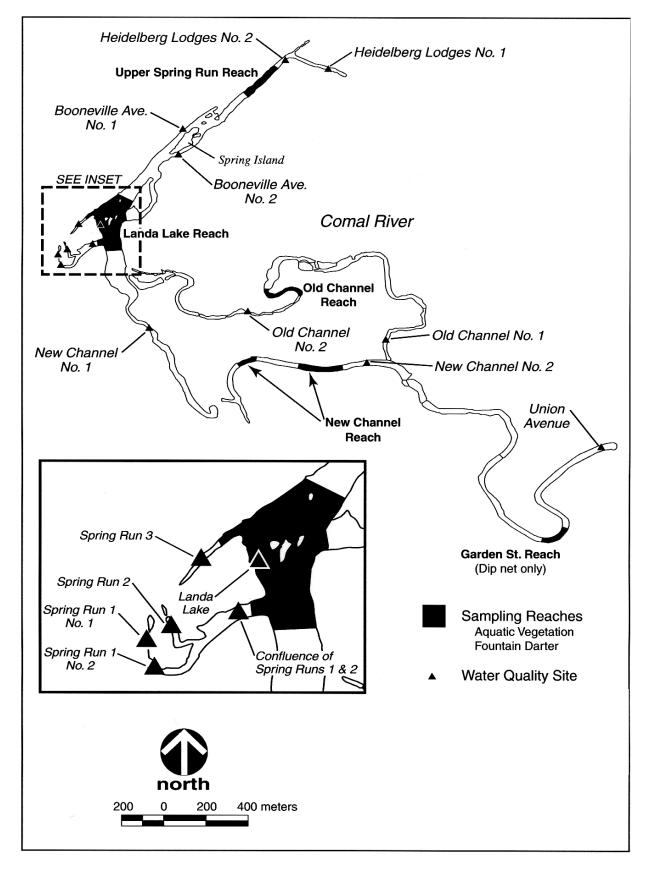


Figure 1. Comal River water quality and biological sampling areas.

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-XRS global positioning system (GPS) unit with real-time differential correction capable of submeter accuracy. The GPS unit was linked to a Fujitsu Stylistic 2300 laptop computer with Aspen software that displays field data as it is gathered and improves efficiency and accuracy. The GPS unit and computer were placed in a 3-meter (m) Perception Swifty kayak with the GPS unit antenna mounted on the bow. The aquatic vegetation was identified and mapped by gathering coordinates while maneuvering the kayak around the perimeter of each vegetation type at the water's surface. Vegetation stands 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.

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium* primarily in the upper Spring Run and Landa Lake) were included in all 2003 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 darter and other fish species. The design of the net is such that it encloses a known area (2 square meters [m²]) and allows a thorough sample by preventing escape of fishes occupying that area. A large dip net (1 m²) is used within the drop net and is swept along the length of the river substrate 15 times to ensure complete enumeration of all fish trapped within the net. For sampling during this study, 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 quarterly event from a grid overlain on the most recent map (created with GPS the previous week) of that reach.

At each location the vegetation type, height, and areal coverage were recorded, along with substrate type, mean column velocity, velocity at 15 centimeters (cm) above the bottom, water temperature, conductivity, pH, and dissolved oxygen. In addition, vegetation type, height, and areal coverage, along with substrate type, were noted for all adjacent 3-m cell areas. Fountain darters were identified, enumerated, measured for standard length, and returned to the river at the point of collection. The same measurements were taken for all other fish species, except for abundant species where only the first 25 individuals were measured; a total count was recorded for a drop net sample beyond the first 25 individuals in such instances. Fish species not readily identifiable in the field were preserved for identification in the laboratory. All live giant ramshorn snails were counted, measured, and destroyed,

while a categorical abundance was recorded (i.e., none, slight, moderate, or heavy) for the exotic Asian snails (*Melanoides tuberculata* and *Thiara granifera*) and the Asian clam (*Corbicula* sp.). A total count of crayfish (*Procambarus* sp.) and grass shrimp (*Palaemonetes* sp.) was also recorded for each dip net sweep.

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.

Visual Observations

Visual surveys were conducted using SCUBA in Landa Lake to verify the continued fountain darter and Comal Springs salamander use of habitat in deeper portions of the lake. The locations of these time-constrained surveys were deeper than conventional sampling methods for the darters would allow. Observations were conducted in the early afternoon for each effort.

An additional component to these surveys was a grid (0.6 m x 13 m) added in summer 2001, and subsequent sampling 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, and larger rocks were moved at the substrate surface to expose any fountain darters.

Gill Parasite Evaluation

The objectives of this study component were to examine the variation in spatial and temporal concentration of *Centrocestus formosanus* drifting in the water column and infection intensity on the gills of caged and resident fountain darters among eight sites in the Comal River. Each of the three methods of estimating parasite infestation was also assessed for viability as a long-term monitoring tool. Data collected in 2003 were added to that presented in the 2002 annual report to finalize the description of the study findings. The study is described in more detail in Cantu's thesis (2003; Texas State University) entitled: "Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas." Future efforts will

include using the best method (described in the results section) for long-term monitoring of parasite densities in the same study areas.

The first method evaluated for monitoring was filtering the water column to measure the concentration of drifting *C. formosanus*. A preliminary diel filtration experiment with samples conducted every 3 hours over a 24-hour period revealed that the highest parasite densities occurred between 0930 and 1230 hours; therefore, all study samples occurred during this time period. Another preliminary exercise revealed that "fixing" the sample with a 0.1% formalin solution greatly increases the percentage of parasites recovered. Thus, all 5-L samples of river water were fixed with a 0.1% formalin solution after being collected. A fiberglass adjustable rod (3.5m) attached by a clamp to a 10-L bucket was used to collect samples while standing on the river bank. During preliminary trials it was discovered that the presence of sediment in samples lowered the percentage of parasites recovered. After the water sample was poured through the filtration apparatus (see Cantu [2003] for description), the filter was removed from the apparatus and fixed. Each filter was stored in separate labeled containers until it could be examined under a dissecting microscope (100X).

The second method evaluated for monitoring was caged fountain darters placed in each of the eight sample locations for 7 days. During preliminary trials it was determined that mortalities started to occur after 7 days, possibly due to starvation. In each site, three cages were wired together, separated by 1-m, weighed down and cleaned of debris and algae every other day. Ten fish were placed in each cage and all were removed on day 7, euthanized with FINQUEL, rinsed, preserved and transported to the San Marcos NFHTC for examination. All gill arches were removed from the right side of the fish and examined for parasites using a dissecting microscope (100X). The total number of cysts per fish was estimated by doubling the number counted on the right-side gill arches.

The third method evaluated for monitoring was evaluation of resident fish. For each sample effort 10 resident fountain darters were collected (or as many as possible up to 10 during a 30-minute interval) using a 40 X 40 cm (1.6 mm mesh) dip net. The collection preceded the placement of cages in the same area, which occurred on the following day. Collected fish were euthanized and cysts counted as described above

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 2003 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 downstreammost edge of the sampling area and involved turning over rocks located on the substrate surface within the Spring Run while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. The Comal Springs salamander locations were noted, along with time and water depth. In order to maintain consistency between samples, all surveys were initiated in the morning and terminated by early afternoon.

Within Spring Run 1, surveys were conducted from the Landa Park Drive bridge up to 9 m below the head spring orifice. Spring Run 3 was surveyed from the pedestrian bridge closest to Landa Lake up to 9 m below the head spring orifice. In the Spring Island area, surveys were conducted within the entire

spring reach including approximately a 15-m radius from each Spring Run outfall. These two areas include the spring outfall on the east side of Spring Island (closest to Edgewater Drive) and the area north of Spring Island (upstream).

Macroinvertebrate Sampling

The macroinvertebrate portion of the study was modified in 2003. Drift nets that had been placed in the Spring Runs in 2000-2002 were moved to the spring openings to obtain a more direct observation of movement of organisms out of the aquifer. The drift net sampling in the Spring Runs provided important information because these samples have explored the movement of organisms through the Spring Runs. Drifting downstream is one of the most important methods for dispersal by benthic invertebrates (Smock 1996). That portion of the study yielded drift rates, densities, and patterns of selected aquatic invertebrates in the spring runs. With a shift in focus to the spring openings we are interested in evaluating the frequency which the primarily spring-adapted (troglobitic) species of concern (Comal Springs riffle beetle, Comal Springs dryopid beetle and Peck's cave amphipod) are expelled from the aquifer.

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 consisted of a 0.45 m by 0.30 m rectangular opening, which connected to a net made of 350 µm mesh. The tail of the net was connected to a detachable 0.28-m-long cylindrical bucket (300 µm mesh). The buckets were removed at 3-hour intervals, and the cup contents were sorted in the field. Except for voucher specimens all Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle, all organisms were identified and returned to their spring of origin. Voucher specimens included fewer than the 20 living specimens of each. 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).

Drift rate (the number of organisms drifting out of the spring orifice per 24-hour-period) and drift density (the number of organisms per 100 cubic meters (m³) of water) were calculated for each taxa in all samples. Drift rate was calculated by summing the total number of organisms collected in a net over a 24-hour period. Drift density was calculated using the following formula:

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

Where N is the number of macroinvertebrates in a sample; t, length of time of sampling (hours), W, net width (m); H, height of water column in the net mouth (m); and V is the mean velocity of water at the mouth of the net (m/s). Drift density was expressed as the number of macroinvertebrates drifting per 100 m³ of water sampled (Smock 1996).

In addition to drift nets placed over spring openings, Comal Springs riffle beetle (*Heterelmis comalensis*) populations were surveyed in January 2003 and during each of the three quarterly sampling effort in 2003. These surveys used a 60-cm X 60-cm quadrat, which was placed over the substrate in areas known to have high quality habitat (based on a preliminary survey) and all rocks of approximately

1-cm and larger were examined for the species. The sides and bottom of each rock was carefully examined and beetles were identified as either Comal Springs riffle beetles or the similar *Microcylloepus pusillus* using a dissecting microscope. Identified beetles were held until the survey was complete and then returned to the sample area. Each survey included 4 quadrats within the sample area to expend the same effort across surveys. There were 3 sample areas including the shallow edge of Spring Run 3 (northwest shoreline) between the two pedestrian bridges, a small area along the western shoreline of Landa Lake across from the city golf course, and an area just upstream of spring island where upwelling springs provide habitat on the bottom of Landa Lake. The number of observed Comal Springs riffle beetles is presented in the results section.

Exotics / Predation Study

This sampling component was not included the quarterly samples of 2003 but will be included in future low-flow sampling efforts.

OBSERVATIONS

The BIO-WEST project team conducted the 2003 sampling events as shown in Table 1.

Table 1. Components of 2003 sampling events.

EVENT	DATES	EVENT	DATES
Sprii	ng Sampling	Fall	Sampling
Vegetation Mapping	Apr 16 - 18	Vegetation Mapping	Nov 4 - 6
Fountain Darter Sampling	Apr 21 - 23	Fountain Darter Sampling	Nov 10 - 12
Comal Salamander Observations	Apr 24	Comal Salamander Observations	Nov 6
Macroinvertebrate Sampling	Jun 12 - 13	Macroinvertebrate Sampling	Nov 23 - 24
Sumi	mer Sampling		
Vegetation Mapping	Aug 13 - 15		
Fountain Darter Sampling	Aug 19 - 21		
Comal Salamander Observations	Aug 22		
Macroinvertebrate Sampling	Sept 6 - 7		

Springflow

The flooding that occurred in July of 2002 and a wet fall raised the aquifer to record levels. Daily springflow values in the first half of 2003 were higher than in 2002 by about 50 cfs and remained above 360 cfs during that time (Figure 2). Relatively wet conditions during the summer maintained discharge from Comal Springs above 350 during the period when flows typically decline. These flow levels are substantially higher than normal and nearly as high as conditions in the latter half of the 2002 summer (after the July flood). Table 2 indicates the lowest discharge that occurred in each year of the study and the date on which it occurred. Clearly 2003 was an unusual year with much higher than normal flows and without the typical decline that occurs in late summer through early fall. As seen in Figure 2, a steady decline in discharge occurred into and during the summer in 2001 and 2002, but was ended abruptly with intense rain events in each of those years; there were no substantial declines or peaks in the hydrograph in 2003. Relative to monthly averages during the period of record (Figure 3), all months in 2003 were at least 70 cfs higher than normal.

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

Year	Discharge	Date
2000	138	Sept. 7
2000	243	•
		Aug. 25
2002	247	Jun. 27
2003	351	Aug. 29

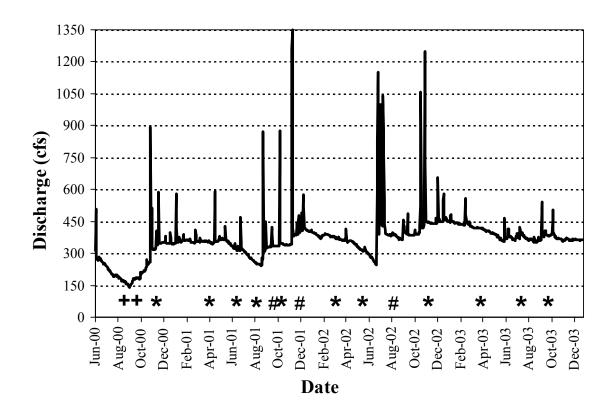


Figure 2. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (*), low (+), and high-flow (#) sampling events are indicated.

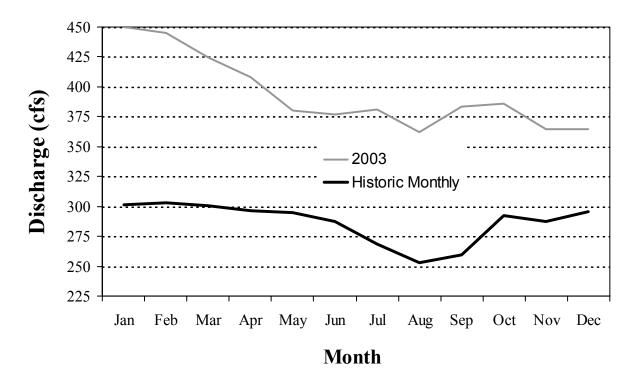


Figure 3. Mean monthly discharge in the Comal River during the 1934-2001 period of record.

Table 3 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 4 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. In 2003, discharge in the Old Channel was increased due to an adjustment in the culvert that regulates flow through this channel. Discharge was greater than 90cfs in each sample, which is significantly higher than the 40cfs estimate for typical conditions by the USFWS (2000). The discharge through the upper section of the Old Channel was approximately 25-28% of the total discharge (measured downstream of the confluence of the Old and New Channels). Spring Runs 1 and 3 (combined) yielded approximately 25% of the total discharge in the Comal River during each sample event in 2003 while Spring Run 2 contributed <2%. Lateral spring flow in Spring Run 3 contributed nearly twice as much water as Spring Run 2 during each sample event in 2003 (10-13cfs compared to 6-7.5cfs respectively). Overall the lateral spring flow from Spring Run 3 contributed about 3% of the total discharge in the Comal River during each sample effort in 2003.

Table 3. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, and 3 and Old Channel reach during each sample effort in 2003.

	Discharge (cfs)		
Location	Spring 2003	Summer 2003	Fall 2003
Total Discharge Comal River (USGS)	405	361	385
Spring Run 1	48.9	42.6	43.1
Spring Run 2	7.4	6.3	5.9
Spring Run 3 (upstream)	40.0	37.4	37.3
Spring Run 3 (downstream)	53.5	49.3	47.4
Old Channel	114.5	93.2	93.2

Table 4. Proportion of total discharge in the Comal River (USGS data) that each Spring Run contributed and proportion that traveled down the Old Channel during each sample effort in 2003.

	Proportion of Total Discharge		
Location	Spring 2003	Summer 2003	Fall 2003
Spring Run 1	12.1%	11.8%	11.2%
Spring Run 2	1.8%	1.7%	1.5%
Spring Run 3 (upstream)	9.9%	10.4%	9.7%
Spring Run 3 (downstream)	13.2%	13.7%	12.3%
Old Channel	28.3%	25.8%	24.2%

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 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 1°C or less, except for a few acute peaks/troughs. The low "spikes" in the temperature graphs occurred during times when a cold front dropped temperatures and brought cold rain; however, such a sharp decline did not occur at other times under similar conditions and suggests that the data logger may have been temporarily exposed (to compare water temperatures with air temperatures and precipitation, see Appendix B). That was clearly the case in the Old Channel reach when the graph spiked to 28°C and above on two occasions.

Overall, the Heidelberg site in the Upper Spring Run Reach (Appendix B), the deep portions of Landa Lake (Appendix B), and the Spring Runs (Figure 4) maintained a narrow range of temperatures that did not exceed 26°C in 2000-2003. 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 to reach 25°C or more during the hottest months. The data for the Spring Island west channel site show that temperatures have not exceeded 26°C between September 2000 and March 2003. Beginning in January 2003 the thermistor readings started to drift upward and reached a peak in March at 26.4°C. However, this data appears to be inaccurate since flows were very high in 2003 and could not have resulted in a temperature increase at that time of year under such conditions. Also, the thermistor was replaced in May and subsequent readings were much lower and more realistic for the conditions.

Downstream of Landa Lake, temperature variations were greater – most values were between 20°C and 26°C (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 2000-2003. The temperature at which fountain darters are believed to have reduced fecundity is 27°C (Bonner et al. 1998) and the lethal limit for the fountain darter is 34.8°C (Brandt et al. 1993). The greatest fluctuation in water temperature occurred in Blieders Creek (upstream of the Spring Runs and Landa Lake) where the temperature increased to nearly 28°C in both 2002 and 2003 (possibly also in 2001, but the thermistor did not function correctly during that period).

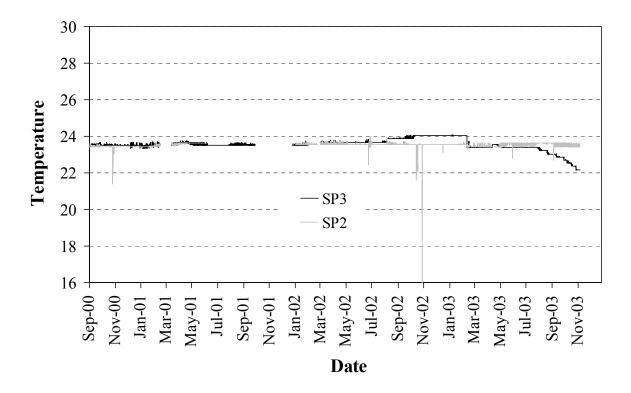


Figure 4. Thermistor data from Spring Runs 2 and 3.

Blieders Creek also registered much lower water temperatures during the winter than areas more directly influenced by springflow, but water temperature reached the lowest values in the the furthest downstream sites. Minimum temperatures occasionally dropped below 18°C downstream of Landa Lake but usually remained above 20°C (Appendix B). While the wide fluctuations in temperature found in Blieders Creek do not appear suitable to provide habitat for fountain darters during a portion of the year, the effects of these water conditions are highly localized. The lower end of Blieders Creek is typically a stagnant pool that does not flow into the Comal River and temperatures observed in the Upper Spring Run Reach were within a much more narrow temperature range (Heidelberg site; Appendix B).

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.

During drop-net sampling, point measurements of water quality were made at each sample. Only one point measurement of dissolved oxygen fell below the water quality standard of 5.0 milligrams per liter (mg/l) in 2003. This site was very shallow (<0.5 ft) in a backwater area with little water circulation, so it is not representative of true conditions. Other measurements in the same vicinity were above the 5.0 mg/l threshold during that sample effort. Dissolved oxygen concentrations recorded at the sites located in the upper portion of the Comal River (Blieders Creek, Heidelberg, and Spring Island sites) were lower than concentrations recorded in the Spring Run sites and downstream (Appendix B). Runoff and flow from Blieders Creek affects water quality conditions at the Heidelberg site to a greater extent than at the other Comal River sites.

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 a trend of decreasing aquatic vegetation coverage in 2003. This is in contrast to 2002 during which there was an increase in virtually all vegetation types between the first (winter) and last (fall) samples. One possible explanation for the different trends in 2002 and 2003 is that the flood in July 2002 provided a scouring effects that stimulated growth of most types of vegetation and the relatively constant discharge in 2003 (though higher than normal) has not provided the kind of flushing flow conditions that stimulate growth.

Cabomba coverage decreased from the high value observed in the summer and fall of 2002 (26.9 m²) during the spring and into the summer before rebounding slightly in the fall 2003. Despite the small increase in the fall 2003 sample, the total coverage (13.5 m²) remained below the lowest value observed in 2002 (18.3 m²). Ludwigia and Limnophila also declined sharply in total coverage during 2003 to fall sample values of 5.0 m² and 7.0 m² respectively, which are less than half of the lowest observations in 2002 (12.1 m² and 25.0 m²).

Hygrophila also decreased dramatically during 2003, from a high of 992.1 m² in the fall of 2002 to a low of 291.4 m² in the fall of 2003. This last observation was the lowest observed since the winter of 2001. Thus, despite the higher than normal flows that have occurred throughout 2003 (at least 70 cfs greater than normal for each monthly average) the total coverage of Hygrophila at the end of the year was reduced. 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. A substantial decrease in Hygrophila, though probably offset in this case by the increase in bryophytes, might have a negative impact on the fountain darter population. If the bryophytes continue to decline and the Hygrophila coverage remains low, there could be a decline in the fountain darter population in the Upper Spring Reach over time.

Bryophytes and *Sagittaria* each continued a trend of increasing coverage begun in 2002 to a peak in the summer of 2003, but both declined in the fall of 2003. [In the 2001 and 2002 annual reports, *Riccia* is the only bryophyte (moss) mentioned; however, we have since determined that there is an additional type, *Amblystegium*, which is abundant in the Upper Spring Run Reach (relative to *Riccia*) and present (but less common than *Riccia*) in Landa Lake. The 2003 map legends were changed from *Riccia* to a new category "Bryophytes" that encompasses both types of moss]. The higher flows that have occurred during the latter half of 2002 and throughout 2003 have clearly had a positive affect on the growth of the bryophytes and to a lesser degree on *Sagittaria*. Although a decline was observed in the bryophyte coverage between the summer 2003 and the fall 2003 samples, there was still nearly double the highest amount observed in 2002. 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. If the bryophytes continue to remain in such high coverage in this reach, fountain darters should respond by increasing population numbers. *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.

Landa Lake Reach

Landa Lake is a 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 did not change much in total coverage from 2000-2003, although some scouring and re-growth was evident in certain areas and some areas were filled in with bryophytes (particularly during late 2002 and 2003). This plant species has flourished since the 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.

The large patch of pure *Hygrophila* in the shallow area between the three islands exhibited the most response of any vegetation type to the flushing flows of 2001-2002 and appears susceptible to paddleboats during the summer and fall. In 2003, the *Hygrophila* in this shallow area was very patchy in the summer and fall despite a lack of any major scouring flows. Conditions during this period (higher flows, but not flushing flows) should have been excellent for growth of most macrophytes. There were two occasions in the fall of 2003 in which discharge increased to above 500 cfs, but this value is substantially lower than many peak discharge events in 2001-2002 and should not have been significant enough to scour the vegetation. 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.

Similar to *Hygrophila*, the *Ludwigia* that is immediately downstream of the *Hygrophila* exhibited some response to the scouring effects of flooding in 2001-2002 and despite the lack of flooding conditions in 2003, also declined substantially between the summer and fall. As with the *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 (some filamentous algae is present in water too deep to sample). There was relatively little direct influence (scouring) on the bryophytes in Landa Lake resulting from flooding in 2001-2002. Although flooding did not scour the bryophytes, the increased flows since the floods may have stimulated growth of bryophytes in Landa Lake in 2003. The coverage of bryophytes increased dramatically after the second high flow event in 2001 and both discharge and bryophyte coverage have remained high ever since.

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 CO₂ levels resulting from higher flows stimulated the growth, but the lab study showed that growth of the two bryophytes (*Riccia* and *Amblystegium*) was not related to CO₂ level. 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 m² and decreased slightly during the summer and fall 2003 sampling events to a low of 3,305 m². 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.

Sagittaria and Nuphar were minimally impacted by high flows in 2001-2002 and did not fluctuate much through the first two samples of 2003 (spring and summer); however, there was a moderate decline in the coverage of each in the fall 2003 sample. Cabomba was one of the few plant types that did not decrease between the summer and fall samples of 2003. As mentioned above, there were no substantial flooding events during this period that would account for the widespread decline in most species. The presence of paddleboats may help explain the patchiness of those plants found in shallow water, but the decline in total bryophyte coverage (mostly in deeper areas), Sagittaria, and Nuphar are not easily explained. These data show that the vegetation community is ever changing, even with 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.

Old Channel Reach

Until 2003, the Old Channel Reach was the most stable habitat with a structure (culvert) that regulates flow through this section at approximately 40 cfs (P. Connor, USFWS, pers. comm.). In 2000-2002 this reach was dominated by filamentous algae, which provided the highest density of fountain darters of any vegetation type. Also common was and aquarium species of the genus *Ceratopteris*. In 2003, the *Ceratopteris* abundance remained approximately the same as in previous years with some fluctuation in total coverage, but the filamentous algae virtually disappeared beginning in the fall of 2002 and remained very low throughout 2003. At the same time, small patches of *Hygrophila* started to become established by the fall 2002 sample and some *Ludwigia* appeared by the spring 2003 sample. Both species appear to be growing at about the same rate, but *Hygrophila* remains slightly more abundant since it started to establish a few months before the *Ludwigia* appeared. These plants have largely covered the areas that had previously been covered with filamentous algae. Part of the reason for these changes in the plant community is probably the higher flows through the channel. The culvert was adjusted at some point in late 2002 or early 2003 and as a result, discharge measurements made by BIO-WEST were 114.5 cfs in the spring 2003 sample and over 93 cfs in each of the latter two samples of 2003.

The changes in this channel are significant because of the importance of filamentous algae to fountain darters. Future sampling of fountain darters in the new vegetation will determine whether the channel will continue to support the densities of fountain darters that had been sampled in 2000-2002 or if the new vegetation types will reduce the densities to those observed in other areas. 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. The filamentous algae was very susceptible to flushing flows during flood events and has remained low while flows are higher than normal in 2003. If flows return to the 40cfs estimate made by the USFWS, conditions may allow for the return of filamentous algae in the reach. It will also be important to monitor the relative proportion of each of the two "new" plant types because *Ludwigia* supports a much greater density of fountain darters than *Hygrophila*. If, like in other areas, the *Hygrophila* eventually comes to dominate the reach, the habitat quality for fountain darters will decrease substantially compared to conditions observed in 2000-2002.

New Channel Reach

Hygrophila dominates the New Channel Reach. The total coverage of Hygrophila did not fluctuated by much between 2000-2003. There has been some minor decrease in total coverage due to scouring after floods, but the bare patches are quickly re-vegetated. Cabomba decreased some following flooding but appears to be well positioned to avoid scouring by flooding in this reach, as almost all patches remained after flooding in 2002 and most had increased in size. This is probably due to it growing within dense stands of Hygrophila that provide some protection to increased flows. Overall, Cabomba has generally increased during 2000-2003 and reached its highest abundance in this reach in the final sample of 2003 (fall).

Until the fall 2003 sample, *Ludwigia* had not been present in this reach during the study. There was some *Ludwigia* found in the upper section that was sampled in 2000-2002, but the first time it was observed in this lower section was in the fall of 2002. Since then, it has increased slightly in total coverage, but remains very low overall.

Fountain Darter Sampling

Drop Nets

The number of drop net sites and vegetation types sampled per reach is presented in Table 5.

The drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sample event. The data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively.

Table 5. Drop net sites and vegetation types sampled per reach in 2003.

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes ^a (2)	Bryophytes ^a (2)	<i>Hygrophila</i> (2) ^b	Bare Substrate (2)
Sagittaria (2)	Hygrophila (2)		Filamentous Algae (2)
Hygrophila (2)	Cabomba (2)		Ceratopteris (2)
	<i>Vallisneria</i> (2)		
	Ludwigia (2)		
Total (6)	Total (10)	Total (2)	Total (6)

^a Switched from Open to Bryophytes, summer 2001.

As in previous sampling, we found a wide range of suitability of the various vegetation types in both the Comal and San Marcos Rivers (Figure 5). Compared to the San Marcos River, the Comal River has a greater diversity of habitats available to fountain darters, and we have observed a wider range of suitability. The densities of fountain darters in the Comal River vegetation types ranged from 2.4 per m² in Sagittaria to nearly 30 per m² in filamentous algae (the type found in the Old Channel Reach [Comal] and Spring Lake Reach [San Marcos], which differs from the finer algae found in the Landa Lake and Upper Spring Run Reaches). The bryophytes (Riccia and Amblystegium) also contained high numbers of fountain darters (>20 per m²), a substantial increase compared to data presented in the 2002 annual report. As mentioned in that document, Riccia increased rapidly and dramatically during 2002 and the fountain darters may have had a lag period before taking advantage of the greater availability of resources. Thus, the lower densities observed in 2002 were probably a result of fountain darters being

^b Upper section removed starting fall 2002; only two sites in lower section were conducted in 2003.

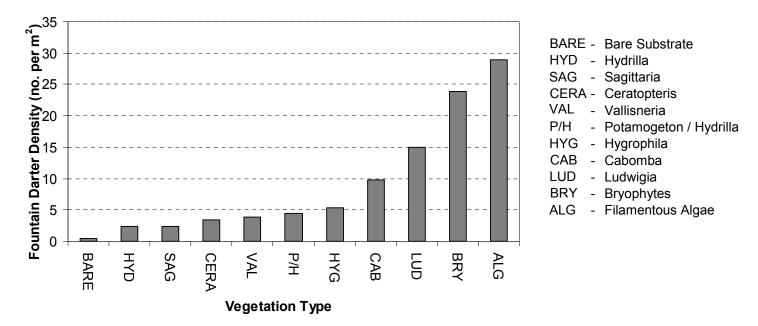


Figure 5. Density of fountain darters collected by vegetation type in the Comal and San Marcos Springs/River ecosystems combined (2000-2003).

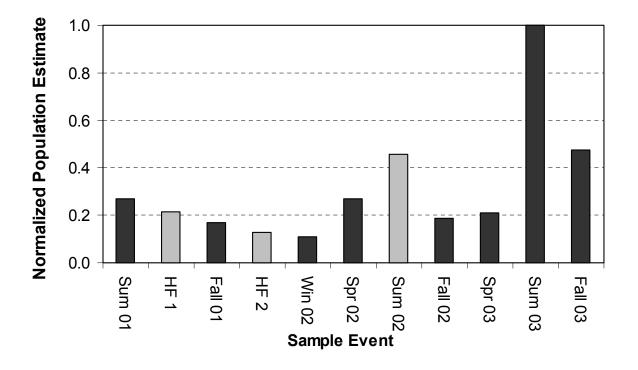


Figure 6. Population estimates of fountain darters in the Landa Lake Reach; values are normalized to the maximum sample (summer 2003). Light bars represent high-flow (flood) events, which were triggered by flows that exceeded a 0.5% likelihood of occurrence based on the period of record. Data prior to Summer 2001 did not have bryophytes included and would not provide an accurate estimate for comparison.

dispersed more widely throughout the new vegetation. The abundant *Riccia* observed at the end of 2002 remained abundant throughout 2003 and the densities of fountain darters observed increased, suggesting that the fountain darters have increased in numbers to occupy the new habitat. Estimates of population abundance based on drop-net data (Figure 6) reveal that the abundant bryophytes coupled with increasing density observed in 2003 resulted in a significant increase over the previous high estimate in the summer of 2002.

The two vegetation types that support the highest densities of fountain darters, bryophytes and filamentous algae, provide cover at the substrate level and high numbers of amphipods, an important food item for fountain darters. In addition to these two vegetation types, *Ludwigia* provides high quality habitat (~15 per m²). *Hygrophila* is similar in structure to *Ludwigia*, but this exotic species found in both the San Marcos and Comal Rivers provides substantially lower-quality habitat than the native *Ludwigia*. *Ludwigia* remains relatively uncommon in Landa Lake and is susceptible to wide fluctuations in total coverage; it tends to be scoured with flushing flows and may be susceptible to mechanical disturbance by boats (described in the vegetation mapping section). Recently, *Ludwigia* has appeared in the Old Channel Reach and is growing rapidly, but its appearance there has accompanied a reduction in filamentous algae, which results in a net loss of fountain darter habitat suitability.

Prior to 2003, the density of fountain darters observed in Sagittaria was only 0.55 per m², but samples in Sagittaria in 2003 had very high numbers of darters. With samples that included 11, 16 and 17 fountain darters per m², the data from 2003 was enough to increase the density calculation for Sagittaria (over the entire study period) to 2.4 per m². This increase appears to be a result of a dramatic increase in the bryophytes found in the Upper Spring Run Reach (where Sagittaria is sampled). The bryophytes (both types) became dense in the open areas surrounding the Sagittaria and Riccia also settled down within the stands of Sagittaria. Because the bryophytes support much greater densities of fountain darters, the numbers of individuals observed in these habitats increased. This phenomenon also occurred in Hygrophila, Ludwigia and Vallisneria in the Landa Lake Reach and consequently, the density calculation for each of those plant types increased by similar amounts. Therefore, these vegetation types become much more favorable to fountain darters with Riccia interspersed within the stand and that the fountain darter population is able to increase as a result. Based on these results, population estimates would be greatly affected by the presence of *Riccia* and the difference in suitability of a given plant type with and without the *Riccia*. These data, coupled with the increased density of fountain darters in the abundant Riccia, suggest that fountain darters have exploited the favorable changes in habitat conditions in Landa Lake with an expansion of the population, but the trend would presumably be reversed if and when the *Riccia* diminishes.

The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem in 2003 is presented in Figure 7 (all data collected from 2000-2003 is presented in Appendix B) and is a statistically normal distribution that is typical of a healthy fish assemblage. Sample sizes were similar in 2002 and 2003, but the overall distribution is differed slightly between the two years. In 2002, the size-class distribution was more evenly spread between 16 mm and 32 mm with no apparent peak in the data. In 2003, the distribution had a clearly defined peak at 30mm with the greatest number of individuals in a much narrower range, between 24 mm and 34 mm. The size-class distribution of 2003 observations was very similar to the overall distribution observed for 2000-2003 but the peak for the latter data was between 22 mm and 26 mm. This shift in 2003 to larger individuals occurred primarily in the upper spring run and Landa Lake reaches where there appeared to be a boom in the population as a result of increased habitat suitability (more Riccia). Many of those individuals grew to a large size by the low

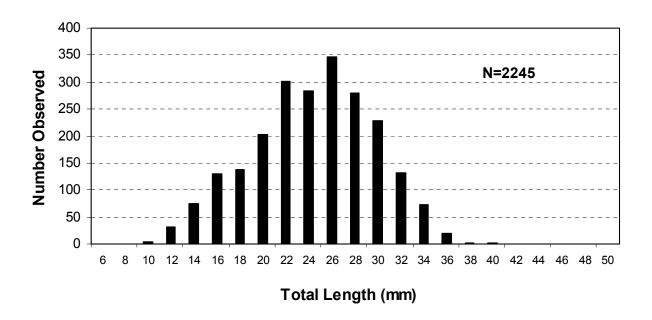


Figure 7. Fountain darter size-class distribution among all drop-net sampling events in the Comal River in 2003.

summer (fountain darters grow 0.2 mm per day according to Brandt et al. [1993]) and large individuals comprised an even greater portion of the population in the fall 2003 sample. Improved conditions and intra-specific competition in the new habitat may have also contributed to a rapid growth rate in some individuals.

Although many more large fountain darters were observed in 2003 than observed in 2000-2002, there were substantially fewer fish under 20 mm in 2003 (n=110) than in 2002 (n=692). The smallest fountain darter observed in 2003 was 14 mm (n=4) while in 2002 there 24 individuals observed that were 10 mm or less. Part of the reason for fewer small fish may be that many small fountain darters were observed in the winter sample of 2002, but no winter sample occurred in 2003 (there were only three sampling events in 2003). Also, small fountain darters were frequently captured in the filamentous algae (in the Old Channel Reach) in 2001 and 2002, but there was very little algae in all 2003 samples.

In 2002, the size-class distributions in the Old Channel and Upper Spring Run Reaches were proportionally similar in each of the four seasonal samples, but many more fountain darters were observed in the Old Channel Reach. In 2003 (Figure 8), the two reaches were again proportionally similar during each sample effort but the number of observations in the Old Channel Reach was substantially lower than in 2002 and was slightly lower than the number of observations in the Upper Spring Run Reach. Fountain darter observations in the Old Channel Reach were lower because of the substantial decrease in filamentous algae, while observations were higher in the Upper Spring Run Reach due to the increase in bryophytes. In both years, these two reaches had the greatest number of small fountain darters in the spring (excluding the winter 2002 sample). A peak in the 16-25mm range during the spring and corresponding shift to the 26-35mm range during the summer is also evident in both reaches during each year. The fall sample was also similar in the two reaches in each year, but in

2002, there were a few individuals in the smallest size category whereas in 2003, there were no small individuals observed in either reach.

Observations of seasonal variation in fountain darter abundance in the Comal River in 2003 differed from that observed in 2000-2002. The greatest number of fountain darters was observed in the summer (n=919) sample followed closely by the fall sample (n=749); the lowest numbers overall were observed in the spring (n=577). The greatest number of small fountain darters (<16 mm) was observed during the spring (n=119). This contrasts to the previous observations of high numbers of fountain darters in spring samples and lower numbers in the fall and highlights the variability that occurs in the system and with this type of biological sampling. Although there does not seem to be a strong inter-annual pattern in population abundance, there does appear to be relatively high seasonal variation that may be due in part to flooding and variation in other physicochemical parameters.

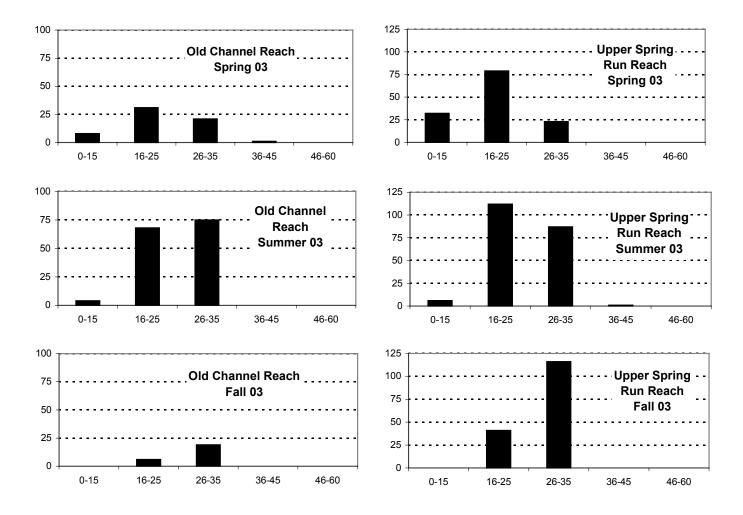


Figure 8. Size-class distributions of fountain darters by date (2003 only) in the Old Channel and Upper Spring Run Reaches.

Although the fewest fountain darters were collected in spring during 2003 there was still the greatest indication of recent reproduction during that time (presence of small darters). The Old Channel Reach had a peak of reproduction in spring 2003 as in spring 2002, but had fountain darters from the smallest size class (thus continuous reproduction) in each sample during those years. Competing theories have been reported in the literature regarding wild fountain darter reproductive cycles; some researchers support continuous spawning (Strawn 1955, Hubbs 1985), while others have noted peaks in reproductive activity (Schenck and Whiteside 1977). Our data support both theories and suggest that the mode of reproduction may depend upon local conditions. The high quality conditions in the Old Channel have allowed for continuous reproduction during the study period. Sites with lower-quality habitat, such as those in the San Marcos River, tend to have reproduction only when conditions are favorable.

Observations of potential low-flow impacts on fountain darter population dynamics are still limited because of inadequate opportunities to conduct low-flow samples. However, the seasonal variation and year-to-year variation in fountain darter populations re-emphasizes the importance of detailed examination of a system to determine the dynamics that dictate both the total population numbers and densities of fountain darters under various conditions rather than a one-time sampling event when low-flows occur.

In addition to fountain darters, a total of 18 fish species and 11,046 individuals were collected from the Comal ecosystem in 2003; of these, 7 species are considered exotic (introduced) (Table 6).

Table 6. Fish species and the number of each collected during 2003 drop-net sampling.

COMMON NAME	SCIENTIFIC NAME	STATUS	TOTAL NUMBER
Rock bass	Ambloplites rupestris	Introduced	8
Yellow bullhead	Ameiurus natalis	Native	13
Mexican tetra	Astyanax mexicanus	Introduced	44
Rio Grande perch	Cichlasoma cyanoguttatum	Introduced	47
Roundnose minnow	Dionda episcopa	Native	47
Fountain darter	Etheostoma fonticola	Native	2245
Gambusia	Gambusia sp.	Native/Introduced	8117
Suckermouth catfish	Hypostomus plecostomus	Introduced	6
Redbreast sunfish	Lepomis auritus	Introduced	1
Warmouth	Lepomis gulosus	Native	2
Bluegill	Lepomis macrochirus	Native	2
Longear sunfish	Lepomis megalotis	Native	3
Spotted sunfish	Lepomis punctatus	Native	139
Sunfish	Lepomis sp.		66
Spotted bass	Micropterus punctulatus	Native	1
Largemouth bass	Micropterus salmoides	Native	5
Texas shiner	Notropis amabilis	Native	3
Sailfin molly	Poecilia latipinna	Native	293
Tilapia	Tilapia sp.	Introduced	4

The giant ramshorn snail was also recorded and measured at each drop net location. Figure 9 shows the densities of live giant ramshorn snails observed in 2003 in the Comal ecosystem by vegetation type, which were generally lower than in past years. The greatest density was 0.68 snails per m² in *Ludwigia* and only 0.17 snails per m² in Vallisneria, which is substantially lower than 2002 findings. In 2002, there were 1.13 snails per m² observed in *Vallisneria* and 0.95 per m² in *Ludwigia*. The greatest density in 2000-2001 was Ludwigia (2.38 per m²). Hygrophila was lower in 2003 (0.43 per m²) compared with 2000-2001 (0.89 m²) and 2002 (1.06 per m²). The two preferred fountain darter vegetation types (bryophytes and filamentous algae) had essentially no giant ramshorn snails present. In the past, the giant ramshorn snails were observed in far greater densities than those reported here. For perspective, the maximum value on the y-axis of Figure 9 is 10 m², which was among the lowest concentrations reported in the early 1990s when the giant ramshorn snail population was booming (T. Arsuffi, Texas State University aquatic ecologist, pers. comm.). During that period the greatest giant ramshorn snail density was near 400 snails per m², and the vegetative community was literally being devoured (T. Arsuffi, Texas State University aquatic ecologist, pers. comm.). By all indications the densities of giant ramshorn snails observed in the Comal ecosystem during the study period to date (including the 2000 low-flow events) pose no serious threat to the aquatic vegetative community. However, because of the impact that this exotic species can have substantial impacts at higher densities, close monitoring should continue.

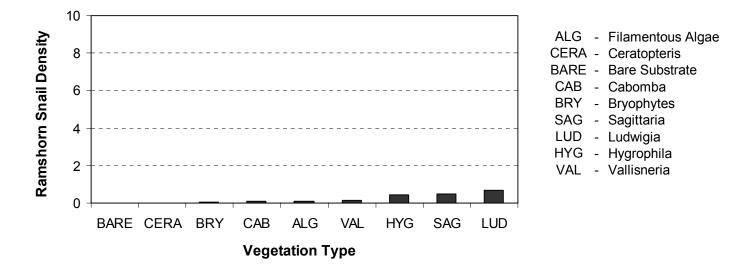


Figure 9. Density of giant ramshorn snail by vegetation type (averaged across all sites).

Dip Nets

The boundaries for each section of the dip net collection efforts are depicted on Figure 10. Data gathered using dip nets for all sections are graphically represented in Appendix B. High water and turbid conditions prevented the collection activities from the New Channel and Garden Street sites on a few occasions in 2000-2002, but these areas were sampled during each quarterly effort in 2003.

As in 2002, there were periods in 2003 in which the Old Channel Reach had high levels of reproduction and periods in which reproduction was less, but there have been darters of the smallest size group (5-15 mm) observed in each sample in this reach (Figure 11). This indicates that there is some reproduction

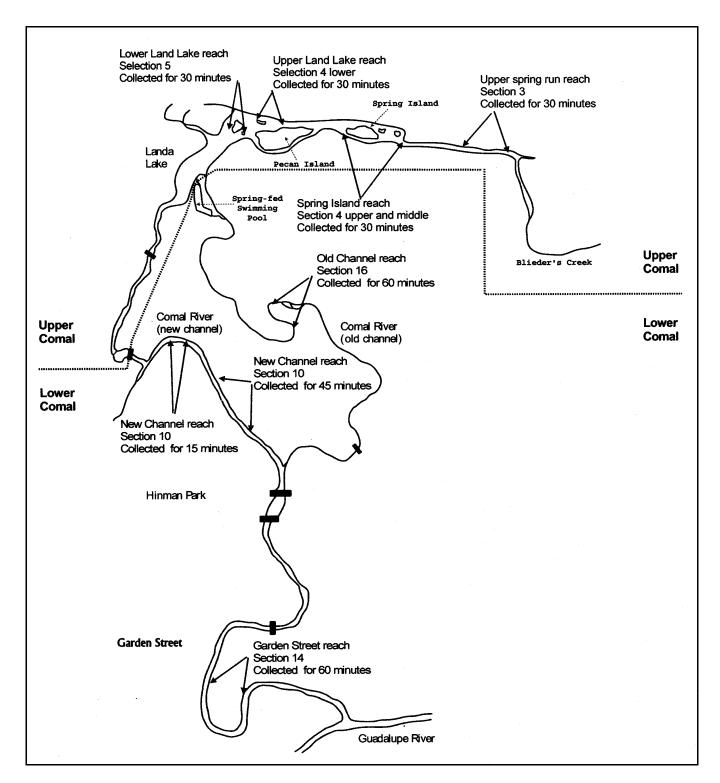


Figure 10. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

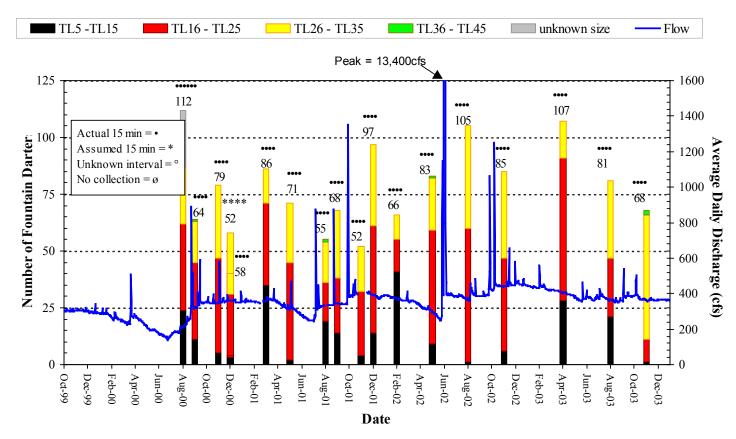


Figure 11. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

occurring year-round. The greatest number of observations of fountain darters in the smallest size class (5-15 mm) occurred in the spring and summer and only one was observed in the fall. This correlates well with the drop net results, in which there were some fountain darters of the smallest size class in both the spring and summer and primarily large fountain darters (26-35mm) in the fall. There was a spike in the number of small individuals in the late winter to early spring of each year of the study to date (2001-2003). In the spring of 2003, all reaches had some fountain darters of the smallest size class, including three that had the greatest number of such observations during the entire study (Upper Spring Run, Spring Island, and New Channel Reaches). This contrasts with 2002 data since several sites in that year had the fewest observations of small fountain darters in the spring. However, the Old Channel, New Channel and Garden Street Reaches have had the highest number of small darters observed during the spring throughout the study.

Some reaches that had a peak in small darters (evidence of recent reproduction) in the spring in 2003 had a more even distribution of small darters among all samples in 2002. One hypothesis for the lack of a typical spring peak in 2002 is a connection to the flushing flows that occurred in 2001-2002. Such conditions may have stimulated reproduction in some individuals immediately after flooding (in fall 2001 and summer 2002) and otherwise disrupted natural stimuli in others that would have triggered a spring reproductive peak. In the Old Channel, the near-complete scouring and rapid regrowth of filamentous algae after the fall 2001 flood event may have been a stimulus to initiate reproduction in most fountain darters in that reach. In 2003, under higher flow conditions, reproduction was evident in the Old Channel Reach in both the spring and summer but very little evidence was observed in the fall.

It is possible that the reduction in filamentous algae has and will continue to modify reproduction in this reach since most algae was gone in the fall and other plants covering the areas it had previously occupied (i.e., *Ludwigia* and *Hygrophila*). This shift in vegetation coverage appears to be associated with post-flood conditions (from the July 2002 flood) and subsequent long-term period of higher flows. Prior to this change, it had appeared that year-round reproduction was occurring in the Old Channel Reach, but if the habitat composition begins to resemble other areas, reproduction may also become more seasonal like in those reaches.

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 has remained consistently high. The quantitative sampling (started in summer 2001) 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 7 shows the number of fountain darters observed in the 7.8 m² grid per sampling event.

Table 7. The number of fountain darters observed per grid/sampling event.

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

These quantitative 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 in the use of since the highest numbers were observed during the fall of each year despite the fact that *Riccia* coverage was lower in the fall of 2002 and 2003 than the spring of either year. Despite this trend, observations in 2003 were relatively similar among sample dates. This similarity may be due to the relatively constant conditions with no scouring events to disturb the

dense *Riccia* coverage that supports the fountain darters in the sample area. 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 this study are described in more detail in Cantu's thesis (2003; Texas State University) entitled: "Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma fonticola*) in the Comal River, Texas." However, a brief summary of the study is provided here.

There was a statistically significant difference among sites (spatial variation) in each of the three methods for sampling parasites. In filtered river water, the site at Spring Island had significantly higher concentrations of *C. formosanus* than in all other sites (18.2±3.1 SE cysts/fish compared to <5 in all other sites). Caged fish also had significantly more parasites per fish in that site than in all others (64.4±16.7 SE cysts/fish compared to <10 in all other sites). There was a significantly greater frequency of infected fish at Spring Island than in the two Spring Run sites (1 and 3) and the Houston street (Upper Spring Run) site. In resident fish, the greatest number of parasites per fish occurred downstream of the confluence of Spring Runs 1 and 2 (significantly more than all other sites) but there was no significant difference among sites in the frequency of infected fish.

There were fewer statistically significant differences among seasons (temporal variation) than among sites during the study. In filtered river water, summer and spring had the highest numbers of *C. formosanus*, but the only significant difference was between summer and winter. In caged fish, there was no significant difference among seasons in the number of parasites per fish, but summer and spring were higher. There was a significant difference between winter (lower) and all of the other seasons for the frequency of infection of caged darters. In resident fish, there were no significant differences between seasons in number of parasites per fish or number of infected fish, although in both instances, the numbers were highest in the fall after a period of moderate rainfall and high discharge.

The findings also show a negative relationship between flow and concentration of the parasites, which likely contributes to the observed spatial variation. The site with the highest concentrations (near Spring Island) had little or no current velocity; whereas, in the sites with the greatest current velocities (Spring Runs) the concentrations were low. Webbe (1966), Stables and Chappell (1986) and Theron et al. (1977) suggest that higher current velocities result in lower risk to fish as intermediate hosts for the parasite. This relationship is likely strongly related to snail density in the area as well, but that has not been evaluated yet. Anecdotally, it does appear that snails are most abundant in areas with low current velocities, and the combination of the two factors has probably contributed to the observations of high parasite density in these areas. Accordingly, future efforts to reduce parasite populations should focus on low-velocity habitats that harbor dense snail populations and allow *C. formosanus* to accumulate.

Among the three methods evaluated for use in future monitoring efforts, filtering river water provides the greatest benefit for the effort. Each method had pros and cons; the dip-net method for resident fish provides the only means of surveying the actual infection status of fountain darters in the river, but does not provide fish with a known location history or infection history. The caged-fish method restricts fish to the conditions at a specific site for a known amount of time, but this requires the greatest amount of effort and expense by far and would be difficult to repeat on a regular basis (and is also subject to

vandalism). The river water filtering technique has the benefit of sampling a particular place and time and can be conducted at any time with a moderate effort. The standard error was also substantially lower with this method than with the others. Finally the filter method had a strong correlation with the caged-fish method, which might otherwise be considered the most accurate way to assess current infection rates in the fish.

Overall, the results show that *C. formosanus* are present in the water column and infect fountain darters year-round in the Comal River. Information on spatial and temporal variability will provide important data that can be used to focus efforts on reducing the host snails in "hot spots" that may substantially improve conditions for resident fountain darters. Time of year does not appear to be a critical factor that might influence removal efforts, but any efforts would probably be most valuable prior to or during reduced flows when parasite concentrations may be highest. Future monitoring of parasites in the water column in these study sites will provide a continuous dataset for evaluating future trends in parasite concentrations, including information on low-flow conditions. As with other components of the study, more data are needed from 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. 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. Since the inception of the project, Comal Springs salamanders have been observed in each sample location during each sample period. The total number of Comal Springs salamanders observed at each survey site during each sampling event is presented in Table 8.

The greatest cumulative number of Comal Springs salamanders observed for all sites during any one sampling event was 67, which occurred during summer 2002; 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 (17.7 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.

In 2002, the winter sampling event supported the preliminary theory that under situations of acute disturbance/habitat modification, Comal Springs salamanders simply retreat into the substrate and return to the surface when conditions are again suitable. The disturbance was due to high flows that moved deposited a substantial amount of sediment in the Spring Runs. In 2003, there were no disturbances and salamander observations remained high in the areas that had been affected by siltation in 2002. Salamanders may have moved laterally in the Spring Run channel or downward into the substrate when silt covered the substrate. Individuals moved back to the sample area and remained there during all samples in 2003. The three samples had relatively similar results in all sample locations. However, one difference did appear significant; there was a high number of Comal salamanders observed in Spring Run 3 during the spring. That observation was greater than any other sample, but there was no observable change in habitat conditions to explain it.

Table 8. 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	25	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	34	9	7	3	53
Spring 2002	36	15	6	5	62
High Flow 2002	41	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
Average	17.7	10.3	6.6	7.2	

Macroinvertebrate Sampling

In 2003, 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 216.6 hours of sample time occurred among the three drift net sites at Comal Springs and 13 species were captured (Table 9). Among species of concern, an average of 10.6 *Stygobromus pecki* (Peck's cave amphipod; many small *Stygobromus* were unidentifiable to species), 1.2 *Heterelmis comalensis* (Comal Springs riffle beetle), and 0.3 *Stygoparnus comalensis* (Comal Springs dryopid beetle) were retrieved during each 24-hour period at all sites. *Stygobromus pecki* was found in all three locations (fewest in Spring Run 1), but *H. comalensis* was not found in the upwelling along the western shoreline of Landa Lake and *S. comalensis* was not found in Spring Run 3 during any sample. There was also a new species of the family Ingolfiellidae found during the November sample in Spring Run 3. The same orifices at the heads of each spring run were netted during all collection events; water quality data is presented in Table 10.

Table 9. Total numbers of invertebrate species collected in drift nets from 12 June to 24 November, 2003 (three sample dates). Federally endangered species are designated with (E). A = adult beetles. L = larvae.

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	72.1	72.5	72	216.6
Crustaceans				
Amphipoda				
Gammaridea				
Crangonyctidae				
Stygobromus pecki (E)	6	43	47	96
Stygobromus russelli	1			1
Stygobromus sp.	163	206	188	557
Hadziidae				
Mexiweckelia hardeni	26	15		41
Sebidae				
Seborgia relicta	1	6	2	9
Artisiidae (=Bogidiellidae)				
Artesia subterranea	3			3
Ingolfiellidea				
Ingolfiellidae				
new species		1		1
Isopoda				
Asellidae				
Lirceolus (2 spp.)	81	26	9	116
Cirolanides texensis	1	2	3	6
Insects				
Coleoptera				
Carabidae				
terrestrial larva	1			1
Dytiscidae				
Comaldessus stygius (adults)	1	3		4
Dryopidae				
Stygoparnus comalensis (E)	2(1A, 1L)		1(A)	3
Elmidae				
Heterelmis comalensis (E)	10(9A, 1L)	1(A)		11

Table 10. Results of water quality measurements from drift net sampling locations at Comal Springs.

	Spring Run 1		S	Spring Run 3			Upwelling		
Date	June	Sept	Nov	June	Sept	Nov	June	Sept	Nov
Temperature (°C)	23.0	23.0	23.1	23.1	23.1	23.3	23.6	23.9	23.4
Conductivity (mS)	0.572	0.575	0.542	0.572	0.576	0.541	0.575	0.578	0.545
рН	7.0	7.0	6.9	7.0	7.0	6.9	7.1	7.0	6.9
Dissolved Oxygen (mg/L)	5.1	5.5	5.8	5.0	5.6	6.0	4.9	5.3	5.7
Velocity (m/s)	0.60	0.80	0.61	0.43	0.41	0.40	0.87	0.52	0.55
Depth (m)	0.36	0.34	0.34	0.60	0.58	0.64	0.64	0.66	0.55

Species of the two genera Stygobromus and Lirceolus were the most abundant at all sites during each sample event (Table 9). Thus, any attempts to establish a springflow-biological response relationship should probably focus on these groups. Stygobromus pecki was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long, possibly because they were dislodged easier than the larger ones. This may be a method of dispersal. Those individuals that were too small to identify to species were recorded as *Stygobromus* sp. and most likely consist of both S. russelli and S. pecki. In previous drift net sampling (downstream of the spring opening in the spring runs) all *Stygobromus* sp. were not identified to species but were not *S*. pecki (T. Arsuffi, Texas State University, personal communication). It appears that the federally endangered S. pecki are restricted to spring openings and areas immediately adjacent while S. russelli (presumably the unidentified Stygobromus sp. in early drift net samples) occurs throughout the spring run. A similar observation was made in Hueco Springs where S. pecki are found in the spring openings but an intensive survey of the downstream spring run habitat revealed only a single S. russelli (J. Krejca personal communication). A qualitative observation of the two Stygobromus species is that many S. pecki have been observed to be orange in coloration while all S. russelli individuals have been white. This may be a result of food type since S. pecki appear to differ from S. russelli in habitat usage and are most common on leafy debris and roots.

Estimates of drift rate (Table 11) and drift density (Table 12) were calculated for each family/genus/species that were identified in the three sample efforts. However, because velocity measurements varied dramatically according to position of instrument within each spring orifice (making reproducible measurements difficult to achieve) drift density values may be somewhat inaccurate. The drift density values are highly dependent on these velocity measurements to calculate total volume of water that passes through the net to determine the number of macroinvertebrates drifting per 100 m³ of water sampled.

Overall there appeared to be a similar relative abundance of several species among the three sample periods. However, there was a wide range of individuals of a given species captured across sample events. For instance, the fewest *Stygobromus* sp. (32) and fewest *Lirceolus* sp. (1) were caught in the June sample in Spring Run 3 and the highest number of each (115 and 17 respectively) was captured in September. Thus, the number of captures of a given species differed dramatically between samples, but the proportion relative to the other species remained similar. This suggests that some density-independent factor is influencing each species equally. The most logical factor would be springflow; however, total discharge in the Comal Springs/River ecosystem was similar during each sample event. It may be possible that slight changes in total spring flow from the Comal Springs/River ecosystem result in more dramatic differences among spring openings. For instance a slight decrease in aquifer water level may reduce the amount of flow in a higher elevation spring (i.e., Spring Run 3) while correspondingly adding some discharge to a nearby lower elevation spring (i.e., Spring Run 1). Further springflow measurements during low-flow periods are needed to investigate this hypothesis.

Table 11. Drift rate of each taxa identified in 2003 drift net sampling by sample location and date. Two additional values are presented (bold numbers): the mean drift rate for each taxa during all 2003 samples and the mean drift rate of all organisms captured by date.

		Spring Run 1				Spring Run 3				
	June	Sept	Nov	Mean	June	Sept	Nov	Mean		
Stygobromus pecki	2.9	2.0	1.0	2.0	10.8	15.0	17.0	14.2		
Stygobromus russelli	0.0	1.0	0.0	0.3	0.0	0.0	0.0	0.0		
Stygobromus sp.	34.3	66.5	62.0	54.3	20.6	100.0	85.0	68.2		
All Stygobromus	37.2	69.5	63.0	56.6	31.3	115.0	102.0	82.4		
Mexiweckelia	12.7	12.1	1.0	8.7	1.0	6.0	8.0	5.0		
Seborgia	0.0	0.0	1.0	0.3	1.0	4.0	1.0	2.0		
Artesia	2.0	1.0	0.0	1.0	0.0	0.0	0.0	0.0		
Lirceolus (2sp.)	27.4	38.3	15.0	27.0	1.0	17.0	8.0	8.6		
Cirolanides	0.0	1.0	0.0	0.3	0.0	1.0	1.0	0.7		
Comaldessus	0.0	0.0	1.0	0.3	1.0	2.0	0.0	1.0		
Stygoparnus	0.0	2.0	0.0	0.7	0.0	0.0	0.0	0.0		
Heterelmis	1.0	6.0	3.0	3.3	0.0	1.0	0.0	0.3		
All Troglobitic & endangered fauna	96.0	142.0	93.0	110.5	35.3	150.0	123.0	102.3		

	Upwelling - West Shoreline				All Sites Combined				
	June	Sept	Nov	Mean	June	Sept	Nov	Mean	
Stygobromus pecki	26.0	16.0	5.0	15.7	13.2	11.0	7.7	10.6	
Stygobromus russelli	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	
Stygobromus sp.	52.0	60.0	76.0	62.7	35.5	75.3	74.3	61.6	
All Stygobromus	78.0	76.0	81.0	78.3	48.7	86.7	82.0	72.3	
Mexiweckelia	0.0	0.0	0.0	0.0	4.6	6.0	3.0	4.5	
Seborgia	0.0	0.0	2.0	0.7	0.3	1.3	1.3	1.0	
Artesia	0.0	0.0	0.0	0.0	0.7	0.3	0.0	0.3	
Lirceolus (2sp.)	1.0	6.0	2.0	3.0	9.9	20.3	8.3	12.8	
Cirolanides	1.0	0.0	2.0	1.0	0.3	0.7	1.0	0.7	
Comaldessus	0.0	0.0	0.0	0.0	0.3	0.7	0.3	0.4	
Stygoparnus	1.0	0.0	0.0	0.3	0.3	0.7	0.0	0.3	
Heterelmis	0.0	0.0	0.0	0.0	1.0	1.3	1.3	1.2	
All Troglobitic & endangered fauna	81.0	82.0	87.0	83.3	70.7	124.3	101.0	98.5	

Table 12. Drift density of each taxa identified in 2003 drift net sampling by sample location and date. Two additional values are presented (bold numbers): the mean drift rate for each taxa during all 2003 samples and the mean drift rate of all organisms captured by date.

		Spring Run 1				Spring Run 3				
	June	Sept	Nov	Mean	June	Sept	Nov	Mean		
Stygobromus pecki	0.04	0.04	0.01	0.03	0.11	0.30	0.27	0.20		
Stygobromus russelli	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00		
Stygobromus sp.	0.47	1.27	0.58	0.71	0.21	2.00	1.34	0.97		
All Stygobromus	0.51	1.33	0.59	0.74	0.32	2.30	1.61	1.17		
Mexiweckelia	0.18	0.23	0.01	0.11	0.01	0.12	0.13	0.07		
Seborgia	0.00	0.00	0.01	0.00	0.01	0.08	0.02	0.03		
Artesia	0.03	0.02	0.00	0.01	0.00	0.00	0.00	0.00		
Lirceolus (2sp.)	0.38	0.73	0.14	0.35	0.01	0.34	0.13	0.12		
Cirolanides	0.00	0.02	0.00	0.00	0.00	0.02	0.02	0.01		
Comaldessus	0.00	0.00	0.01	0.00	0.01	0.04	0.00	0.01		
Stygoparnus	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.00		
Heterelmis	0.01	0.12	0.03	0.04	0.00	0.02	0.00	0.00		
All Troglobitic & endangered fauna	1.33	2.71	0.88	1.44	0.36	3.00	1.94	1.45		

	<u>Upwelling - West Shoreline</u>				All Sites Combined				
	June	Sept	Nov	Mean	June	Sept	Nov	Mean	
Stygobromus pecki	0.35	0.33	0.07	0.25	0.16	0.22	0.12	0.15	
Stygobromus russelli	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	
Stygobromus sp.	0.70	1.23	1.13	0.99	0.44	1.51	1.13	0.87	
All Stygobromus	1.05	1.56	1.21	1.24	0.60	1.74	1.25	1.02	
Mexiweckelia	0.00	0.00	0.00	0.00	0.06	0.12	0.05	0.06	
Seborgia	0.00	0.00	0.03	0.01	0.00	0.03	0.02	0.01	
Artesia	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	
Lirceolus (2sp.)	0.01	0.12	0.03	0.05	0.12	0.41	0.13	0.18	
Cirolanides	0.01	0.00	0.03	0.02	0.00	0.01	0.02	0.01	
Comaldessus	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	
Stygoparnus	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	
Heterelmis	0.00	0.00	0.00	0.00	0.00	0.05	0.02	0.02	
All Troglobitic & endangered fauna	1.09	1.68	1.30	1.32	0.87	2.50	1.54	1.39	

Comal Springs Riffle Beetle

There was a moderate amount of variability in the presence/absence of the Comal Springs riffle beetles in individual quadrat (0.36 m²) samples in 2003, but there were always at least 5 individuals captured in the four quadrat samples combined from each sample site. A maximum of 43 beetles was observed in one quadrat in the Spring Island area during the January 2003 sample. Most of the beetles (32) in that sample were found on a small stick (1.5 ft long, 0.2 ft wide) that had settled on top of the spring upwelling. The stick was not in the same spot in the following sample and substantially fewer individuals were observed there. In another location a smaller stick also had a few beetles on it. In the Spring Run 3 and western shoreline sample locations, individuals were most often found among leaves at the water's edge, particularly where a seep flowed over rocks and provided an area where leaves accumulated.

The total number of individuals observed in all four quadrats was divided by the cumulative area of the quadrats (1.44 m²) to calculate density for comparison between sample locations and among dates (Table 13). The population along the western shoreline is typically small since the area sampled is not much bigger than the total area sampled with the quadrats. This population appears isolated since sampling in upstream and downstream locations along the shoreline has not yielded any other individuals. The Spring Run 3 area has a greater diversity of habitat and high numbers were frequently found in samples there. The upwelling areas near Spring Island have provided a wide range of observations. It appears that individuals congregate in particular habitat conditions (e.g., the stick) and when these habitats are found, high numbers are observed. Because of this tendency for individuals to congregate in favorable habitats, we intend to modify the sampling strategy in 2004 to maximize efficiency. A preliminary effort has revealed that placing small pieces of cotton fabric (e.g., from a handkerchief) will attract Comal Springs riffle beetles if left in place for an extended time (3 weeks). By using the same size piece of fabric and leaving it in place for the same period of time, this design will provide a means of accurately comparing across samples and also provide a more accurate measure of the current population using each sample site during the 2004 quarterly samples.

Table 13. Number of Comal Springs riffle beetles observed per m^2 in each sample location. This number was calculated by dividing the total number of observations in all 4 quadrat samples by the cumulative area (1.44 m^2).

SAMPLE DATE	Spring Island	Spring Run 3	Western Shoreline
January 03	32.6	45.1	4.9
March 03	6.9	22.2	3.5
September 03	29.2	6.9	10.4
November 03	12.5	11.1	6.3
Average	20.3	21.4	6.3

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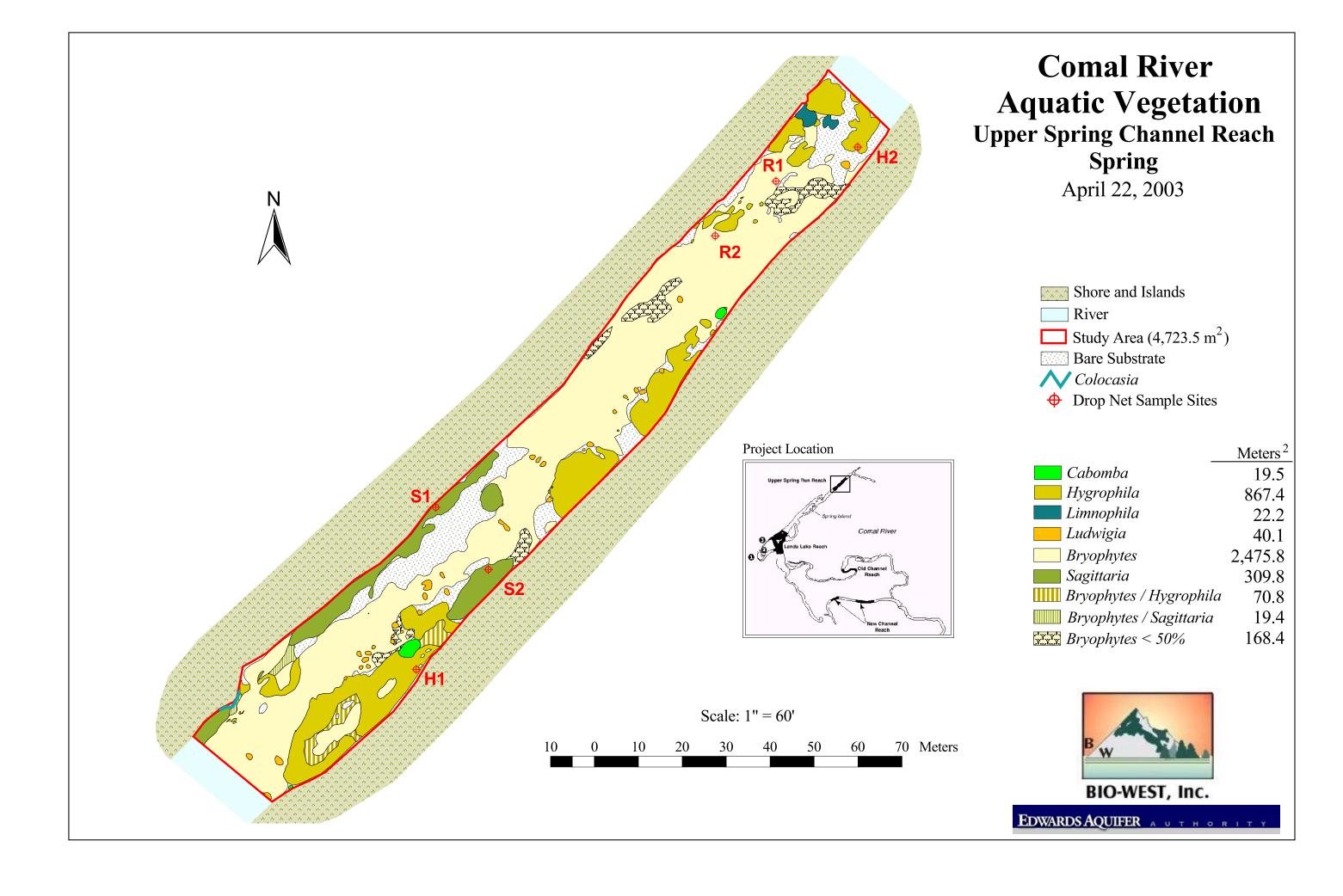
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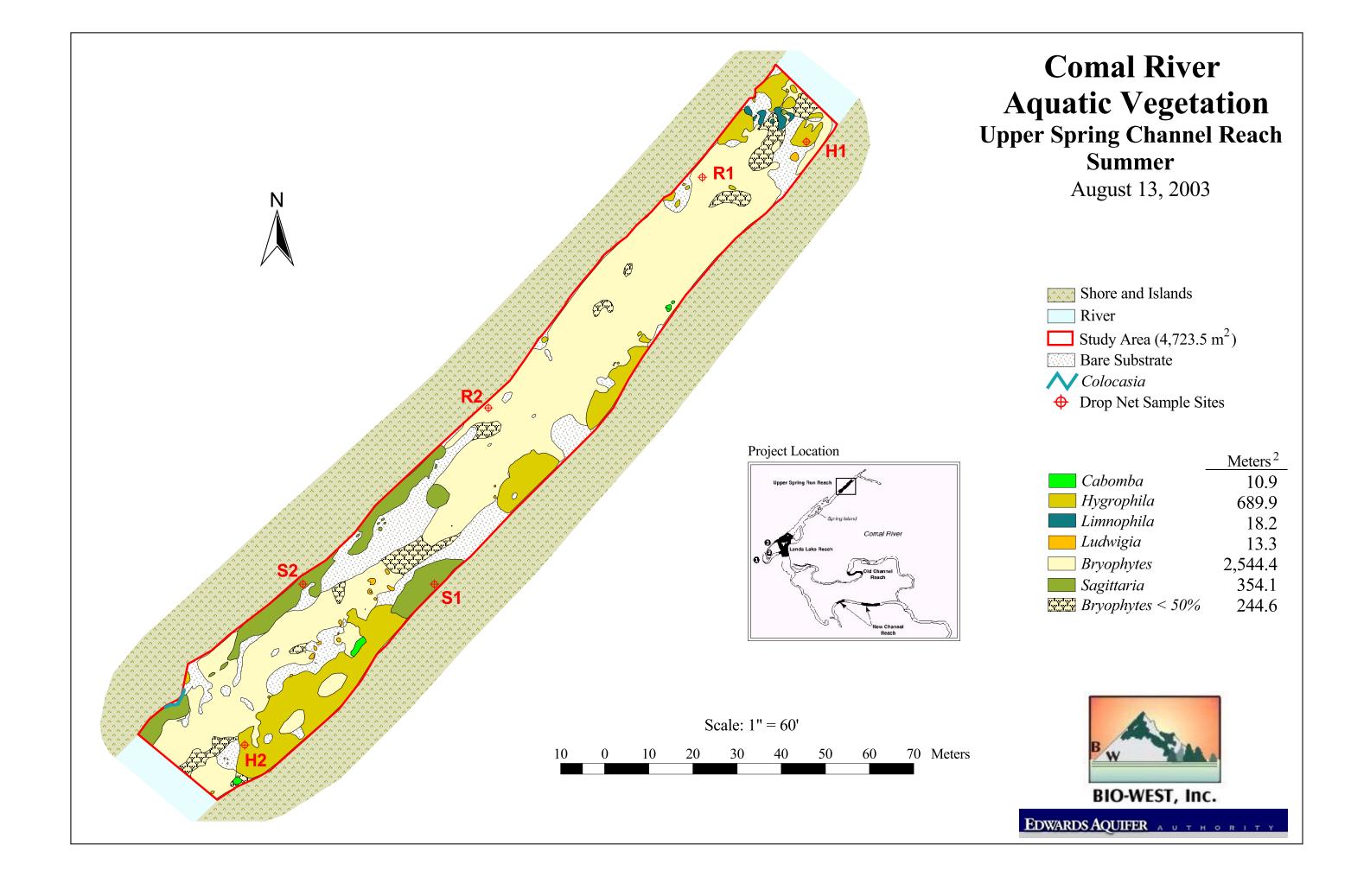
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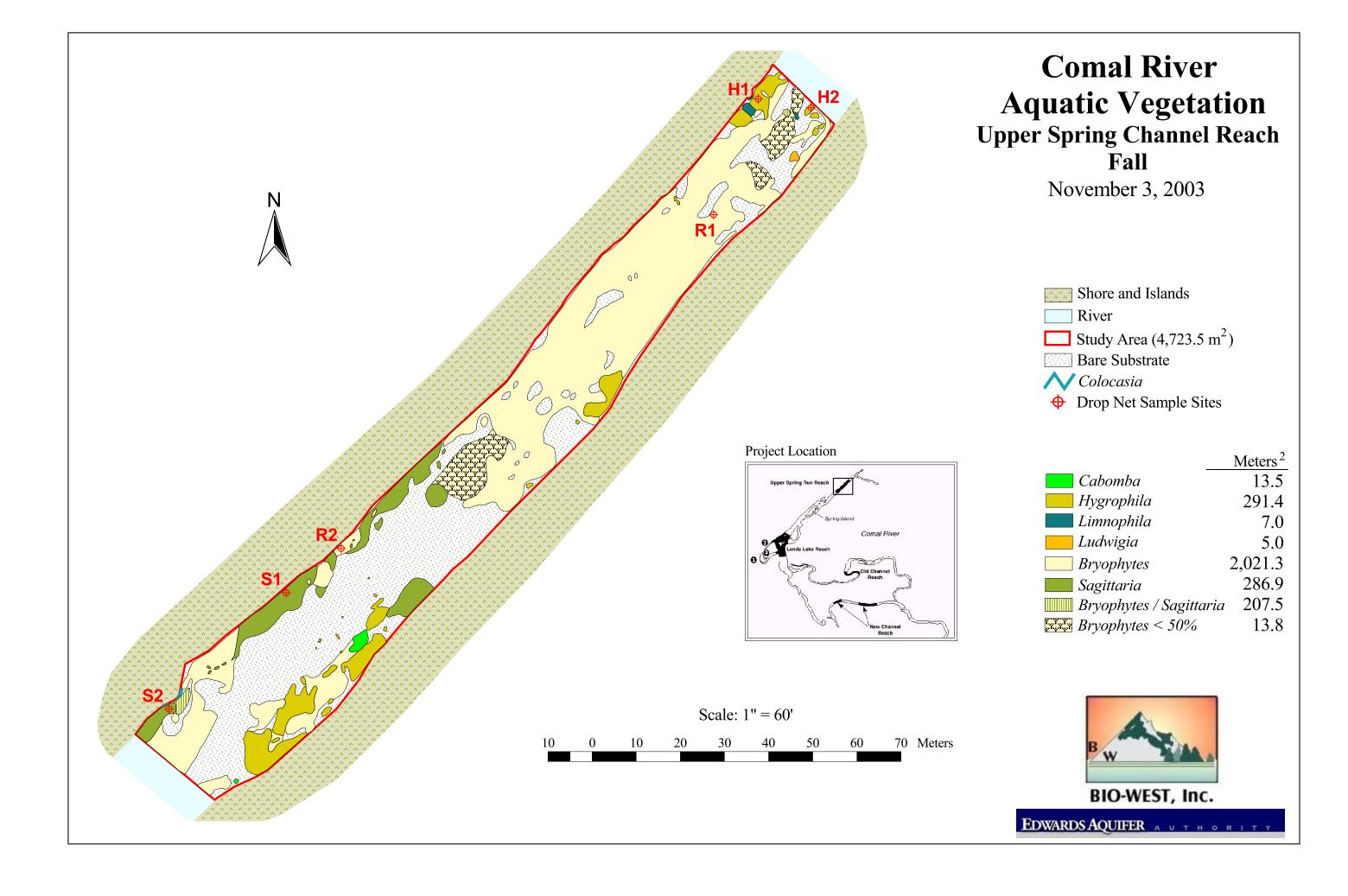
APPENDIX A: AQUATIC VEGETATION MAPS

Upper Spring Run Reach

Spring 2003 Summer 2003 Fall 2003

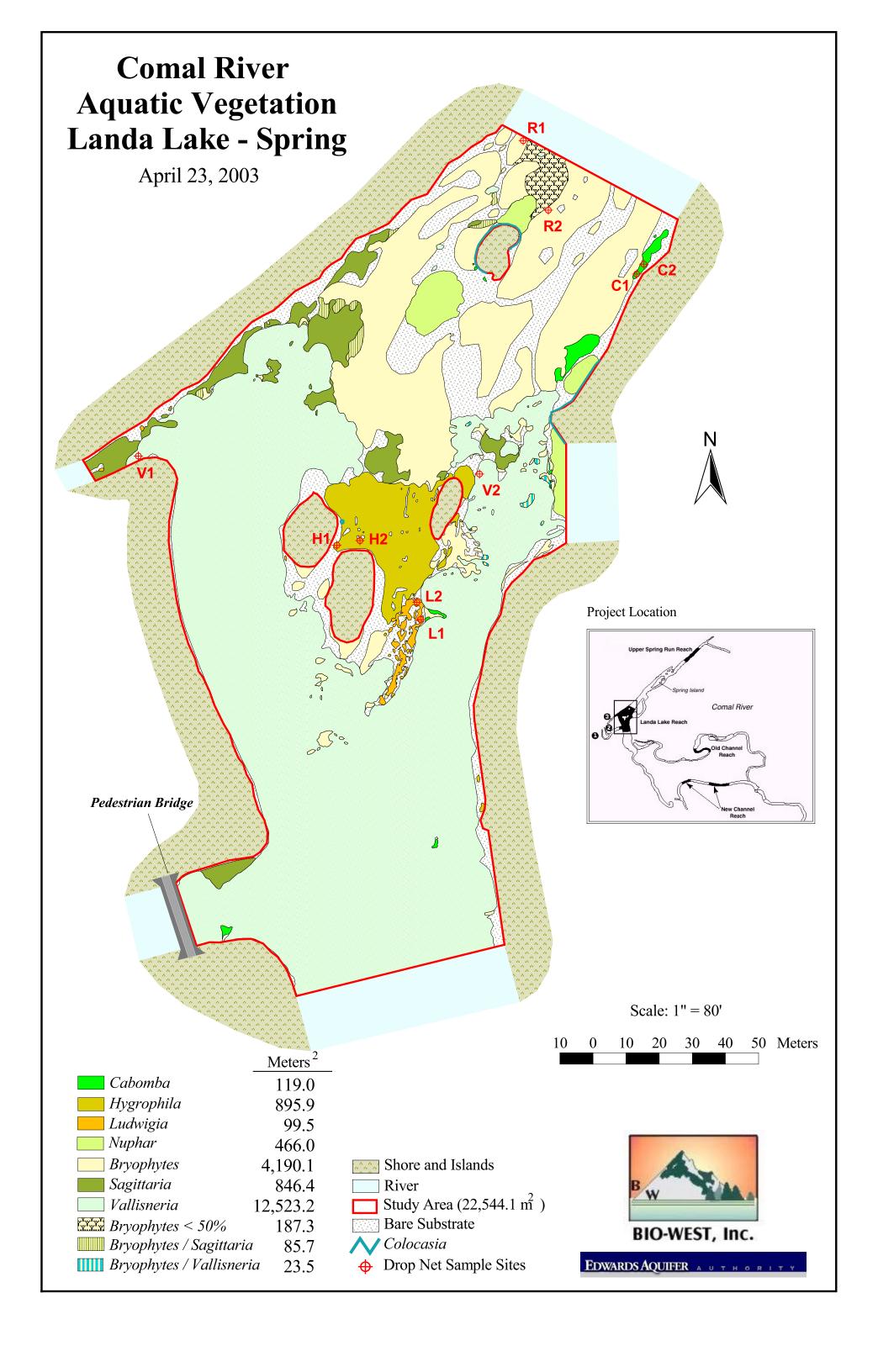


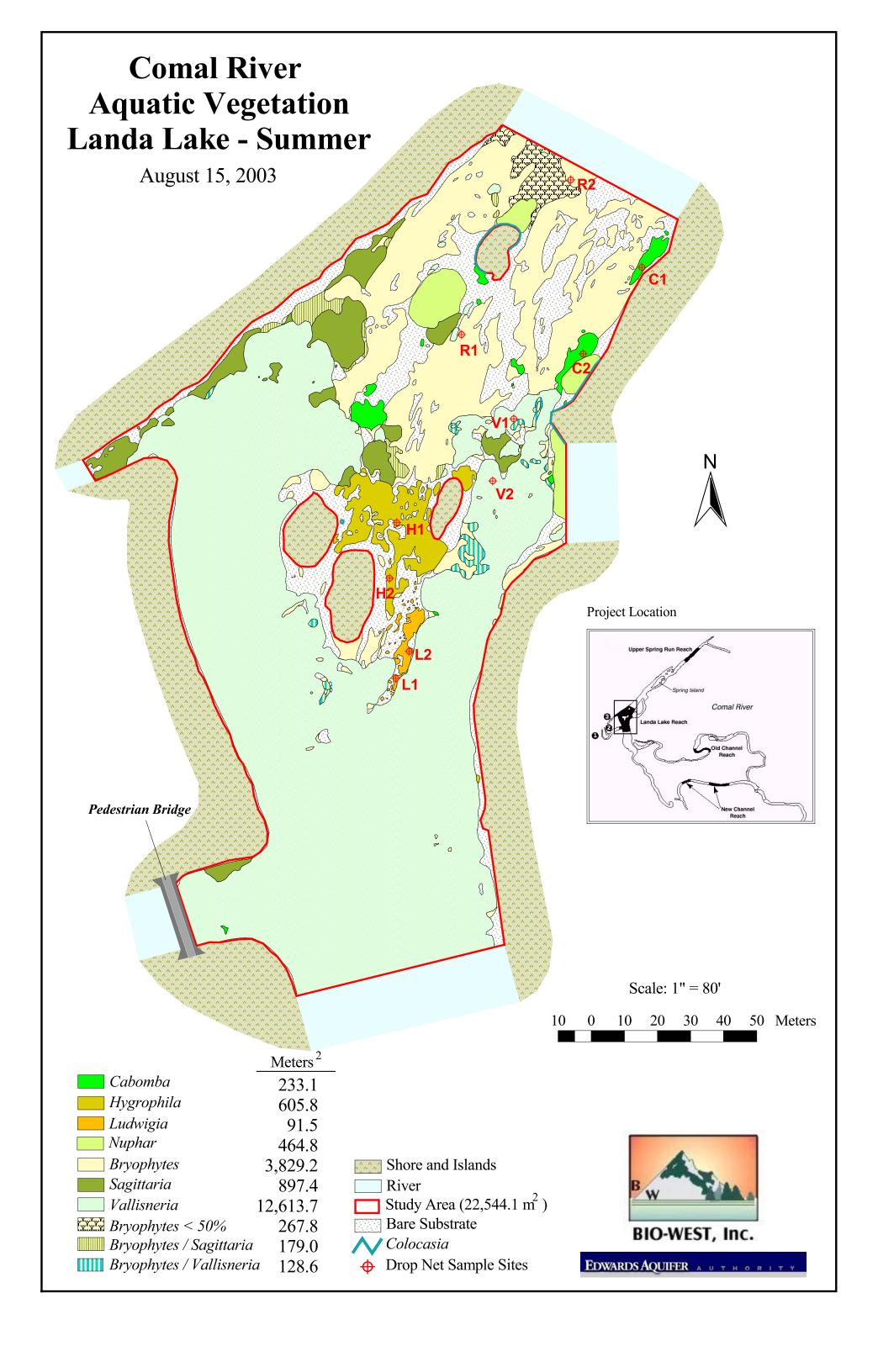


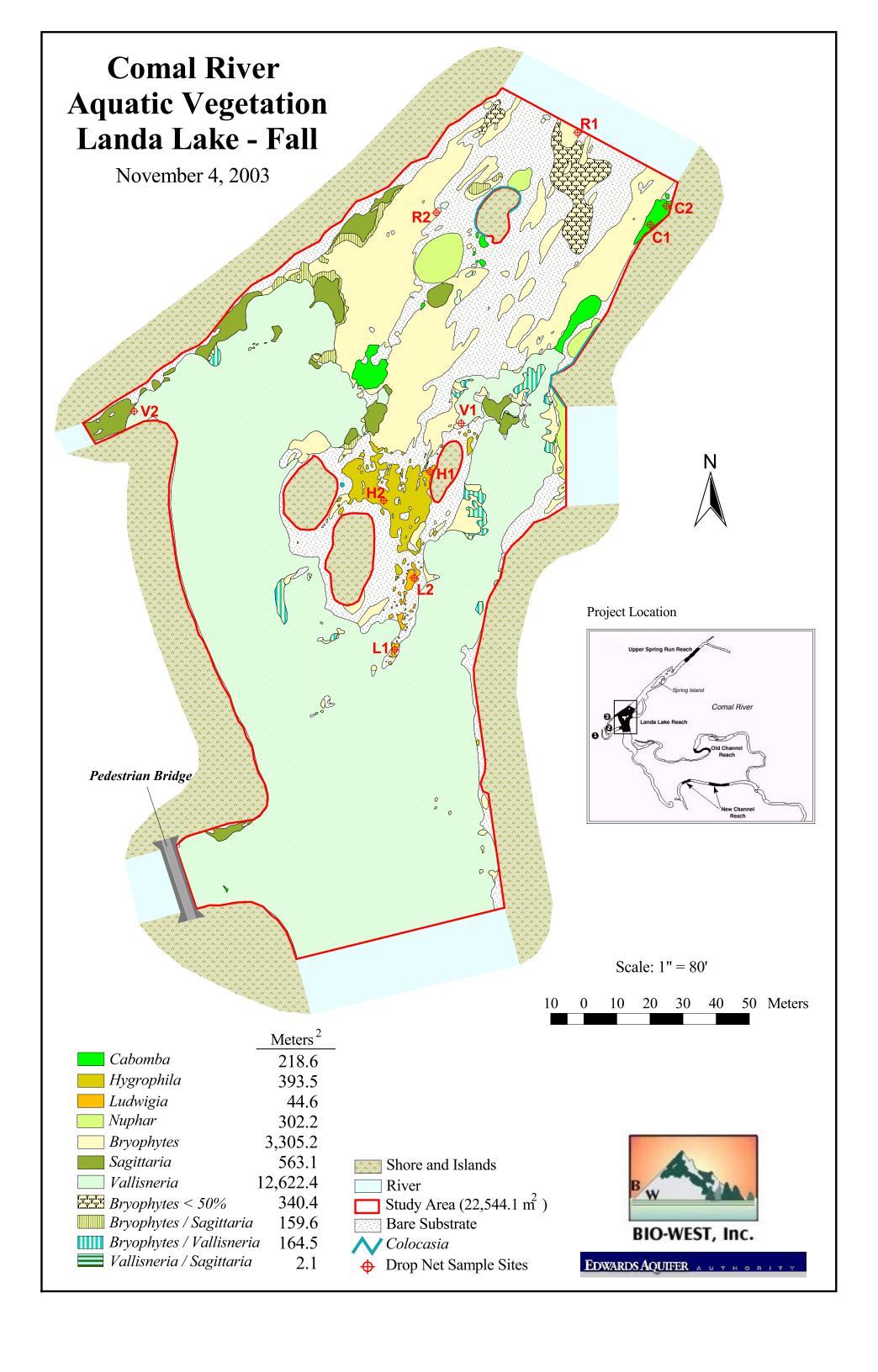


Landa Lake Reach

Spring 2003 Summer 2003 Fall 2003





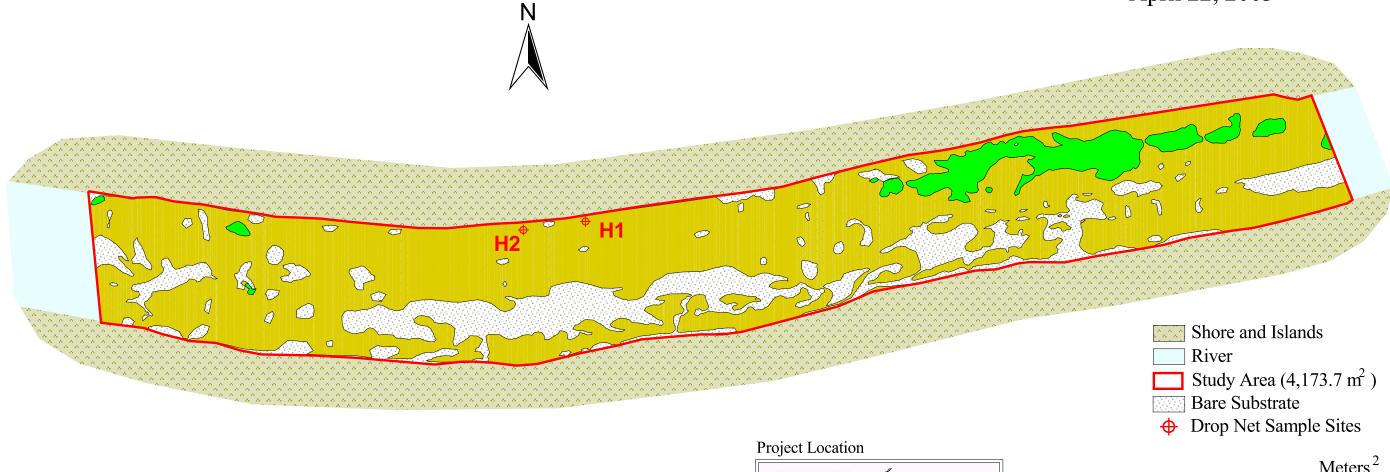


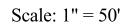
New Channel Reach

Spring 2003 Summer 2003 Fall 2003

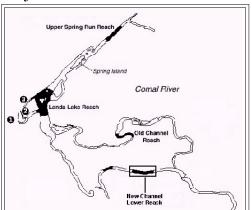
Comal River Aquatic Vegetation New Channel Lower Reach Spring

April 22, 2003





10	0	10	20	30	40	50	60	70 Meters



	Meters ²
Cabomba	247.5
Hygrophila	3,011.5

0.4



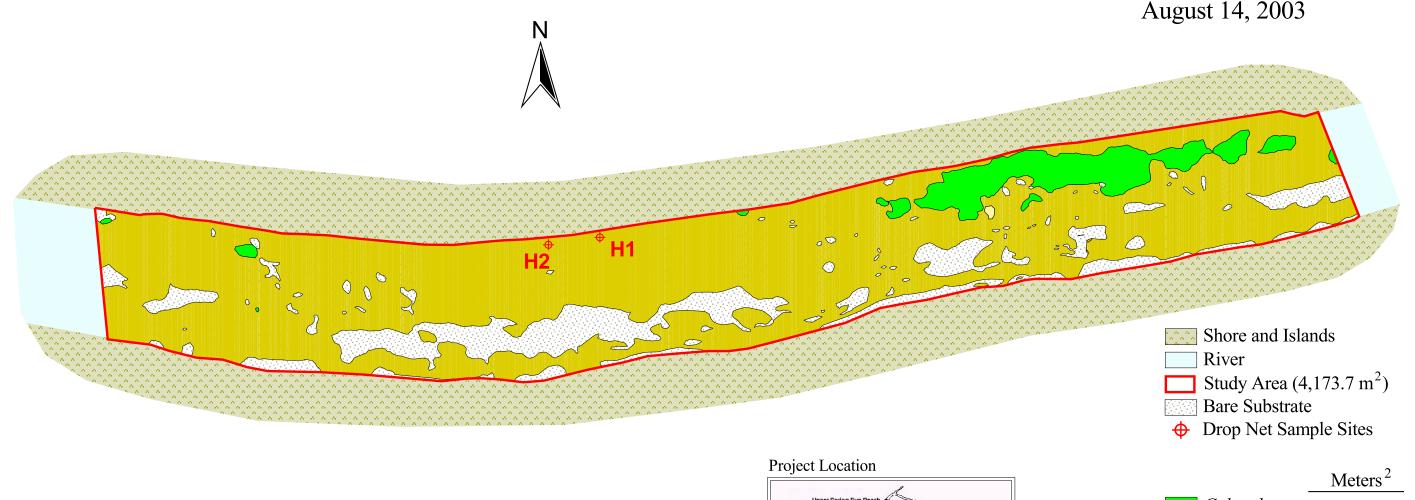
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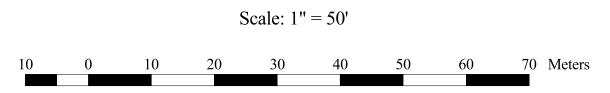
BIO-WEST, Inc.

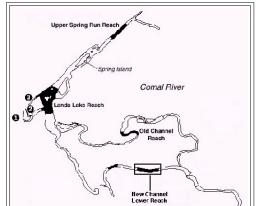
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Comal River Aquatic Vegetation New Channel Lower Reach Summer

August 14, 2003







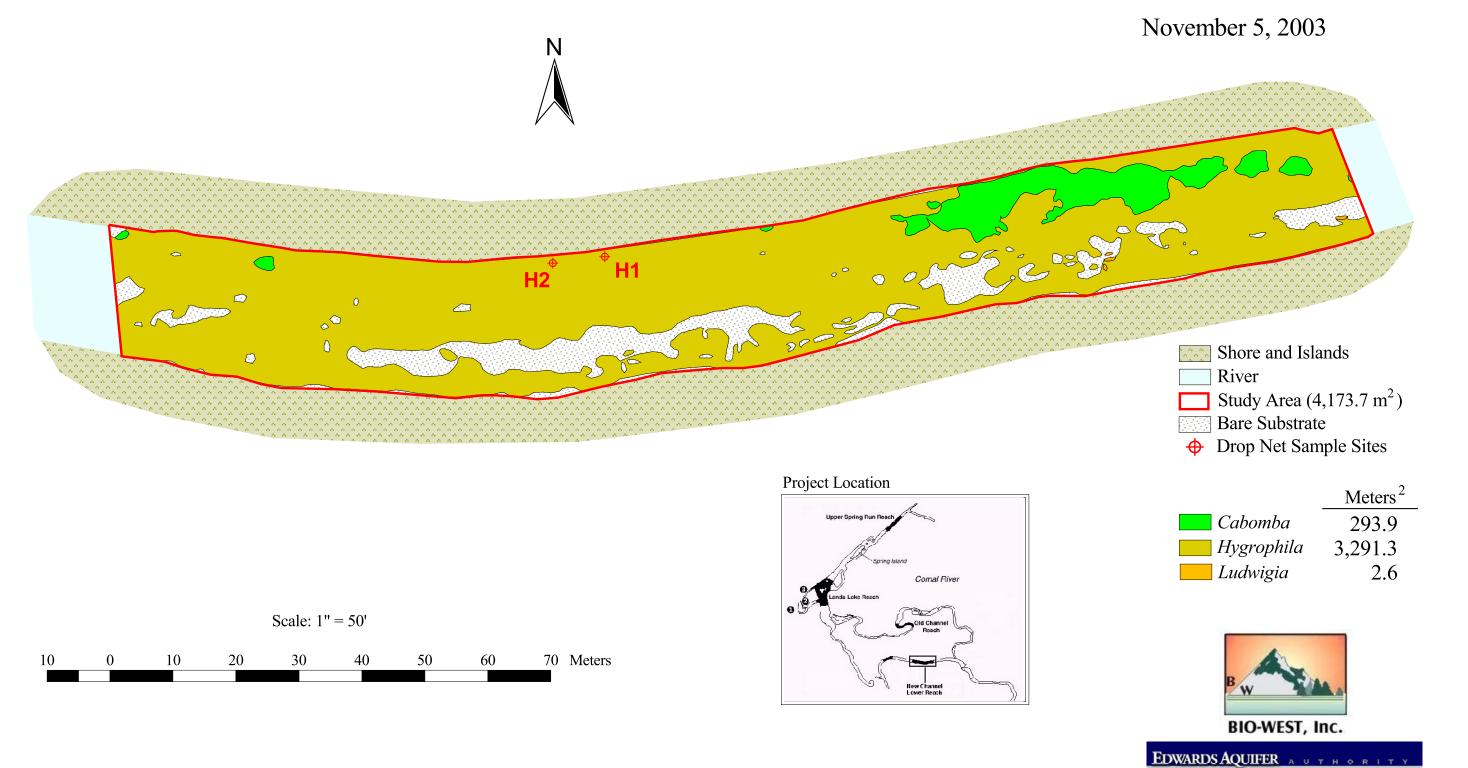
	Meters -
Cabomba	281.2
Hygrophila	3,228.8
Ludwigia	0.3
Bryophytes	2.4



BIO-WEST, Inc.

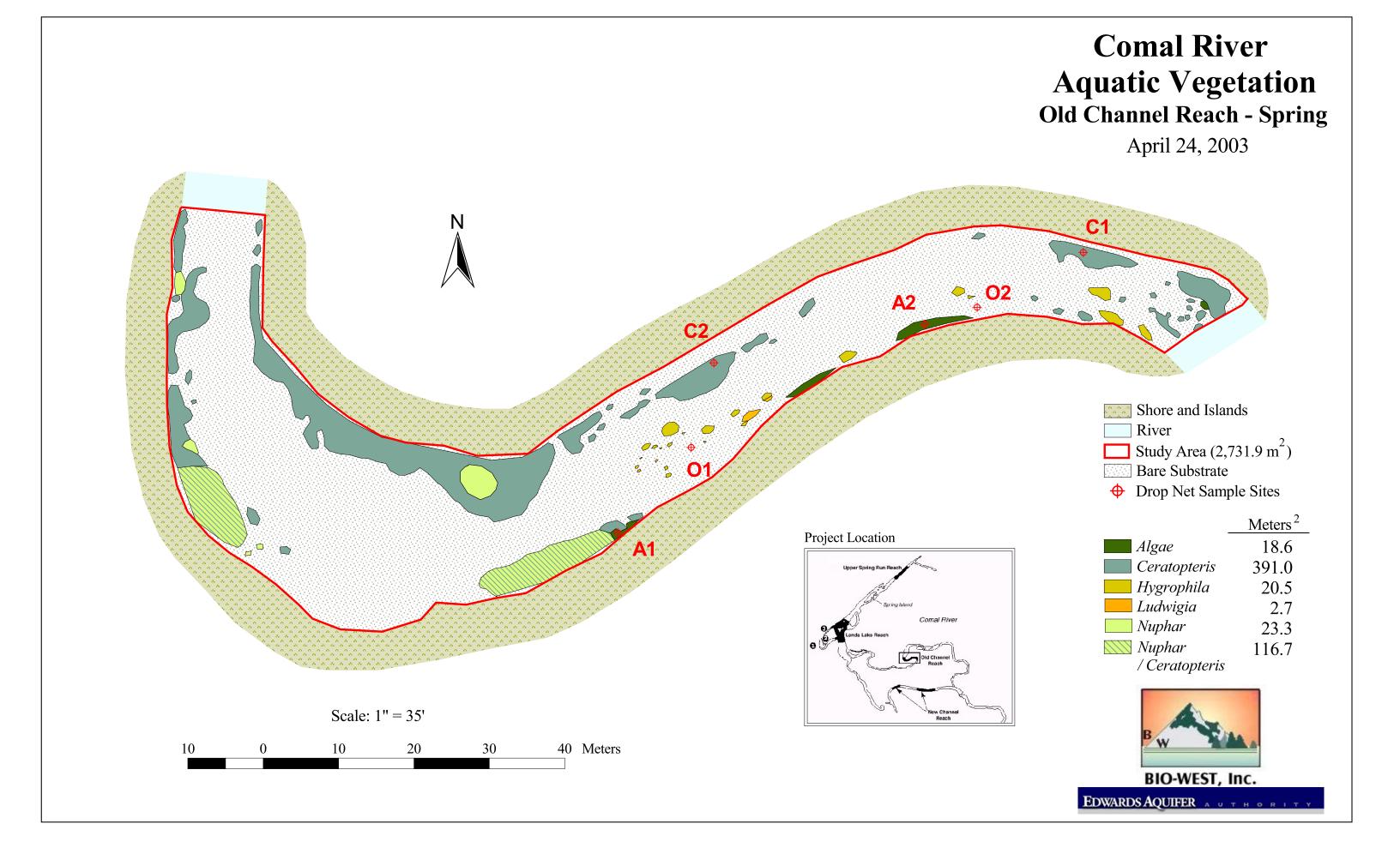
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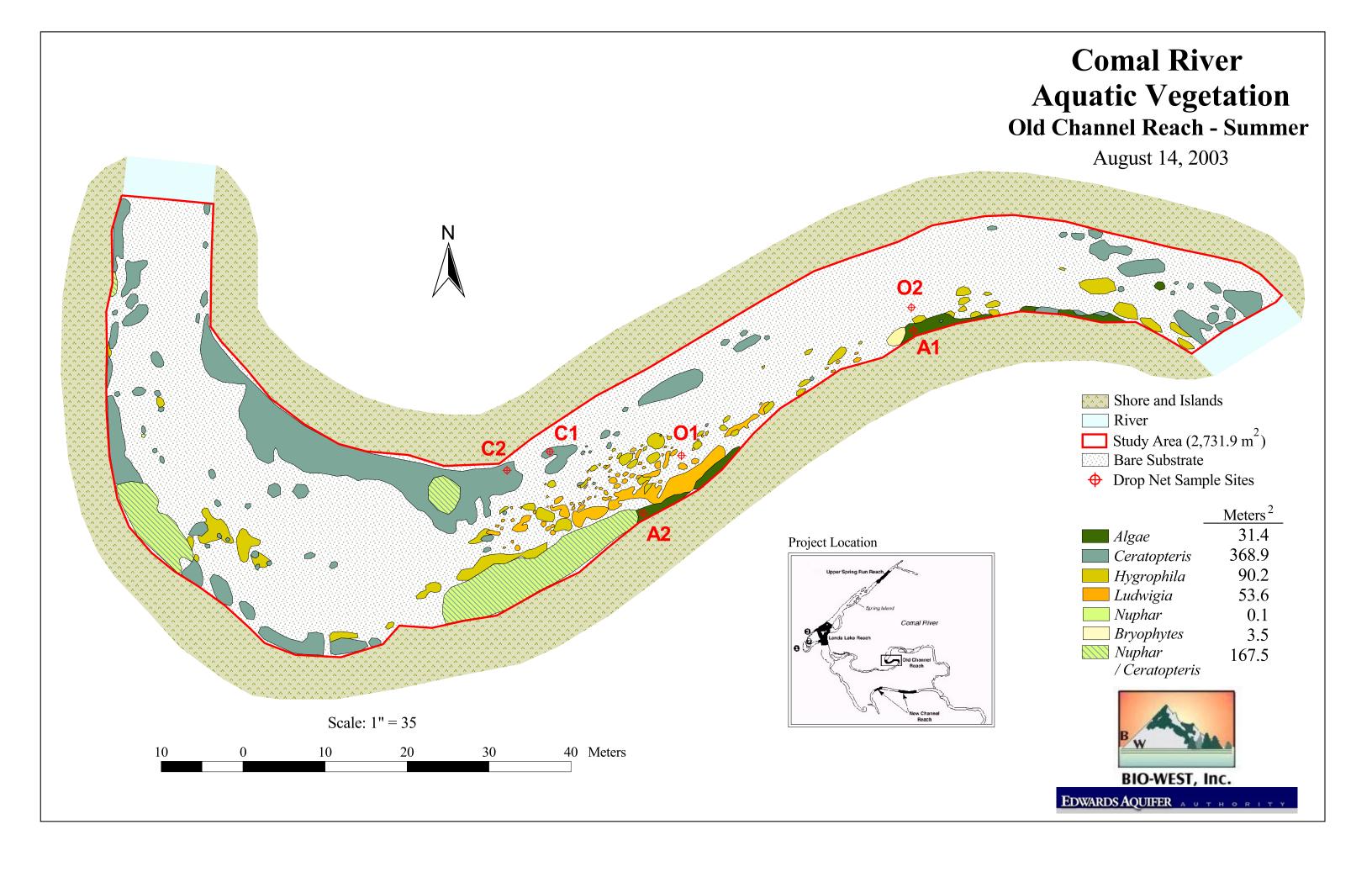
Comal River Aquatic Vegetation New Channel Lower Reach Fall

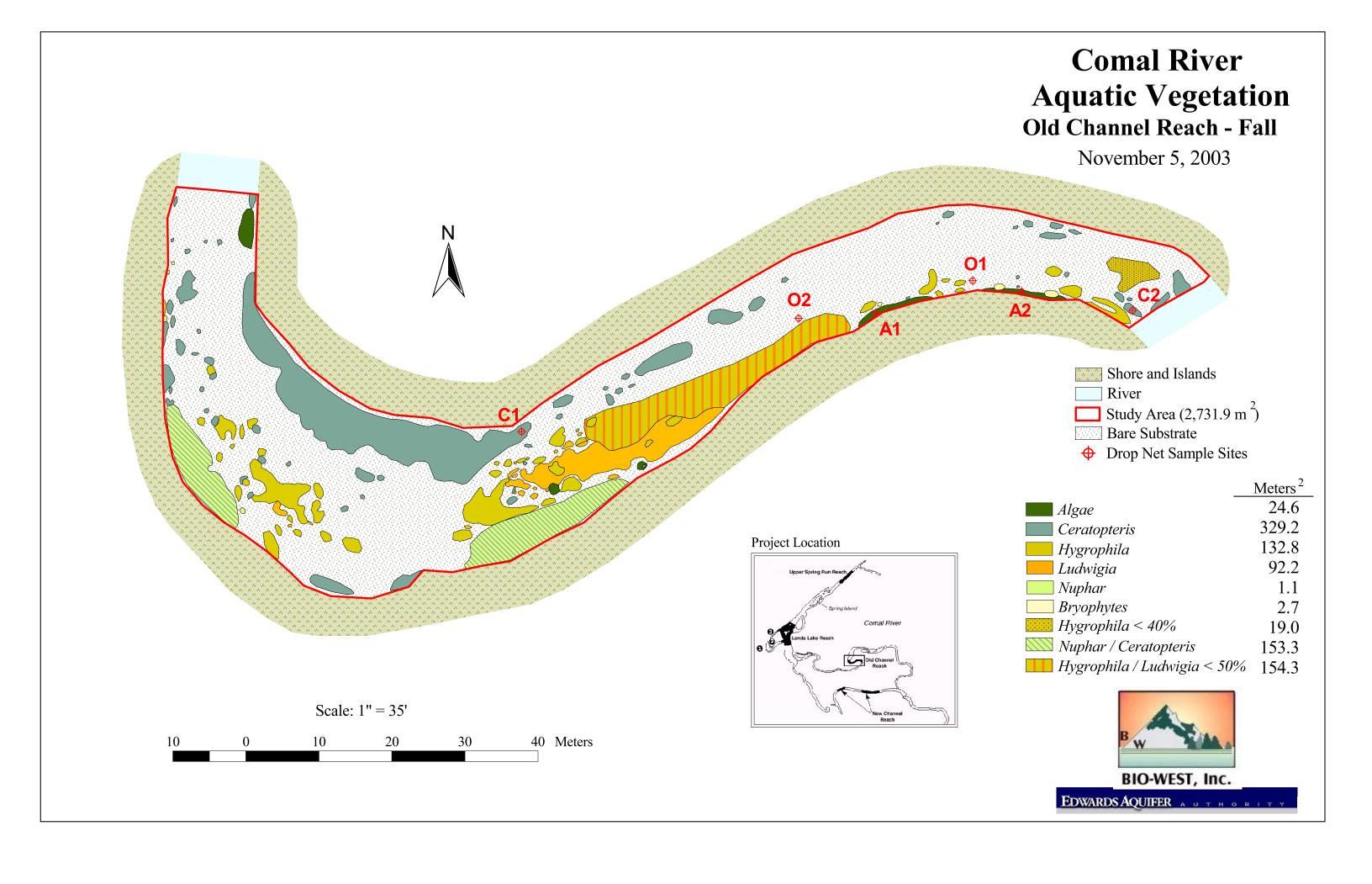


Old Channel Reach

<u>Spring 2003</u> <u>Summer 2003</u> <u>Fall 2003</u>



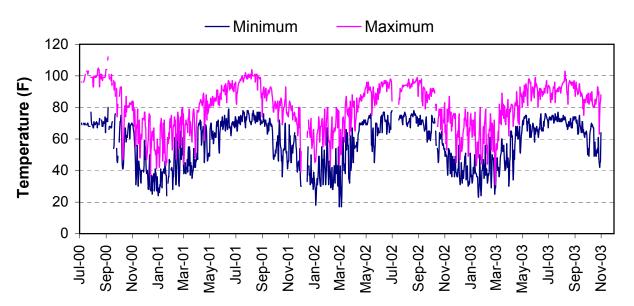




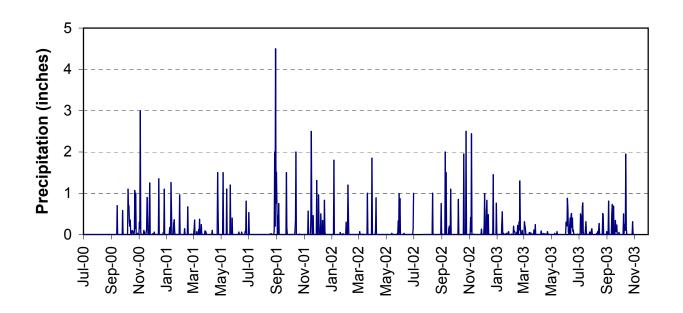
APPENDIX B: DATA AND GRAPHS

Water Quality Data and Thermistor Graphs

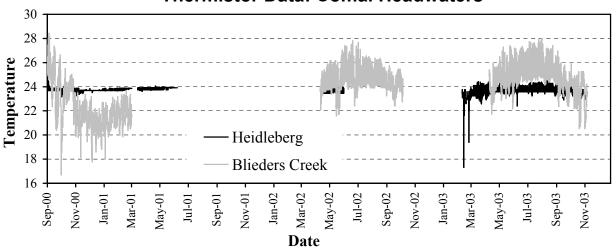
Daily Air Temperature Data for New Braunfels, Texas



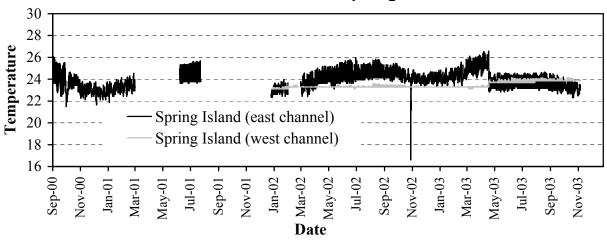
Daily Precipitation Data for New Braunfels, Texas



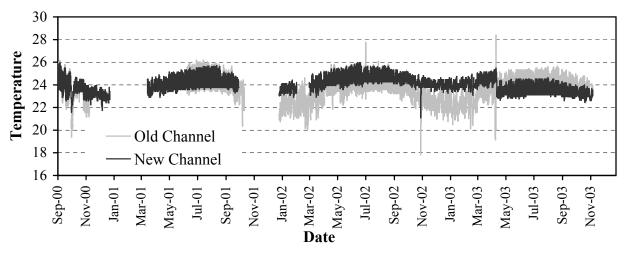
Thermistor Data: Comal Headwaters



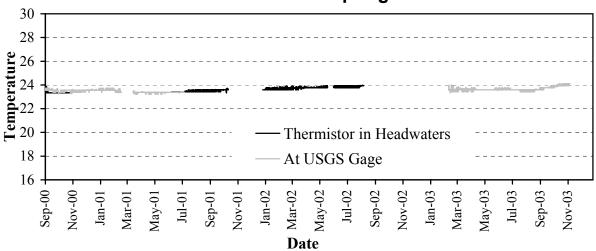
Thermistor Data: Spring Island



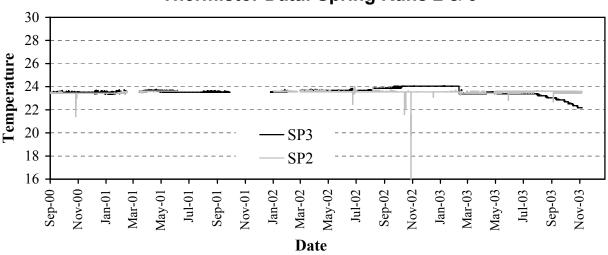
Thermistor Data: Old & New Channels



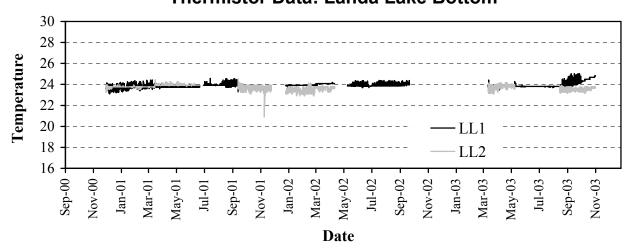
Thermistor Data: Spring Run 1



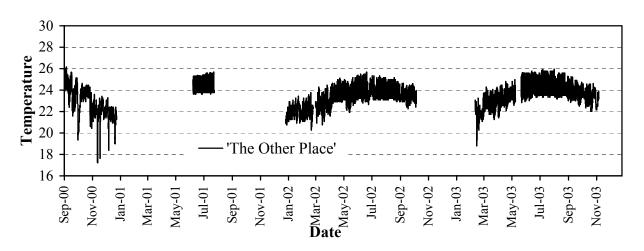
Thermistor Data: Spring Runs 2 & 3

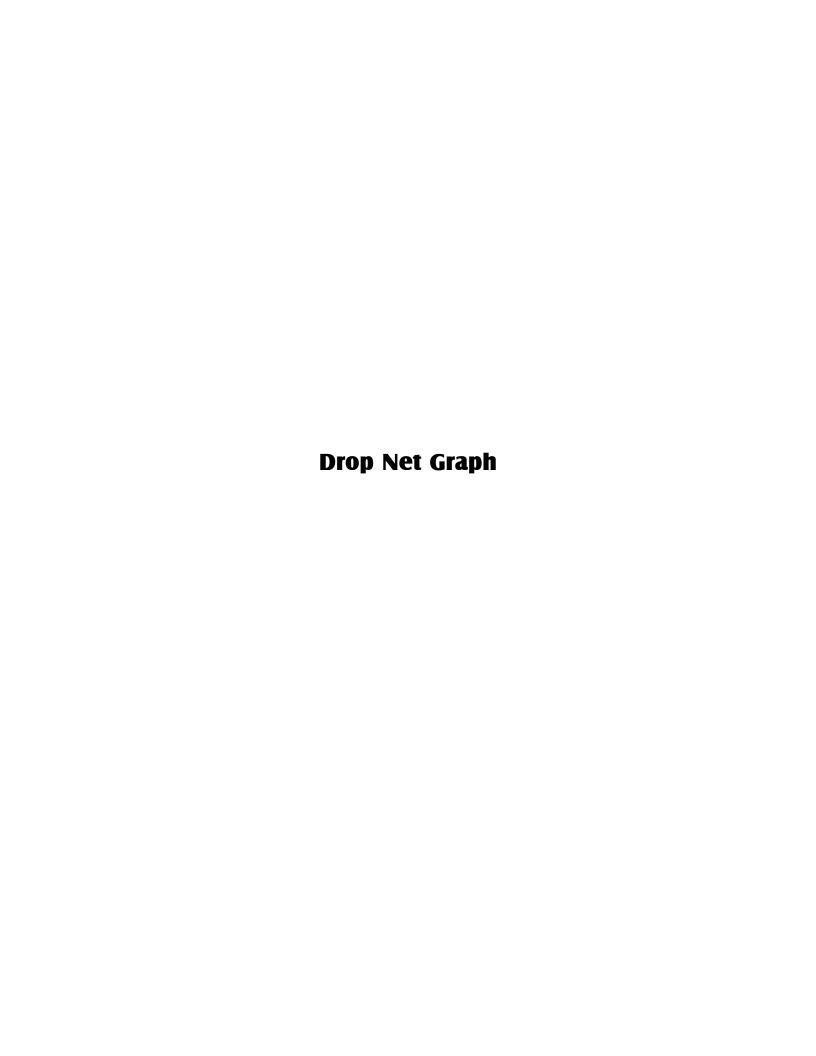


Thermistor Data: Landa Lake Bottom

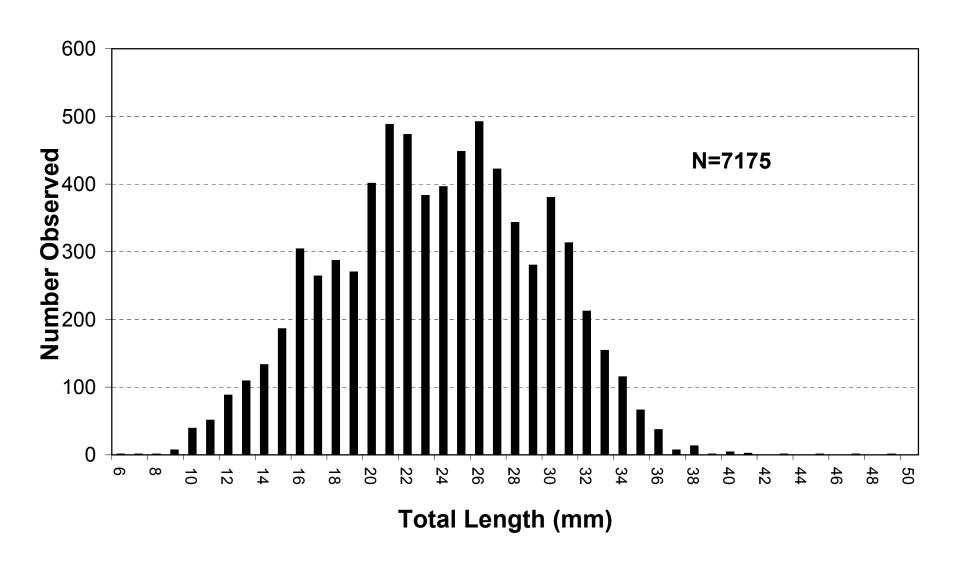


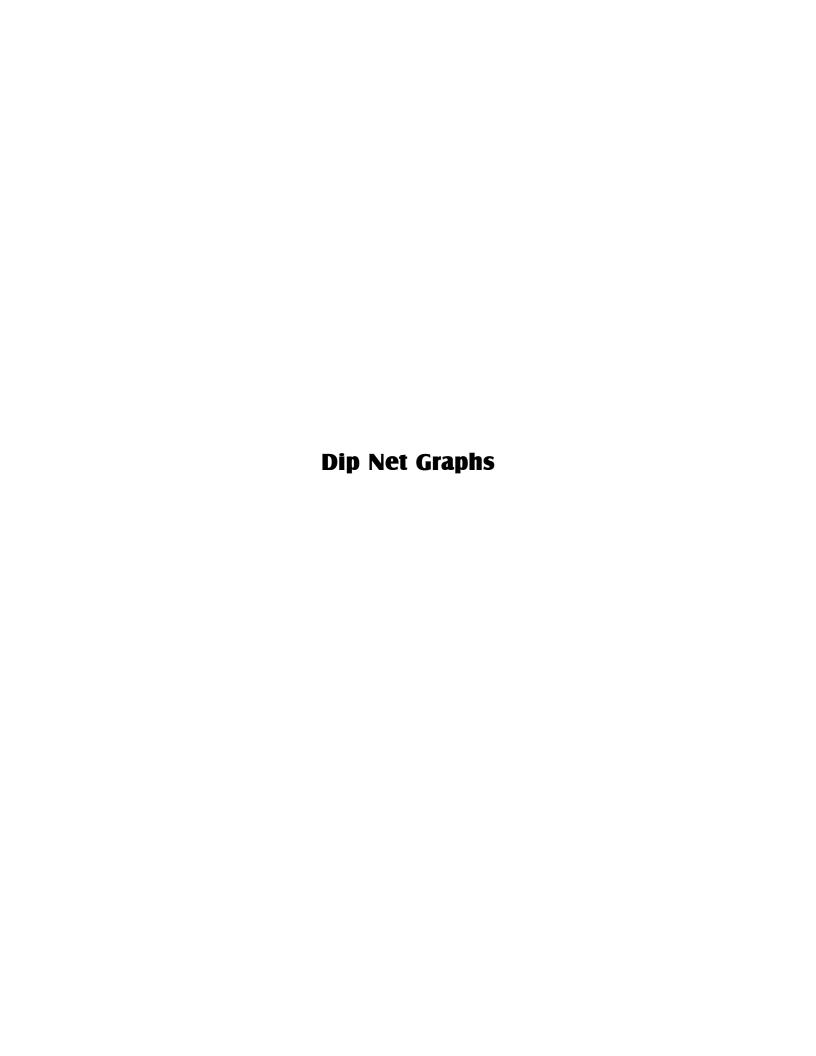
Thermistor Data: Other Place



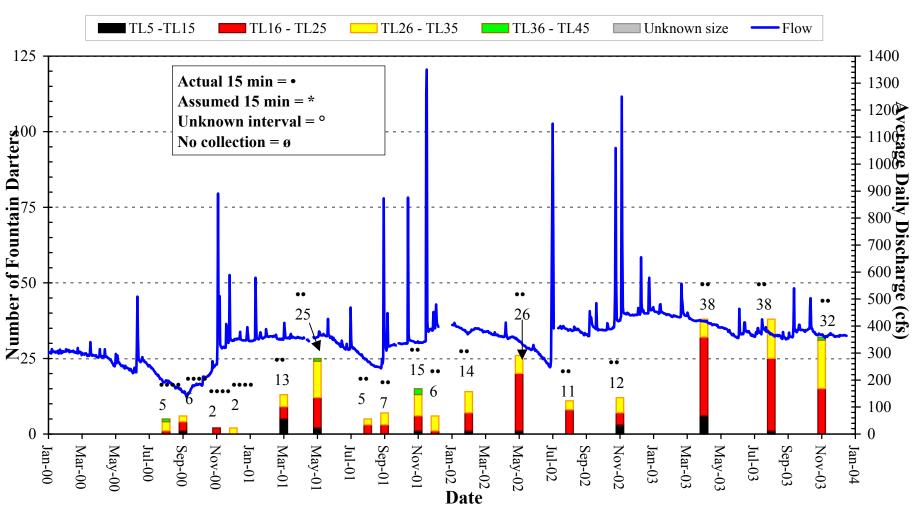


Drop Net Results 2000-2003 in the Comal River

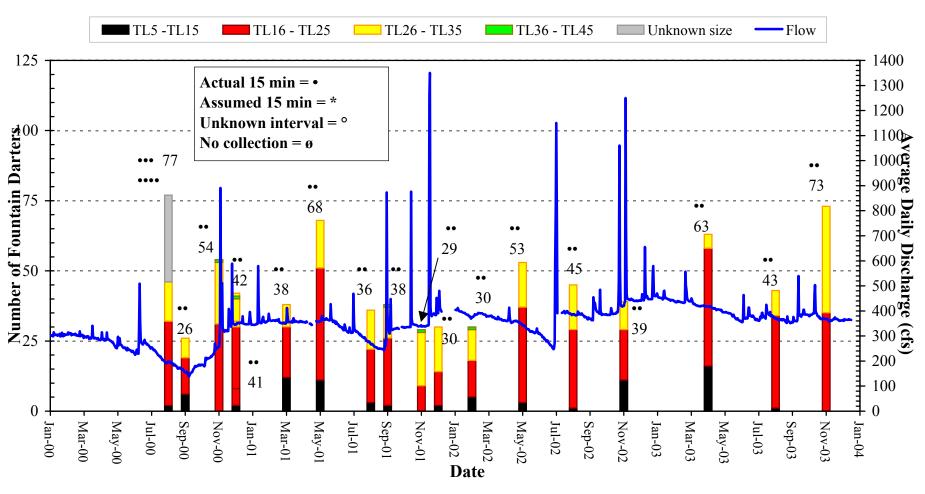




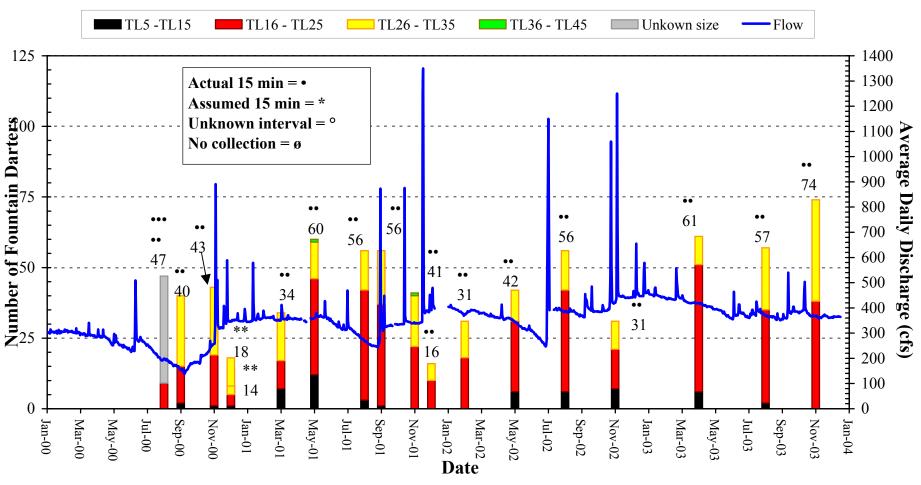
Fountain Darters Collected from the Upper Spring Run Reach (Section 3) Dip Net Results - Comal River



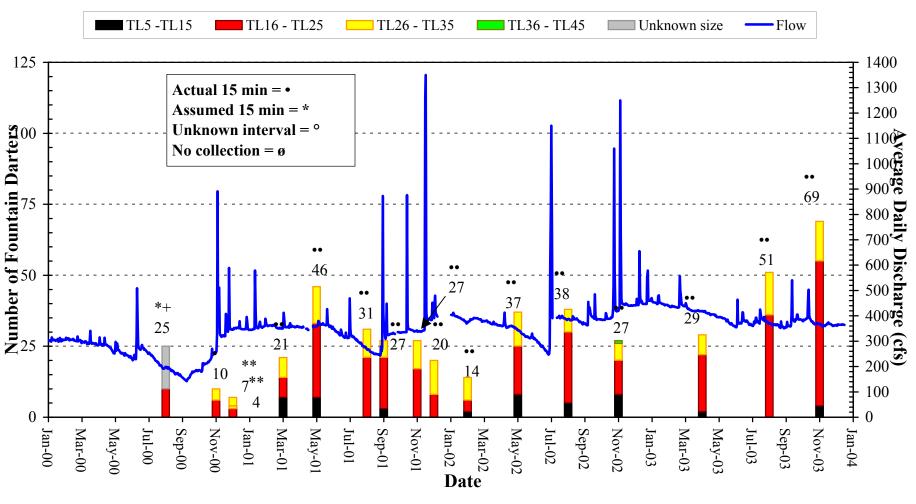
Fountain Darters Collected from the Spring Island Area (Section 4U-M) Dip Net Results - Comal River



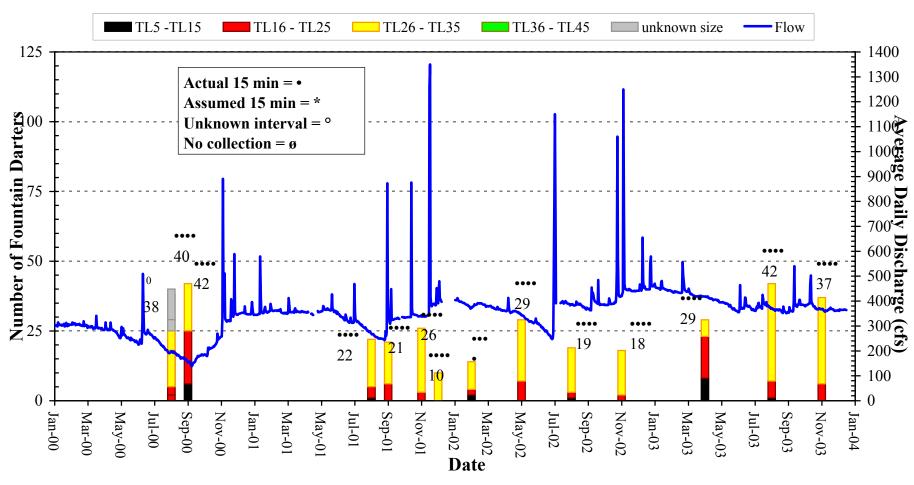
Fountain Darters Collected from the Landa Lake Reach (Section 4L) Dip Net Results - Comal River



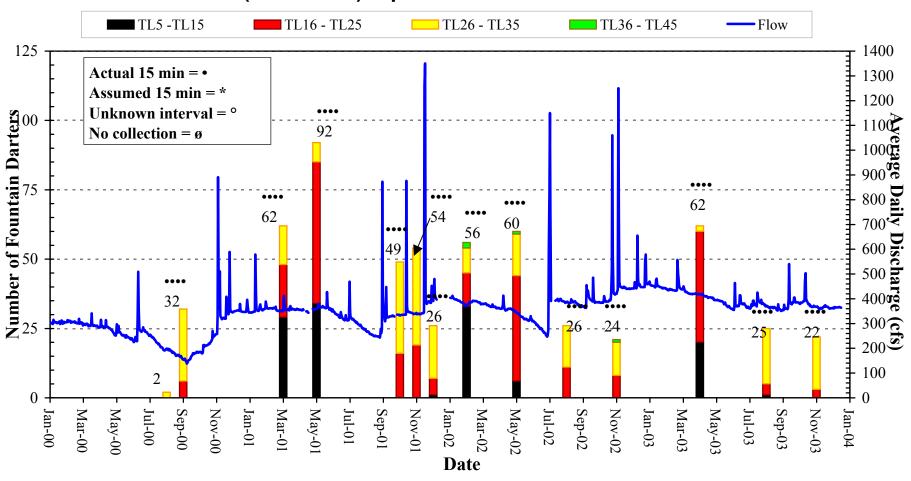
Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River



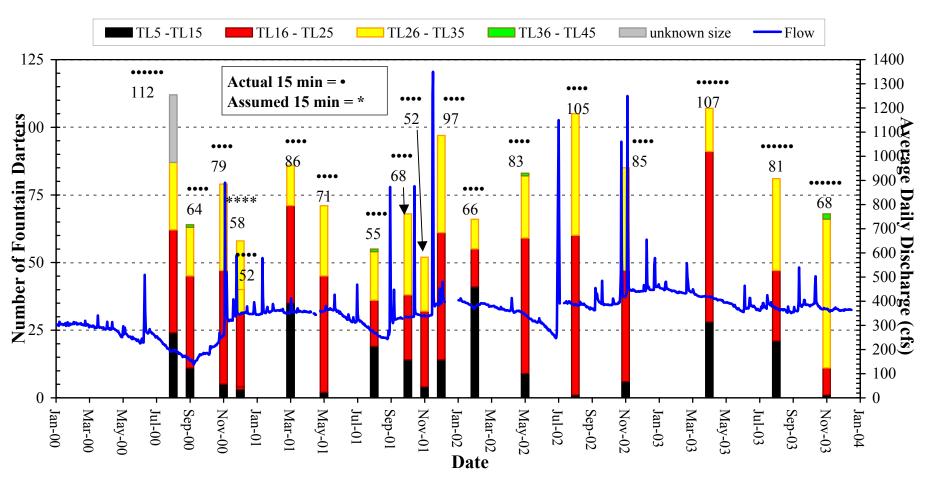
Fountain Darters Collected from the New Channel Reach (Section 10L-U) Dip Net Results - Comal River



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



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



APPENDIX C: DROP NET RAW DATA

(not available online)