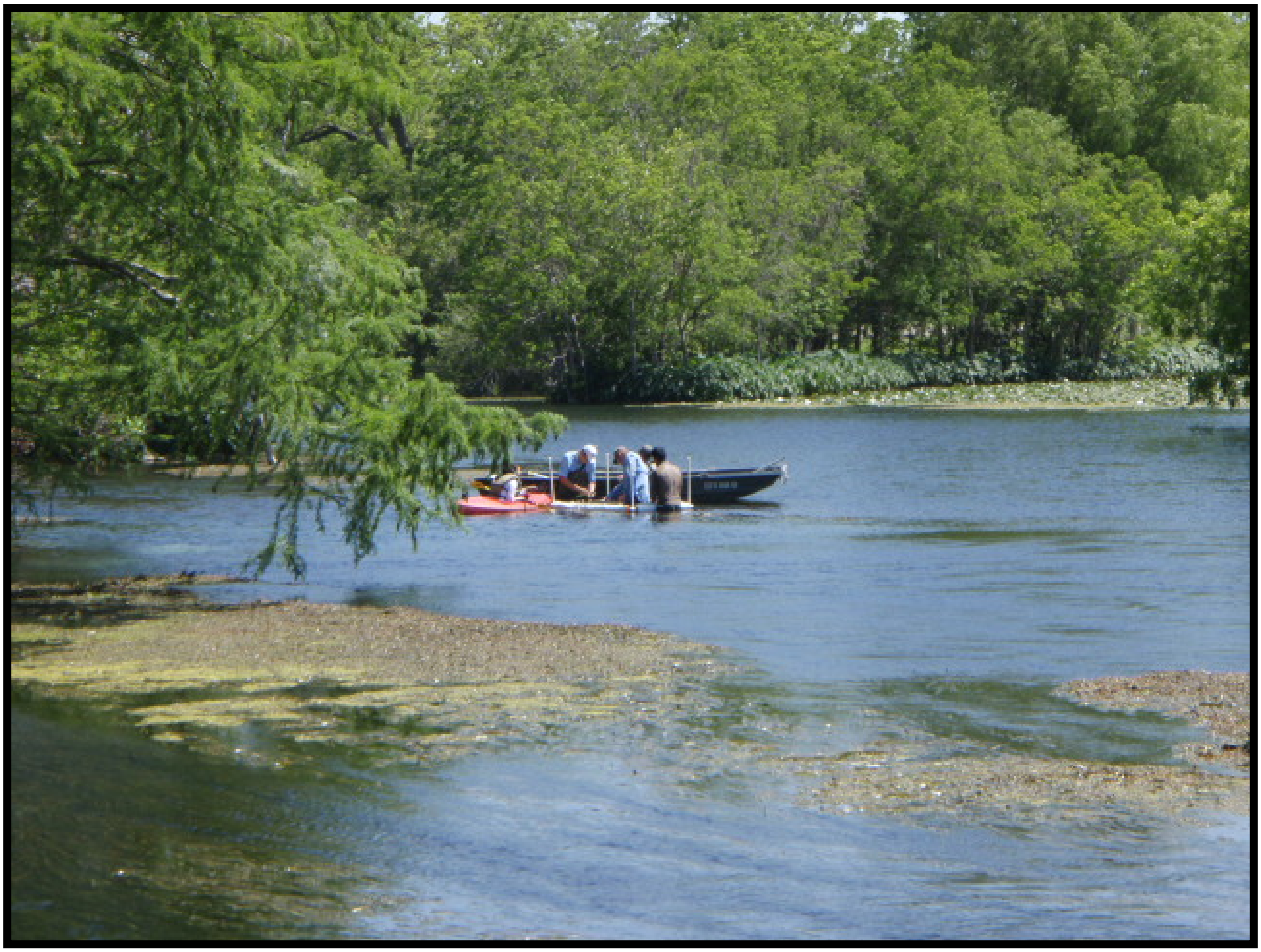
Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem

FINAL 2008 ANNUAL REPORT



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EXECUTIVE SUMMARY

This annual summary report presents a synopsis of methodology used and an account of sampling activities, including sample conditions, locations and raw data obtained during two sampling events (Comprehensive Monitoring Effort) conducted on the Comal Springs/River ecosystem in 2008. For ease of comparison, the data are reported here in an annual report format similar to previous reports (BIO-WEST 2001-2008).

Discharge in the Comal River was lower in 2008 compared to 2007, but no Critical Period events were triggered. The highest discharge occurred on the first day of 2008 (412 cubic feet per second [cfs]), while the lowest (260cfs) was recorded in June. Even with the extreme drought in central Texas throughout the majority of 2008, flows ended the year just slightly below the historical average. Spring inputs remained relatively constant throughout the year. Of the three spring runs, Spring Run 3 continued to contribute the most water due to the multitude of springs flowing into it. As in previous years, the highest water temperatures were observed at Blieder's Creek because it does not receive spring contributions that can stabilize temperatures. At the spring orifice, water temperatures at Spring Run 1 varied less than 1 °C for the entire year. The highest temperatures observed within the river itself were near 30 °C at the downstream site in the New Channel. Thermistor data continues to provide good baseline information for air temperature and precipitation effects within the study area, and will yield important information during low-flow events.

As in previous years, non-native aquatic vegetation flourished in some reaches, while native plants grew well in others. Native bryophytes continued to dominate the upstream end of the Upper Spring Reach. In spring, tall columns of the mosses formed blanketing the substrate, but by fall they became fragmented possibly due to an early fall precipitation event. *Vallisneria* was the dominant vegetation in Landa Lake in 2008, where it was mostly found in deeper areas of the lake. Non-native *Hygrophila* dominated the central section of the reach near several islands. Native *Ludwigia* fragmented more in 2008 and only a handful of small plants remain in Landa Lake. This trend has continued for several years and may have an important effect on fountain darter (*Etheostoma fonticola*) populations as sampling has shown that fountain darters prefer *Ludwigia* to *Hygrophila*. Another preferred fountain darter habitat, *Cabomba*, changed little in 2008.

Competition between native and non-native plants also occurs in the Old Channel Reach where *Hygrophila* and *Ludwigia* grow adjacent (and sometimes mix) to each other. *Ludwigia* and *Hygrophila* surface areas both increased slightly in 2008, but *Hygrophila* continued to dominate most of the reach. Filamentous algae covered enough area in the Old Channel Reach in 2008 to justify sampling for fountain darters within it. This is important to note as conditions have not allowed this for several years. Filamentous algae have consistently had the highest density of fountain darters of any vegetation type. This reach will continue to be closely monitored to observe the interaction between native and non-native plants, and the fountain darter populations that depend on them. With few precipitation events, vegetation at the New Channel Reach increased substantially in 2008. This highly channelized reach is very susceptible to scouring due to high flows following precipitation events. *Hygrophila* is still dominant where it covers most of the river right portion of this reach. Native *Cabomba* increased substantially this year where it is second only to *Hygrophila* in surface area. Bryophytes and *Ludwigia* were also present in the New Channel Reach.

Drop-netting continues to be the best method for sampling fountain darters in varied vegetation types and in 2008 (as previously mentioned) two filamentous algae sites were sampled in the Old Channel Reach for the first time in several years. Native vegetation (filamentous algae, bryophytes, *Ludwigia*) contained the highest numbers of fountain darters across all reaches combined in 2008. Filamentous algae and bryophytes also contain high numbers of amphipods, a common food item for fountain darters, which likely explains the fountain darters preference for these vegetation types. Normalized population estimates for spring 2008 were the highest recorded since 2003. As in previous years, length-frequency distribution data reflected a spring reproductive peak for fountain darters. Presence/absence dip netting yielded similar proportions of fountain darters in 2008 compared to previous years.

Spring Run 1 and Spring Run 3 yielded almost twice the number of salamanders in 2008 compared to 2007. On the contrary, Spring Island salamander counts were considerably less in 2008. No salamander observations at the Spring Island Spring Run for the third straight sampling session is likely because of the removal of fist-size rocks (preferred habitat) at this site.

The macroinvertebrate drift net sampling yielded a total of 10 taxa at the three drift net sites in Comal Springs during 2008, compared to 14 the previous year. Similar to 2007, species of the genus *Stygobromus* and *Lirceolus* continued to be most abundant at all sites. Peck's cave amphipod (*Stygobromus pecki*) was the dominant amphipod at all sites. There were substantially more Comal Springs riffle beetles (*Heterelmis comalensis*) collected in 2008 than in 2007. However, as in previous years, beetles tended to be patchily distributed with wide ranges of abundance between sites and seasons.

Water quality data collected by volunteers in the Master Naturalist Program provided insight into weekly changes in carbon dioxide (CO_2) concentrations and pH levels at several sites in the Comal River. These data continue to track how CO_2 levels are highest near spring inputs and decrease with distance from the spring orifices. Recreational use data indicates that people continue to occupy the New Channel location during the summer more than any of the other sites.

Near average flows in the Comal River in 2008 continued to maintain fountain darter populations in this unique springs system. Non-native and native vegetation continued to compete at several reaches, but with the exception of *Ludwigia* at Landa Lake, surface areas were similar to 2007. The presence of bryophytes in some of the reaches will continue to harbor large populations of fountain darters. If the central Texas drought continues into 2009, resulting flow decreases may trigger low-flow Critical Period sampling events. These events, although undesirable for the public and environment should provide much needed information on biotic responses to more extreme conditions.

METHODS

Study Location

Comal Springs, which consists of many spring openings, is the largest spring system in Texas. The clear, thermally constant water issues from the downthrown side of the Comal Springs Fault Block. The Comal River extends 5 kilometers to its confluence with the Guadalupe River. Although Comal Springs reportedly has the greatest discharge of any springs in the Southwest, the flows can diminish rapidly during drought conditions and the springs completely ceased to flow for several months in the summer and fall of 1956 during the drought of record. Despite this fact, Comal Springs is home to several extremely rare, listed species. This study includes monitoring and applied research efforts directed toward these species including one fish, the fountain darter, and three invertebrates, Comal Springs dryopid beetle (*Stygoparnus comalensis*), Comal Springs riffle beetle, and Peck's cave amphipod. One additional species that was monitored during this study was the undescribed Comal Springs salamander (Eurycea sp.)

Two full comprehensive sampling efforts were conducted (spring and fall) in 2008 with additional dipnetting for fountain darters collected in the summer, and volunteer assistance (initiated in 2006) with weekly water quality measurements and recreational counts on the Comal system. A full comprehensive event includes the following sampling components and volunteer activities:

<u>Water Quality</u>
Thermistor Placement
Thermistor Retrieval
Fixed Station Photographs
Weekly standard parameters (volunteer)

<u>Aquatic Vegetation Mapping</u>

<u>Fountain Darter Sampling</u>
Drop Nets
Dip Nets
Visual Observations

<u>Salamander Observations</u>

<u>Macroinvertebrate Sampling</u>
Drift Nets
Point Water Quality Measurements
Comal Springs Riffle Beetle Surveys

<u>Recreation Observations</u>
Weekly Recreation Counts (volunteer)

Comal Springflow

Total discharge data for the Comal River were acquired from United States Geological Survey (USGS) water resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2008). 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, 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. Finally, spot water velocity measurements were taken during each sampling event using a SonTek® FlowTracker with handheld unit.

Low-Flow Sampling

There were no low-flow sampling events on the Comal Springs / River system in 2008.

High-Flow Sampling

There were no high-flow sampling events on the Comal Springs / River system in 2008.

Water Quality Sampling

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. Due to the consistency in water quality conditions measured over the first several years of sampling, the water quality component of this study was reduced in 2003. However, two important components for maintenance of long-term baseline data, temperature loggers (thermistors) and fixed station photography were collected in 2008. In addition, conventional physico-chemical parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen, water depth at sampling point, and observations of local conditions) were taken at the surface, mid-depth, and near the bottom (when applicable) in all drop-net sampling sites using a Hydrolab Quanta. When conditions trigger Critical Period events in the future, the full spectrum of water quality sampling parameters will be measured, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

In October 2008, new thermistors (Tidbit v2) were employed as a replacement for all existing thermistors at each site. This periodic replacement is designed to prevent temperature drift which has been documented in long-term employment of such devices. Thermistors are placed in select water quality stations along the Comal River and continue to be downloaded at regular intervals to provide continuous monitoring of water temperatures in these areas. Thermistors were also placed in deeper locations within Landa Lake using SCUBA and set to record temperature data every 10 minutes. The thermistor locations will not be described in detail here to minimize the potential for thermistor tampering.

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

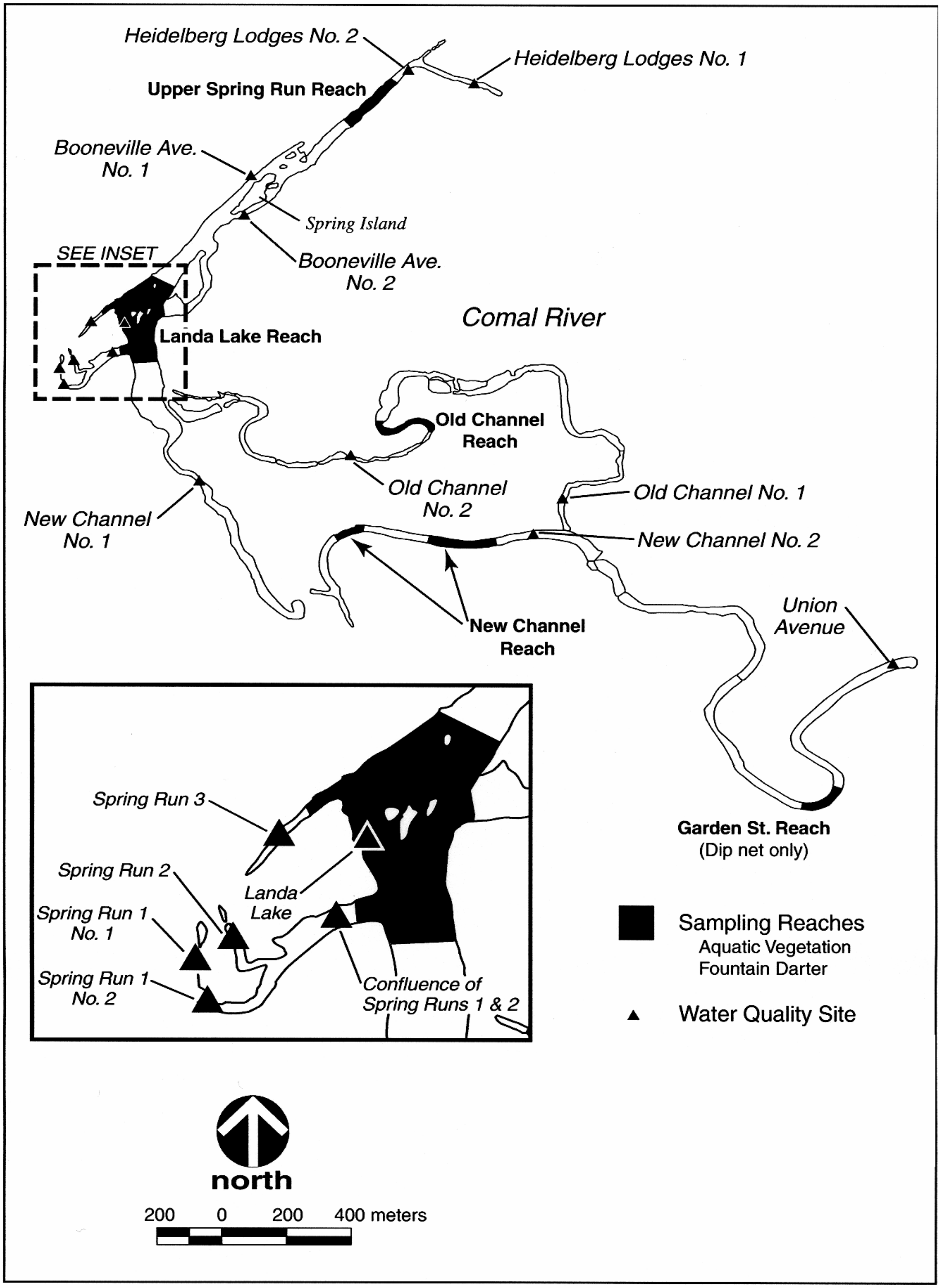


Figure 1. Comal River water quality and biological sampling areas.

Aquatic Vegetation Mapping

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

Hygrophila in the Old Channel Reach

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium*; primarily in the Upper Spring Run and Landa Lake) were included in all 2008 sampling events. Difficulties with mapping these vegetation types (patchiness, bryophytes were easily obscured by filamentous algae, etc.) precluded their inclusion during previous studies and early on in this project; however, these vegetation types were documented as important fountain darter habitat and have been included in all sample events since the summer of 2001.

Fountain Darter Sampling Methods

Drop Nets

A drop net is a type of sampling device previously used by the United States Fish and Wildlife Service (USFWS) to sample fountain darters and other fish species. The design of the net is such that it encloses a known area (2 square meters [m^2]) and allows a thorough sample by preventing escape of fishes occupying that area. A large dip net (1 m^2) is used within the drop net and is swept along the length of the river substrate 15 times to ensure complete enumeration of all fish trapped within the net. For sampling during this study, a drop net was placed in randomly selected sites within specific aquatic vegetation types. The vegetation types used in each reach were defined at the beginning of the study as the dominant species found in that reach. Sampling sites were randomly selected per dominant vegetation type for each sampling event from a grid overlain on the most recent map (created with GPS-collected data during the previous week) of that reach.

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

Drop Net Data Analysis

The fisheries data collected with drop nets were analyzed in several ways. Calculations of fountain darter density in the various vegetation types during 2000-2008 provide valuable data on species/habitat relationships. These average density values were also used with aquatic vegetation mapping data on total coverage of each vegetation type by sampling effort to create estimates of the population abundance in each reach (fountain darter density within a vegetation type x total coverage of that vegetation type in the given reach). Because there were generally only two drop net samples in each vegetation type within each reach, density estimates between sampling efforts had great variation and population estimates based on those densities are greatly influenced by this variation. Part of the variation would be due to changes in environmental conditions (discharge, temperature, etc.) that had occurred since the last sample, but part is due to natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints) it is difficult to tell how much of the variation is attributed to each source within a given sampling effort. Using the average density of fountain darters across all samples for a given vegetation type does not account for changes in density across samples (differences associated with changes in environmental conditions), but the increased sample size substantially reduces the high natural variability. This type of comparison between samples, where density values are held constant across all samples, is based entirely upon changes in vegetation composition and abundance between sampling efforts. Because these abundance estimates use the same density values across sites and seasons, and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Dip Nets

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

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

Dip Net Data Analysis

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

Presence/Absence Dip Netting

In 2008, presence/absence dip netting was conducted on the Comal River during the spring (April 23) and fall (November 6) sampling events. During each sample, fifty sites were distributed among the 4 sample reaches based on total area, diversity of vegetation, previous fountain darter abundance estimates, and overall biological importance of each reach. The distribution of sample sites in 2008 was similar to previous years and included sites in each of the dominant vegetation types in a given reach. In most cases, sites were randomly selected from a grid overlain on the most recent vegetation map of that reach. However, occasionally, where certain vegetation types exhibited limited coverage, sites were chosen to fall within the selected vegetation type. Four dips were conducted at each site. After each dip, presence or absence of fountain darters was noted and the entire contents of the net were placed into a plastic tub with river water to avoid recapturing organisms. After all dips were completed at a site, all organisms were released and time of day was recorded.

Although this technique does not allow for detailed analysis, it does provide a quick and less destructive method of monitoring large-scale trends in the fountain darter population using easily collected presence/absence data.

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 in areas too deep to allow efficient drop net or dip net techniques. Observations were conducted in the early afternoon for each effort. An additional component to this effort was a grid (0.6 m x 13.0 m) survey added in summer 2001, and subsequent sampling. The grid was used to quantify the number of fountain darters using these deeper habitats. To sample the area, all fountain darters within the grid were counted. A much more labor-intensive effort would be required to develop an estimate of the true population size in the sample area, but the data are useful in providing an indication of the relative abundance of the fountain darters that are found in areas similar to those sampled. This data also provides insight into trends in population dynamics that may occur over time.

Comal Springs Salamander Visual Observations

In addition to the visual observations made in the deeper portions of Landa Lake for fountain darters and Comal Springs salamanders, the BIO-WEST project team performed presence/absence surveys for the Comal Springs salamanders in the spring reaches located at the head of the Comal River during both 2008 sampling events. Surveys were conducted in Spring Run 1, Spring Run 3, and the Spring Island area (Figure 1) and performed by two people in each spring reach. Each survey began at the downstream-most edge of the sampling area and involved turning over rocks located on the substrate surface within the Spring Run while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. The Comal Springs salamander locations were noted, along with time and water depth. In order to maintain consistency between samples, all surveys were initiated in the morning and terminated by early afternoon.

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

Salamander survey in Spring Run 1

Macroinvertebrate Sampling

In 2008, drift nets were placed in spring openings during the spring and fall comprehensive sampling efforts. Drift nets were placed over the openings of Comal Spring Runs 1 and 3 and a moderate-sized spring upwelling along the western shoreline of Landa Lake. The nets were anchored into the substrate directly over the spring opening, with the net face perpendicular to the direction of flow of water. The nets had a 0.45-m by 0.30-m rectangular opening and mesh size of 350 micrometers (µm). The tail of the net was connected to a detachable 0.28-m long cylindrical bucket (300-µm mesh). The buckets were removed at 4-hour intervals, and the cup contents were sorted in the field. Except for voucher specimens of Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle, all organisms of these three species were identified and returned to their spring of origin. Voucher specimens included fewer than the 20 living specimens (identifiable in the field) of each species. All other invertebrates were preserved in 70% ethanol for later identification. Water quality measurements (temperature, pH, conductivity, dissolved oxygen, and current velocity) were taken at each drift net site using a Hydrolab multiprobe and DataSonde (model 2) and a Marsh McBirny portable water current meter (model 201D).

In addition to drift nets placed over spring openings, surveys of the endangered Comal Springs riffle beetle, were conducted in the two comprehensive sampling efforts in 2008 (May and November). These samples were conducted in three disjunct areas of Landa Lake on the Comal River, in locations that were previously identified (BIO-WEST 2002) to have the highest densities of Comal Springs riffle beetles. The three sites included Spring Run 3, the western shoreline of the lake, and upstream of Spring

Island. Samples were collected using the same methodology as in previous years. Bed sheets (60% cotton, 40% polyester) were cut into 15-cm x 15-cm squares. At each of the three study sites, 10 springs found in potential habitat were selected and sampled using this method. Depth (ft), current velocity (m/s), and landmark distance measurements were taken at each spring. Each square had the corners folded inward and placed in the spring with rocks loosely stacked over top to keep it in place. Approximately four weeks later, squares were located and removed followed by depth and current velocity measurements. Beetles were identified, counted, and returned to their spring of origin. Other spring invertebrates collected on the squares were also noted.

Master Naturalist Monitoring

Volunteers with the Texas Master Naturalist program continued their monitoring efforts in 2008 at select locations along the Comal Springs/River system. The Texas Master Naturalist Program is a partnership among the Texas Cooperative Extension, Texas Parks and Wildlife Department, and numerous local partners designed to provide natural resource education, outreach, and other services through volunteer efforts. To become a Master Naturalist, an individual must complete an approved training course and complete at least 40 hours of volunteer service per year. The program currently supports over 2,750 volunteers across the state of Texas (http://masternaturalist.tamu.edu).

Since the summer of 2006, Master Naturalist volunteers have assisted BIO-WEST by collecting weekly water quality and recreation data on the Comal Springs/River ecosystem. Volunteers collected data at five sites (Figure 2) on a weekly basis (typically on a Friday afternoon). At each site, an Oakton Waterproof pHTestr 2 was used to assess pH, and a LaMotte Carbon Dioxide Test Kit was used to measure carbon dioxide concentrations in the water column. In addition to water quality measurements, recreational use data was collected at each site by counting the number of tubers, kayakers, anglers, etc. using the area at the time of sampling. Photos were taken at each site and any other notes on recreational use or condition of the river were recorded during each sampling event.

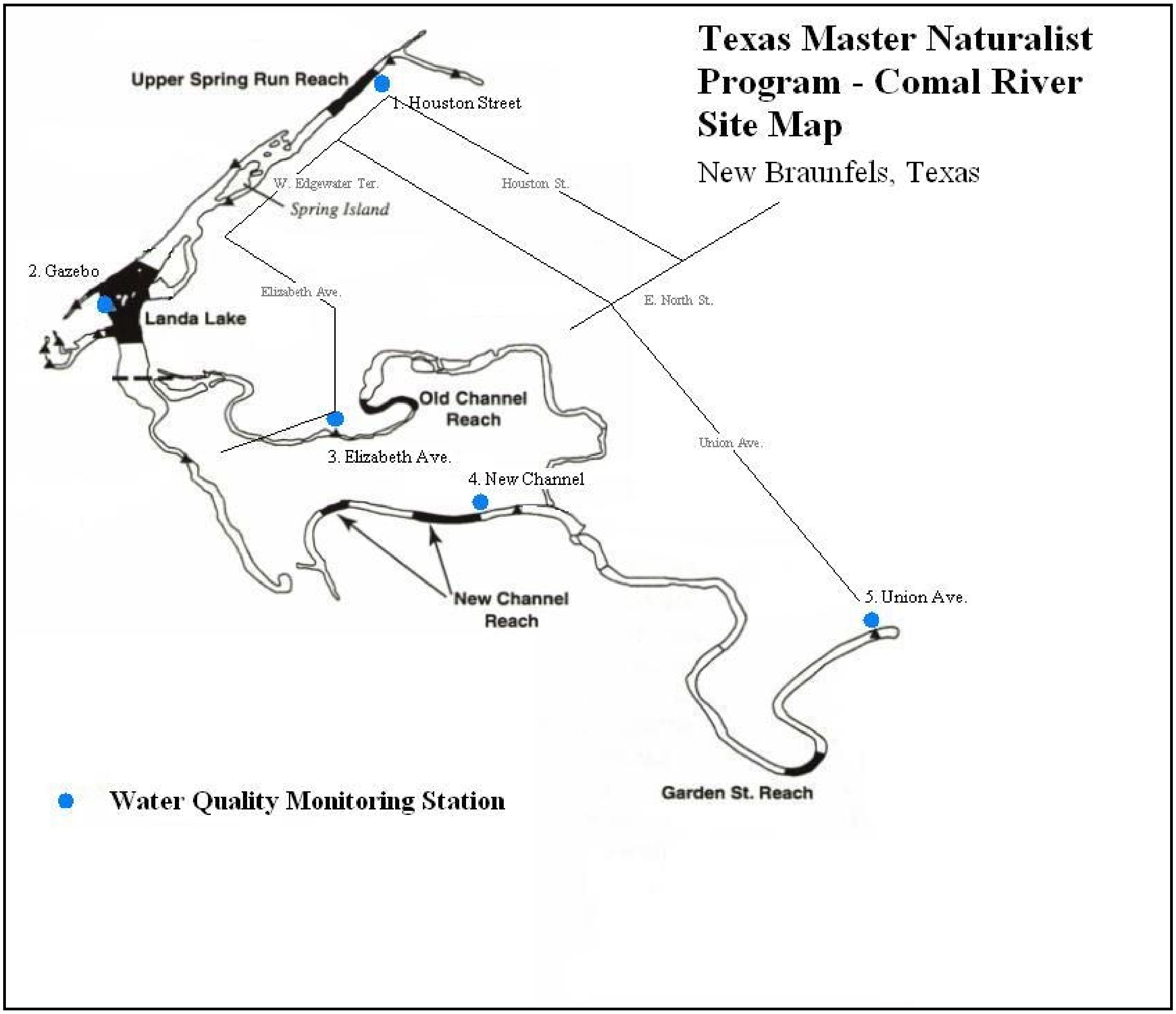


Figure 2. Weekly water quality / recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers.

Exotics / Predation Study

This sampling component was not included in 2008 as no critical period sampling efforts were triggered.

OBSERVATIONS

The BIO-WEST project team conducted the 2008 sampling events as shown in Table 1.

Table 1. Components of the 2008 Comal Springs / River sampling events.

EVENT	DATES	EVENT	DATES
Spring Sampling		**Fall Sampling**	
Vegetation Mapping	Apr 17 – 18, Apr 21 - 22	Vegetation Mapping	Oct 23 – 24, Oct 27 - 28
Fountain Darter Sampling	Apr 30 – May 2	Fountain Darter Sampling	Nov 3 - 5
Comal Salamander Observations	Apr 30	Comal Salamander Observations	Nov 5
Macroinvertebrate Sampling	May 14 - 15	Macroinvertebrate Sampling	Nov 20 - 21
Summer Sampling			
Fountain Darter Sampling	July 31		

Comal Springflow

Discharge in the Comal River decreased through most of 2008 with a slight temporary reversal in August and September. The lowest recorded discharge occurred in June when flow was at 260 cfs (Table 2). Prior to June flows in the river were above their historical averages, and then again in August and September before falling back below for the remainder of the year (Figures 3 and 4). A precipitation event in late summer led to a peak, but afterwards the extended period of drought contributed to decreased flows for the remainder of 2008. The minimum flow in the Comal River in 2008 was the highest minimum since 2005. As in the San Marcos River, the highest recorded daily average flow occurred on January 1 (412 cfs).

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

Year	Discharge	Date
2000	138	Sept. 7
2001	243	Aug. 25
2002	247	Jun. 27
2003	351	Aug. 29
2004	335	May 28
2005	349	July 14
2006	202	Aug. 25
2007	251	Mar. 8-10
2008	260	June 30

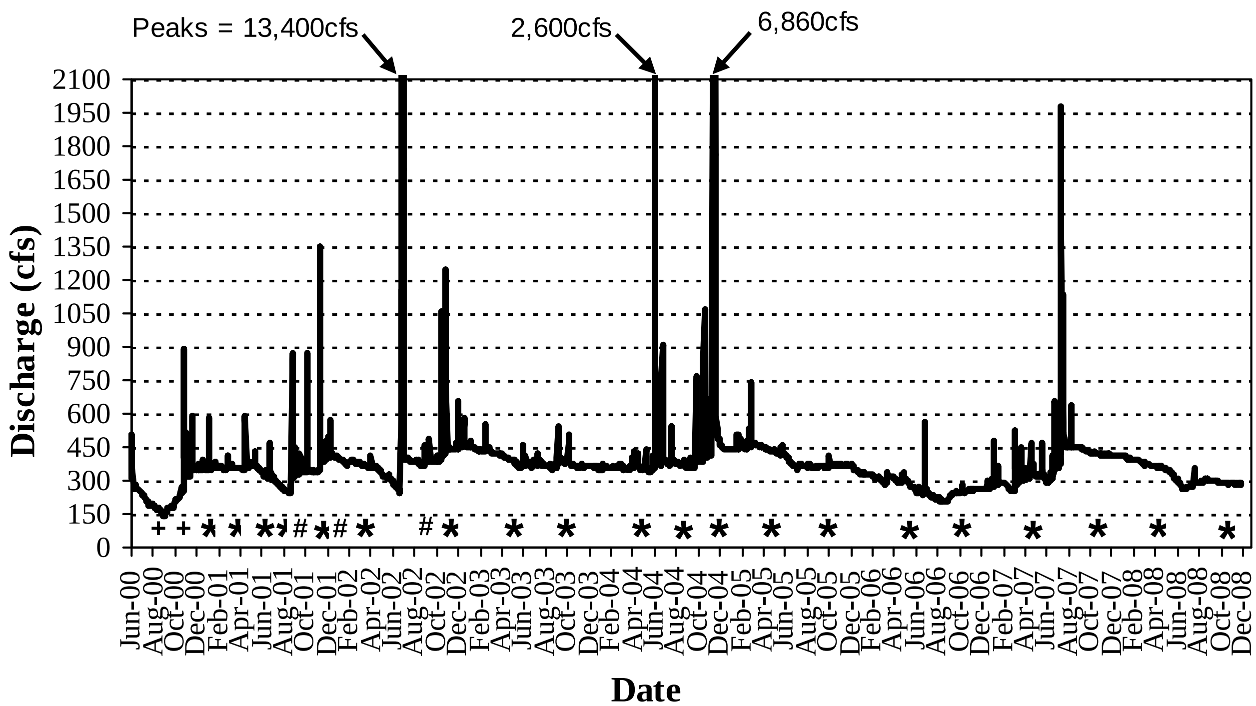


Figure 3. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (*), low (+), and high-flow (#) sampling events are indicated.

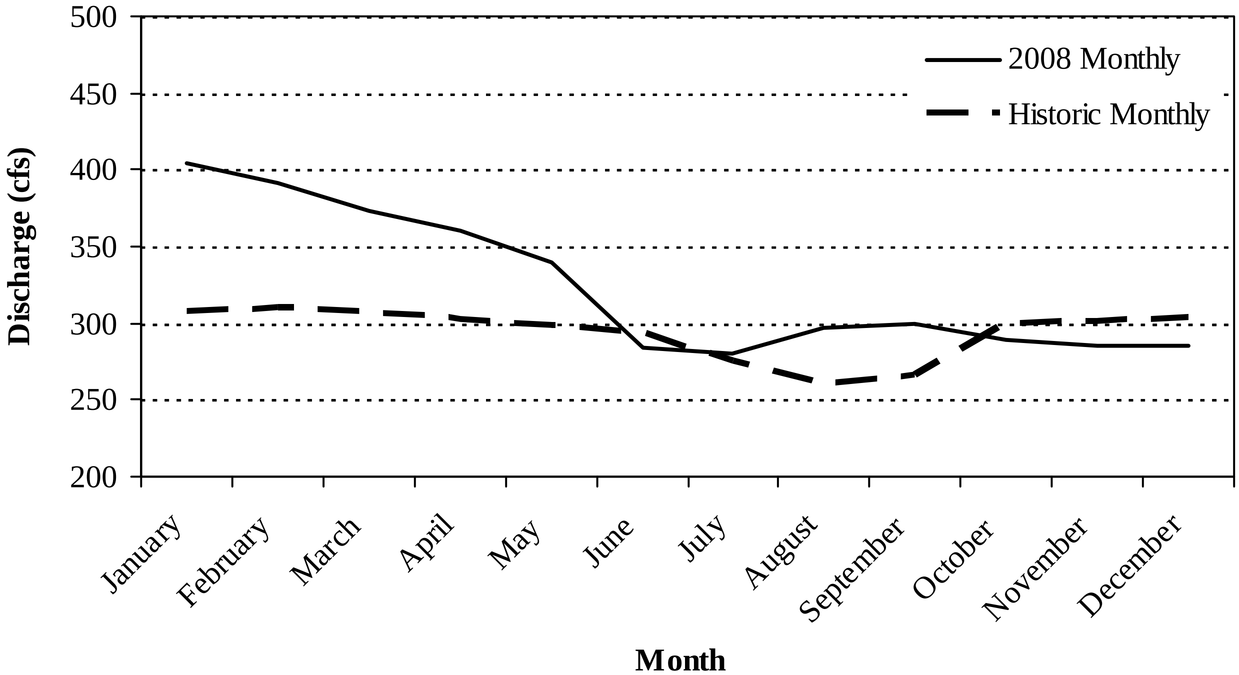


Figure 4. Mean monthly discharge in the Comal River during the 1934-2008 period of record.

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 discharge that traveled down the Old Channel during each sample effort. All measured flows in fall 2008 were lower than in fall 2007. However, only at Spring Run 3 (upstream) and the Old Channel were flows lower in spring 2008 than in spring 2007. With very little precipitation falling over the Edwards Aquifer in 2008 flows decreased or were similar at all sites by fall 2008 (except the Old Channel). During this period the proportion of flows only decreased at Spring Run 1 indicating that as overall flow in the river dropped, the proportion from the springs becomes more important to the overall flows in the system. This is not surprising considering flows from non spring-fed sources (e.g. Blieder's Creek, Dry Comal Creek) decrease as precipitation events become sparser.

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 2007 - 2008.

	Discharge (cfs)			
Location	**Spring 2007**	**Fall 2007**	**Spring 2008**	**Fall 2008**
Total Discharge Comal River (USGS)	330	424	354	286
Spring Run 1	31.9	42.2	35.6	22.7
Spring Run 2	5.1	6.4	6.0	6.1
Spring Run 3 (upstream)	16.0	14.9	13.0	12.0
Spring Run 3 (downstream)	40.0	52.1	41.5	37.0
Old Channel	57.3	54.6	48.7	57.3

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 (New Channel not included) during each sample effort in 2007 - 2008.

	Proportion of Total Discharge			
Location	**Spring 2007**	**Fall 2007**	**Spring 2008**	**Fall 2008**
Spring Run 1	9.7%	10.0%	10.0 %	7.9 %
Spring Run 2	1.5%	1.5%	1.7 %	2.1 %
Spring Run 3 (upstream)	4.8%	3.5%	3.7 %	4.2 %
Spring Run 3 (downstream)	12.1%	12.3%	11.7 %	13.0 %
Old Channel	17.4%	12.9%	13.8 %	20.0 %

Water Quality

The continuously recorded water temperature data have provided a good view of the thermal conditions experienced by fountain darters and other species throughout the Comal Springs ecosystem from 2000 - 2008. Water temperatures are most constant at or near the spring inputs, and become more variable downstream as other inputs (streams, runoff, and precipitation) become more influential. At times, precipitation can have acute impacts (typically very cold rainfall) in some locations, but these are generally short-lived and the overall relationship at these sites is more directly associated with air temperature (air temperatures also strongly influence precipitation temperatures).

A representative graph of thermistor data for the Comal Springs/River ecosystem in 2008 is presented in Figure 5; additional graphs for all years can be found in Appendix B. Gaps in readings present on some graphs are indications of theft or thermistor failure, and in the latter case, these readings were excluded because they may not be entirely accurate. All existing thermistors were replaced with new Tidbits® at all sites in October 2008. As in past years, water temperatures at Blieder's Creek exhibited the most variation within the Comal system (21.7 – 29.3 °C). Although this site is located at an utmost upstream location of Comal Springs, it is not springfed and thus, vulnerable to temperature fluctuations because most of its input comes from precipitation. Without spring contributions, this creek often becomes stagnant when flows (and precipitation events) are reduced causing elevated temperatures. With these fluctuations in temperature it is unlikely that fountain darters would find suitable year-round habitat in Blieder's Creek.

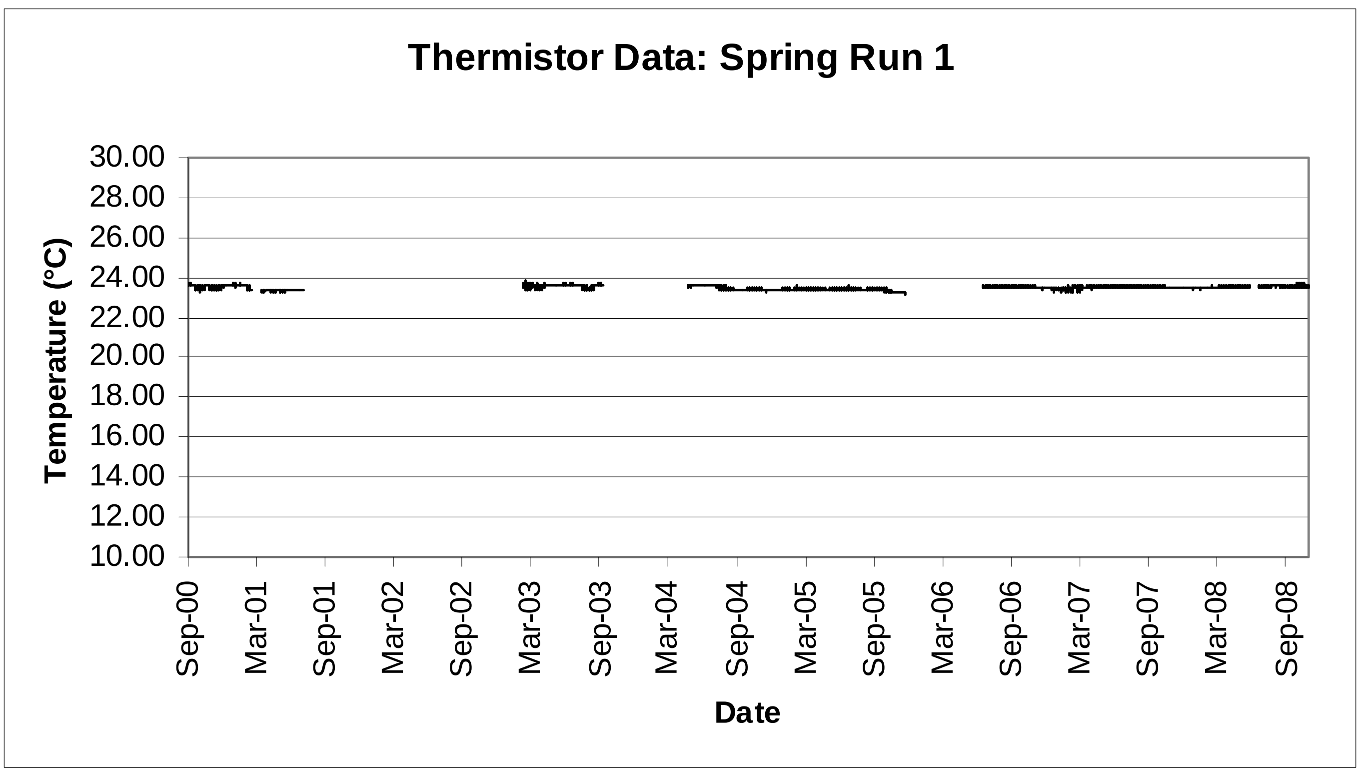


Figure 5. Thermistor data from Spring Run 1.

As in previous years, water temperatures at the spring runs varied less than 1 °C in 2008 (Figure 5). Although a sharp increase is observed in the Spring Run 2 data (Appendix B), it is likely caused by "drift" which is indicative of a temperature logger failing. Any time this was observed in the data, thermistors were immediately replaced. The highest temperatures observed within the river itself were near 30 °C at the downstream site in the New Channel. This site is far from spring inputs (which would help stabilize water temperatures) and more prone to fluctuations due to precipitation events. Only this site and Blieder's Creek exceeded the Texas Commission on Environmental Quality (TCEQ) water quality standards value of 26.67 °C. Temperature data at the Old Channel exemplifies typical seasonal fluctuations with the highest water temperatures recorded during summer. The thermistor at the Other Place site failed and we do not have any current 2008 water temperature data from that site.

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

Total vegetation coverage in this most upstream reach continued to increase by spring 2008 (4,255.8 m^2). Bryophytes continued to be the dominant vegetation covering much of the upper half of the Upper Spring Run Reach (2,760.0 m^2). As discharge declined in 2008, bryophyte coverage also decreased. By fall 2008 much of the large sections of this vegetation had become fragmented and decreased in surface area by 62% (1,057.4 m^2). In addition, areas where bryophytes grew vertically in the water column in the spring were reduced to small plants near the substrate of the river. These plants will need to be closely monitored in 2009 to examine whether this decrease is part of the natural cycle of the bryophytes (described in previous annual reports), or if the changes are due to the flow regime. The non-native plant *Hygrophila* continued to flourish in this reach with the majority of coverage located in the downstream end in spring 2008 (717.3 m^2). However, by fall this plant had decreased by over 50% when much of it became fragmented in this downstream stretch of the reach.

Native plants are an important habitat for fountain darters in this reach. Unfortunately, *Cabomba* is no longer found here and *Ludwigia* surface area has been reduced to only a few plants. By fall 2008 *Ludwigia* surface area was reduced to 2.1 m^2 with only 4 plants remaining in the reach. Although, we do not sample fountain darters in this plant type in the Upper Spring Run Reach, it has been shown to be an important habitat to the endangered fish in other reaches (Landa Lake, Old Channel Reach). *Sagittaria* is found along much of the western bank in the downstream section of this reach. It increased slightly in 2008 from 692.7 m^2 in spring to 729.3 m^2 in fall. Although native plants appear to be declining in this reach (*Cabomba*, *Ludwigia*), bryophytes continue to provide important fountain darter habitat because of the structure they offer throughout the reach.

Landa Lake Reach

Vallisneria is the most ubiquitous plant in this reach because it dominates the deeper portions of the lake. It increased from fall 2007 (13,412.6 m^2) to spring 2008 (13,784.8 m^2), and then decreased slightly by fall 2008 (13,690.6 m^2). Although this plant appears to have a solid foothold in this reach, it is closely monitored because of the giant ramshorn snail (*Marisa cornuarietis*) herbivory that occurred in the early 1990s and additionally, the suckermouth catfish (*Hypostomus sp.*) has been observed in several "gaps' in the vegetation indicating that its presence may have the potential to affect this plant. The non-native plant *Hygrophila* was still prevalent in the Landa Lake Reach in 2008. This plant decreased slightly in 2008 (598.8 m^2 to 515.2 m^2) when it became somewhat fragmented in the middle section of this reach. The native plant, *Ludwigia* which grows adjacent to *Hygrophila* in this reach decreased from fall 2007 (37.1 m^2) to spring 2008 (27.1 m^2). This plant continues to fragment in the middle section of this reach. It seems likely that the non-native plant *Hygrophila* is outcompeting *Ludwigia* here. By fall 2008, the *Ludwigia* became more fragmented (17.4 m^2) and only occupied a few small stands adjacent to *Hygrophila* and *Vallisneria*. *Ludwigia* is an important habitat for fountain darters and its decline may have effects on the population of this endangered fish.

Another native plant, *Cabomba*, also decreased by the spring of 2008 (272.5 m^2 in fall 2007 to 158.7 m^2 in spring 2008). This is another vegetation type that is important to the endangered fountain darter. Much of this decline in surface area is due to the large plant in the upstream end of the reach shrinking. While there are many smaller plants scattered in the reach, these plants are in deeper areas that are not amenable to fountain darter sampling, so it is unknown if they also harbor higher numbers of fountain darters. By fall 2008, *Cabomba* increased slightly (178.4 m^2) with several plants in deeper portions of the lake growing in size. Along with *Ludwigia* this native plant will be closely monitored because of its importance to fountain darter populations. *Sagittaria* also flourished in the lake in 2008. In spring it occupied 1,445.8 m^2 in the deeper areas in the middle of Landa Lake. It continued to grow and by fall occupied 1,506.9 m^2 of the reach. Although, this native plant is not sampled for fountain darters in this reach it may become more important if other native plant species continue to decline.

Bryophytes continued to be prevalent in this reach in 2008 occupying much of the upstream section. In spring this plant had increased to 3,363.8 m^2 where it covered much of the deeper portions of the lake. A dramatic decline was observed by fall when pure stands of bryophytes only occupied 176.3 m^2 of the lake. Green algae mixed very thoroughly with bryophytes on the upper west side of the lake. Much of these algae covered up the bryophytes so that it was only seen when moving off this top layer. In the deeper section of the reach bryophytes disappeared completely possibly due to a minor flushing event during late summer. However, this was more likely the boom, than bust cycle that this plant has experienced in this reach during below, average, and above average flow conditions over the past 8 years. Carbon dioxide concentrations have also been tied to the spatial and temporal bryophyte abundance in the Comal system. Therefore, if drought conditions persist, and flows decrease further in 2009, close monitoring of the bryophyte community is imperative to better understand causes of this variation in this important vegetation type.

Old Channel Reach

Until 2003, the Old Channel Reach maintained the most stable aquatic vegetation community with a structure (culvert) that regulates flow through this section. Later, that culvert was reconstructed to increase its capacity which resulted in increased and more variable discharge that has affected aquatic communities downstream. Non-native vegetation continued to dominate this reach in 2008. Although *Hygrophila* decreased in surface area from 2007 (1,518.7 m^2) to 2008 (1,349.1 m^2), it is still the dominant vegetation type here. With declining flows, *Hygrophila* increased only slightly by fall (1,350.4 m^2). *Ceratopteris* (another non-native plant) increased by 61% in 2008 (45.4 m^2 to 116.1 m^2), although it did not appear to colonize any areas used by native plants.

Native aquatic vegetation struggled again in 2008, but filamentous algae have recolonized some parts of the Old Channel Reach. By fall 2008 it occupied 29.2 m^2 and drop-net sampling for fountain darters in this vegetation type (which had been suspended since 2006 due to limited areal extent) was conducted. Considering the Old Channel flow distribution (Table 3), the flow levels observed since spring 2007 (ranging from 48-57 cfs) are similar to what was observed during the pre-reconstructed culvert era which exhibited abundant filamentous algae. Continued monitoring in 2009 will help determine whether 2008 was a temporary occurrence or if permanent re-establishment is starting. *Ludwigia* continued to fragment by spring 2008 when several small patches only totaled 28.0 m^2. By fall 2008 some of the plants grew larger (43.8 m^2), but this plant is still being out competed by *Hygrophila* in this reach. Bryophytes also flourished in 2008 reaching a high of 95.5 m^2 by fall 2008. This is encouraging for fountain darter populations considering this is a preferred habitat for their populations.

The interaction between these plants in this reach is very important to fountain darter populations. With *Hygrophila* dominating more of this reach, fountain darter populations have decreased because this vegetation type provides lower quality habitat than the native vegetation it replaced. However, a re-establishment of filamentous algae and the influx of bryophytes would be a great start in reversing this decline. This ebb and flow of non-native versus native vegetation areas will likely have lasting implications on fountain darter populations in this unique reach.

New Channel Reach

The lack of flushing rain events in 2008 contributed to an explosion of vegetation growth in the New Channel Reach. Total vegetation area increased to 1,861.5 m^2 by spring 2008 after most of it was completely eliminated during high flows in early 2005. *Hygrophila* still dominates the reach (1,340.4 m^2) extending along almost the entire river right side bank. It increased by 37% by fall (2,130.8 m^2). The other common plant that is recolonizing this reach is the native *Cabomba*. Although this reach is too deep for standard drop-net sampling to assess fountain darter populations, it is likely important for darter populations that this native plant flourish here. During spring 2008 this plant had fragmented in many areas and only occupied 218.2 m^2 mostly along the north wall. However, by fall this plant more than tripled to 751.2 m^2, the highest area observed in years. Another native vegetation type, bryophytes, was very common in spring 2008 (295.3 m^2). Over the course of the year, *Cabomba* filled in many areas that the bryophytes had occupied, and by fall bryophytes (6.0 m^2) were virtually gone. *Ludwigia* maintained several small patches in 2008 for a maximum surface area of 13.3 m^2 by fall 2008. Although *Hygrophila* continues to be the dominant vegetation in this reach, the growth of native plants may be an important factor in sustaining fountain darter populations in the New Channel.

Fountain Darter Sampling Results

Drop Nets

A total of 628 drop net samples were conducted during 2000-2008 in the Comal Springs/River ecosystem. Forty-four of these samples (22 in spring and 22 in fall) were conducted in 2008. The number of drop net sites and vegetation types sampled per reach is presented in Table 5. Drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sample event, and data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. There were some changes over the course of the study including a shift from sampling two bare substrate sites during each sampling event in the Upper Spring Run and Landa Lake in 2000-2001 to sampling two bryophytes sites in those reaches beginning in the summer of 2001. In 2004, there was a change in the sample design for the Old Channel Reach in response to the dramatic shift from a vegetation community dominated by filamentous algae and *Ceratopteris* to one dominated by *Hygrophila* and *Ludwigia*. Also, in 2005 the New Channel Reach was removed from the drop net sampling effort as vegetated areas had become too deep to sample. In spring 2008, coverage of filamentous algae was very limited in the Old Channel Reach, therefore, the six sample sites were split evenly between *Ludwigia* (3) and *Hygrophila* (3). However, by fall 2008, coverage of filamentous algae had increased enough to allow for placement of two drop net sites in this vegetation type.

Drop net data collected from 2000-2008 show that average densities of fountain darters in the various vegetation types ranged from 3.6/m^2 in *Ceratopteris* to 25.3/m^2 in filamentous algae (Figure 6). Native vegetation types which provide thick cover at or near the substrate (i.e., filamentous algae, and bryophytes [23.0/m^2]) tend to have the highest fountain darter densities. Filamentous algae and bryophytes also contain high numbers of amphipods, a common food item for fountain darters. In contrast, exotic vegetation (*Ceratopteris* and *Hygrophila* [6.9/m^2]), and native vegetation with simple leaf structures (*Vallisneria* [4.1/m^2] and *Sagittaria* [5.2/m^2]) which provide little cover near the substrate tend to have fewer darters. In the Comal River, the native vegetation types *Cabomba* and *Ludwigia* exhibit intermediate fountain darter densities.

Filamentous algae and bryophytes, which provide the best fountain darter habitat, are also the most susceptible to scouring during high flow events and have shown considerable fluctuation in coverage over the study period. Filamentous algae was once the dominant vegetation type in the Old Channel Reach, however, it has been replaced in recent years by *Hygrophila* and *Ludwigia*. This has resulted in an overall decrease in the abundance of fountain darters in this reach (see dipnet data). Although densities are slightly less in bryophytes than in filamentous algae, bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake reaches, and thus make up a significant portion of the available habitat. Bryophytes have also increased in the Old Channel Reach since fall 2006. *Cabomba* and *Ludwigia* are also relatively common in the Comal River/Springs ecosystem and therefore provide substantial amounts of fountain darter habitat. Although fountain darter densities are relatively low in *Hygrophila*, it is an important habitat component because it is abundant in all sample reaches.

Table 5. Sample distribution among drop net sites and vegetation types of the Comal Springs / River sampling reaches in 2008.

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

[a] Switched from Open to Bryophytes, summer 2001.
[b] Areas with vegetation are currently too deep to sample.
[c] Three *Ludwigia* and three *Hygrophila* sites were sampled in spring 2008 to make up for the absence of filamentous algae.
[d] Filamentous algae were not sampled in spring 2008 due to limited coverage, but two sites were sampled in fall 2008.

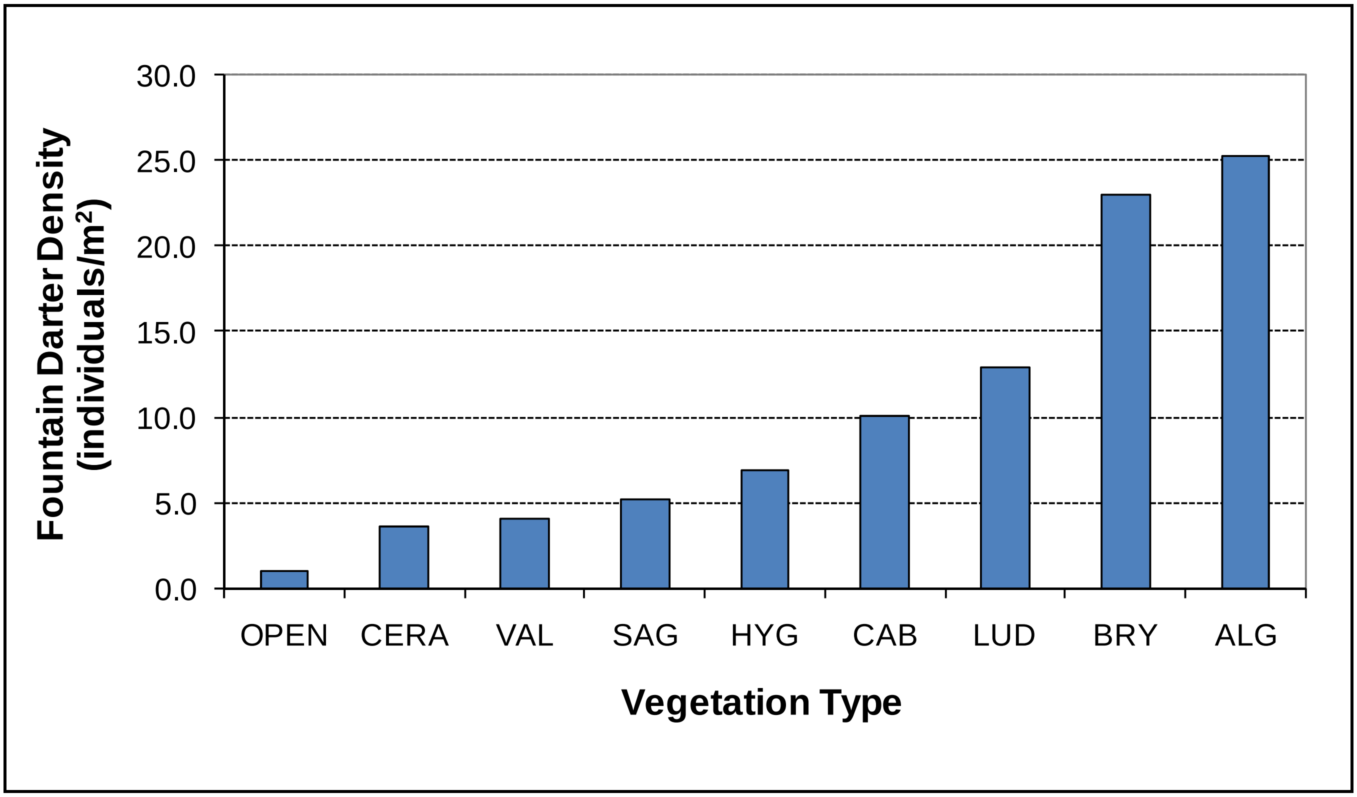


Figure 6. Density of fountain darters collected by vegetation type in the Comal River / Springs ecosystem from 2000-2008. CERA – *Ceratopteris*, VAL – *Vallisneria*, SAG – *Sagittaria*, HYG – *Hygrophila*, CAB – *Cabomba*, LUD – *Ludwigia*, BRY – Bryophytes, ALG – Algae.

Estimates of fountain darter population abundance in all reaches (Figure 7) were based on the changes in vegetation composition and abundance and the average density of fountain darters found in each, as described in the methods section. The vegetation type that had the greatest influence on these estimates was the bryophytes because of the size of the Landa Lake Reach (where most of the bryophytes were mapped) and the density of fountain darters found there. Thus, as coverage of bryophytes in this reach fluctuate, so do fountain darter population estimates. Estimates of reach adjusted population abundance were highest in spring 2003 when coverage of bryophytes peaked in Landa Lake (Figure 7). Estimates for spring 2008 were the highest recorded since 2003 (Figure 7). Although fall 2008 estimates were not as high, they easily fell within the range of variation observed during the study period. Population estimates in fall 2000, winter 2001, and spring 2001 were low because mapping at the time did not include algae in the Old Channel Reach or bryophytes in the Landa Lake Reach. All high-flow Critical Period samples during the study period have shown a decrease in population estimates relative to the previous sample; however there was an increase in the subsequent sample each time. This is most likely related to scouring of important vegetation types resulting in fountain darters becoming more scattered at high flows.

Drop netting efforts in 2008 resulted in collection of 776 fountain darters in the Comal River/Springs ecosystem. The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem during each sample period in 2008 is presented in Figure 8 (all data collected in previous years is presented in Appendix B). As in previous years, small fountain darters are more abundant in the spring sample suggesting a peak in reproduction during this time. However, at least some reproduction seems to occur year-round as evidenced by the presence of a few small darters in fall samples.

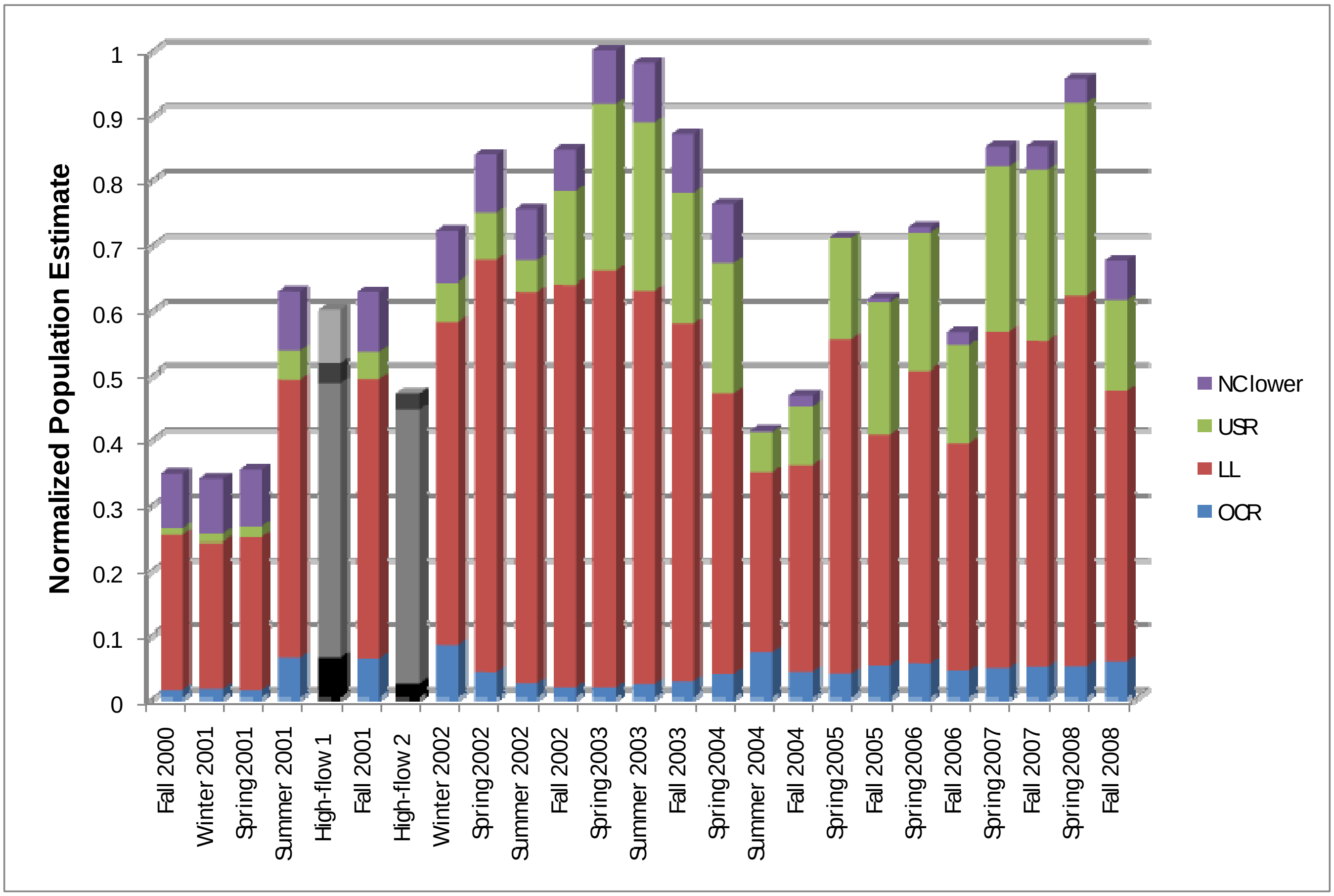
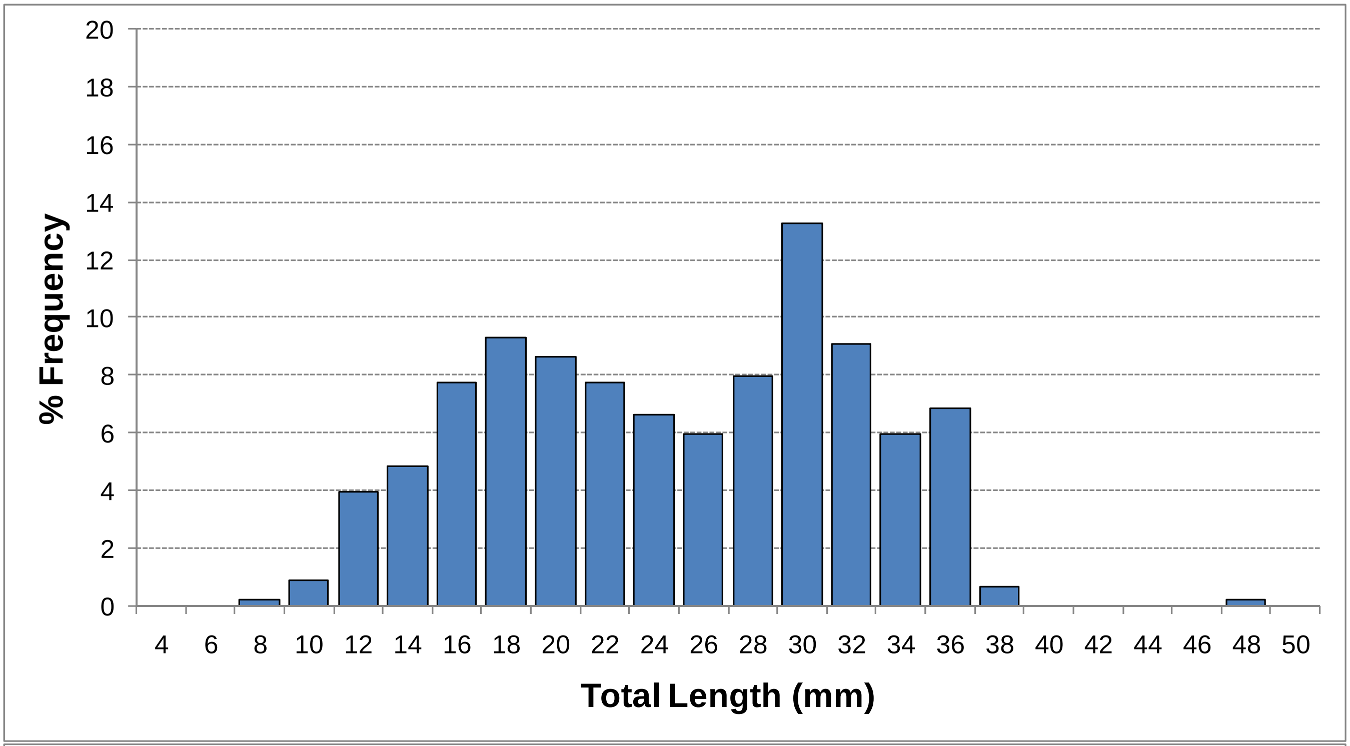


Figure 7. Population estimates of fountain darters in all four sample reaches (NClower = New Channel lower, USR = Upper Spring Run, LL = Landa Lake, OCR = Old Channel) combined (2000-2008); values are normalized to the maximum sample. Black and gray bars represent critical period sampling events.

There is less reproduction occurring at the Old Channel Reach compared to the Upper Spring Run Reach (Figure 9). At the Old Channel no fountain darters less than 16 mm (TL) were captured in the spring 2008 sampling effort. In contrast, in the Upper Spring Run 15 darters in this size class were captured during the drop-netting effort. As a result, the size class distribution in the fall in the Old Channel is similar to the spring whereas the distribution shifts to larger individuals in the fall at the Upper Spring Run (Figures 9 and 10). This lack of (or lessened) amount of reproduction at the Old Channel Reach may be a result of an abundance of less preferred vegetation types (*Hygrophila*) prevalent in this reach.



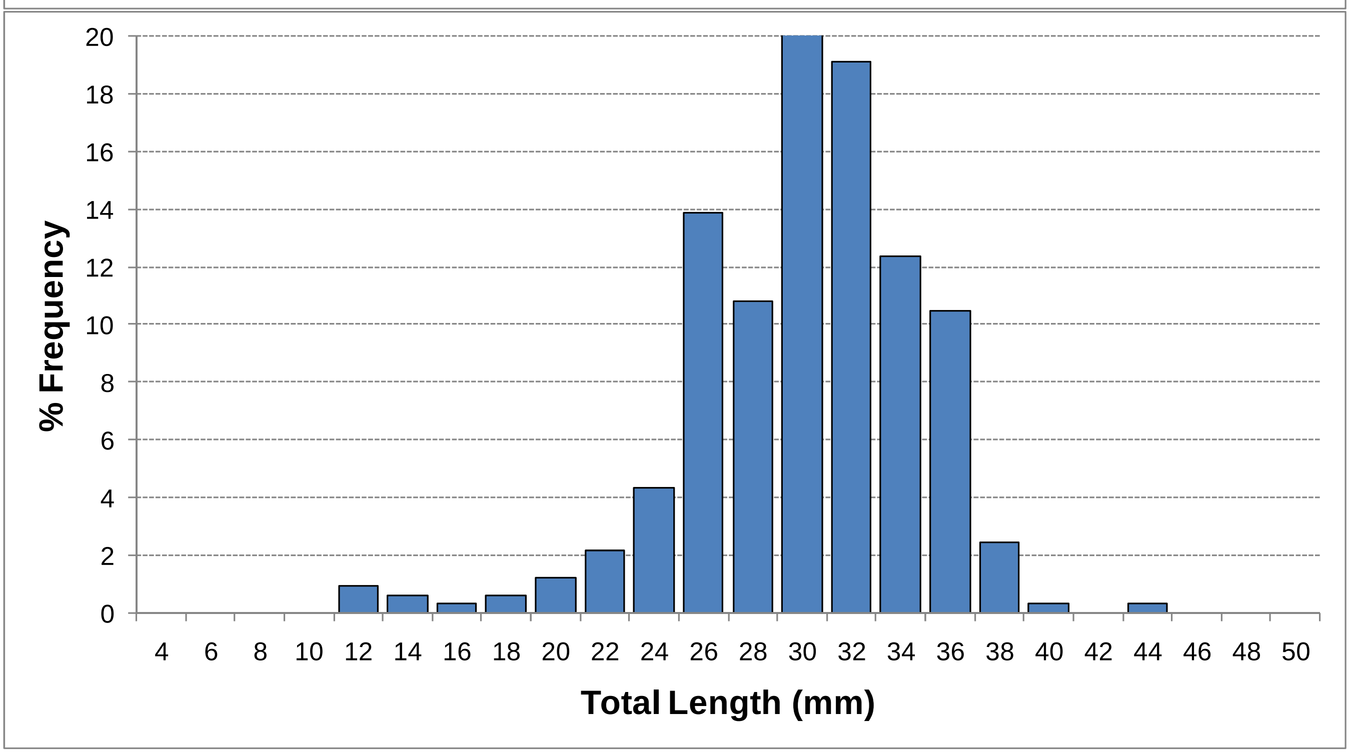


Figure 8. Length frequency distribution of fountain darters collected from the Comal River by drop-netting in spring (top) and fall (bottom) 2008.

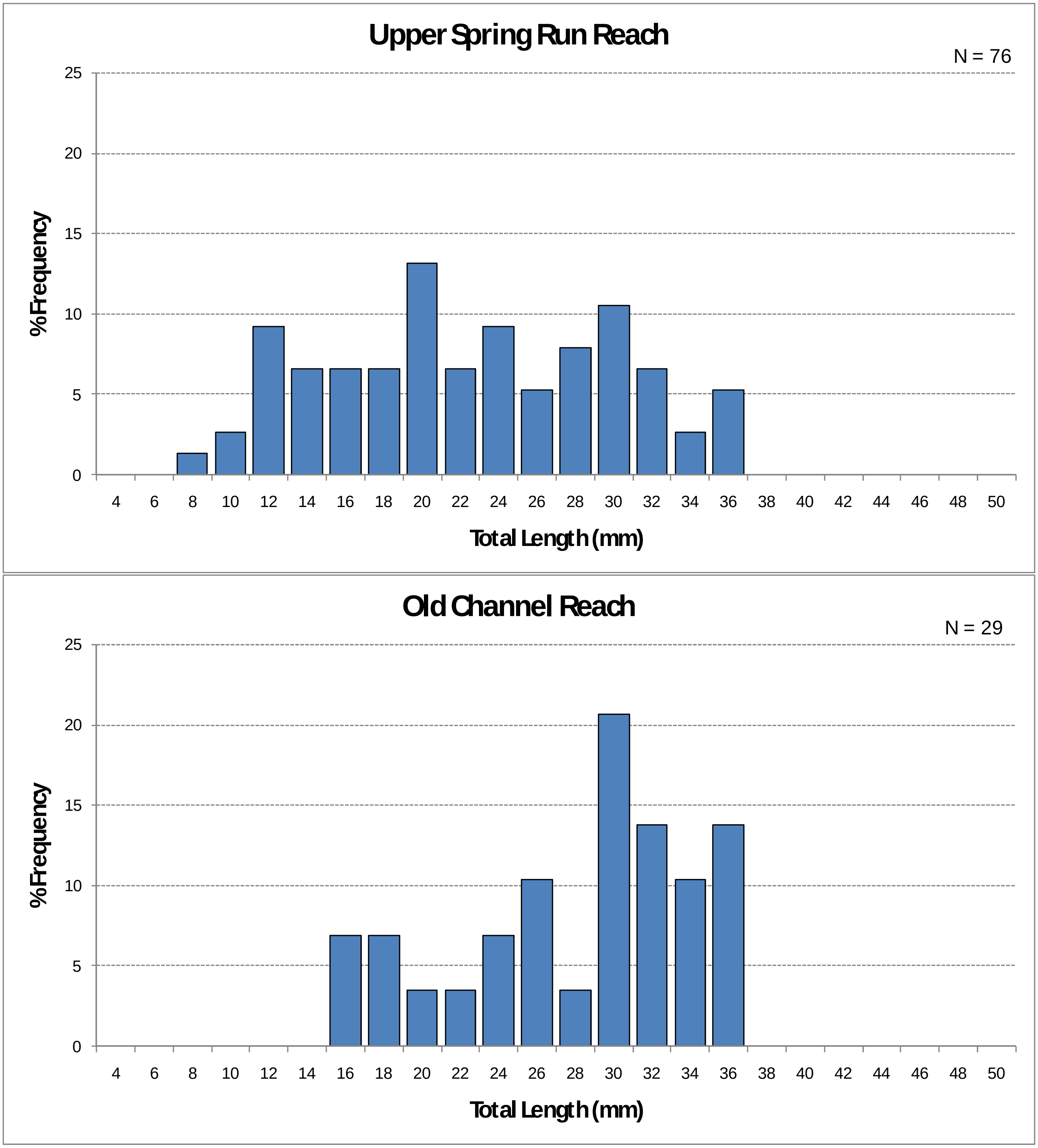


Figure 9. Length frequency distribution of fountain darters collected from the Upper Spring Run Reach and the Old Channel Reach in spring 2008.

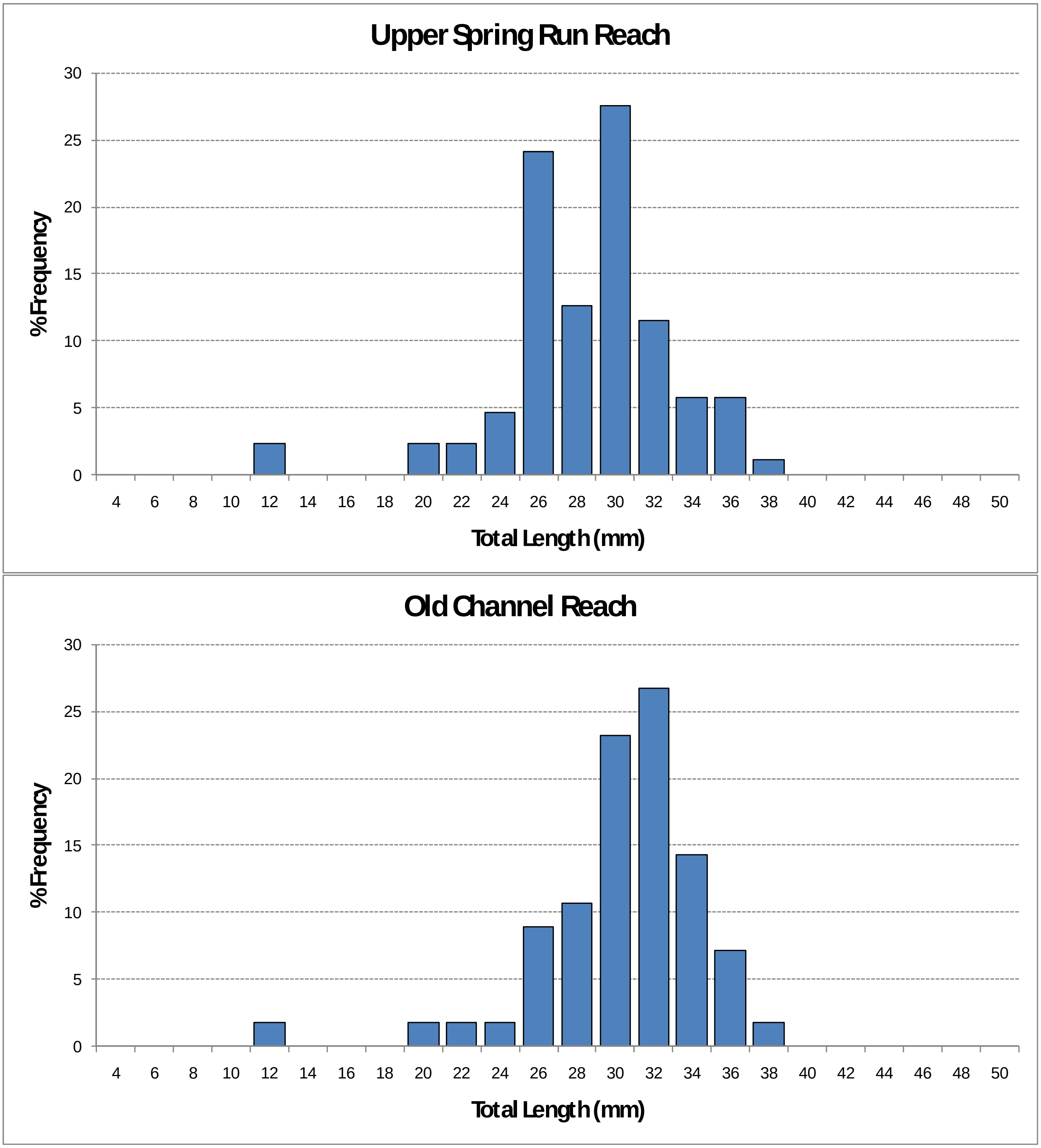


Figure 10. Length frequency distribution of fountain darters collected from the Upper Spring Run Reach and the Old Channel Reach in fall 2008.

In addition to fountain darters, 83,327 specimens representing 23 other fish taxa have been collected by drop netting from the Comal Springs/River ecosystem during the study period (2001 - 2008); of these, seven are considered exotic or introduced (Table 6). Although several of these species are potential predators of fountain darters, previous data collected during this study suggested that predation by both native and introduced predators is minimal during average discharge conditions. The impact of predation will be further evaluated under low discharge.

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

Another exotic species which has had considerable impact on the vegetation community in the Comal River/Springs ecosystem in the past is the giant ramshorn snail. In the early 1990s, giant ramshorn snails became very dense and caused substantial destruction to the vegetation community in the Comal River. However, numbers have since declined. Over 100 giant ramshorn snails were collected in drop net sampling in both 2000 and 2001. In the last three years, only one live giant ramshorn snail has been collected from the Comal Springs/River ecosystem during drop net sampling. Yet, because this exotic species can have considerable impacts at higher densities, close monitoring of their populations will be continued.

Table 6. Fish taxa and the number of individuals collected during drop net sampling on the Comal River/Springs ecosystem during the study period.

COMMON NAME	SCIENTIFIC NAME	STATUS	NUMBER COLLECTED	
			2008	2001-2008
Rock bass	*Ambloplites rupestris*	Introduced	0	18
Black bullhead	*Ameiurus melas*	Native	0	1
Yellow bullhead	*Ameiurus natalis*	Native	4	89
Mexican tetra	*Astyanax mexicanus*	Introduced	0	285
Central stoneroller	*Campostoma anomalum*	Native	0	1
Rio Grande cichlid	*Cichlasoma cyanoguttatum*	Introduced	36	391
Guadalupe roundnose minnow	*Dionda nigrotaeniata*	Native	1	261
Fountain darter	*Etheostoma fonticola*	Native	776	11242
Greenthroat darter	*Etheostoma lepidum*	Native	5	60
Gambusia	*Gambusia sp.*	Native	3768	76001
Suckermouth catfish	*Hypostomus plecostomus*	Exotic	0	60
Redbreast sunfish	*Lepomis auritus*	Introduced	0	132
Green sunfish	*Lepomis cyanellus*	Native	0	10
Warmouth	*Lepomis gulosus*	Native	0	24
Bluegill	*Lepomis macrochirus*	Native	0	30
Longear sunfish	*Lepomis megalotis*	Native	1	39
Redear sunfish	*Lepomis microlophus*	Native	0	1
Redspotted sunfish	*Lepomis miniatus*	Native	86	1161
Sunfish	*Lepomis sp.*	Native/Introduced	16	679
Spotted bass	*Micropterus punctulatus*	Native	0	1
Largemouth bass	*Micropterus salmoides*	Native	1	87
Texas shiner	*Notropis amabilis*	Native	27	61
Mimic shiner	*Notropis volucellus*	Native	1	29
Sailfin molly	*Poecilia latipinna*	Introduced	199	3888
Blue tilapia	*Oreochromis aurea*	Exotic	0	18
TOTAL			4921	94569

Dip Nets

Data gathered using dip nets are graphically represented in Figure 11 (Old Channel Reach) and in Appendix B for all reaches. The boundaries for each section of the dip net collection efforts are depicted in Figure 12.

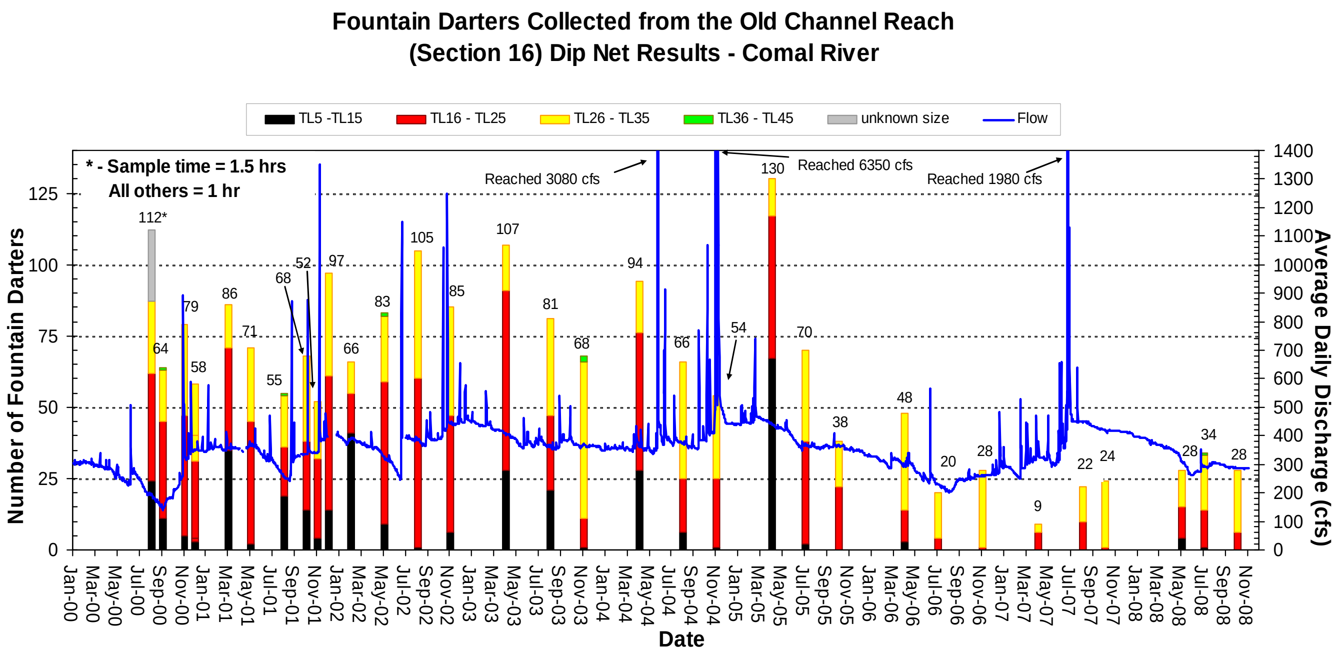


Figure 11. Number of fountain darters collected from the Old Channel Reach (Section 16) of the Comal River / Springs ecosystem using dip nets (2000-2008).

Dip net figures in Appendix B provide a good example of how changes in vegetation community can affect fountain darter population dynamics. These graphs indicate how numbers of fountain darters in each reach change in relation to flow (hydrograph) in the Comal River. In 2005 the vegetation community of the Old Channel Reach switched from being dominated by high-quality filamentous algae to one dominated by *Hygrophila*. This switch resulted in a corresponding change in the fountain darter population. Before 2005, the number of darters collected per sample ranged from 54 to 130 and all samples contained small darters (<15 mm) indicating year-round reproduction. Since this change in vegetation, total number of darters per sample has ranged from 9 to 48 and small darters have only been collected in spring months. However, bryophytes have recently become established in the Old Channel. If bryophytes become widespread in the Old Channel, it will likely lead to a rebound in the number of fountain darters collected in this reach.

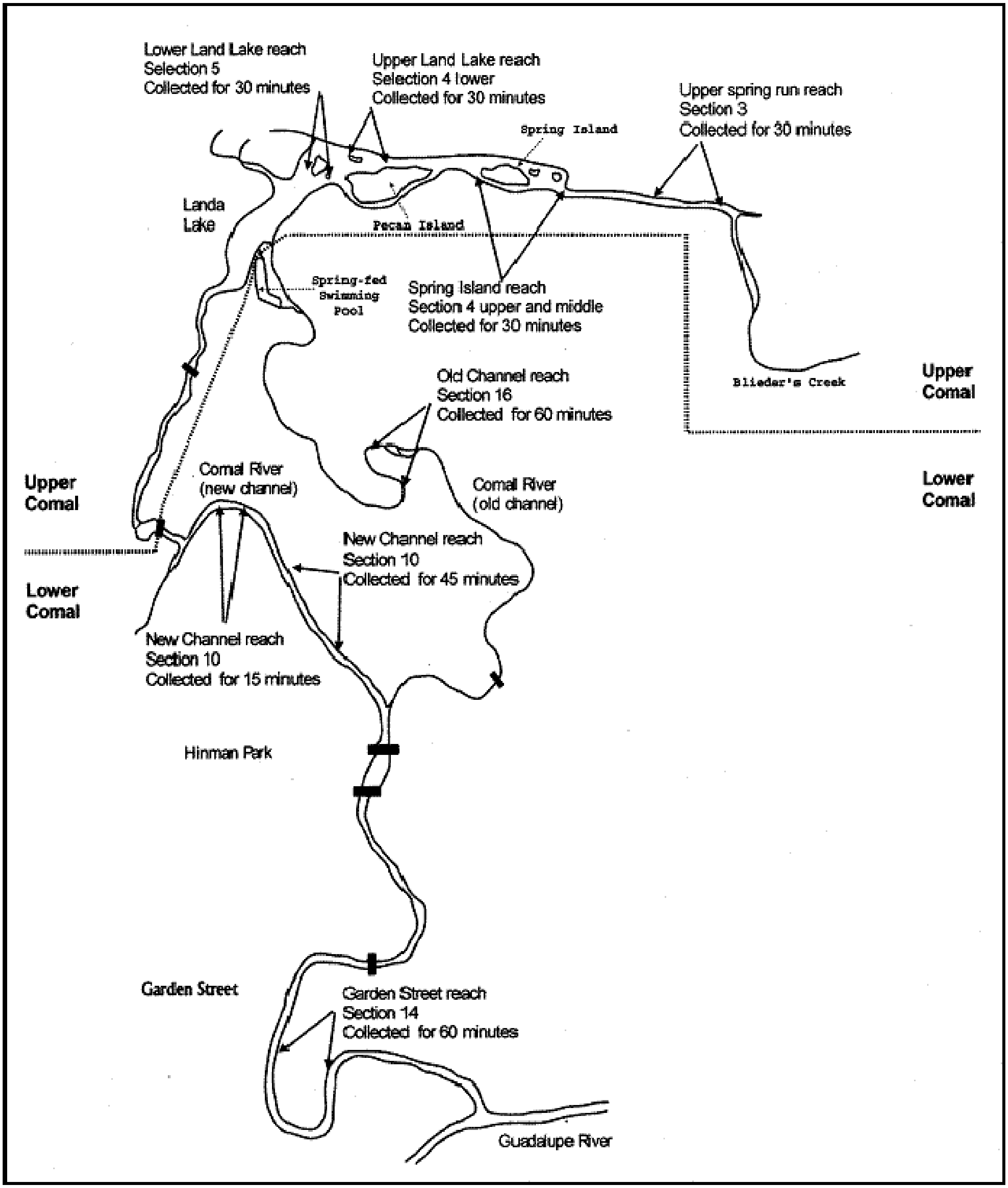


Figure 12. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

Overall, size class distributions of fountain darters from dip netting correlate well with those of drop netting: small fountain darters are most abundant in the spring, and larger darters dominate fall samples (Appendix B). However, small fountain darters are occasionally captured in summer, winter, and fall sample periods as well. This indicates that there is some reproduction occurring year-round, although perhaps on a limited basis and only in certain areas. These areas which exhibit year-round reproduction are relatively close to spring upwellings and contain large amounts of bryophytes, which provide high-quality fountain darter habitat according to drop net density estimates.

Variability in the total number of fountain darters collected by dip netting makes any inference into overall population trends difficult with this method. However, noticeable changes in numbers and size distributions of fountain darters have been observed in several sample reaches and are well correlated with changes in vegetation community. For example, there was a substantial increase in the number of darters collected from the Upper Spring Run Reach in 2003 which corresponded with an increase in bryophytes in this reach at approximately the same time. Similarly, vegetation shifts in the Old Channel Reach described above seem to have resulted in a decrease in the overall numbers of darters collected there since summer 2005.

Presence/Absence Dipnetting

The overall percentage of sites and percentage of dips in which fountain darters were present has remained relatively consistent since initiation of this technique in fall 2005 (Figure 13). The percentage of sites (N = 50) containing darters has varied from 52-70% across seven sample periods, and the percentage of dips (N = 4 @ each site = 50*4 = 200) containing darters has varied from 27-45%. Although the Comal River typically exhibits higher percentages than the San Marcos, the percent of darters present decreased in both spring and fall of 2008 (Figure 14). However, more data is needed to confirm this decrease, which may be due simply to sampling variation.

Although this technique does not provide detailed data on habitat use, and does not allow for quantification of population estimates, it does provide a quick and less intrusive method of examining large-scale trends in the fountain darter population. Therefore, data collected thus far provides a good baseline for comparison in future critical period events.

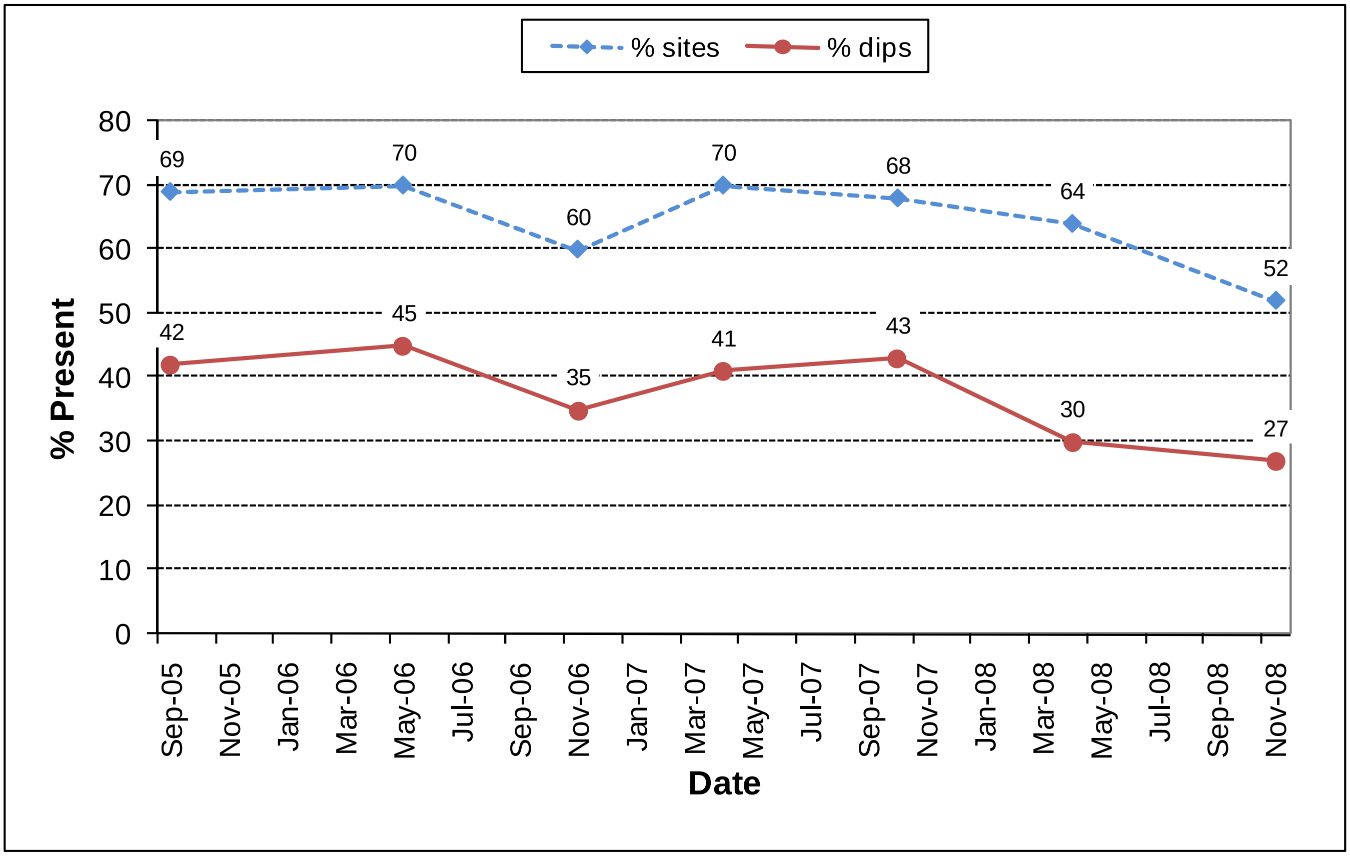


Figure 13. Percentage of sites (n = 50) and percentage of dips (n = 200) in which fountain darters were present during spring and fall 2005-2008.

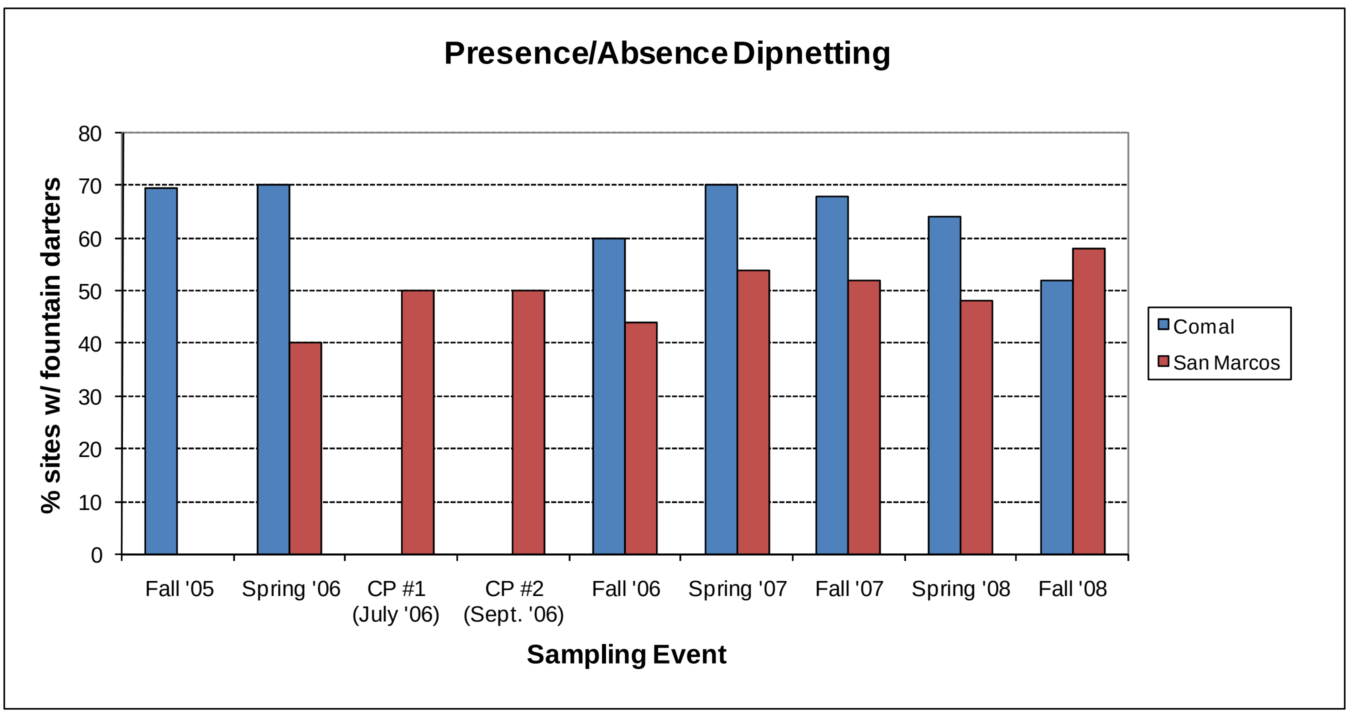


Figure 14. Percentage of sites (n = 50) on the Comal and San Marcos Rivers in which fountain darters were present during 2005-2008.

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. The quantitative sampling results are limited to a single grid per sampling event; therefore an accurate estimate of the true population size within the sample area is not possible. A much more labor-intensive effort would be required to provide such an estimate. These data simply provide an indication of the relative abundance of the fountain darters that are found in areas similar to that sampled and allow some insight into trends that may be occurring over time. Table 7 shows the number of fountain darters observed in the 7.8 m^2 grid per sampling event.

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

SAMPLE DATE	NUMBER OF FOUNTAIN DARTERS	PERCENT BRYOPHYTES WITHIN GRID
Summer 2001	24	50
High Flow 1 2001	31	50
Fall 2001	44	65
High Flow 2 2001	39	60
Winter 2002	50	90
Summer/High Flow 2002	21	40
Fall 2002	88	80
Spring 2003	43	85
Summer 2003	51	90
Fall 2003	56	80
Spring 2004	45	60
Summer 2004	12	15
Fall 2004	48	70
Spring 2005	49	90
Fall 2005	65	95
Spring 2006	32	35
Spring 2007	27	75
Fall 2007	87	95
Spring 2008	65	90
Fall 2008	44	50

Spring 2008 exhibited a slight decrease in the number of darters compared to fall 2007 associated with a small decrease in bryophyte coverage. As bryophytes became fragmented and considerably reduced by fall 2008, the percentage of vegetation decreased to nearly half that present in spring 2008, and the corresponding reduction in fountain darters was evident. These data reinforce the importance of bryophytes as fountain darter habitat.

Comal Springs Salamander Visual Observations

Salamander counts in Spring Run 1 were consistent in spring and fall 2008 (27 and 26, respectively) (Table 8). Good habitat (bryophytes and plentiful fist-sized rocks) at this sampling site was a likely contributor to this increase from 2007. At Spring Run 3 more salamanders were found than ever before in the spring (28). Although the number observed declined by fall (19) it was still indicative of a healthy population at this site. Overall, total salamanders observed in spring (61) and fall (51) were still well above the average (45.9) for this study.

For the third consecutive sampling session (since fall 2007) no salamanders were observed at the Spring Island Spring Run (Table 8). This specific population has been declining at this site we have observed that fist-sized rocks (typical of this salamanders' habitat) are nearly non-existent. It is undocumented, but likely that people have been removing larger rocks from this small spring run. There were also fewer salamanders observed at the Spring Island East Outfall site in 2008 (6 each sampling period). It was observed that a large mat of green algae had covered up rocks that are typically covered in bryophytes. This algal mat may have contributed to a population shift away from this sample area. The mat also made sampling more difficult which likely also contributed to these lower numbers.

Table 8. Total number of Comal Springs salamanders observed at each survey site during each sampling period.

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
August 2000	9	13	11	1	34
September 2000	5	14	6	5	30
Fall 2000	8	4	4	2	18
Winter 2001	16	9	8	1	34
Spring 2001	20	7	17	6	55
Summer 2001	23	15	4	4	46
High-flow 1 2001	31	12	1	6	50
Fall 2001	11	8	13	7	39
High-flow 2 2001	18	2	6	5	31
Winter 2002	18	9	7	3	53
Spring 2002	10	15	6	5	62
High Flow 2002	18	7	3	16	67
Fall 2002	20	10	8	9	47
Spring 2003	20	21	6	13	60
Summer 2003	25	10	3	13	51
Fall 2003	31	10	3	19	63
Spring 2004	36	14	7	12	69
Summer 2004	27	14	4	14	59
Fall 2004	20	2	2	35	59
Spring 2005	18	10	2	11	41
Fall 2005	22	7	0	16	45
Spring 2006	12	13	2	8	35
Fall 2006	14	11	2	29	56
Spring 2007	15	10	2	23	50
Fall 2007	18	13	0	11	42
Spring 2008	27	28	0	6	61
Fall 2008	26	19	0	6	51
Average	**19.2**	**11.4**	**4.7**	**10.6**	**45.9**

Macroinvertebrate Sampling

In 2008, drift net sampling around spring openings and regular monitoring of Comal Springs riffle beetles in several locations were designed to assess habitat requirements and population dynamics of the federally listed invertebrate species.

Drift Net sampling

A total of 10 taxa were captured from 144 hours of sample time at the three drift net sites in Comal Springs during 2008 (Table 9). Table 10 displays the physico-chemical data collected at these sites during sampling. Total discharge in the Comal River was approximately 344 cfs during the May sample and 284 cfs during the November sample. The flows in November were considerably lower than the previous year's fall sampling (418 cfs). Species of the genus *Stygobromus* and *Lirceolus* continued to be most abundant at all sites (Table 9). *Stygobromus pecki* (Peck's cave amphipod) was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long, which suggests that smaller individuals may be more susceptible to expulsion from the aquifer. Those individuals that were too small to identify to species were recorded as *Stygobromus* sp. and most likely consisted of both *S. russelli* and *S. pecki*. A total of 7 (2 larvae, 5 adults) *Heterelmis comalensis* and no *Stygoparnus comalensis* were found in the drift net samples in the Spring Run sites in 2008. It is not clear why *Stygoparnus comalensis* were not located this year after 11 had been observed in 2007.

During the drift survey, what appears to be a troglobitic flatworm was collected alive and transferred to Krista McDermid (Zara Environmental) for proper preservation. There is only 1 described blind flatworm recorded in Texas, *Sphalloplana mohri* from caves and wells in Hays, Kendall, Mason, San Saba, and Travis Counties, and one undescribed, *Phagocata* sp. from the hyporheos of Hondo Creek in Medina County. No specimens of the subterranean amphipod *Parabogidiella* sp. were collected in 2008 (as they were in 2007 and 2006).

Table 9. Total numbers of troglobitic and endangered species collected in drift nets during May and November, 2008. Federally endangered species are designated with (E). A = adult beetles. L = larvae.

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	48	48	48	144
Crustaceans				
Amphipoda				
Crangonyctidae				
Stygobromus pecki (E)	18	26	37	81
Stygobromus russelli	3	1	1	5
Stygobromus spp.	111	154	363	628
All *Stygobromus*	132	181	401	714
Hadziidae				
Mexiweckelia hardeni	3	6	1	10
Sebidae				
Seborgia relicta		2		2
Bogidiellidae				
Artesia subterranea				
Parabogidiella n. sp				
Isopoda				
Asellidae				
Lirceolus (2spp.)	151	34	7	192
Cirolanidae				
Cirolanides texensis	1		1	2
Arachnids				
Hydrachnoidea				
Hydryphantidae				
Almuerzothyas n. sp	13			13
Insects				
Coleoptera				
Dytiscidae				
Comaldessus stygius		2 A		2
Haideoporus texanus				
Dryopidae				
Stygoparnus comalensis				
Elmidae				
Heterelmis comalensis	6 (1L, 5A)	1 L		7

Table 10. Results of water quality measurements conducted in 2008 during drift net sampling efforts at Comal Springs.

	Spring Run 1		Spring Run 3		West Shore Upwelling	
Date	May	Nov	May	Nov	May	Nov
Temperature (°C)	23.1	23.1	23.3	23.3	23.8	23.7
Conductivity (mS)	0.532	0.559	0.5	0.559	0.544	0.559
pH	7.2	7.0	7.2	7.0	7.2	7.0
Dissolved Oxygen (mg/L)	5.7	5.7	5.6	5.6	5.4	5.5
Current Velocity (m/s)	0.5	0.6	0.4	0.6	0.4	0.3

As in previous years, water quality variables remained relatively constant at all sites in 2008, indicating a stable environment for the organisms at the observed discharges.

Comal Springs Riffle Beetle

Comal Springs riffle beetle sampling conducted as part of this study provides basic information on the population dynamics and distribution of the species among sample sites. The total number of beetles collected in 2008 (504) were substantially higher than the number observed in 2007 (Table 11, Figure 15). In past years, cotton lures were often disturbed (e.g. animals) and this was not the case in 2008.

Four elmid pupae were collected (the size suggest that they were *Heterelmis*, but could be *Microcylloepus*) from Spring Island on 2 cotton lures that were not near the shore. Typically, elmids pupate above water line in moist soil, under rocks, or in rotting wood (White and Roughley 2008). These pupae appeared hydrophobic (floating on top of the water tension) and may have been pupating in airspaces created by trapped air bubbling (off-gassing as result of super saturation) through the upwellings.

As in previous years, beetles tended to be patchily distributed with wide ranges of abundance between sites and seasons. Therefore, temporal patterns in overall abundance of Comal Springs riffle beetles are extremely variable (Figure 12). A large increase in abundance of beetles was apparent in spring 2004 when the current method of sampling beetles using cotton lures placed in spring openings was initiated. In 2003, beetles were actively sampled by examining rocks near spring areas, which resulted in much lower catch rates than the current methodology. Since sampling with cotton lures began, the number of Comal Springs riffle beetles has varied between 293 and 648 per sample period. Although this limited amount of data does not allow for detailed analysis of population trends at this time, it will provide critical baseline data for comparison to that collected during potential critical periods in the future.

Table 11. Total numbers of Comal Springs riffle beetles (*Heterelmis comalensis*) at each survey site during each sampling period.

Sample Period	Spring Run 3	West Shore	Spring Island	Total
January 03	65	7	47	**119**
March 03	32	5	10	**47**
September 03	10	15	42	**67**
November 03	16	9	18	**43**
May 2004	88	83	122	**293**
August 2004	169	143	90	**402**
November 2004	170	175	146	**491**
April 2005	119	121	121	**361**
November 2005	262	201	185	**648**
May 2006	256	195	160	**611**
November 2006	185	92	125	**402**
May 2007	59	161	119	**339**
November 2007	204	83	132	**419**
May 2008	155	139	156	**450**
November 2008	144	133	227	**504**

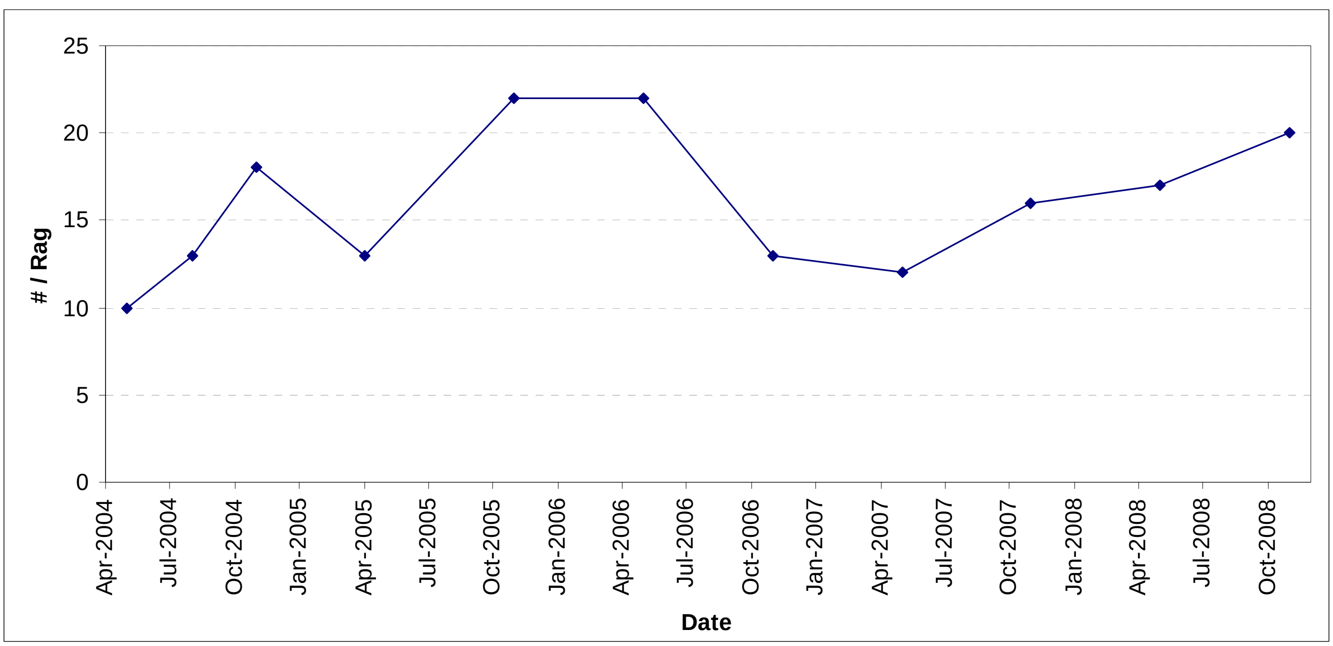


Figure 15. Density (#/cotton lure) of Comal Springs riffle beetles (*Heterelmis comalensis*) combined for each sampling data for 2004 – 2008.

Master Naturalist Monitoring

Since the summer of 2006, Master Naturalist volunteers have assisted BIO-WEST by collecting weekly water quality and recreation data on the Comal River/Springs ecosystem. Volunteers collected data at 5 sites (Figure 16) on a weekly basis (typically on a Friday afternoon). At each site, an Oakton Waterproof pHTestr 2 was used to assess pH, and a LaMotte Carbon Dioxide Test Kit was used to measure carbon dioxide concentrations in the water column. In addition to water quality measurements, recreational use data was collected at each site by counting the number of tubers, kayakers, anglers, etc. using the area at the time of sampling. Photos were taken at each site and any other notes on recreational use or condition of the river were recorded during each sampling event.

Water quality data collected by Master Naturalist volunteers in 2008 showed that CO_2 concentrations were highest near springs (Figure 17), while pH increased as you go downstream (Figure 18). At all sites, CO_2 concentrations were similar between years (2006-2008). Carbon dioxide will continue to be a key parameter to monitoring during Critical Period conditions because of its influence on aquatic vegetation.

Recreational use data continues to document that of the sites evaluated the New Channel is the most heavily recreated. This is not surprising since it is both within a park setting and is heavily used by tubers. The Union Avenue site is the second most heavily used of the recreation sites, notably because it is an exit station for tubers during the summer months. During 2007, these two sites were utilized primarily between the last week in April until the first week in September (82% and 88% of annual utilization, respectively). Similarly in 2008, 81% of the recreationalists at the New Channel and 85% of those at Union Avenue were recorded during this summer period. The gazebo site in Landa Park ranked third in numbers of recreators, but there are more land-based recreation activities that occur at this site (i.e. shore angling, dog walking, etc.) than at the two previously mentioned sites. The gazebo site is also utilized year-round with fewer numbers of people present during each of the sampling days, rather than having mainly summertime tubing-related visitors (as with the New Channel and Union Avenue sites).

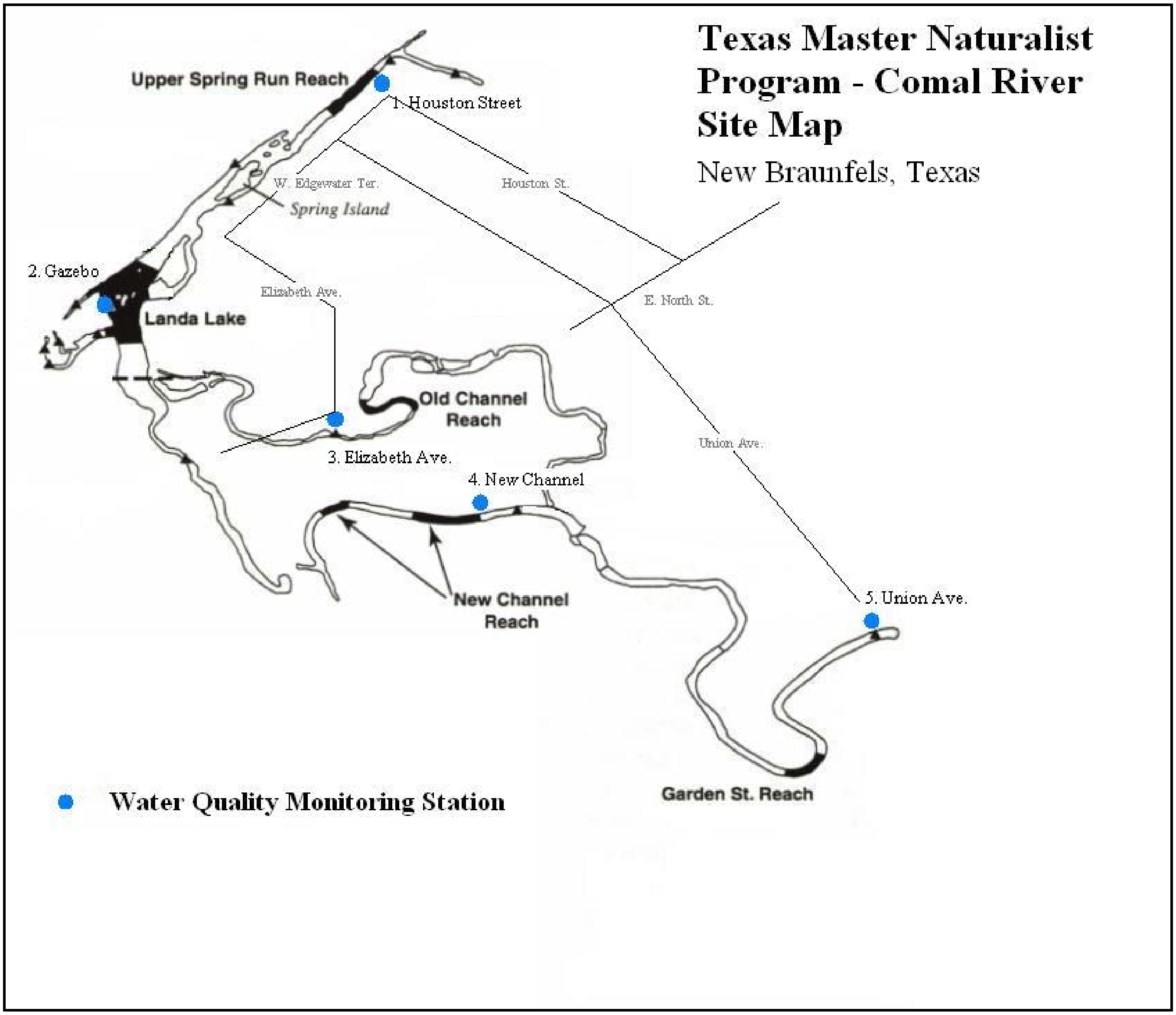


Figure 16. Water quality / recreation sites on the Comal River monitored weekely by Texas Master Naturalist volunteers (2006 – 2008).

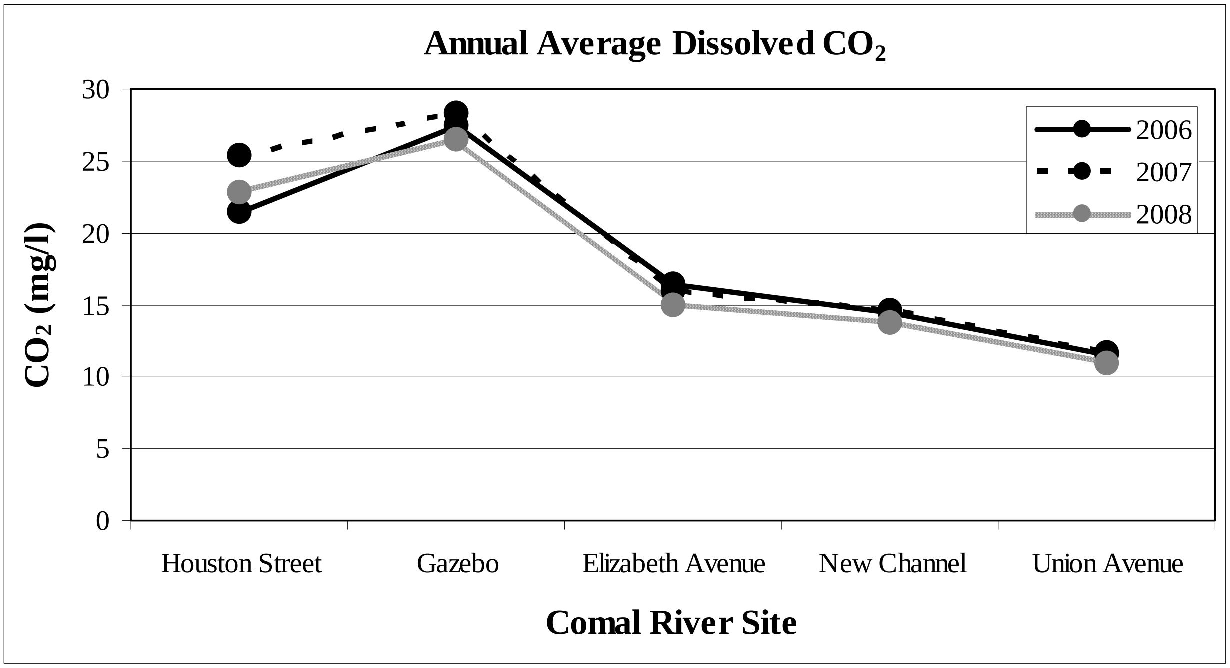


Figure 17. Mean dissolved CO_2 values at all sites from July 2006 – November 2008.

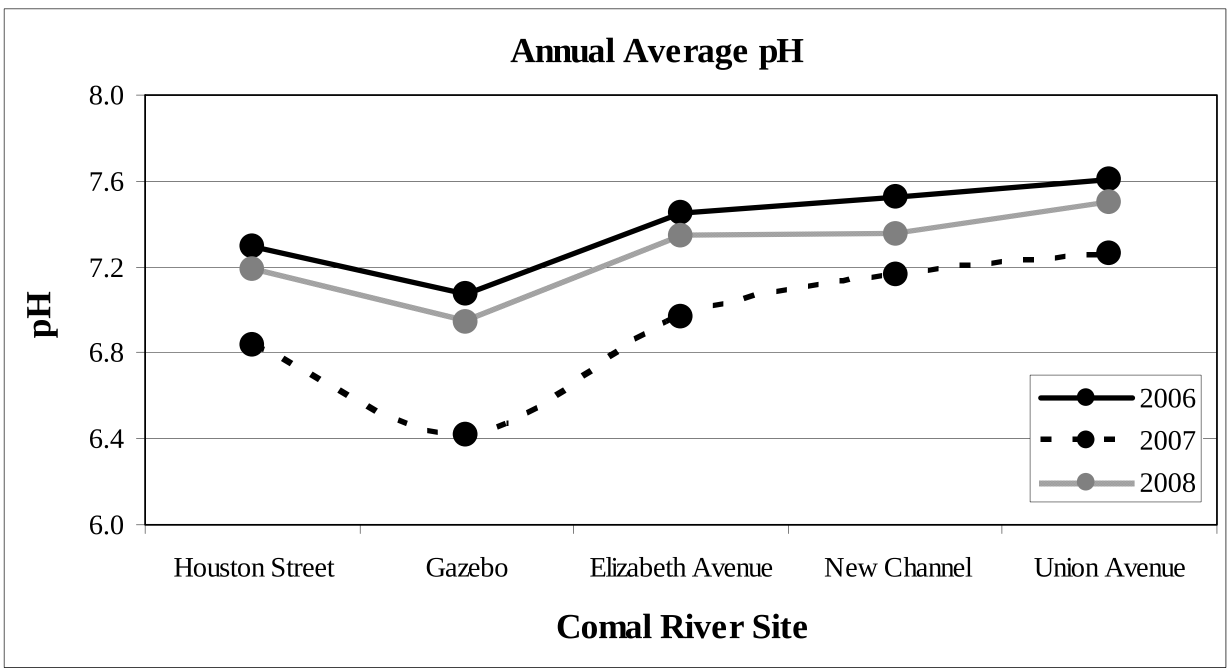


Figure 18. Mean pH values at all sites from July 2006 – November 2008.

Exotics / Predation Study

Because there were no Critical Period events triggered in 2008, no samples were made for the exotics / predation component of this study.

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White, D. S., and R. E. Roughley. 2008. Aquatic Coleoptera - Elmide (Riffle Beetles). Pages 632 *in* R. W. Merritt, K. W. Cummins, and M. B. Berg, editors. An Introduction to the Aquatic Insects of North America, Fourth Edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

APPENDIX A:
AQUATIC VEGETATION MAPS

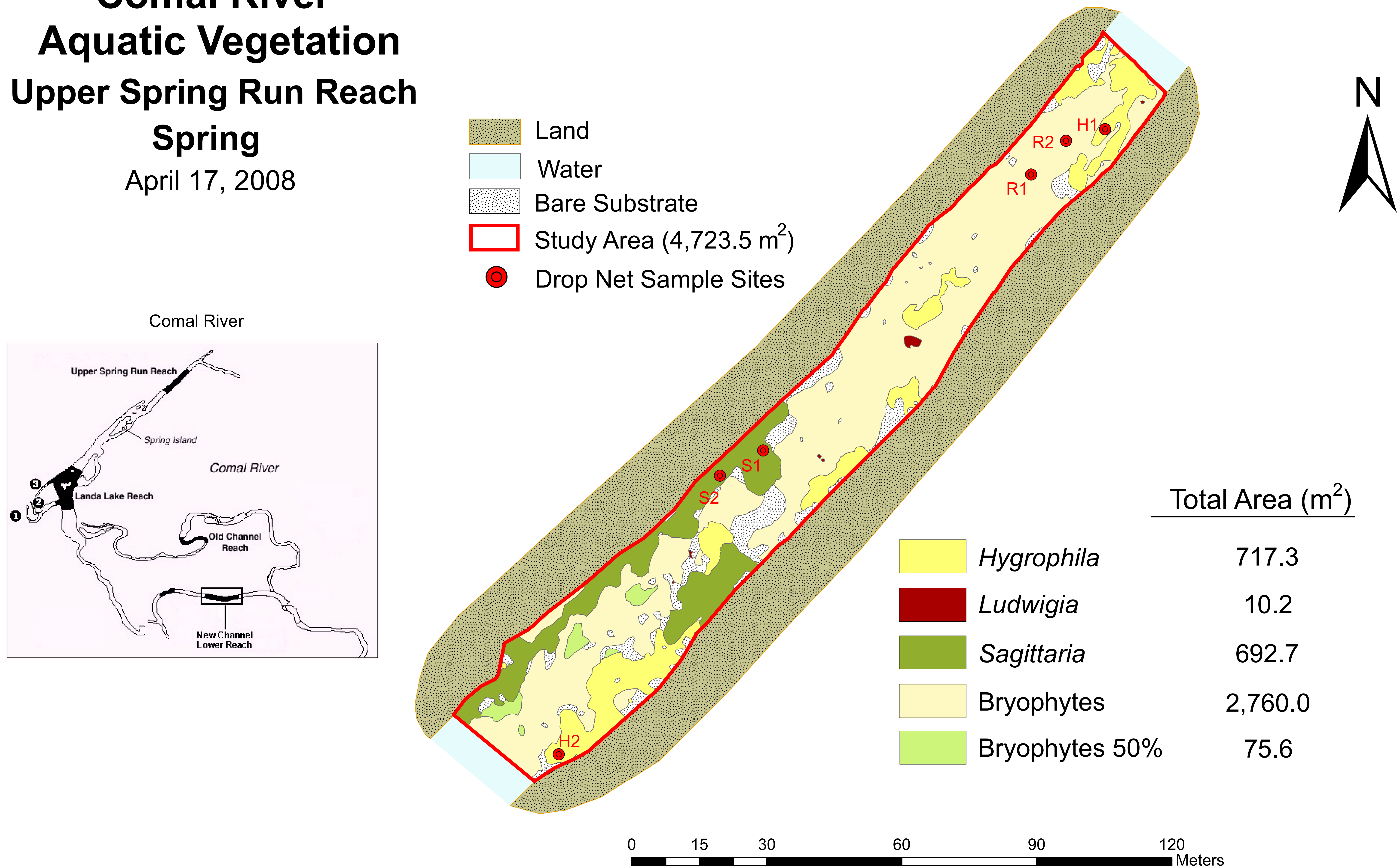
Upper Spring Run Reach

Comal River Aquatic Vegetation

Upper Spring Run Reach

Spring

April 17, 2008

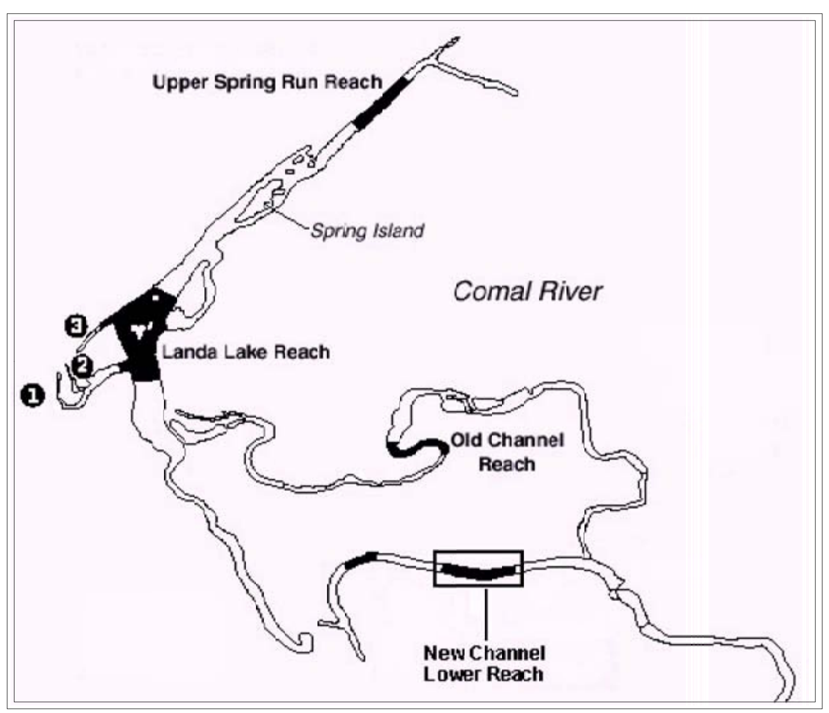


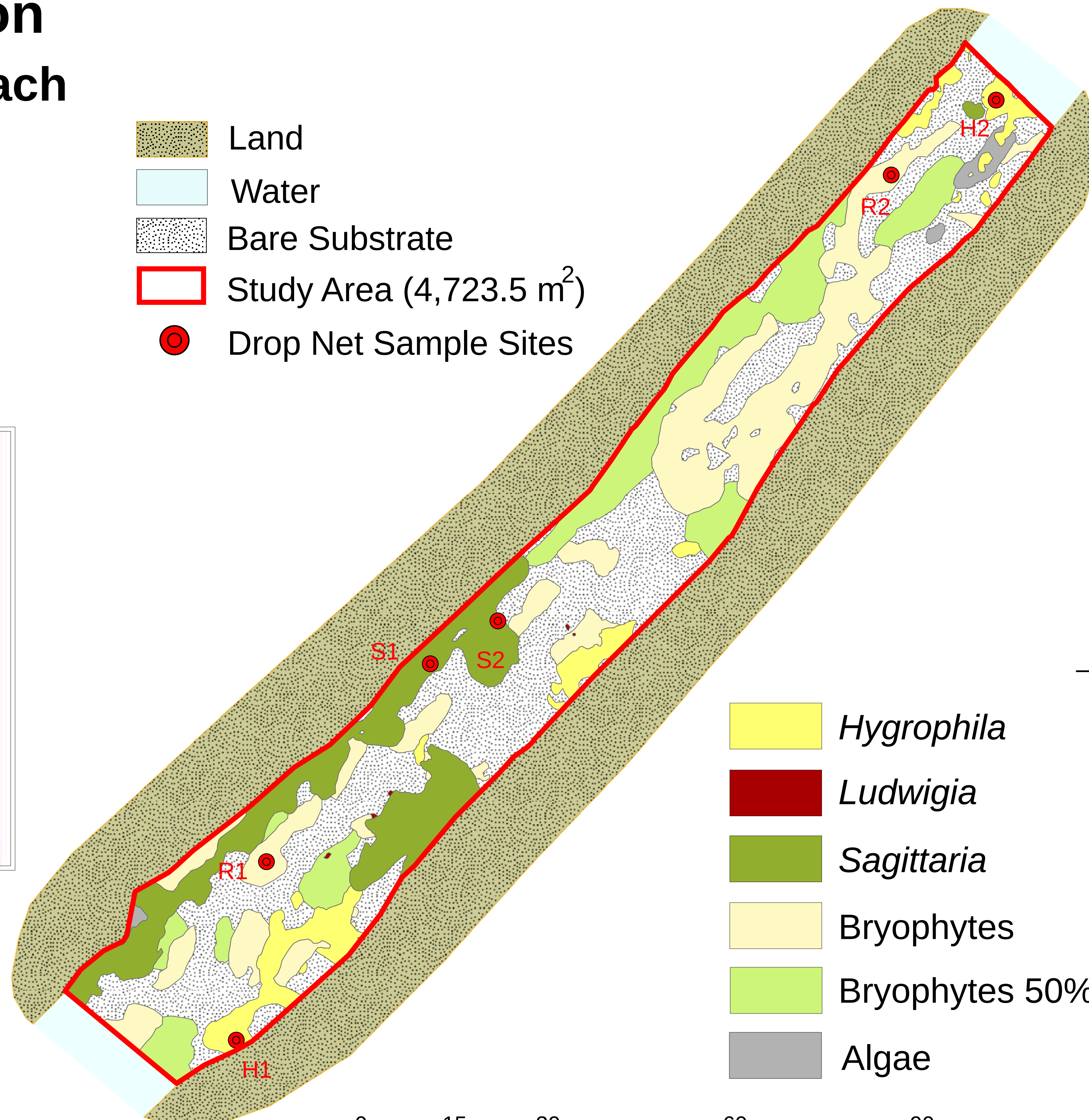
Comal River
Aquatic Vegetation
Upper Spring Run Reach
Fall
October 23, 2008

- Land
- Water
- Bare Substrate
- Study Area (4,723.5 m^2)
- Drop Net Sample Sites

N

Comal River



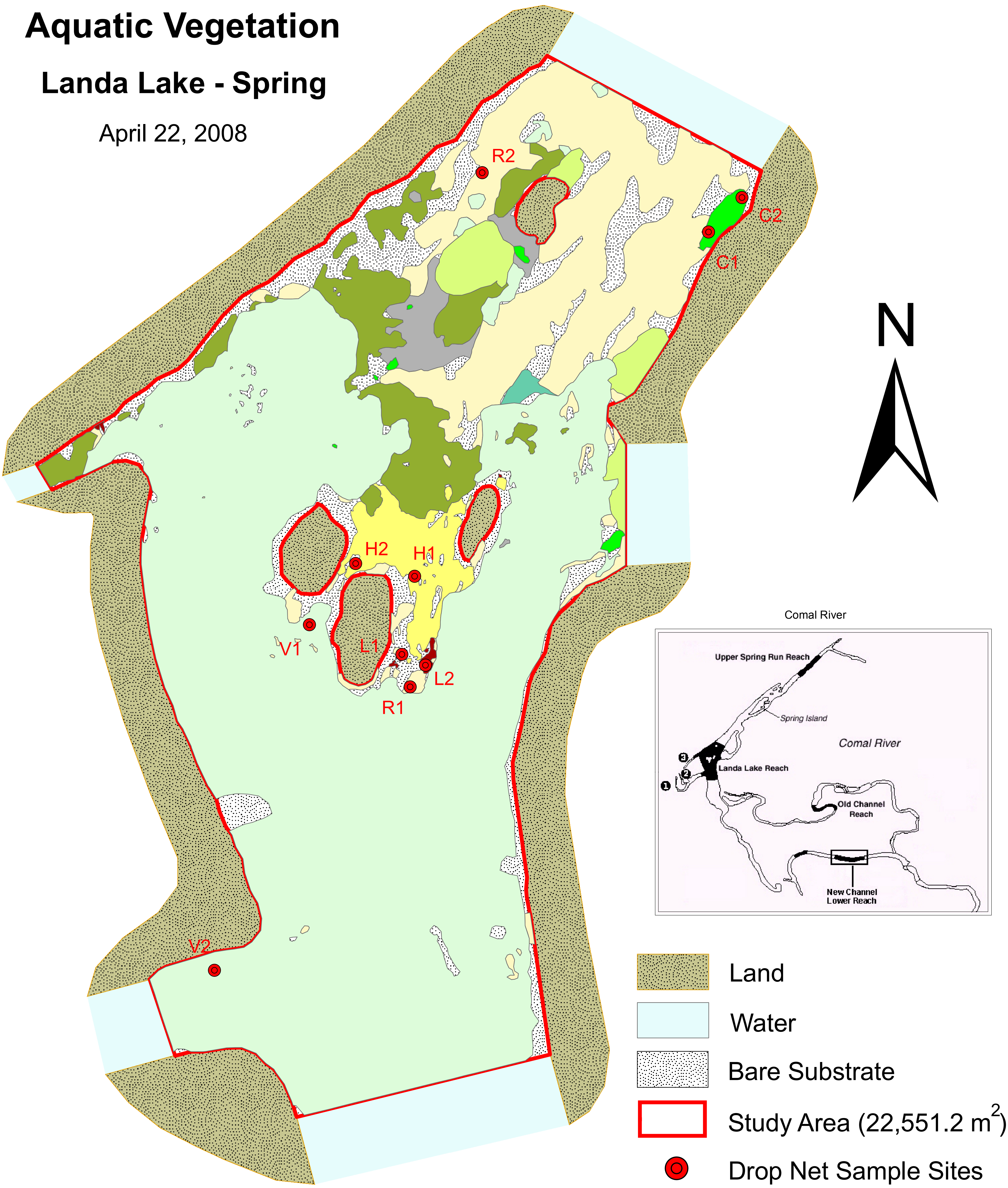


	Total Area (m^2)
Hygrophila	342.5
Ludwigia	2.1
Sagittaria	729.3
Bryophytes	1,057.4
Bryophytes 50%	677.0
Algae	50.1

0 15 30 60 90 120 Meters

Landa Lake Reach

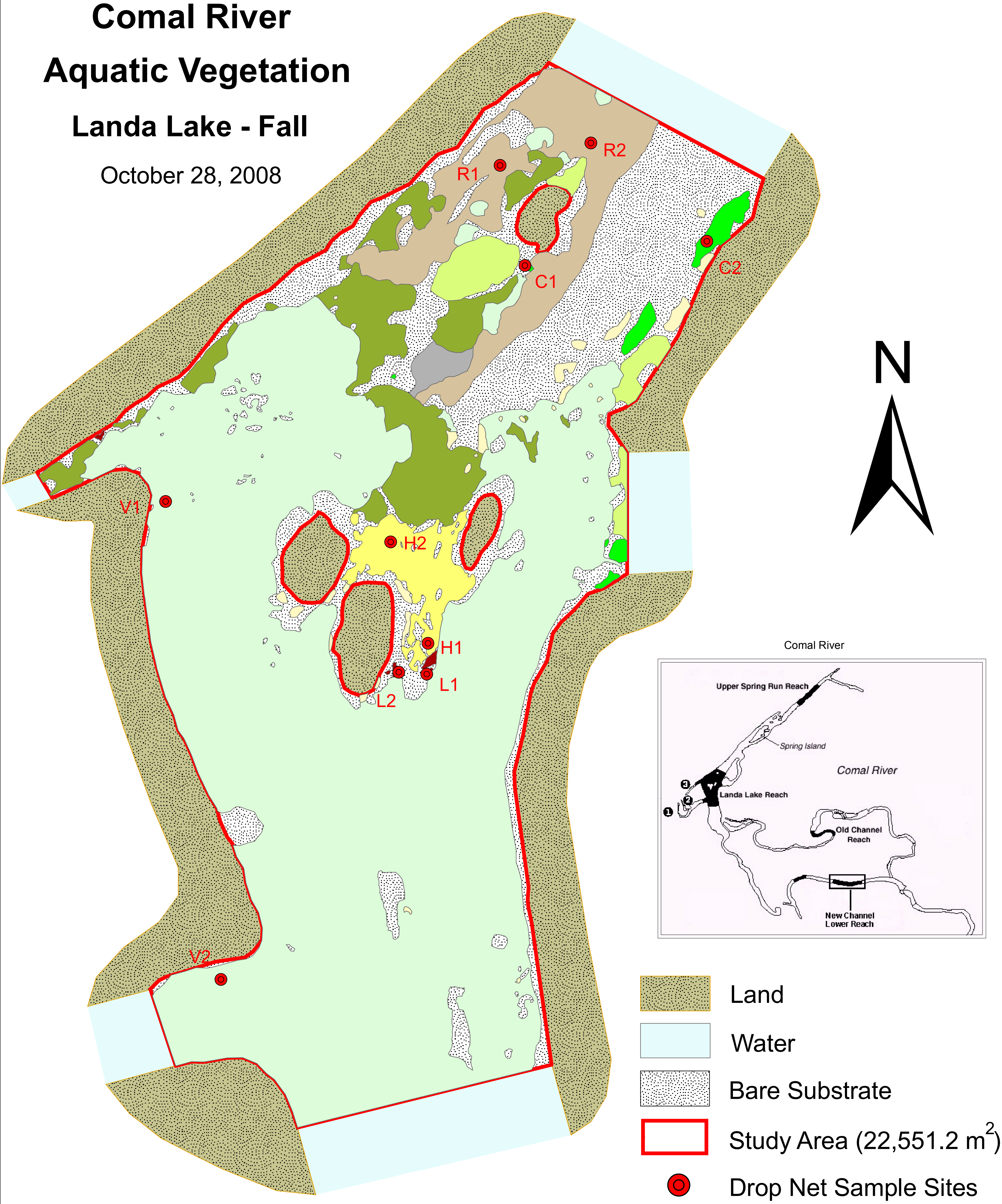
Comal River
Aquatic Vegetation
Landa Lake - Spring
April 22, 2008



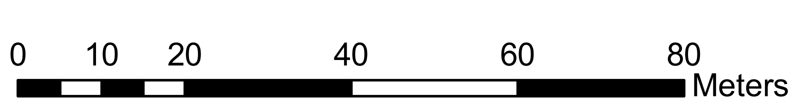
	Total Area (m^2)		Total Area (m^2)
Algae	351.0	*Nuphar*	503.7
Bryophytes	3,363.8	*Sagittaria*	1,445.8
Cabomba	158.7	*Vallisneria*	13,784.8
Hygrophila	598.8	Bryophytes 50%	50.2
Ludwigia	27.1		

0 10 20 40 60 80 Meters

Comal River
Aquatic Vegetation
Landa Lake - Fall
October 28, 2008



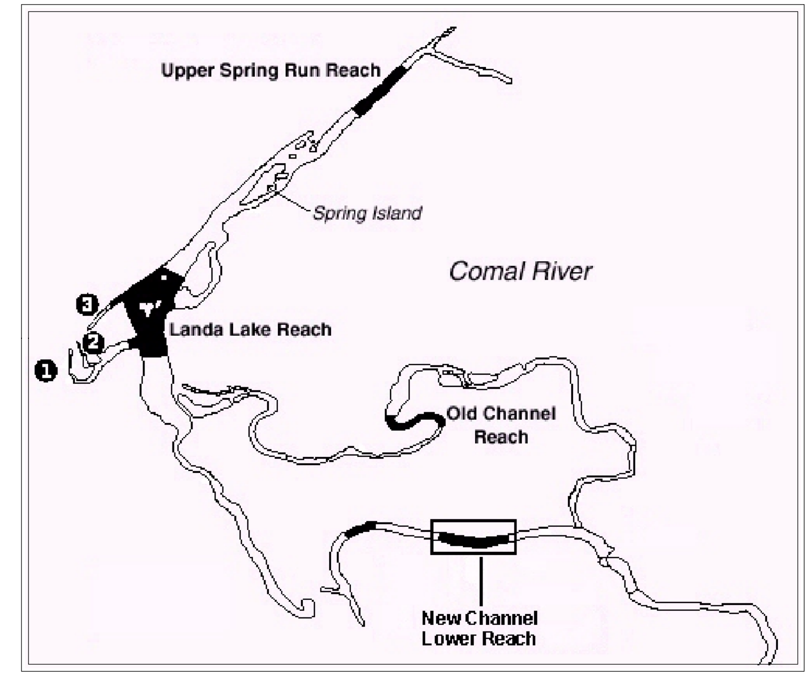
	Total Area (m^2)		Total Area (m^2)
Algae	114.4	*Nuphar*	431.6
Bryophytes	176.3	*Sagittaria*	1,506.9
Cabomba	178.4	*Vallisneria*	13,690.6
Hygrophila	515.2	Bryophytes / Algae	1,587.5
Ludwigia	17.4		



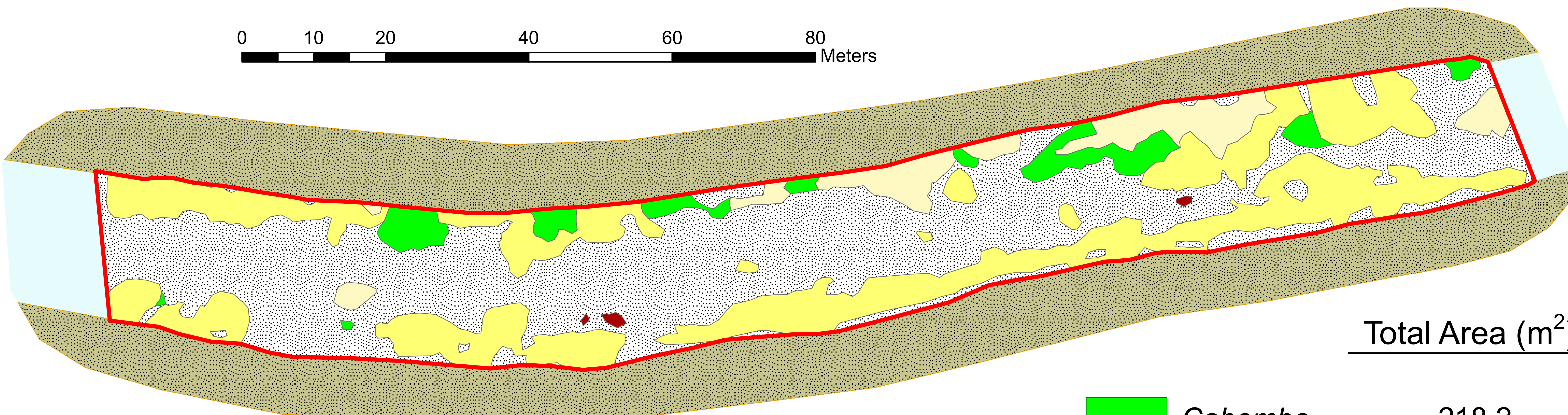
New Channel Reach

Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring
April 18, 2008

Comal River

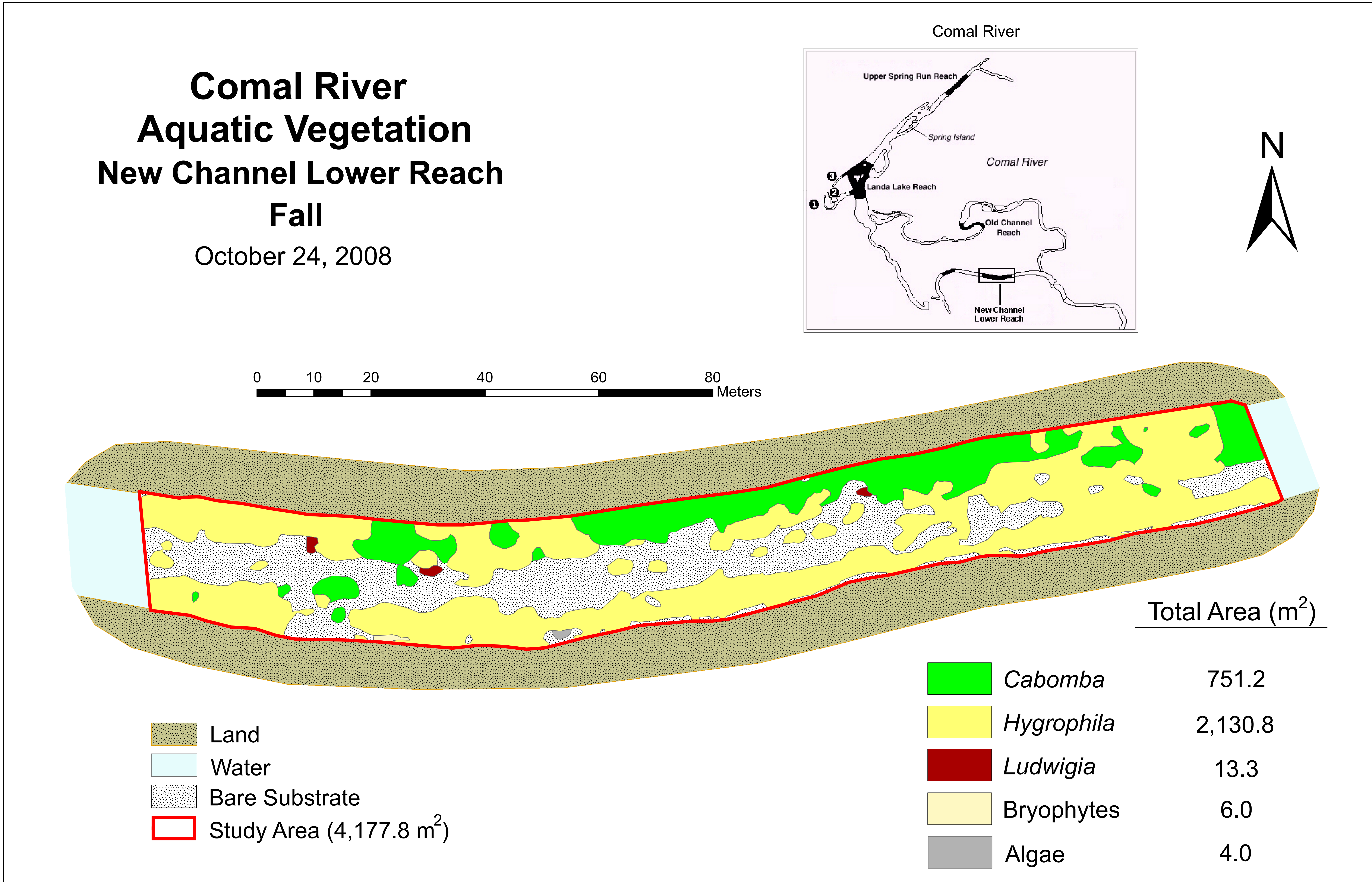


N



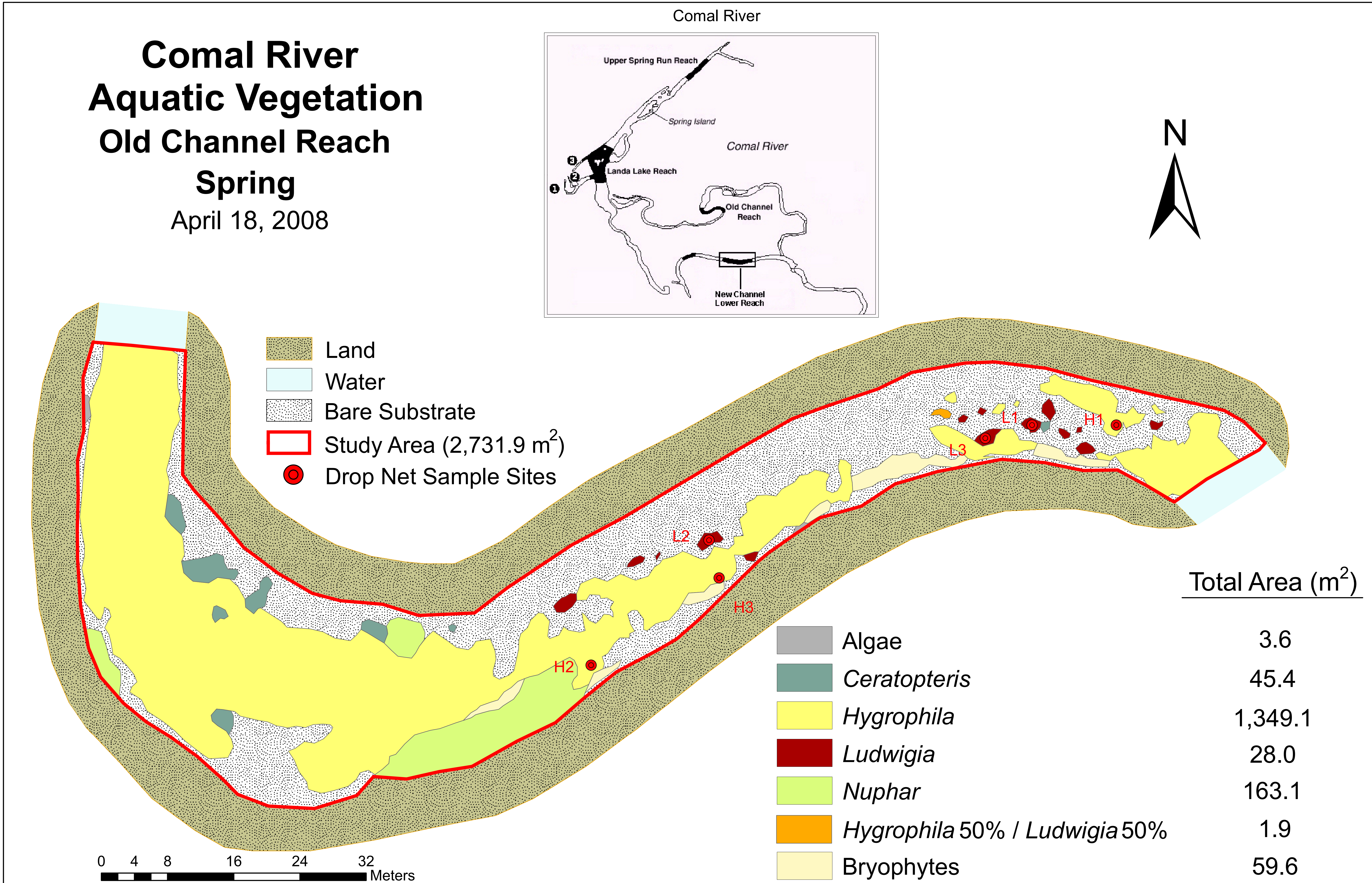
- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m^2)

	Total Area (m^2)
Cabomba	218.2
Hygrophila	1,340.4
Ludwigia	7.6
Bryophytes	295.3



Comal River
Aquatic Vegetation
New Channel Lower Reach
Fall
October 24, 2008
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
0 10 20 40 60 80 Meters
Land
Water
Bare Substrate
Study Area (4,177.8 m²)
Total Area (m²)
Cabomba 751.2
Hygrophila 2,130.8
Ludwigia 13.3
Bryophytes 6.0
Algae 4.0

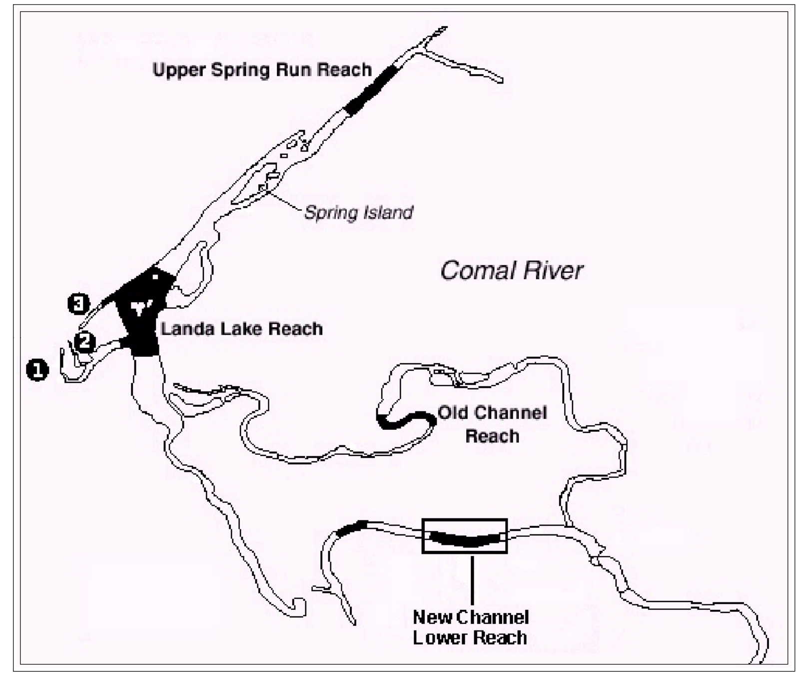
Old Channel Reach

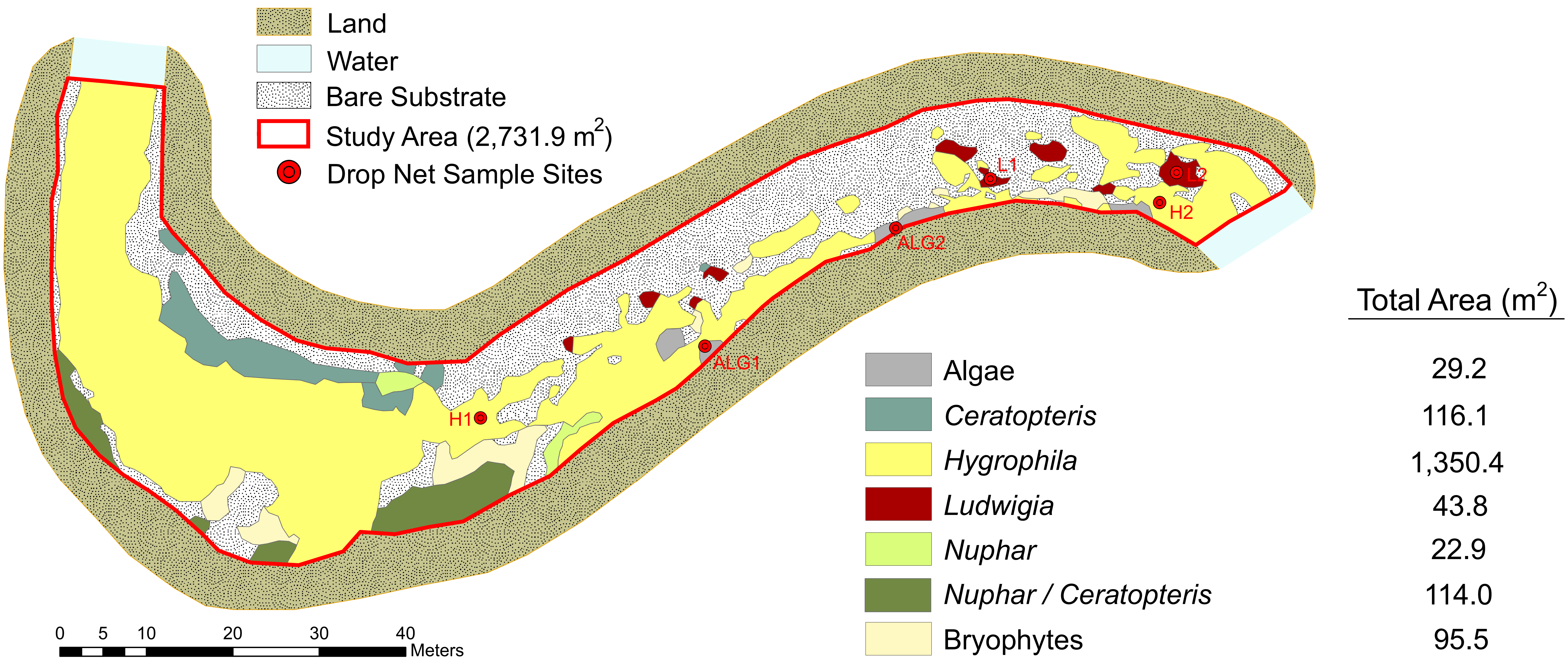


Comal River
Aquatic Vegetation
Old Channel Reach
Spring
April 18, 2008
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
Land
Water
Bare Substrate
Study Area (2,731.9 m2)
Drop Net Sample Sites
L1
H1
L3
L2
H3
H2
Total Area (m2)
Algae 3.6
Ceratopteris 45.4
Hygrophila 1,349.1
Ludwigia 28.0
Nuphar 163.1
Hygrophila 50% / Ludwigia 50% 1.9
Bryophytes 59.6
0 4 8 16 24 32
Meters

Comal River
Aquatic Vegetation
Old Channel Reach
Fall
October 24, 2008

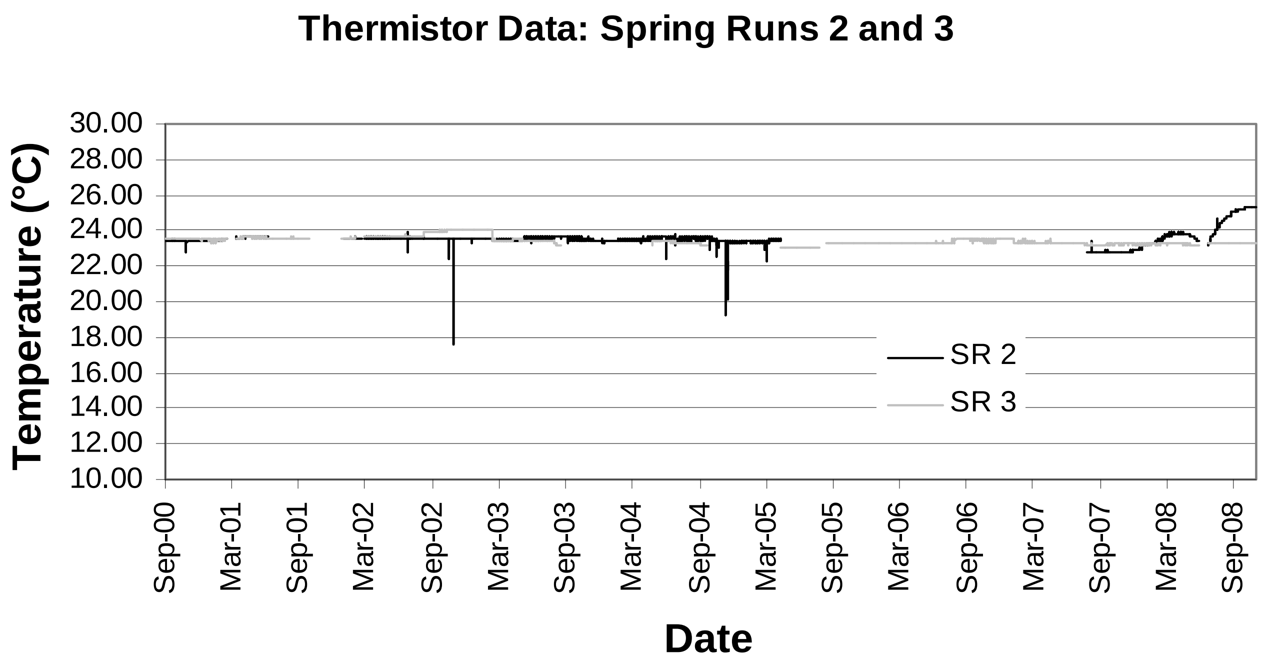
Comal River



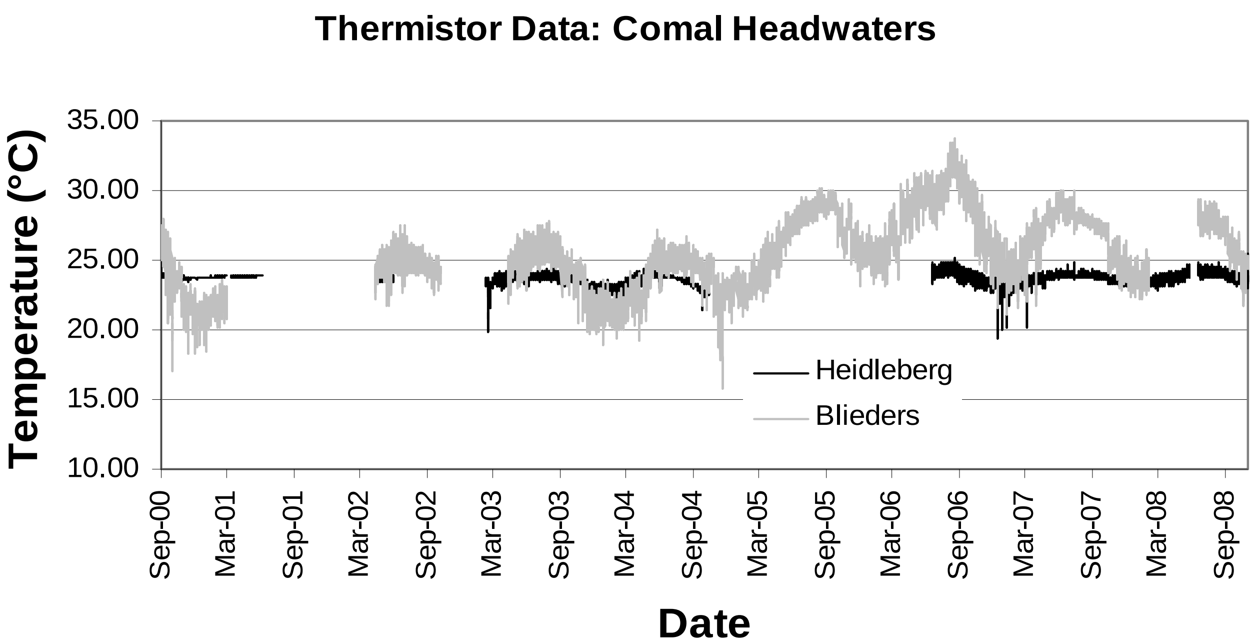


APPENDIX B:
DATA AND GRAPHS

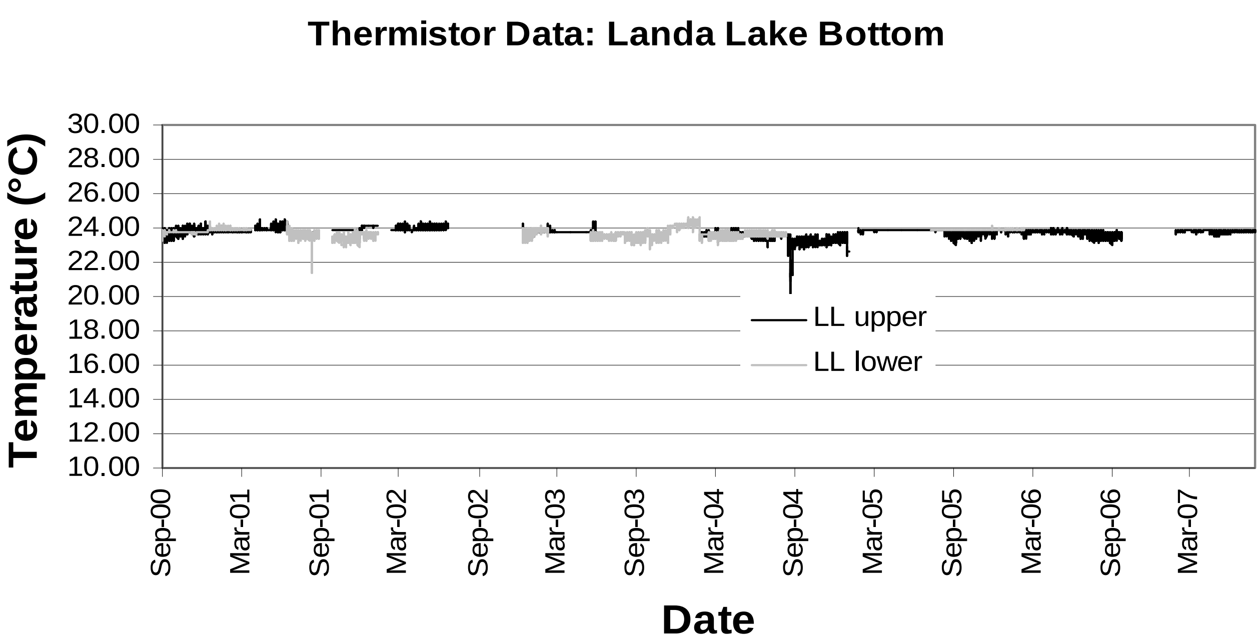
Water Quality Data
and
Thermistor Graphs



Thermistor Data: Spring Runs 2 and 3
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
SR 2
SR 3
Sep-00
Mar-01
Sep-01
Mar-02
Sep-02
Mar-03
Sep-03
Mar-04
Sep-04
Mar-05
Sep-05
Mar-06
Sep-06
Mar-07
Sep-07
Mar-08
Sep-08
Date

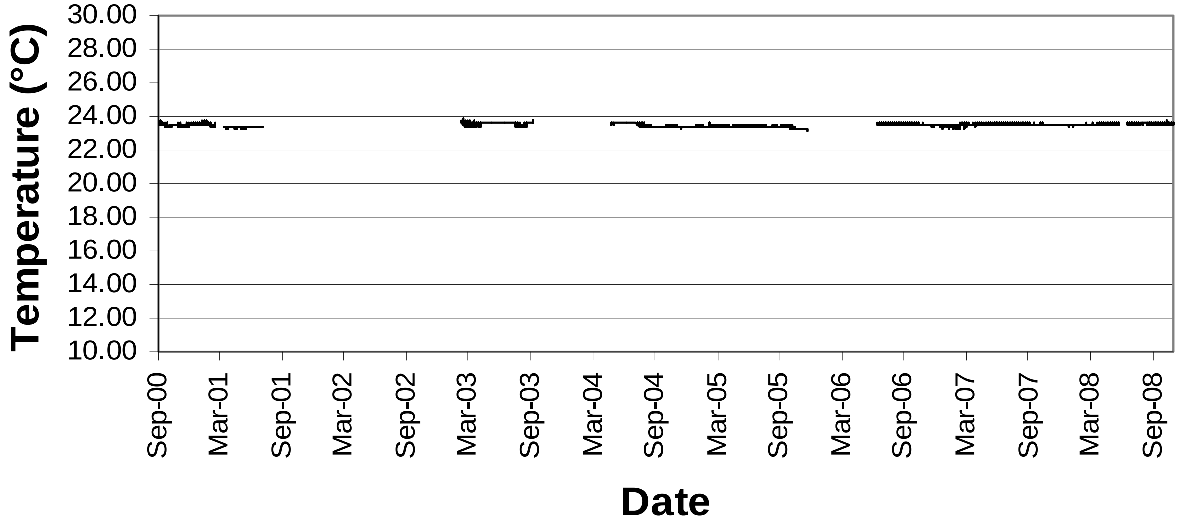


Thermistor Data: Comal Headwaters
Temperature (°C)
35.00
30.00
25.00
20.00
15.00
10.00
Heidleberg
Blieders
Sep-00
Mar-01
Sep-01
Mar-02
Sep-02
Mar-03
Sep-03
Mar-04
Sep-04
Mar-05
Sep-05
Mar-06
Sep-06
Mar-07
Sep-07
Mar-08
Sep-08
Date

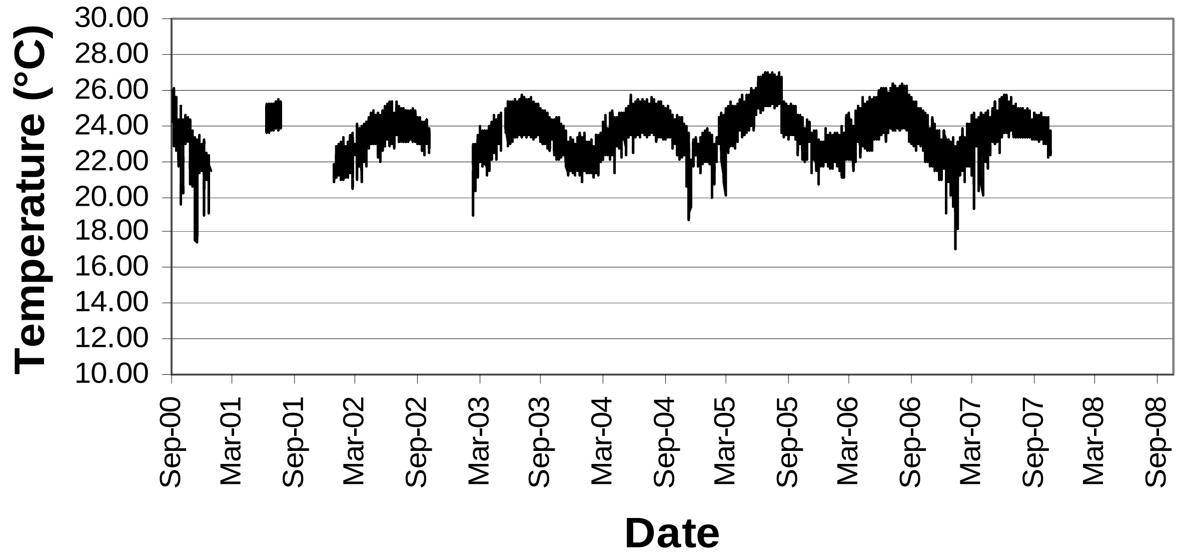


Thermistor Data: Landa Lake Bottom
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
LL upper
LL lower
Sep-00
Mar-01
Sep-01
Mar-02
Sep-02
Mar-03
Sep-03
Mar-04
Sep-04
Mar-05
Sep-05
Mar-06
Sep-06
Mar-07
Date

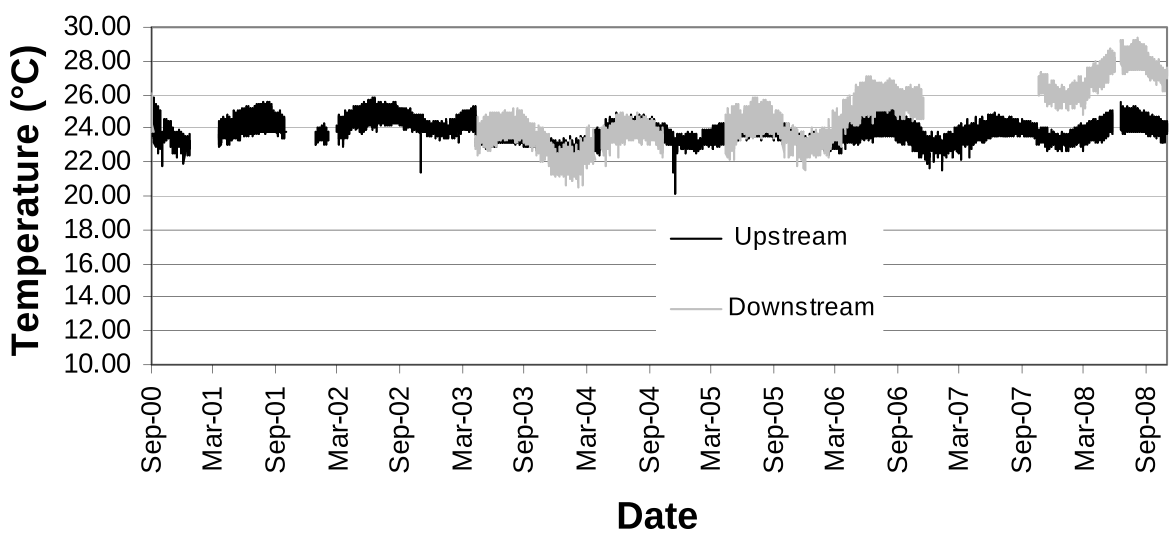
Thermistor Data: Spring Run 1



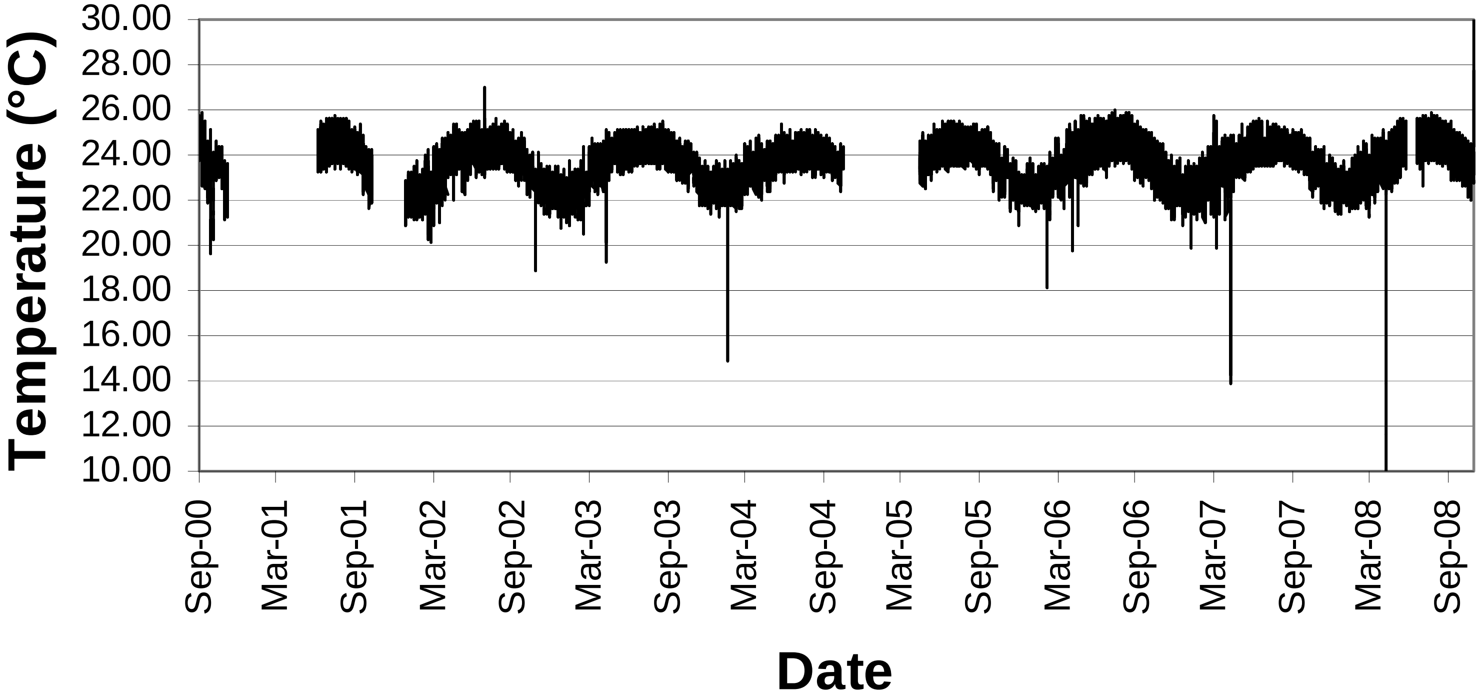
Thermistor Data: Other Place



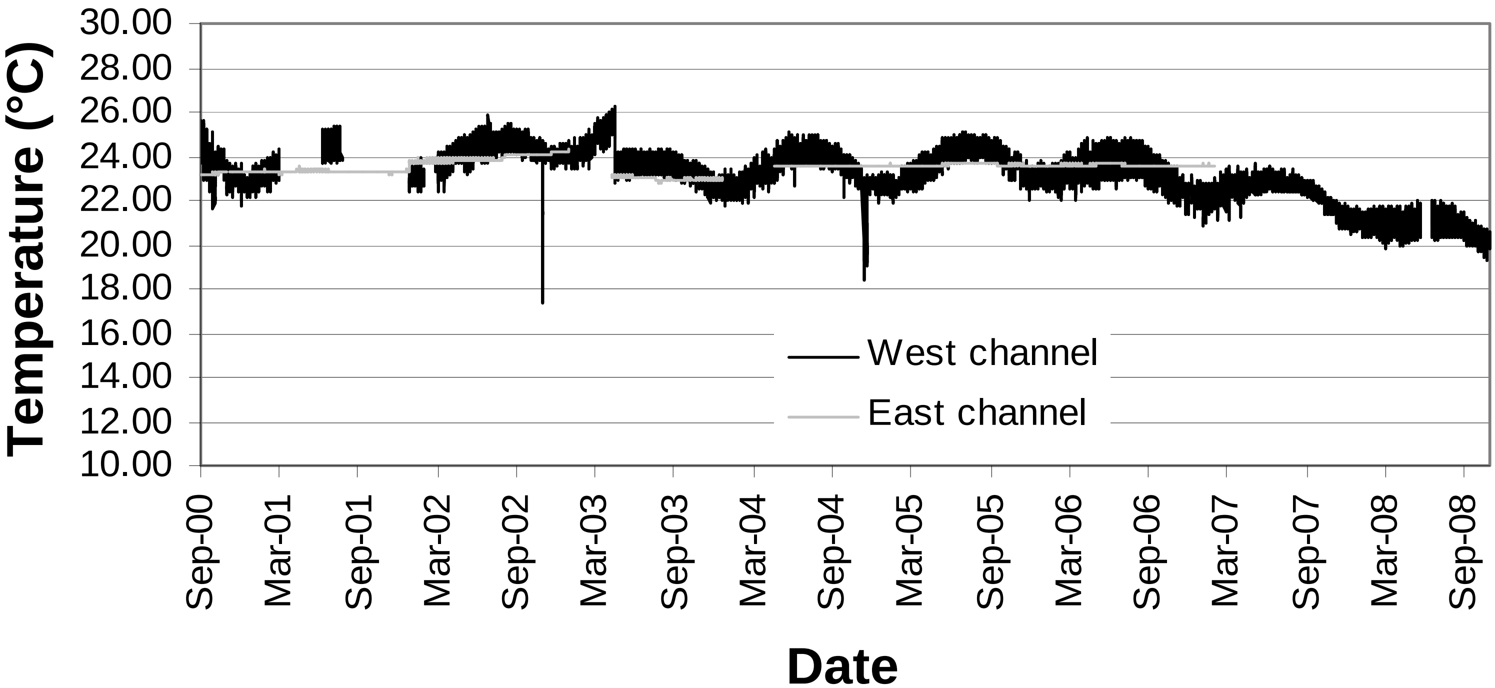
Thermistor Data: New Channel



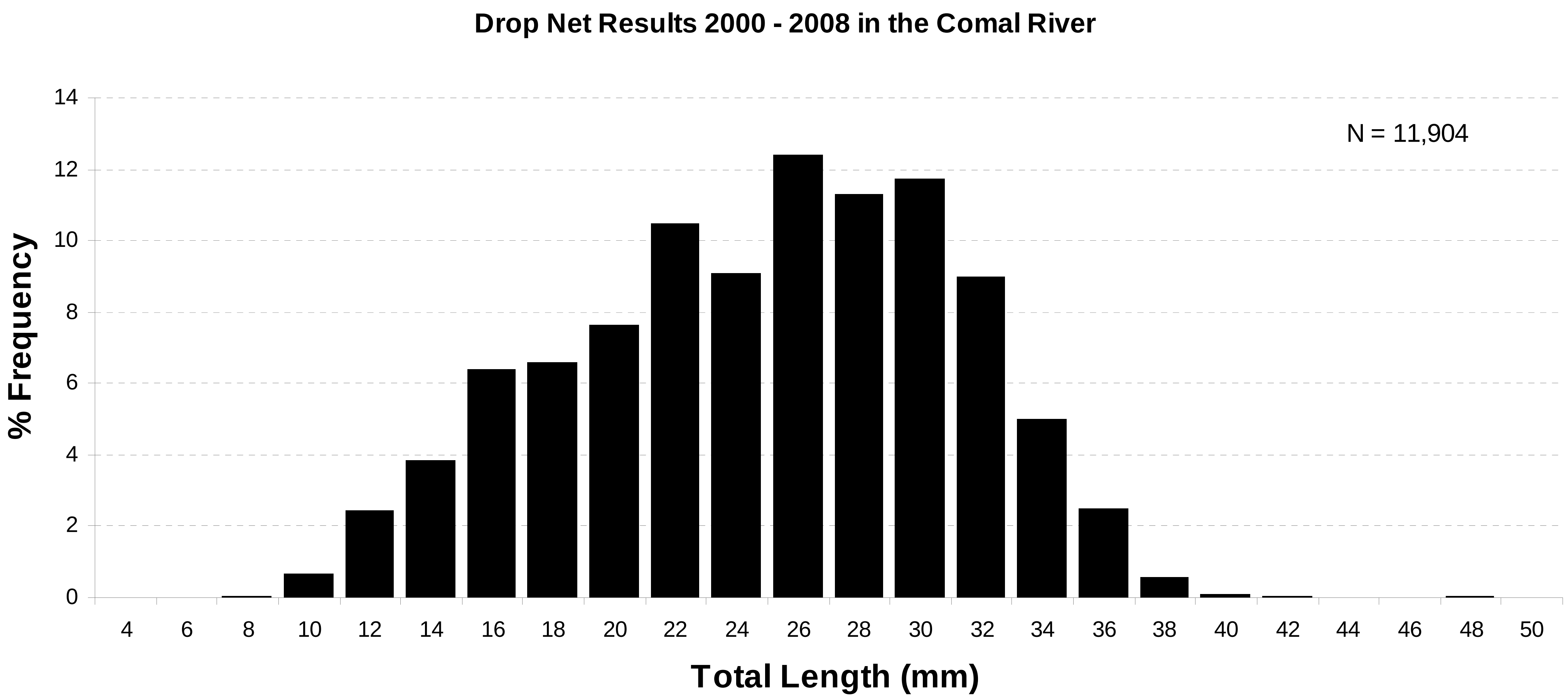
Thermistor Data: Old Channel



Thermistor Data: Spring Island

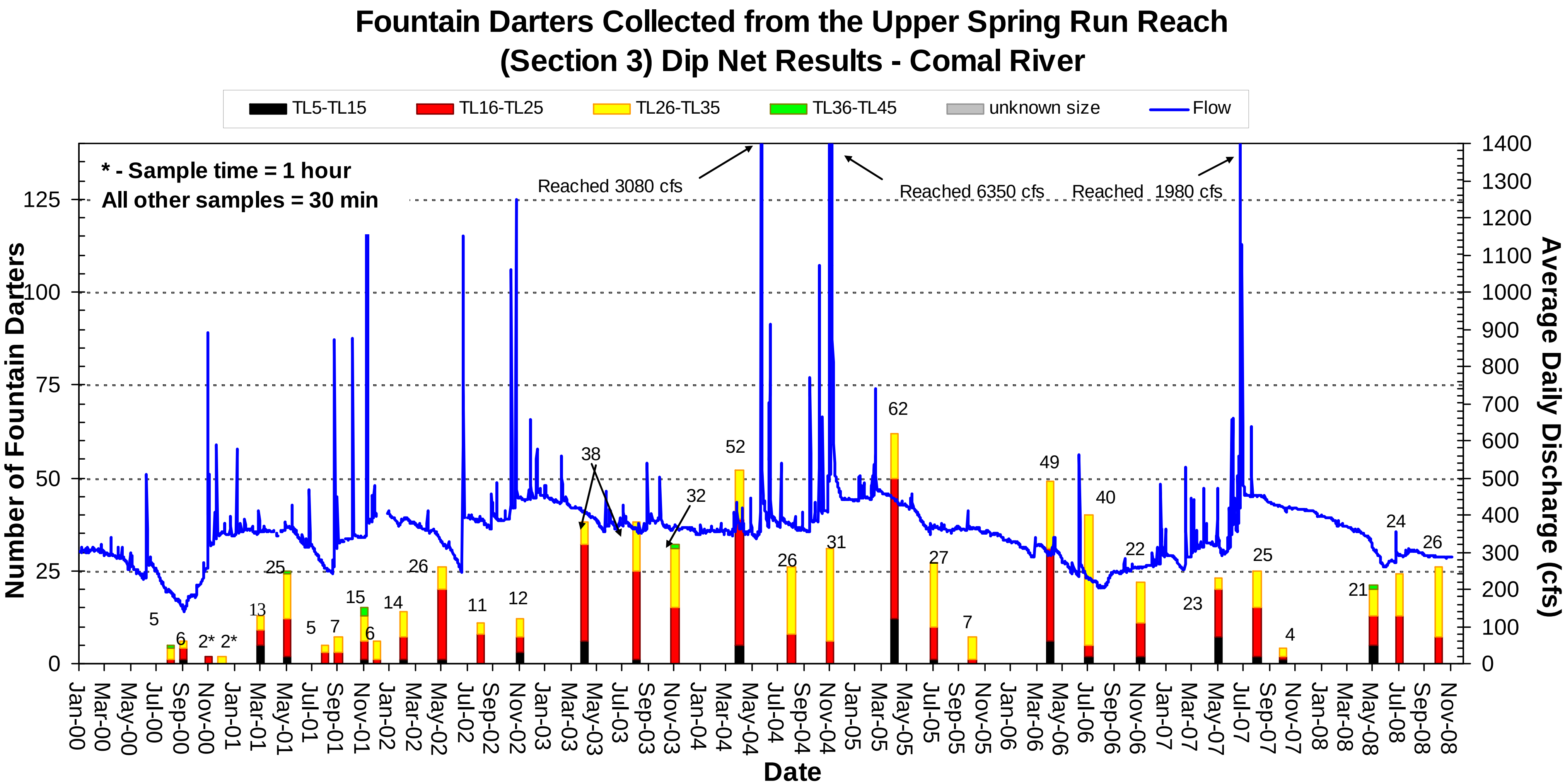


Drop Net Graph



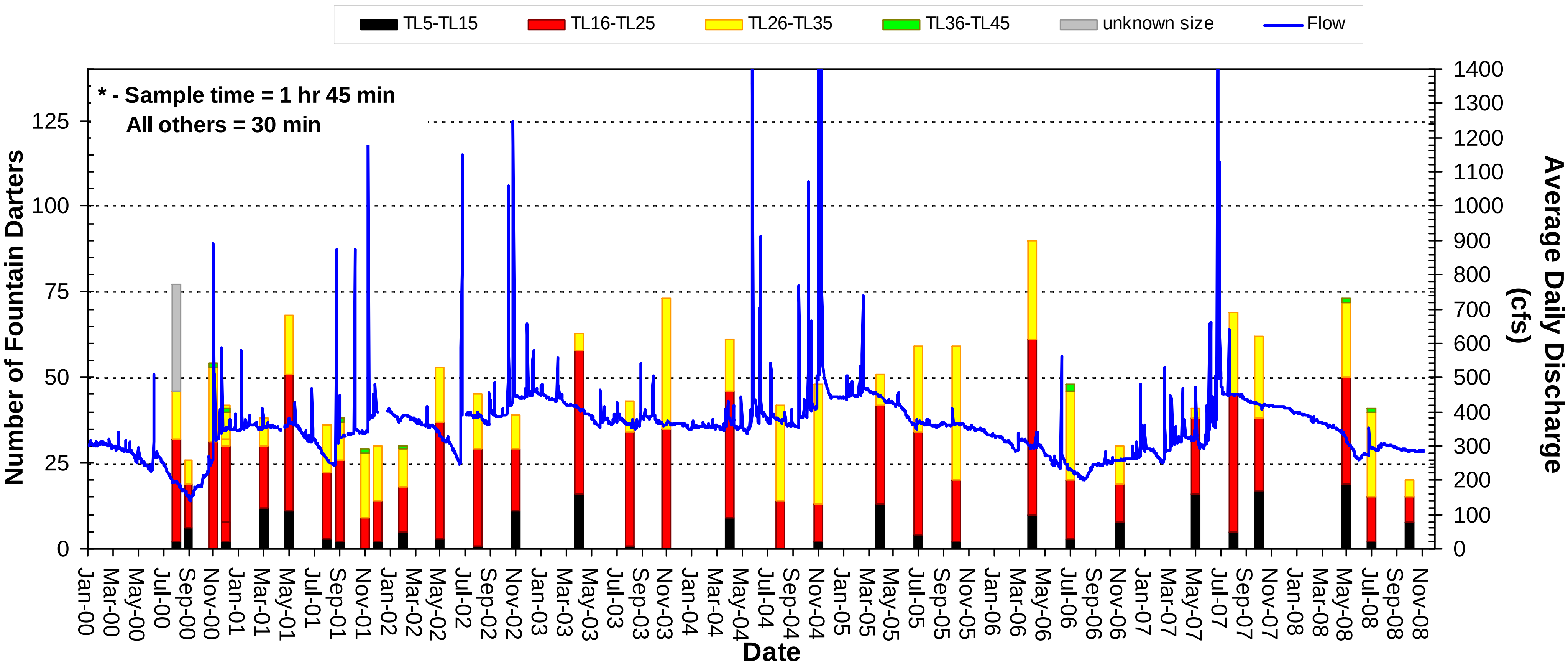
Drop Net Results 2000 - 2008 in the Comal River
N = 11,904
% Frequency
0
2
4
6
8
10
12
14
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
Total Length (mm)

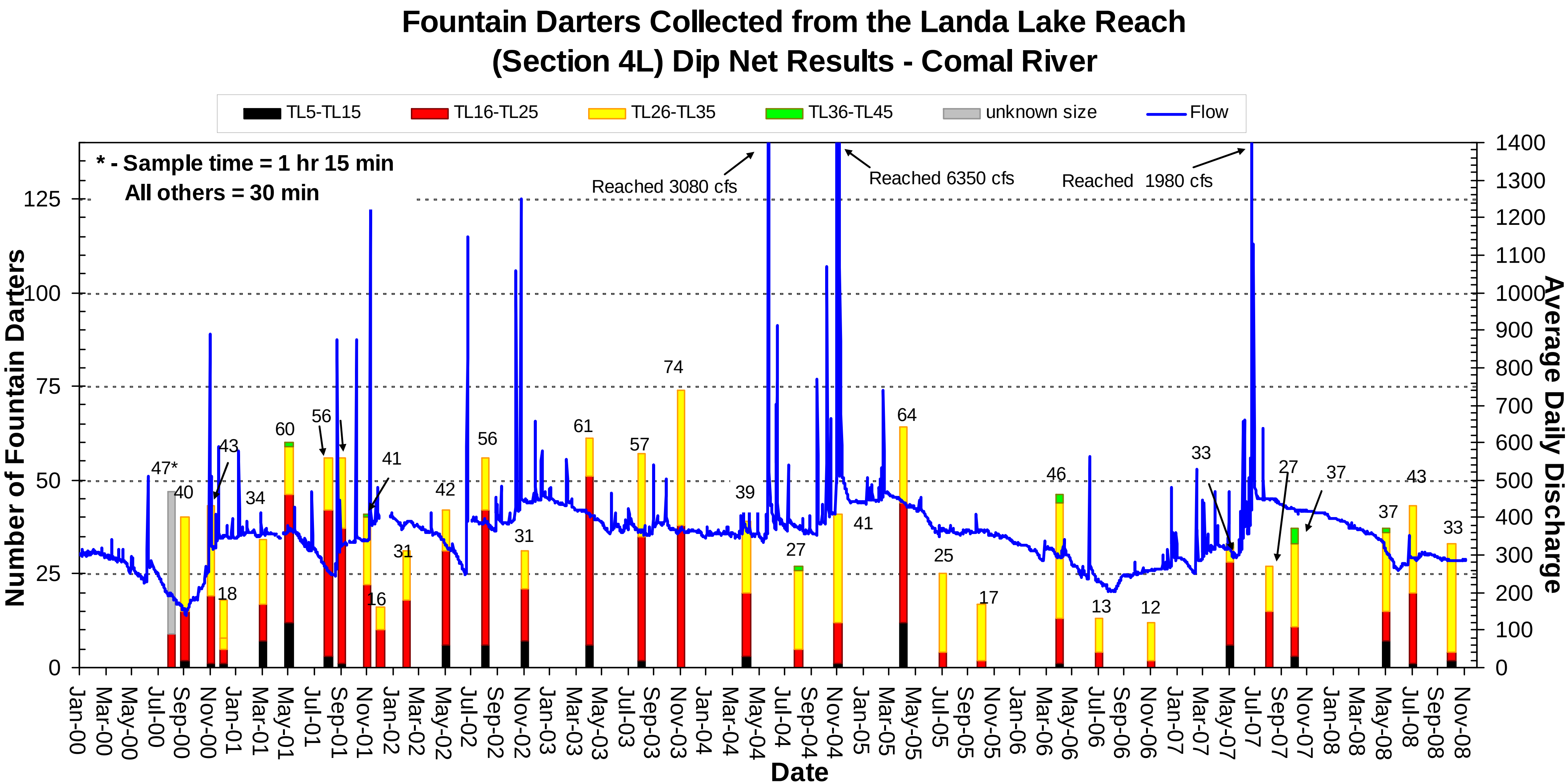
Dip Net Graphs

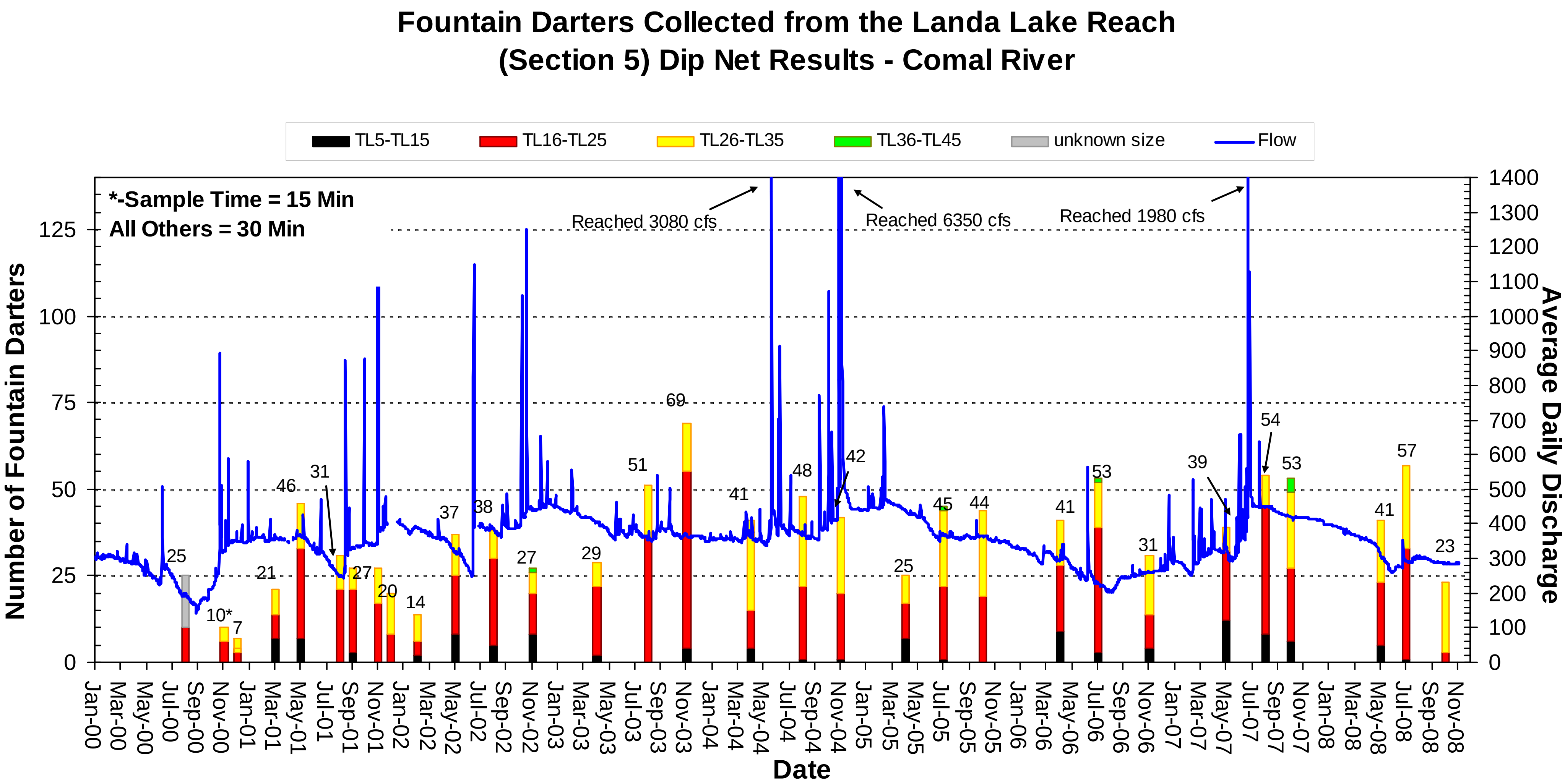


Fountain Darters Collected from the Upper Spring Run Reach
(Section 3) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hour
All other samples = 30 min
Reached 3080 cfs
Reached 6350 cfs
Reached 1980 cfs
Number of Fountain Darters
Average Daily Discharge (cfs)
Date

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

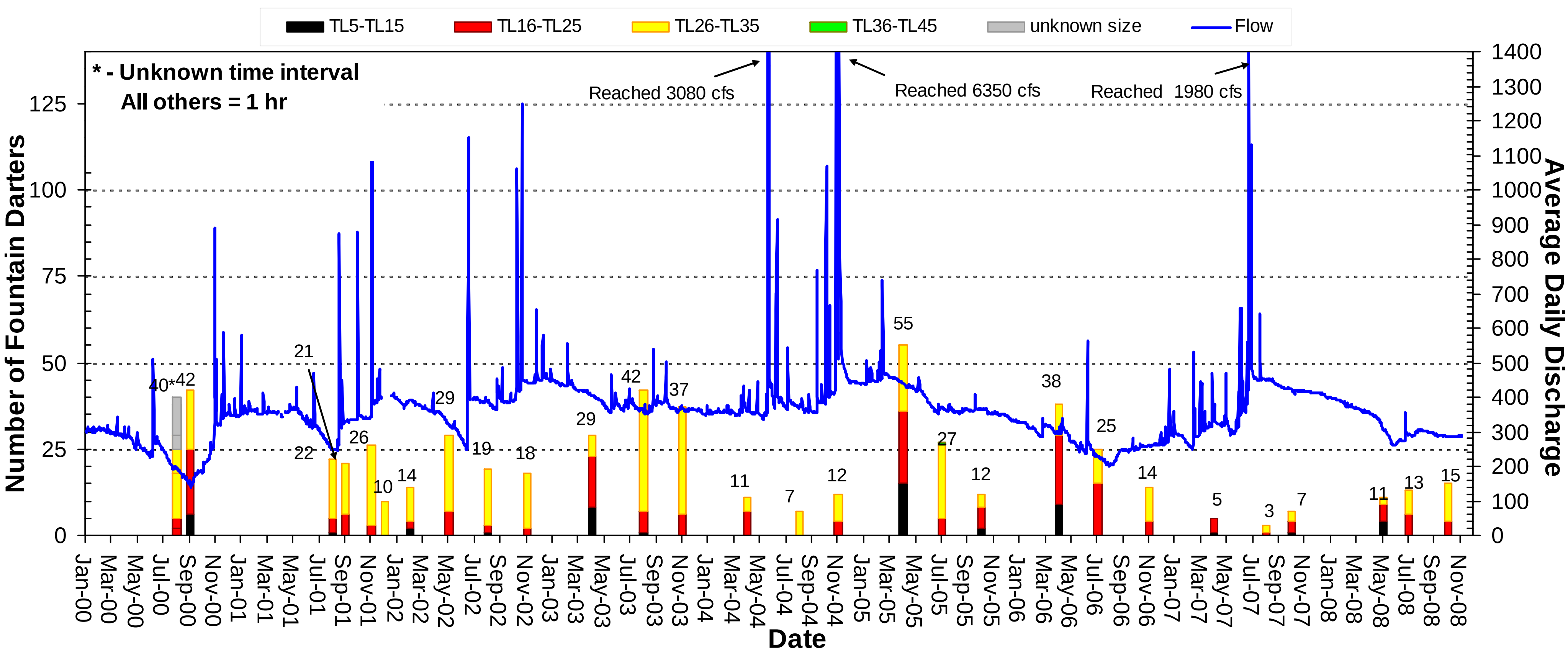



Fountain Darters Collected from the Landa Lake Reach
(Section 4L) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hr 15 min
All others = 30 min
Reached 3080 cfs
Reached 6350 cfs
Reached 1980 cfs
Number of Fountain Darters
Average Daily Discharge
Date
47*
40
43
18
34
60
56
41
16
31
42
56
31
61
57
74
39
27
41
64
25
17
46
13
12
33
27
37
37
43
33

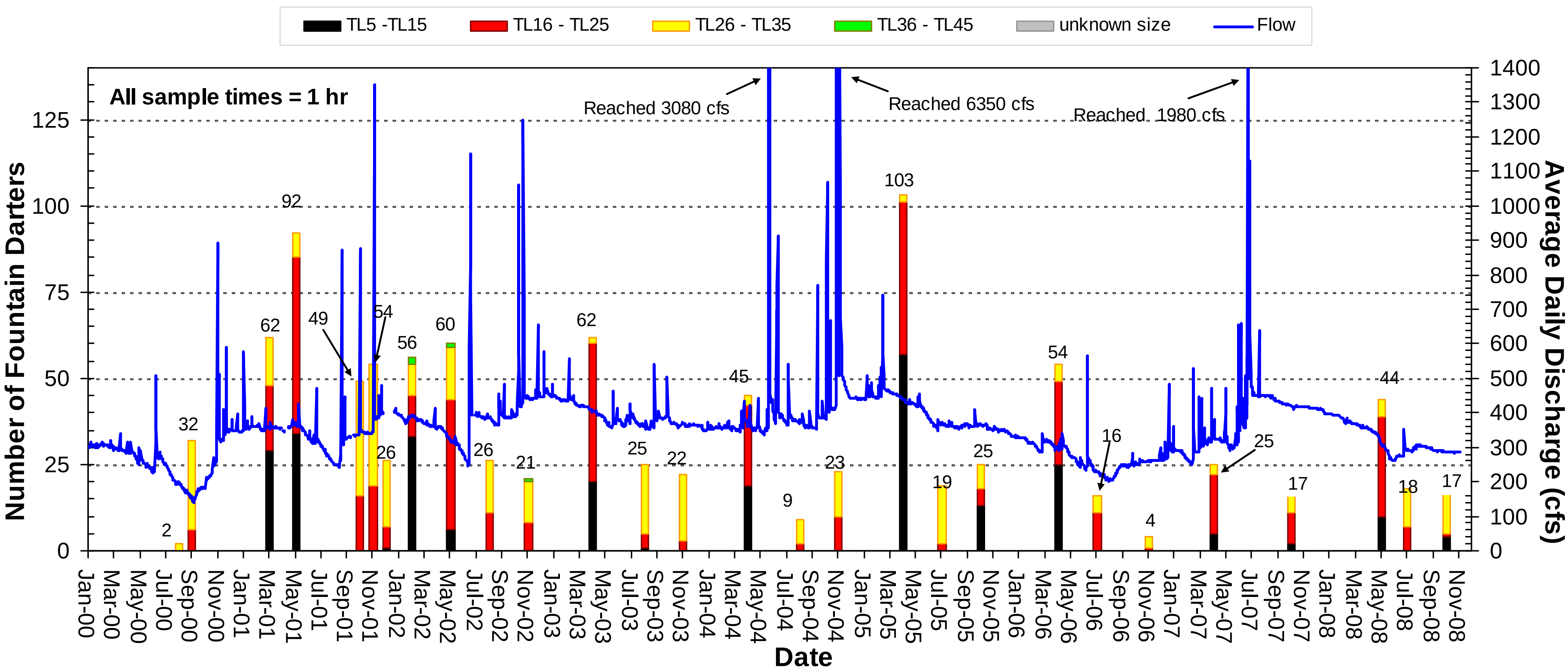


Fountain Darters Collected from the Landa Lake Reach
(Section 5) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
*-Sample Time = 15 Min
All Others = 30 Min
Reached 3080 cfs
Reached 6350 cfs
Reached 1980 cfs
Number of Fountain Darters
Average Daily Discharge
Date

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



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



APPENDIX C:
DROP NET RAW DATA

(not available digitally)