

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

Final 2009 ANNUAL REPORT



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EXECUTIVE SUMMARY

This annual summary report presents a synopsis of methodology used and an account of sampling activities conducted during three sampling events (two Comprehensive Monitoring efforts and one Critical Period low-flow effort) on the Comal Springs/River ecosystem in 2009. For ease of comparison, the data are reported here in an annual report format similar to previous reports (BIO-WEST 2001-2009).

The year 2009 was the culmination of a prolonged drought in central Texas that began in 2007. Discharge in the Comal River was at its lowest since 2000. The lowest total discharge recorded during 2009 was 158 cubic feet per second ([cfs] initial gage data indicated 150 cfs, but were later amended), and flows were below 200 cfs for 83 consecutive days. This resulted in the lowest flows experienced in the individual spring runs since late summer 2000. Flows at the Old Channel remained relatively constant throughout the year because discharge is controlled by culverts at Landa Lake. Precipitation increased in September culminating with a pulse of 4,290 cfs in October, which was the highest flow recorded in the Comal River during 2009.

Surface water grab samples were collected at 15 locations along the Comal River to evaluate conventional water chemistry parameters for the low-flow Critical Period sampling effort in July. Other than temperature, an upstream to downstream pattern in water quality values was not observed during the summer water quality sampling. Values remained fairly constant throughout the system or fluctuated minimally from site to site. Temperature data within the Spring Runs varied little even though flows were the lowest in nine years. Water temperatures were highest and most variable at Blieders Creek because this channel is upstream of the major springs, and strongly influenced by runoff from local precipitation events.

The variable flows of 2009 had a myriad of effects on the aquatic vegetation in all reaches. At the Upper Spring Run Reach, reduced springflow contributed to a dominance of green algae and a resulting reduction in coverage of bryophytes during the spring. In summer (Critical Period), *Hygrophila* and *Ludwigia* coverage was reduced while *Sagittaria* area changed little. A major flushing event from Blieders Creek in October resulted in scouring of the reach and a further reduction in plant coverage. Aquatic vegetation in the Landa Lake Reach followed similar patterns, with green algae expanding its coverage under low flows as spring gave way to summer. *Hygrophila* and *Ludwigia* decreased slightly over 2009, while *Cabomba* increased. The precipitation event in fall considerably reduced bryophyte coverage, but vegetation in protected areas (behind islands, bends in the river) changed little after this event. Relatively constant flows in the Old Channel Reach resulted in little change to the aquatic plant community in this reach, and *Hygrophila* and *Ludwigia* remained the dominant plants. Filamentous algae that were recently absent from this reach have gained a foothold and remained in several spots throughout the year. The most dynamic effects of flow on aquatic vegetation were observed at the New Channel Reach. Between spring and fall of 2009 there was a 94% reduction in aquatic vegetation coverage in this reach. Most of this reduction is due to the precipitation event that occurred prior to the fall sampling effort. Since the New Channel Reach is highly channelized, high discharges can severely scour this reach. The only vegetation remaining was small patches of *Hygrophila* and *Cabomba*.

Aquatic vegetation is an important component of fountain darter (*Etheostoma fonticola*) habitat in the Comal River. Higher densities of these fish are consistently found in native vegetation like bryophytes, filamentous algae, *Ludwigia*, and *Cabomba*. However, darters are also found in less-optimal non-native vegetation types like *Hygrophila*. The lower than average flows of 2009 decreased overall coverage of bryophytes in some areas (mostly due to a blanketing effect of green algae), but coverage of other native vegetation like *Cabomba* and filamentous algae remained relatively constant. *Ludwigia* coverage

continues to be limited in the Comal River with several small patches at Landa Lake and Upper Spring Run. It has remained relatively constant at the Old Channel Reach in recent years, but over the nine year study period it has declined considerably in that reach.

Fountain darter abundance in the Comal River was again variable in 2009, but within the range of previous years observed throughout this study. As described above, native vegetation continues to be the most important type of habitat available to fountain darters in the river. Scouring of vegetation due to a high-flow event prior to the fall sampling effort resulted in lower fountain darter population estimates in fall. Bryophytes and filamentous algae are prone to scouring (neither are well rooted), and when they decline there is a resulting decrease in fountain darter population estimates. In areas near spring upwellings with native vegetation (e.g., bryophytes) there appears to be year-round reproduction. However, dip net data confirm an early spring reproductive peak for these fish in other areas of lower quality habitat.

Although the total number of Comal Springs riffle beetles (*Heterelmis comalensis*) captured decreased slightly from 2008 to 2009, numbers were similar to those observed in 2007. Some seeps along the west side of Landa Lake had no measurable flow during portions of 2009, and this may have contributed to the lower numbers of riffle beetles and Peck's Cave amphipods (*Stygobromus pecki*) collected in this area. Two additional specimens of an undescribed amphipod (*Parabogidiella* sp., first captured in 2008) were captured during 2009 sampling. Additionally, another undescribed amphipod (*Ingolfiella* sp.) was captured during this study in 2009.

The total number of salamanders observed during the spring and Critical Period sampling efforts were the highest since the inception of the study. Lower than average flows in 2009 may have contributed to increased searching efficiency in the spring runs. Although the number of salamanders observed in the Spring Island Spring Run has decreased during the study period, this is mainly due to human-induced habitat modifications (removal of fist-sized rocks) within this spring run. High flows in October 2009 resulted in decreased salamander counts, but this may also be attributed to reduced sampling efficiency due to the higher flows. Overall, the variable flows of 2009 appeared to have little effect on salamander populations in the Comal River.

Flows from Comal Springs in 2009 were the lowest observed since 2000, but impacts on the biota in the ecosystem appeared to be minimal. Continued monitoring over an extended period of lower than average flows is necessary to fully understand the impact of discharge on the varied biota in the system.

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 is monitored during this study is the undescribed Comal Springs salamander (*Eurycea* sp.).

Two full comprehensive (spring and fall) and one Critical Period low-flow (June / July) sampling efforts were conducted in 2009 with additional dip netting for fountain darters collected in the summer. Additionally, volunteers assisted with weekly water quality measurements and recreational counts on the Comal system. A full comprehensive event includes the following sampling components and volunteer activities:

Water Quality

Thermistor Placement
Thermistor Retrieval
Fixed Station Photographs
Weekly Standard Parameters (Volunteer)
Point Water Quality Measurements
Surface Water Grab Samples (Critical Period)

Aquatic Vegetation Mapping

Fountain Darter Sampling

Drop Nets
Dip Nets
Visual Observations

Salamander Observations

Macroinvertebrate Sampling

Drift Nets
Comal Springs Riffle Beetle Surveys

Recreation Observations

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 2009). 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. These data were used to estimate the contribution of each major Spring Run to total discharge in the river, and to estimate the relative proportion of water flowing in the Old and New Channels. Finally, spot water velocity measurements were taken during each drop net sampling event. All discharge and velocity measurements were taken using a SonTek® FlowTracker.

Low-Flow Sampling

Discharge in the Comal River decreased through much of 2009 culminating in a Critical Period event in June / July when initial flow data indicated that discharge dropped to 150 cfs in the river.

High-Flow Sampling

There were no high-flow sampling events on the Comal Springs / River system in 2009.

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 throughout 2009. 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. During the low-flow Critical Period event in summer 2009, the full spectrum of water quality sampling parameters were measured, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

Temperature Thermistors

Thermistors set to record water temperature every 10 minutes 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. 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 allow for temporal habitat evaluations and include an upstream, a cross-stream, and a downstream picture; these were taken at each water quality site depicted in Figure 1.

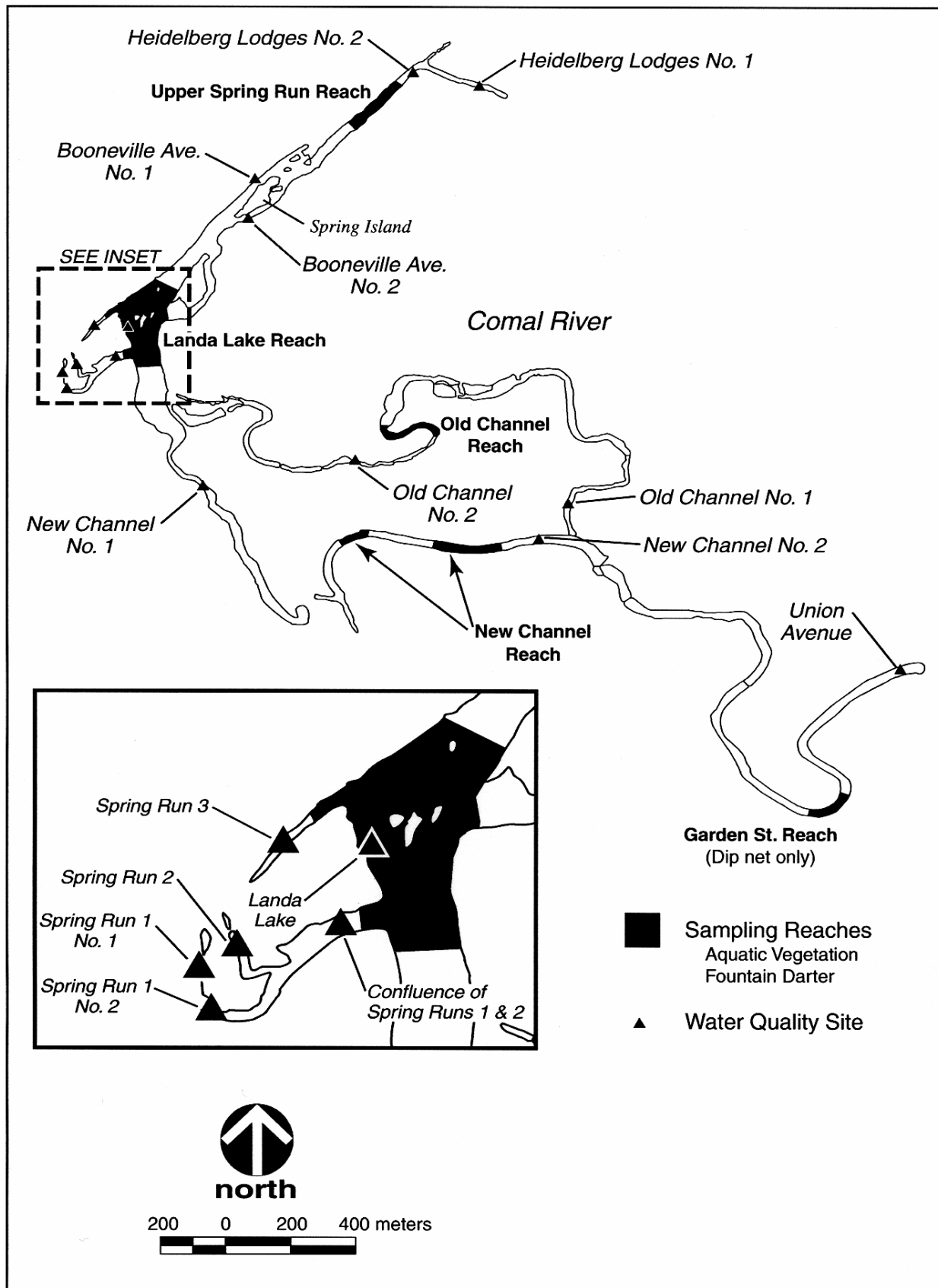


Figure 1. Comal River water quality and biological sampling areas.

Water Quality Grab Samples

Surface-water grab samples were collected at 15 locations along the Comal River to evaluate conventional water chemistry parameters (Figure 1). Sample collection and water chemistry analyses conducted during 2000-2002 sampling events are described in the 2002 annual report (BIO-WEST 2003). Following the same protocols, water quality analysis was conducted during one low-flow Critical Period sampling event in 2009. During sample collection, two 500-mL surface-water samples were collected at each site. One of the two samples was left unpreserved for nitrate, soluble reactive phosphorus (SRP), alkalinity and total suspended solid (TSS) analyses, and the other sample was acidified with sulfuric acid for ammonia, total nitrogen (TN), and total phosphorus (TP) analyses. Turbidity was not determined for water samples in 2009. Chemical analyses of surface water samples for the 2009 sampling events were conducted by the AnalySys, Inc. laboratory in Austin, Texas, where water chemistry parameters were determined utilizing EPA standard methods (Table 1) and are described in more detail below.

Nitrate Nitrogen and Soluble Reactive Phosphorus: Following standard EPA Method 300.1, the concentrations of anions in a 10- μ L sample are determined using an ion chromatography system equipped with a conductivity detector.

Total Nitrogen: Following standard EPA Method 351.2, the sample is heated in the presence of sulfuric acid, potassium sulfate, and mercuric sulfate for two and one-half hours. The resulting residue is cooled, diluted to 25mL and determined by spectroscopy.

Ammonium: Following standard EPA Method 350.2, the sample is buffered at alkaline pH with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, distilled into a solution of boric acid and then determined by spectroscopy.

Total Phosphorus: Following standard EPA Method 365.2, the sample is pretreated to select the phosphorus forms of interest; the forms are then converted to orthophosphate. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex, which is reduced with ascorbic acid to form an intense blue-colored complex. The absorbance of the complex is measured by spectroscopy, and is proportional to the orthophosphate concentration.

Alkalinity: Following standard EPA Method 310.1, an unaltered sample is titrated to an electrometrically determined end point of pH 4.5.

Total Suspended Solids: Following standard EPA Method 160.2, a well-mixed sample is filtered through a glass fiber filter, and the residue retained on the filter is dried to a constant weight at 103-105°C.



Table 1. Water quality analyses performed on surface-water grab samples from 15 sites along the Comal River in 2009, along with the analytical method, technique, and minimum analytical detection levels of each analysis.

PARAMETER	EPA METHOD	TECHNIQUE (2009)	MINIMUM ANALYTIC LEVELS (per liter)
Total Suspended Solids	160.2	Gravimetric	Appropriate
Alkalinity	310.1	Titration	10 mg
Nitrate Nitrogen	300.1	Ion Chromatography	0.05 mg
Ammonium	350.2	Spectroscopy	0.01 mg
Total Nitrogen	351.2	Spectroscopy	0.5 mg
Soluble Reactive Phosphorous	300.1	Ion Chromatography	0.05 mg
Total Phosphorous	365.2	Spectroscopy	0.01 mg

* micrograms.

Master Naturalist Monitoring

Volunteers with the Texas Master Naturalist program continued their monitoring efforts in 2009 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 (TPWD), 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.

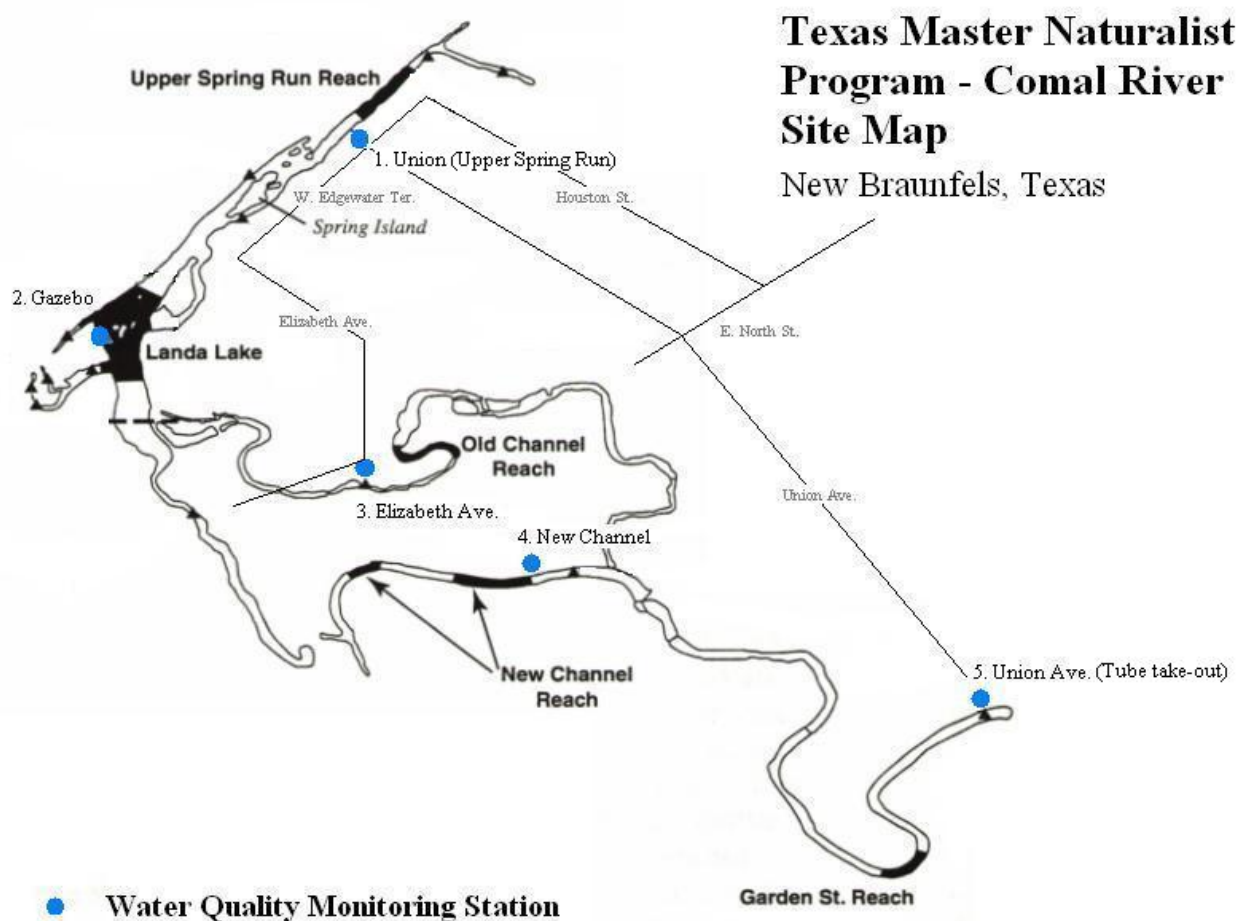


Figure 2. Weekly water quality / recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers.

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 (or similar device) with TerraSync software that displays field data in real time and improves efficiency and accuracy. The GPS unit was placed in a 10-foot (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.

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium*; primarily in the Upper Spring Run and Landa Lake) were included in all 2009 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.



Hygrophila (left) and algae growth within *Hygrophila* (right) in the Old Channel Reach

Fountain Darter Sampling Methods

Drop Nets

A drop net is a 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 drop 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 the adjacent area within three meters of the drop net. Fountain darters were identified, enumerated, measured for total 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 2000-2009 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 a 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 would be due to natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints), it is impossible to tell how much of the variation is attributed to each source within a given sampling effort. Using the average density of fountain darters across all samples for a given vegetation type does not account for changes in density across samples (differences associated with changes in environmental conditions), but the increased sample size substantially reduces the high natural variability. This type of comparison between samples, where density values are held constant across all samples, is based entirely upon changes in vegetation composition and abundance between sampling efforts. Because these abundance estimates use the same density values across sites and seasons, and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Dip Nets

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

To balance the effort expended across samples, a predetermined time constraint was used for each reach (Upper Spring Run - 0.5 hour, Spring Island area - 0.5 hour, Landa Lake - 1.0 hour, New Channel - 1.0 hour, Old Channel - 1.0 hour, Garden Street - 1.0 hour). The areas of fountain darter collection were marked on a base map of the reach. Although information regarding the density of fountain darters per vegetation type was not gathered with this method (as in drop net sampling), it did permit a more thorough exploration of various habitats within each 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). 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. Dip net data were analyzed by visually evaluating graphs of length-frequency distribution for each sample reach.

Presence/Absence Dip Netting

Presence/Absence dip netting was initiated on the Comal River during fall 2005. This method is designed to be a quick, efficient, and repetitive means of monitoring the fountain darter population. Also, since it is much less destructive than drop netting, it can be conducted during extremely low-flow periods without harming critical habitat.

During each sample, fifty sites were distributed among the four sample reaches based on total area, diversity of vegetation, previous fountain darter abundance estimates, and overall biological importance of each reach. Sites were randomly selected within the dominant vegetation types within each reach. 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 near the site of capture.

Visual Observations

Visual surveys were conducted using SCUBA in Landa Lake to verify continued habitat use in deeper portions of the lake by both fountain darters and Comal Spring's salamanders. These time-constrained surveys were conducted in areas too deep for efficient drop netting or dip netting. Observations were conducted in the early afternoon. Since summer 2001, a specially-designed grid (0.6 m x 13.0 m) has been used to quantify the number of fountain darters using these deeper habitats. During each survey, all fountain darters within the grid were counted. A more labor-intensive effort would be required to develop an estimate of the true population size in the sample area, but these data are useful in providing an indication of fountain darter relative abundance in areas similar to those sampled. These data also provide insight into trends in population dynamics that may occur over time.

Gill Parasite Evaluations

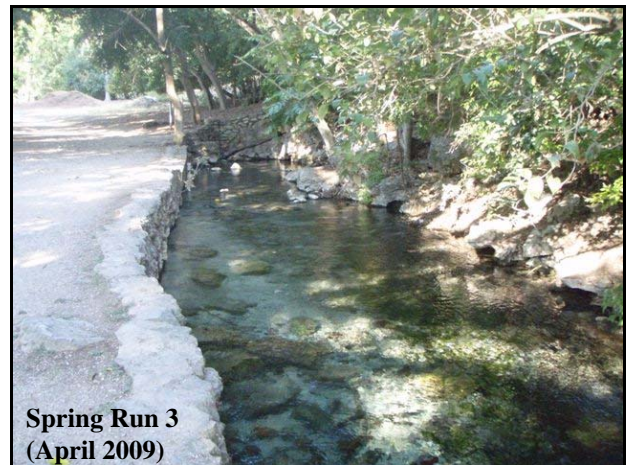
The Asian trematode, *Centrocestus formosanus*, was first discovered on fountain darters in the Comal River during October 1996. Following a 13-month parasite/darter study, the parasite was found to be detrimentally affecting darters in the Comal River. The parasite attaches to the fish's gill filaments causing extensive gill tissue proliferation and damage. *Centrocestus formosanus* was first observed in the Comal River in 1990. The parasite requires three hosts to complete its lifecycle. The adult form of the parasite resides in fish-eating birds. The Green Heron (*Butorides virescens*) has been found infected with the parasite in central Texas. A non-native snail, *Melanoides tuberculatus* that has been in central Texas since 1964, has been confirmed as its central Texas first intermediate host. Numerous fish species have been confirmed as the parasite's second intermediate host.

Monitoring of the parasite in the Comal River has been going on since the late 1990's and has been inconsistent. Parasite presence and numbers can be counted on resident fountain darters, hatchery-reared fountain darters placed in cages within the river, in *M. tuberculatus* collected from the river, and directly from river water. Each type of sampling provides different information on the parasite's population size and fountain darter threat level. Anne Bolick (Texas State University) sampled the numbers of parasites drifting through three Comal River sites every 2 weeks between June 2006 and May 2007 (Bolick 2007). During 2009, sampling was initiated on July 7 and conducted with the same methodologies and at the same three sites used by Bolick. Samples were collected every 2 weeks during July, August, and September. During mid-September, spring flows started increasing and when

flows reached 200 cfs, the time between samplings was increased to every 4 weeks. Spring flows through December 14 have averaged 228 cfs with a range of 161 to 312 cfs.

Comal Springs Salamander Visual Observations

In addition to visual observations made in 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 2009 sampling events. These two-person surveys were conducted in Spring Run 1, Spring Run 3, and the Spring Island area (Figure 1).



Each survey began at the downstream-most edge of the sampling area and involved turning over rocks located on the substrate surface while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. Salamander locations were noted, along with time, and water depth. 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 upstream to 9-m below the head spring orifice. Spring Run 3 was surveyed from the pedestrian bridge closest to Landa Lake upstream to 9-m below the head spring orifice. In the Spring Island area, surveys were conducted within the entire spring reach including an approximately 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 on the north side of Spring Island (upstream).

Macroinvertebrate Sampling

In 2009, 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 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 McBirney 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 2009 (June and December). These samples were conducted in three disjunct areas of Landa Lake, 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 which were placed in spring openings with rocks loosely stacked on top to keep them in place. Approximately four weeks later, squares were removed, and depth and current velocity measurements were taken. Beetles were identified, counted, and returned to their spring of origin. Other spring invertebrates collected on the squares were also noted. At each of the three study sites, 10 springs were sampled using this method.

OBSERVATIONS

The BIO-WEST project team conducted the 2009 sampling events as shown in Table 2.

Table 2. Study components of the 2009 sampling events.

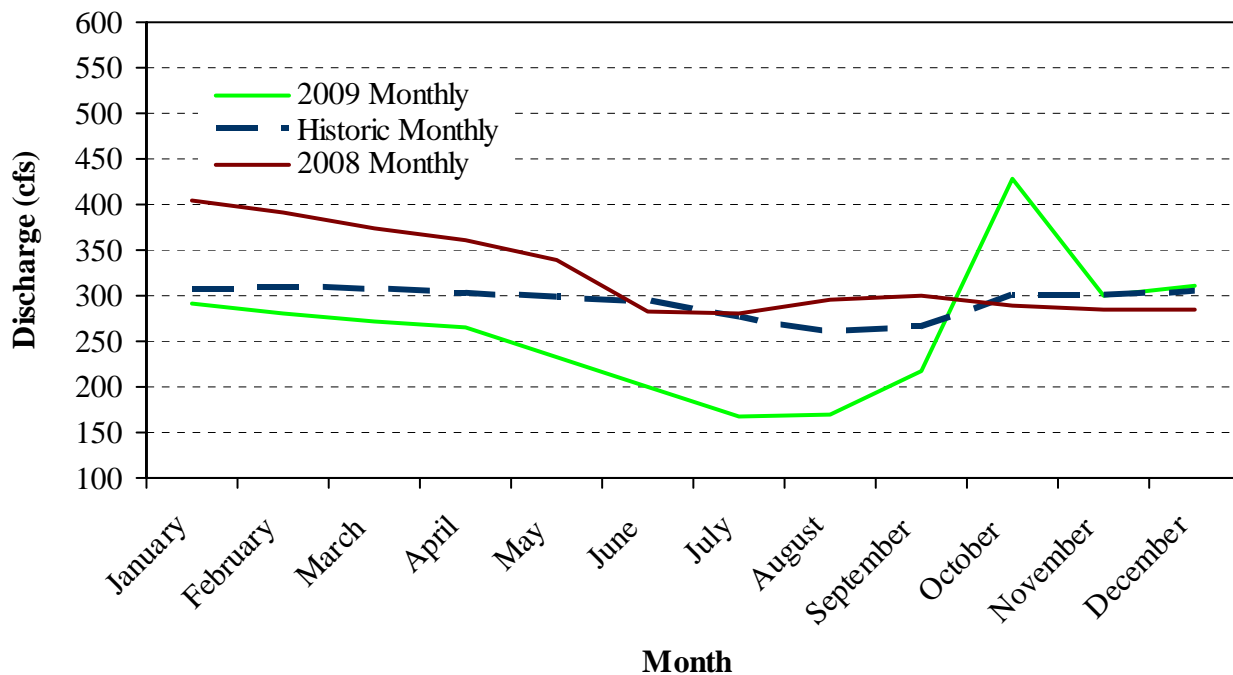
EVENT	DATES
Spring	
Vegetation Mapping	April 22 - 27
Fountain Darter Sampling	May 13 - 15
Comal Salamander Observations	May 15
Macroinvertebrate Sampling	June 11 - 12
Critical Period 1	
Vegetation Mapping	June 24 – July 3
Fountain Darter Sampling	June 29 – July 1
Comal Salamander Observations	July 2
Water Quality Collection	July 2
Fall	
Vegetation Mapping	October 22 - 27
Fountain Darter Sampling	October 21 - 23
Comal Salamander Observations	November 6
Macroinvertebrate Sampling	December 6 - 7

Comal Springflow

A prolonged drought in Central Texas contributed to decreased discharge in the Comal River during much of 2009. Discharge began the year below 300 cfs and did not exceed that number until early September. The lowest discharge occurred on July 2 (158 cfs), which is the lowest discharge recorded since the first year of this study (Table 3). Initial uncorrected gage data (provisional) indicated that flows had decreased below 150 cfs which triggered a Critical Period low-flow event in July. The monthly average flow was well below the historic average until October when it crept above 300 cfs (Figure 3). Significant precipitation beginning in fall led to increased discharge. This included a significant one day event (October 4) when discharge reached 4,290 cfs - the highest discharge observed in the Comal River since 2004.

Table 3. Lowest discharge during each year of the study (2000-2009) 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
2009	158	July 2

**Figure 3. Mean monthly discharge in the Comal River during the 1934 - 2009 period of record.**

As total discharge in the Comal River decreased through the first half of 2009, flows in the Spring Runs generally decreased while discharge in the Old Channel stayed relatively steady (Table 4). At Spring Run 1, discharge was cut in half between May and July, when flows in the river were at their lowest since 2000. Discharge at Spring Run 2 actually increased slightly during this period, although this may represent a measurement error. Flows at Spring Run 3 decreased steadily from fall 2008 through summer 2009. Although flows decreased in the Old Channel Reach, they remained relatively steady because there is a culvert upstream that controls the flow through this section of the river. By October 2009, significant rain events occurred over much of the aquifer and flows increased in the Comal River. Flows at all Spring Runs increased, except for Spring Run 2, which raises questions about the 5.6 cfs measurement taken in July. Flows at the Old Channel only increased slightly even though flows in the river more than doubled since the previous flow measurement. This illustrates that the majority of flow in the Comal River is diverted down the New Channel.

While flows generally decreased over the whole system, the amount contributed from each Spring Run did not fit any particular trend (Table 5). When discharge in the Comal River was at its lowest point in 2009, Spring Runs 1 and 3 (upstream) contributed nearly the same percentage of flow to the river with total Spring Run 3 contributing more (as in all years). By July, the Old Channel contained nearly 30% of the flow in the river. When flows increased significantly in fall, percentages decreased at all sites measured except Spring Run 1 which contributed over 7%. With more water flowing through the system, most of it was diverted down the New Channel because of the culvert structure keeping flow in the Old Channel relatively constant.

Table 4. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, 3, and Old Channel reach during fall 2008 and all sampling efforts in 2009.

Location	Discharge (cfs)			
	Fall 2008	Spring 2009	Critical Period 2009	Fall 2009
Total Discharge Comal River (USGS)	286	224	159	351
Spring Run 1	22.7	15.5	7.6	24.9
Spring Run 2	6.1	3.3	5.6	3.8
Spring Run 3 (upstream)	12.0	11.5	7.4	8.9
Spring Run 3 (downstream)	37.0	25.7	21.7	31.8
Old Channel	57.3	53.2	45.3	49.7

Table 5. Percentage of total discharge in the Comal River (USGS data) that each Spring Run contributed and percentage that traveled down the Old Channel during fall 2008 and all sampling efforts in 2009.

Location	Percentage of Total Discharge			
	Fall 2008	Spring 2009	Critical Period 2009	Fall 2009
Spring Run 1	7.9 %	6.9 %	4.8 %	7.1 %
Spring Run 2	2.1 %	1.5 %	3.5 %	1.1 %
Spring Run 3 (upstream)	4.2 %	5.1 %	4.7 %	2.5 %
Spring Run 3 (downstream)	13.0 %	11.5 %	13.6 %	9.1 %
Old Channel	20.0 %	23.8 %	28.5 %	14.2 %

Water Quality Results

Temperature Thermistors

The continuously recorded water temperature data (Appendix B) have provided a good view of the thermal conditions throughout the Comal Springs ecosystem from 2000-2009. 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. Water temperatures are most constant at or near the spring inputs and become more variable downstream as other factors (runoff, precipitation, and ambient temperature) become more influential. At times, precipitation can have acute impacts (cold winter rainfall) in some locations resulting in large temperature spikes. However, these are generally short-lived, and the overall relationship at these sites is more directly associated with ambient air temperature (air temperatures also strongly influence precipitation temperatures).

The thermistor data for Spring Run 1 are presented in Figure 4, and graphs for all other reaches can be found in Appendix B. Even in lower-flow conditions (like in 2009) the Spring Runs still exhibit little variation in temperature (typically less than 1 °C). The temperature did not exceed 26.67 °C (Texas Commission on Environmental Quality [TCEQ] water quality standard) at any of the spring runs in 2009 even though the minimum flow was the lowest observed since 2000. The TCEQ water quality standard of 26.67 °C was only exceeded at the Blieders Creek site. Water temperatures at Blieders Creek exhibited the highest temperature variation ranging from 16.19 – 30.33 °C. Blieders Creek is not spring-fed, and thus, vulnerable to temperature fluctuations because most of its inputs come from localized precipitation and runoff. This creek often becomes stagnant during dry periods, causing elevated temperatures. Areas near Landa Lake typically exhibit relatively little variation, whereas the Old Channel exhibits stronger seasonal temperature fluctuations due to its distance from spring inputs (Appendix B).

Thermistor Data: Spring Run 1

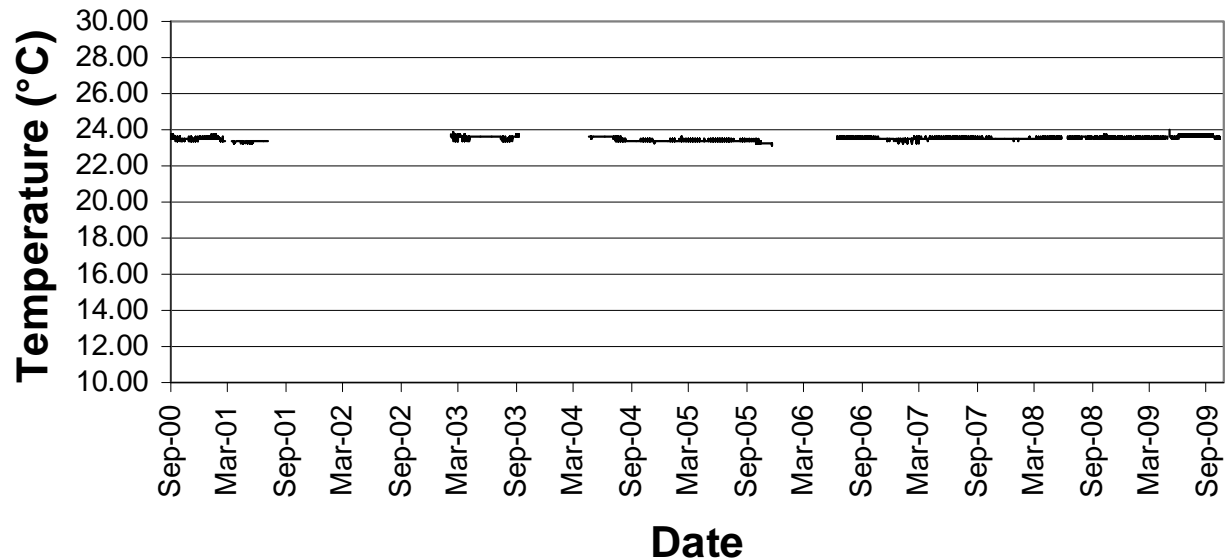


Figure 4. Temperature (°C) data at Spring Run 1 from 2000 – 2009.

Water Quality Grab Samples

The original water quality sampling sites along the Comal River were chosen based on historical locations that have been used during basic limnological sampling conducted at Texas State University. A summary of the water quality results from the original 2000-2002 sampling is presented in BIO-WEST (2003). The same water quality sampling sites, with the addition of a second sample in Spring Run 1, a sample in Landa Lake, and a sample at the confluence of Spring Runs 1 and 2, were sampled during the summertime low-flow sampling event in 2009 (Figure 1). The Comal River sampling site locations were as follows:

- Blieders Creek (Heidelberg lodges Number 1),
- Heidelberg Main Channel (Heidelberg lodges Number 2),
- Island Park, Far Channel (Booneville Avenue Number 1),
- Island Park, Near Channel (Booneville Avenue Number 2),
- Spring Run 1 Number 1,
- Spring Run 1 Number 2,
- Spring Run 2,
- Confluence of Spring Runs 1 and 2,
- Spring Run 3,
- Landa Lake,
- New Channel, upstream (Number 1),
- New Channel, downstream (Number 2),
- Old Channel, upstream (Number 2),
- Old Channel, downstream (Number 1), and
- Union Avenue (tube take-out; replaces The Other Place).

A summary of water quality data for the summer 2009 water quality sampling event is presented in Tables 6 and 7. Generally, an upstream to downstream pattern in water quality values, other than temperature, was not observed during the summer water quality sampling. Values remained fairly constant throughout the system or fluctuated minimally from site to site. Temperatures varied minimally between sites during the water quality sampling event (Table 6). However, continuously sampled temperature thermistor data (Appendix B) provide a more detailed data set than the temperature data collected seasonally with the water quality data or with the summertime grab samples.

Dissolved oxygen (DO) concentrations did not meet the water quality standard of 5.0 milligrams per liter (mg/l) at nine of fifteen Comal River sample sites during the summer sampling event. Blieders Creek is minimally influenced by spring flows and exhibits highly variable flows throughout the year (Appendix B). The Heidelberg site is located upstream of Landa Lake and downstream of Blieders Creek. Runoff and flow from the creek may affect water quality conditions at the Heidelberg site to a greater extent than at the other Comal River sites. Overall, DO concentrations recorded at the sites located in the upper portion of the Comal River (Blieders Creek, Heidelberg, and Spring Island sites) and the spring run sites were lower than concentrations recorded in the downstream sites (Table 6).

Table 6. Summary of Comal River ecosystem physical water quality measurements from a summer 2009 sampling event.

Location	Temperature (°C)	pH	Conductivity (µS/cm)	Dissolved Oxygen (mg/l)
Blieders Creek	25.93	7.36	538	5.66
Heidelberg, Main Channel	23.92	7.43	553	3.83
Island Park, Far Channel	23.37	7.46	557	3.82
Island Park, Near Channel	23.34	7.46	552	4.29
Spring Run 1, upstream	23.42	7.56	551	4.63
Spring Run 1, downstream	23.48	7.43	553	5.66
Spring Run 2	23.46	7.45	552	4.25
Confluence of Spring Runs 1 and 2	23.51	7.41	556	4.78
Spring Run 3	23.47	7.35	552	4.76
Landa Lake	23.70	7.44	554	5.16
New Channel, upstream	23.46	7.64	548	3.95
New Channel, downstream	23.50	7.60	547	6.48
Old Channel, upstream	23.59	7.67	549	4.42
Old Channel, downstream	24.03	7.78	577	5.17
Union Avenue (tube take-out)	23.94	7.82	554	6.48

Table 7. Summary of Comal River ecosystem water quality analytical results from a summer 2009 sampling event.

Location	TSS (mg/l)	Alkalinity (mg/l)	Ammonium (mg/l)	Nitrate (mg/l)	Total N (mg/l)	SRP (mg/l)	Total P (mg/l)
Blieders Creek	<4	230	0.122	1.0	1.91	<0.05	0.0229
Heidelberg, Main Channel	<4	220	0.121	1.51	2.08	0.0775	0.0255
Island Park, Far Channel	<4	230	0.0896	1.46	2.76	<0.05	0.0229
Island Park, Near Channel	<4	240	0.0366	1.57	2.18	<0.05	0.0229
Spring Run 1, upstream	<4	240	0.0656	1.64	2.34	<0.05	0.0102
Spring Run 1, downstream	<4	240	0.0454	1.63	2.2	<0.05	0.0178
Spring Run 2	<4	240	0.121	1.62	2.75	<0.05	<0.01
Confluence of Spring Runs 1 and 2	<4	240	0.0581	1.54	2.31	<0.05	<0.01
Spring Run 3	<4	240	0.0795	1.63	2.74	<0.05	<0.01
Landa Lake	<4	240	0.106	1.59	2.79	<0.05	0.0127
New Channel, upstream	<4	220	0.105	1.5	2.02	<0.05	<0.01
New Channel, downstream	<4	240	0.117	1.49	2.35	<0.05	0.0102
Old Channel, upstream	<4	240	0.0631	1.45	1.91	<0.05	<0.01
Old Channel, downstream	<4	240	0.0997	1.46	2.28	<0.05	0.0102
Union Avenue (tube take-out)	<4	240	0.082	1.47	1.96	<0.05	0.0153

The TSS values were very low (below 4 mg/l; Table 7) at all sites in the river, reflecting the clear water quality of this spring system. Alkalinity was consistent between sites during the summer 2009 sampling (Table 7), with values similar to those measured in 2000-2002 (BIO-WEST 2003).

The SRP concentrations and TP concentrations on the Comal River were well below the TCEQ's screening values of 0.1 mg/l and 0.2 mg/l respectively (Table 7). Only one site had a measurable concentration of SRP (Heidelberg, main channel; Table 7), and the highest values of TP (ranging from 23 to 26 µg/l) were measured at the upstream sites (Blieders Creek, Heidelberg main channel, Island Park far channel, Island Park near channel). Similar high values of TP were measured in 2000-2002 (27 to 30 µg/l; BIO-WEST 2003). Point source discharges include the wastewater treatment plant located on the Dry Comal River which enters near the New Channel Upstream site and the Schlitterbahn Water Park which enters the Old Channel. Non-point source discharges include runoff from urban areas (City of New Braunfels), some agricultural areas and a municipal golf course. Although values are higher at these sites, it should be stressed that these SRP values are well below the TCEQ's screening levels for surface waters.

Nitrate values exceeded the water quality standards screening level of 1.0 mg/l in most cases, whereas, ammonium values were well below the screening level of 1.0 mg/l (Table 7). The TN values for the Comal River are influenced by the high nitrate concentrations. These high values are not the result of

anthropogenic inputs to the immediate surface waters. The spring flow is the most likely source of high nitrate values found at all sites in the Comal River system. The median concentration of nitrate in the Edward's Aquifer ranges from 1.4 to 1.7 mg/l (Bush et al. 1998). Nitrate values in the Comal River were fairly constant throughout the river in 2009, and were similar to values in 2000-2002 (1.3 to 2.5 mg/l; except at two sites during the August 2000 when nitrate values reported were near 6.0 mg/l at the Other Place and Island Park; BIO-WEST 2003). In contrast, ammonium concentrations varied among sites (Table 7), at levels well below the screening level.

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 2) on a weekly basis. Typically, data are collected over a two-hour time span (approximately 20 minutes spent at each site) on a Friday afternoon, although data has been collected between 9am – 4pm and on other days of the week.

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 (CO₂) concentrations in the water column. Water quality data collected by Master Naturalist volunteers in 2009 showed that CO₂ concentrations were highest near springs (Figure 5), while pH increased going downstream (Figure 6). At all sites, CO₂ concentrations were similar between years (2006-2009). Carbon dioxide will continue to be a key parameter to monitoring during Critical Period conditions because of its influence on aquatic vegetation.

In addition to water quality measurements, recreational use data was collected at each site by counting the number of swimmers, shore anglers, boat anglers, recreational boaters (e.g., kayaks, paddle boats, etc.), people with dogs, and other recreationists 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. To compare recreational use at the various sites, weekly counts of recreation users were converted to monthly averages and plotted over the survey period (Figures 7-11). Recreational use data shows that the Elizabeth Street site is very infrequently used (Figure 7), while the Upper Spring Run at Union Avenue is used by a few people usually during the summer months (Figure 8). The Landa Lake park gazebo area is used for recreation regularly during all months of the year (Figure 9), although it does not attract as many people as the New Channel park site. The New Channel site is the most heavily recreated site, with recreation concentrated from March until September (Figure 10). Tubing is the dominant recreational activity at this site, especially between May and September. This is not surprising since it is both within a park setting and is heavily used by tubers as a launching point. The Union Avenue site is the second most heavily used of the recreation sites, because it is an exit station for tubers during the summer months (Figure 11). During 2009, recreational use at the Union Avenue site appeared to be concentrated between May and September. At four of the five sampling locations (excluding the Upper Spring Run site), recreational use appeared to be higher in 2009 than in 2007 or 2008.

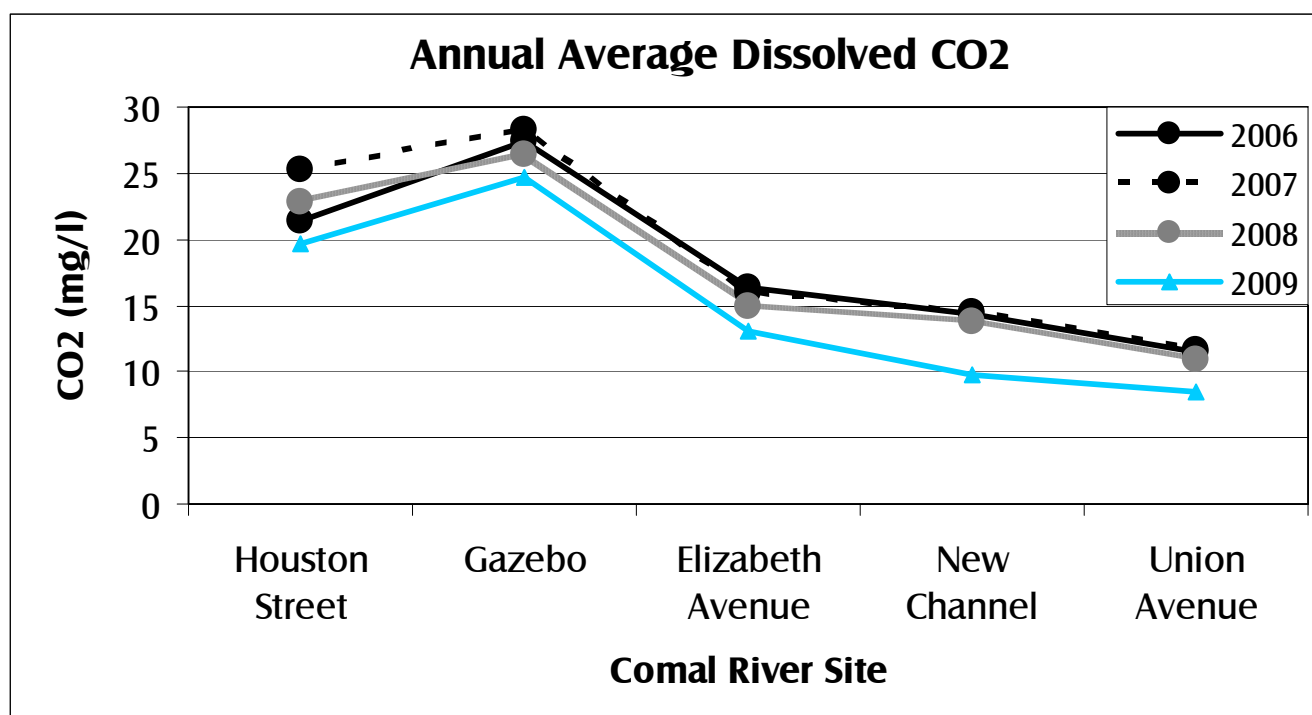


Figure 5. Annual average dissolved carbon dioxide (CO₂) concentrations at five sites on the Comal River system (2006-2009).

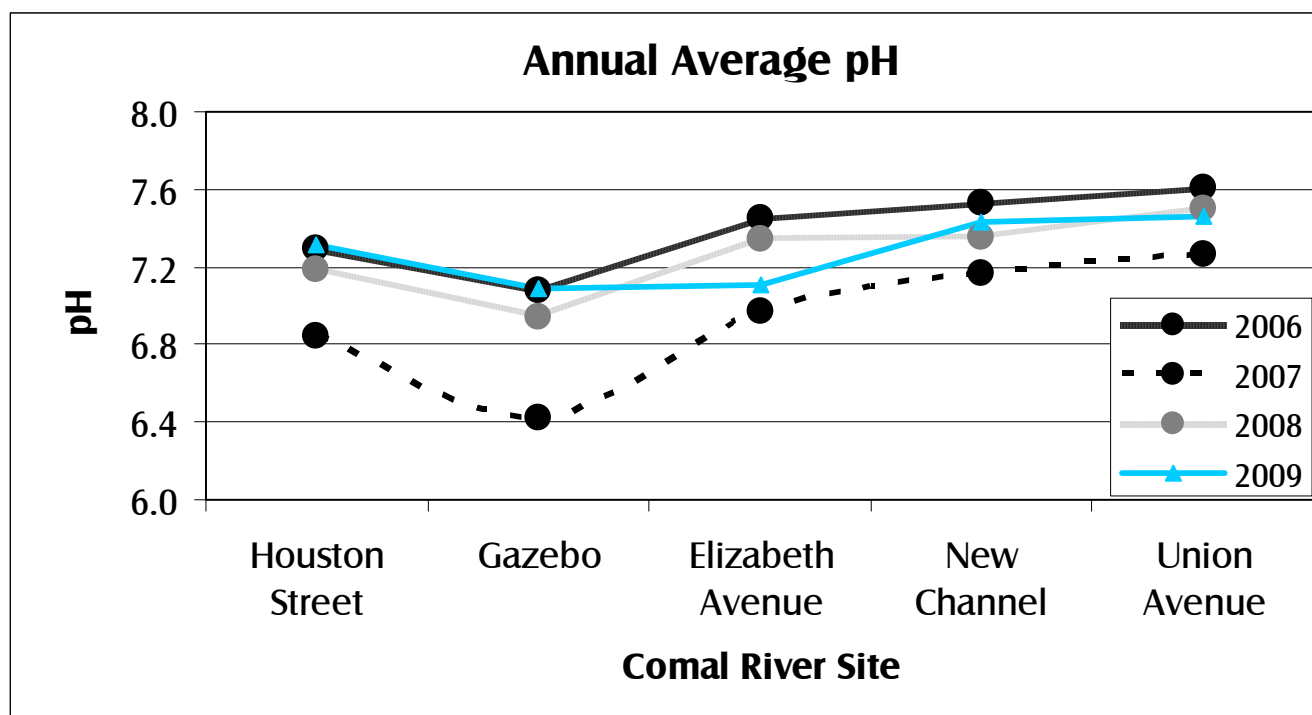


Figure 6. Annual average pH values at five sites on the Comal River system (2006-2009).

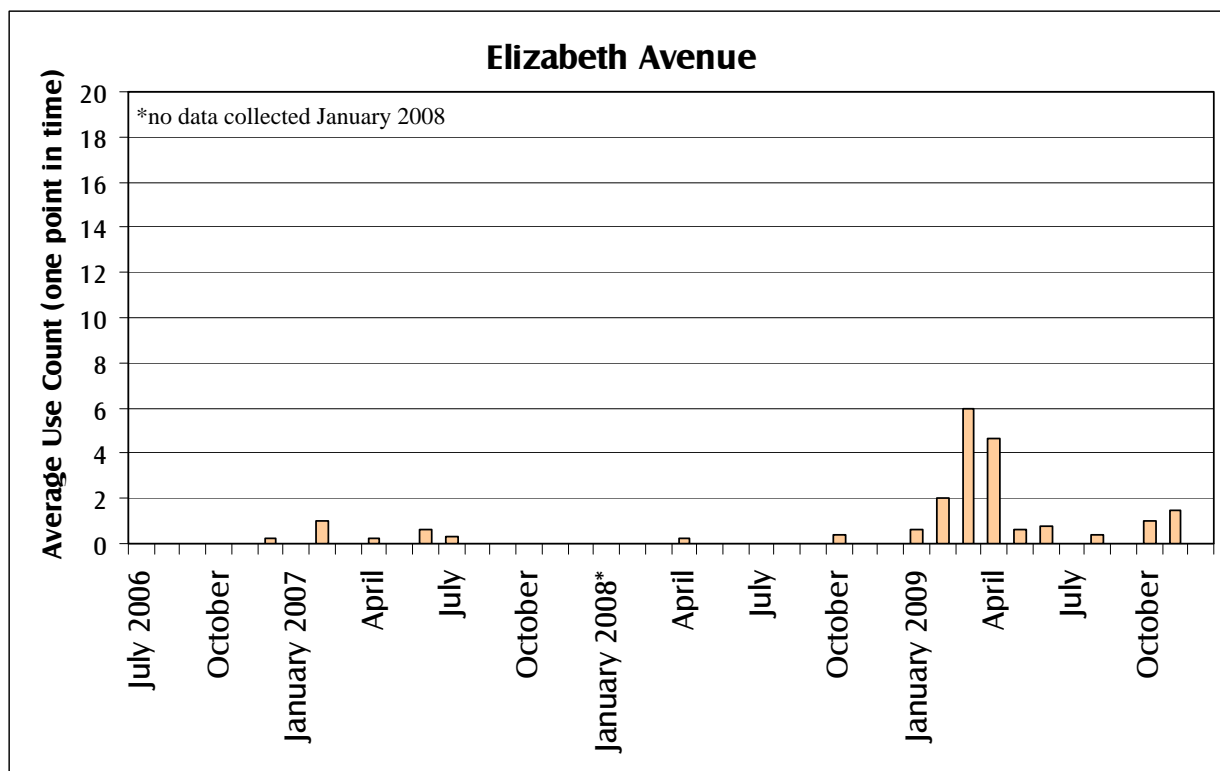


Figure 7. Average recreational use counts at the Elizabeth Avenue site (2006-2009).

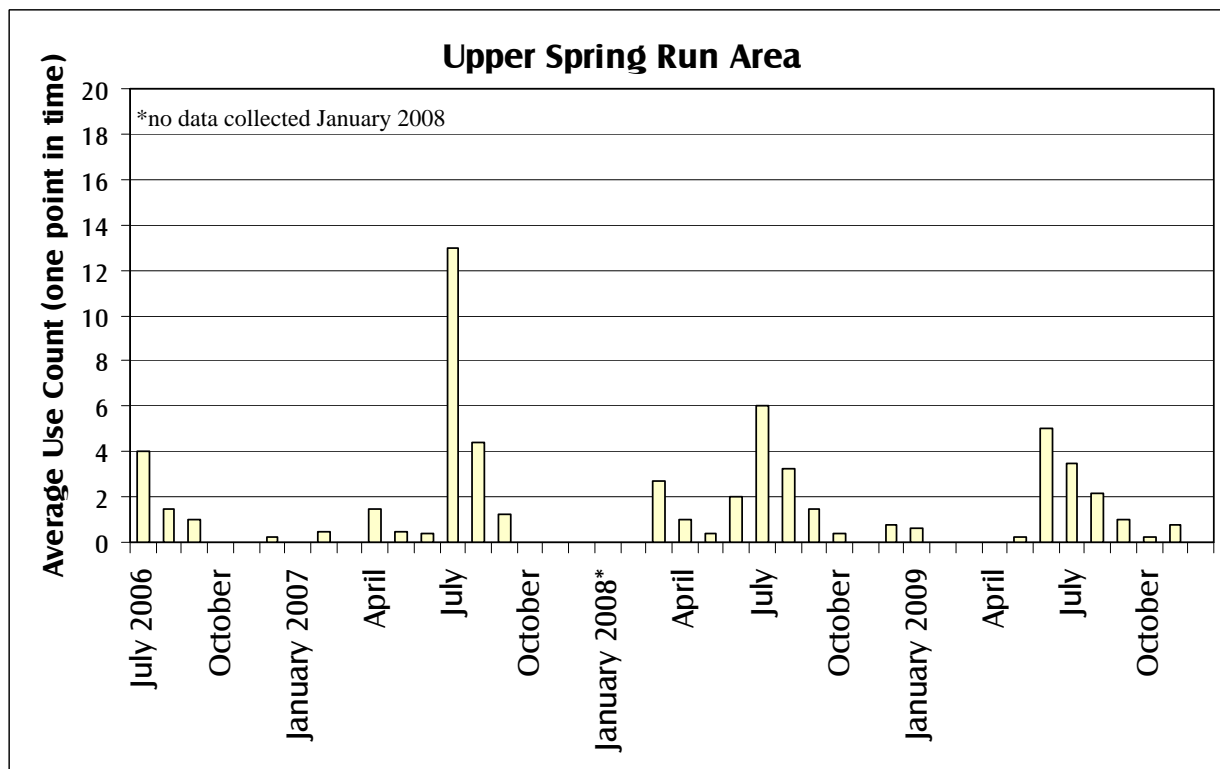


Figure 8. Average recreational use counts at the Upper Spring Run area (2006-2009).

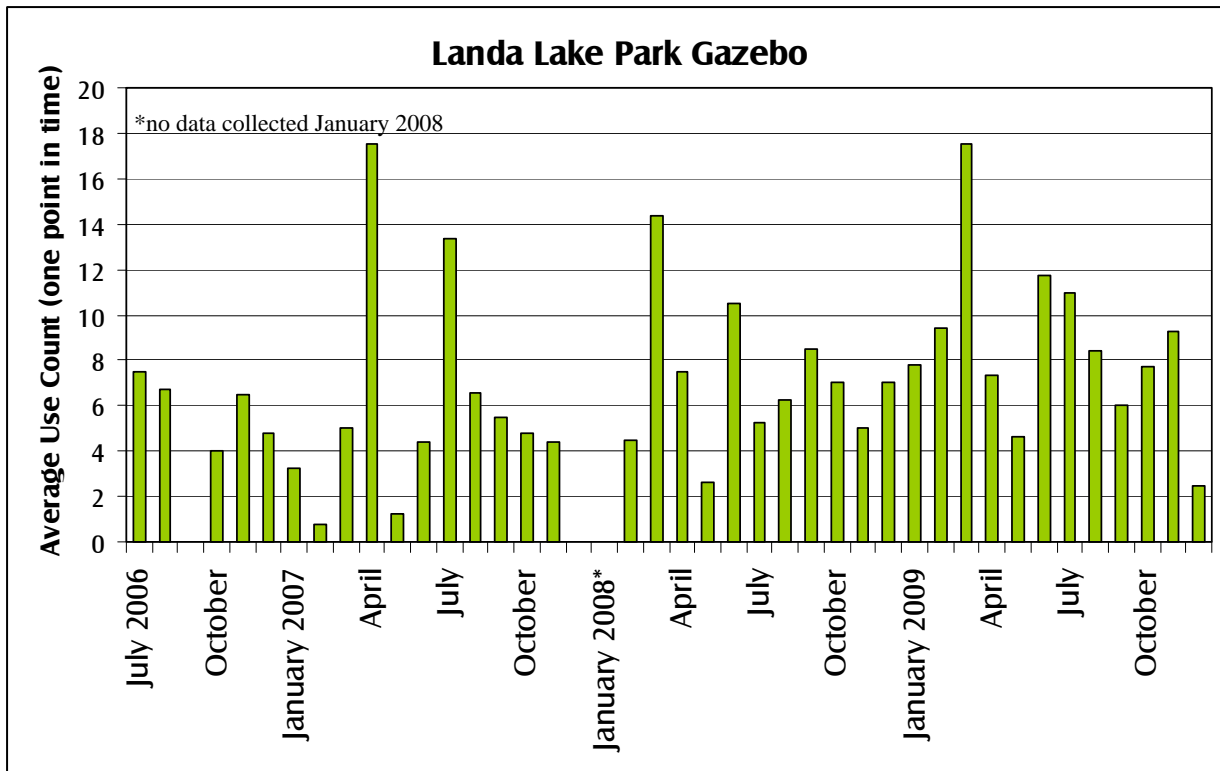


Figure 9. Average recreational use counts at the Landa Lake Park Gazebo site (2006-2009).

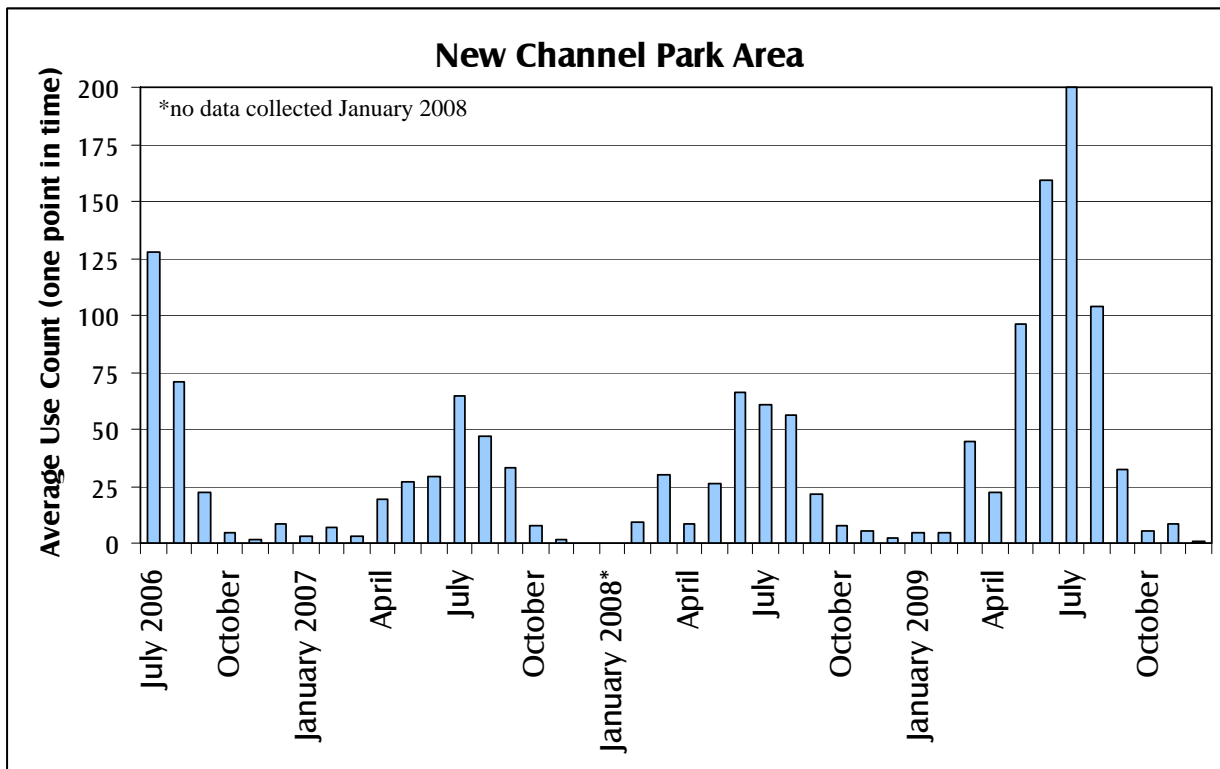


Figure 10. Average recreational use counts at the New Channel site (2006-2009).

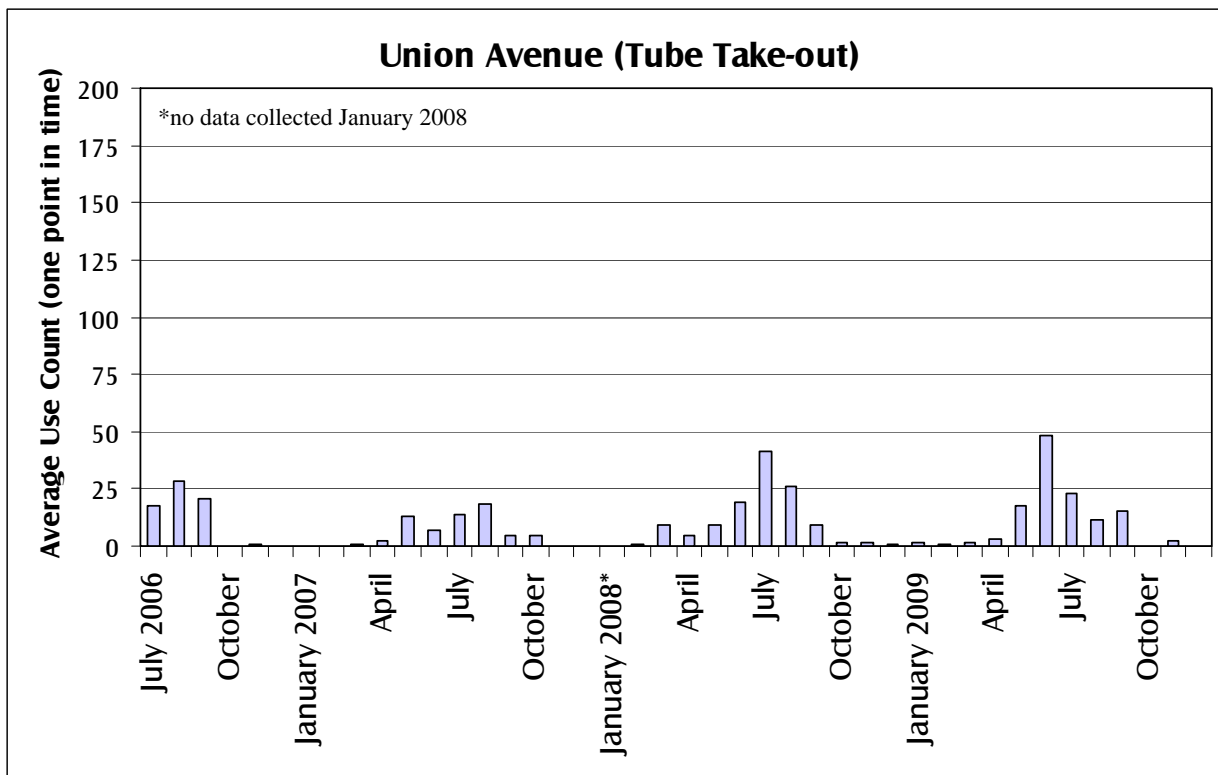


Figure 11. Recreational use counts at the Union Avenue site (2006-2009).

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

The continued drought in Central Texas contributed to reduced flows in the Comal River and its tributaries. Reduced springflow in the Upper Spring Run Reach led to changes in the aquatic vegetation community there. Green algae dominated this reach in spring covering much of the lower half of the reach (1,568.4 m²) where bryophytes are usually dominant. However, bryophytes were still present in much of the upper half of the reach (1,067.8 m²). As flows continued to decrease into the summer, the green algae (54.0 m²) became reduced throughout the entire reach, while bryophytes (1,636.4 m²) dominated both the upper and lower portions. Coverage of *Hygrophila* became patchier as the year continued decreasing from 529.0 m² (spring) to 314.3 m² (Critical Period 1). The native plant *Ludwigia* continued its tenuous grasp in this reach with a few plants still present in the middle of the river. The final aquatic vegetation mapping effort occurred on October 13, 2009, which was 9 days after a major precipitation event resulted in flows of over 4,000 cfs in the Comal River. As a result, plants that are not firmly rooted to the substrate like algae and bryophytes had a difficult time remaining in the reach. When the Upper Spring Run Reach was mapped in the fall, algae were no longer present and bryophytes had decreased nearly in half (853.4 m²) with much of it only remaining in the lower half of the reach. Only small patches remained in the upper half of the reach near Blieders Creek (an extremely flashy

stream). *Hygrophila* also decreased substantially (131.0 m^2), while only a few patches of *Ludwigia* remained (2.8 m^2).

Native plants are an important habitat for fountain darters in the Comal River. The highest concentrations are found in *Cabomba*, *Ludwigia*, and bryophytes. However, *Cabomba* is no longer present in this reach, and *Ludwigia* is too patchily distributed to contribute much high quality fountain darter habitat. Although *Sagittaria* is abundant in this reach and less prone to flushing events (more firmly rooted), it doesn't harbor the numbers of fountain darters that other native plants do because of its simple leaf structure. Overall, the non-native *Hygrophila* contains fewer darters, but it does appear to be of some importance to these fish within the Upper Spring Run Reach. 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

The reduced flows in the Comal River affected the Landa Lake Reach in similar ways to the Upper Spring Run Reach. Native vegetation like *Cabomba* and bryophytes changed little during the first half of the year. *Cabomba* increased slightly (173.8 m^2 to 180.0 m^2) by July (Critical Period 1), while bryophytes decreased in coverage ($2,788.9 \text{ m}^2$ to $2,347.3 \text{ m}^2$) during that time. By fall, *Cabomba* increased slightly (181.0 m^2) even though a major flushing event had occurred in the system prior to mapping. *Cabomba* prefers slower-moving habitats like behind islands and along river-right of the upper portion of Landa Lake where it is protected from much of the flow. However, bryophytes are found in the open water in this reach where they are highly susceptible to flushing events. As a result, the total areal coverage was reduced to 385.6 m^2 by the fall mapping effort. These drastic reductions in bryophytes are not unusual and appear to be a seasonal fluctuation as it happens in most years. *Hygrophila* coverage exhibited a similar trend as bryophytes decreasing over the course of the year (605.3 m^2 [spring], 497.2 m^2 [Critical Period 1], 474.5 m^2 [fall]). *Ludwigia* remained patchily distributed decreasing slightly from spring (23.4 m^2) to fall (17.8 m^2).

As in the Upper Spring Run Reach, native plants are important to the populations of fountain darters found in Landa Lake. Similarly, *Cabomba* and bryophytes yield high numbers of these fish, but unlike the Upper Spring Run Reach *Cabomba* is perennially found in several patches within the lake. While these patches are located in relatively deep water we are able to sample them and confirm their importance as fountain darter habitat. As stated above these *Cabomba* patches are located in relatively protected areas making their continued presence extremely important to these fish. Bryophytes are also an important habitat for darters, as confirmed by drop net density data. *Vallisneria* is the most common native plant in this reach, but it yields low numbers of fountain darters because of its simple leaf structure. A diverse community of native vegetation in Landa Lake will continue to be invaluable to fountain darter populations in the Comal River.

Old Channel Reach

Non-native plants cover a majority of the river in the Old Channel Reach. Here, *Hygrophila* is abundant, and the aquarium plant, *Ceratopteris*, has gained a foothold. Native plants that were once dominant here are patchily distributed. Although flows decreased across the system through the beginning of 2009, discharge was relatively static in this reach because of a culvert that controls the flow through the Old Channel. As a result, total areas and the relative proportions of each species varied little over 2009. *Hygrophila*'s dominance in the reach continued increasing slightly from spring ($1,526.1 \text{ m}^2$) to fall ($1,569.1 \text{ m}^2$). Areal coverage of *Ludwigia* increased as flows decreased (23.3 m^2 [spring] to 48.4 m^2 [Critical Period]) and by fall (39.2 m^2) was only slightly less than in 2008 (43.8 m^2 [BIO-WEST 2009]). As in previous years, *Ludwigia* is almost entirely confined to the shallower upstream end of the

reach. Unlike previous years, filamentous algae remained relatively constant in 2009. By July, areal coverage of filamentous algae had increased to 39.1 m² while flows continued to decrease in the Comal River. Although it decreased by more than half by fall of 2009 (19.2 m²) it was still more prevalent than in the spring (12.6 m²).

The regulated flow in this reach allows a unique opportunity to monitor the interaction between native (filamentous algae, bryophytes, *Ludwigia*, *Nuphar*) and non-native (*Hygrophila*, *Ceratopteris*) plants in a relatively non-flashy channel. As 2009 progressed, flows decreased in the Comal River and slower velocities led to somewhat stagnant conditions in many of the reaches. This was not the case in the Old Channel Reach, and as a result areal coverage of all plants changed little. Although the amount of native plants remained relatively constant in 2009, they have still decreased substantially since the inception of this study. Close monitoring is necessary to understand the interactions between native and non-native plants in this reach and how they affect fountain darter populations.

New Channel Reach

Because the New Channel Reach is highly channelized it is the most susceptible to flashy flows. Concrete walls confine most of this reach and leave plants highly vulnerable to scouring during localized precipitation events. Although *Cabomba* decreased slightly from 2008 (751.2 m² to 680.3 m²) to 2009 it still flourished along the river right side where there is more protection from higher velocities due to the slight bend in the river. Similarly, *Hygrophila* (the other dominant plant in this reach) decreased slightly from 2008 (2,130.8 m²) to 2009 (1,991.1 m²). *Ludwigia* continued its tenuous hold on this reach decreasing from spring (23.1 m²) to July (6.9 m²) 2009 where it was reduced to a single patch in the upper portion of the reach. The fall high-flow event that produced flows in excess of 4,000 cfs in the Comal River decimated the aquatic vegetation community in the New Channel Reach. By October, only *Cabomba* (73.0 m²) and *Hygrophila* (100.0 m²) remained in this section of the river. It will likely take a significant amount of time for this reach to see the amount of aquatic vegetation that was present prior to October 2009.

Fountain Darter Sampling Results

Drop Nets

A total of 66 drop net samples were conducted during 2009 in the Comal Springs/River ecosystem. The number of drop net sites and vegetation types sampled per reach is presented in Table 8. 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 are 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. Again in fall 2009, filamentous algae were very sparse in the Old Channel Reach, and *Hygrophila* was substituted for both algae sites.

Table 8. Drop net sites and vegetation types sampled per reach in 2009.

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

^a Switched from Open to Bryophytes, summer 2001.

^b Due to limited coverage, filamentous algae were not sampled in fall 2009. Two additional *Hygrophila* sites were sampled.

The number of fountain darters captured in each drop netting event in 2009 varied from a low of 532 in fall to a high of 610 in summer. Excluding collections from the New Channel Reach since it is no longer sampled; the number captured during each event over the course of the study has varied from 224 to 901. Figure 12 demonstrates the number of fountain darters collected in each drop net event overlain on a hydrograph showing mean daily discharge. Due to the extremely variable nature of the discharge data, no discharge-abundance relationships are obvious from this analysis. Although fountain darter abundance varies considerably, a linear trendline suggests a rather stable population throughout the study period with no long term trend.

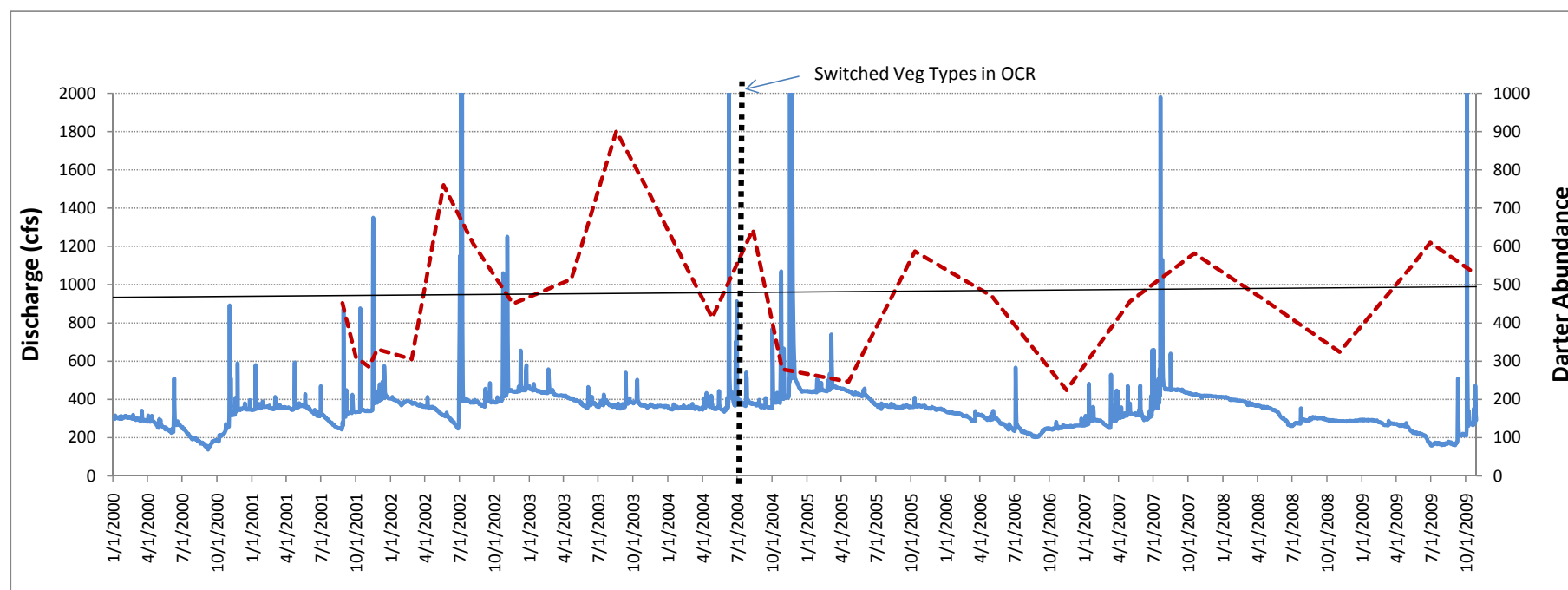


Figure 12. Abundance of fountain darters from each drop net sampling event (red dashed line) plotted over a hydrograph of mean daily discharge from the USGS gauge on the Comal River at New Braunfels (blue line).

Drop net data collected from 2000-2009 show that average densities of fountain darters in the various vegetation types ranged from 3.6/m² in *Ceratopteris* to 23.9/m² in bryophytes (Figure 13). Native vegetation types which provide thick cover at or near the substrate (i.e., bryophytes and filamentous algae [23.5/ m²]) 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* [7.0/m²]), and native vegetation with simple leaf structures (*Vallisneria* [5.0/m²] and *Sagittaria* [4.7/m²]) 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 (9.9 and 13.4/ m², respectively).

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 to a lesser extent, *Ludwigia*. This has resulted in an overall decrease in the abundance of fountain darters in this reach (see dipnet data). Bryophytes are a key habitat component because they typically 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, 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.

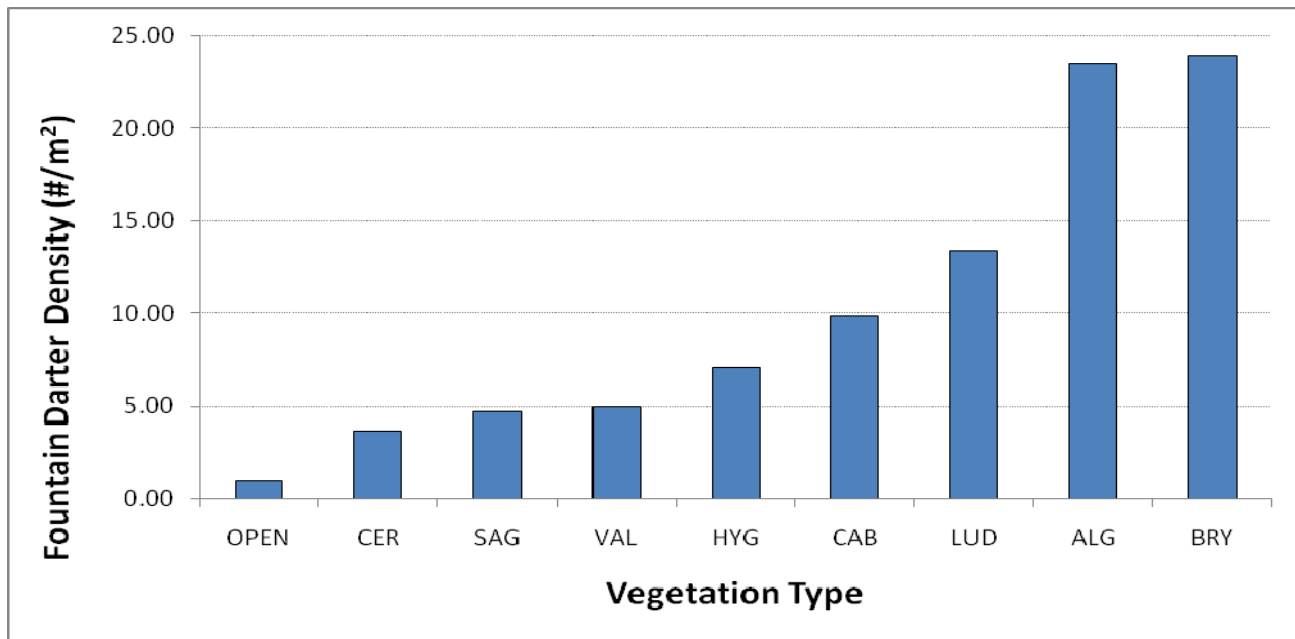


Figure 13. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem from 2000-2009. CER - *Ceratopteris*, SAG - *Sagittaria*, VAL - *Vallisneria*, HYG - *Hygrophila*, CAB - *Cabomba*, LUD - *Ludwigia*, ALG - Filamentous Algae, BRY - Bryophytes.

Estimates of fountain darter population abundance in all reaches (Figure 14) 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 population abundance were highest in spring 2003 when coverage of bryophytes peaked in Landa Lake (Figure 14). Population estimates in fall 2000, winter 2001, and spring 2001 are low because mapping at the time did not include algae in the Old Channel Reach or bryophytes in the Landa Lake Reach. All high-flow Critical Period samples during the study period showed a decrease in population estimate 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. A high-flow event immediately prior to the fall 2009 sampling event resulted in scouring of bryophytes in Landa Lake, and therefore, a low population estimate. Although population estimates for fall 2009 decreased considerably from previous estimates, all population estimates from 2009 fell within the range observed over the study period.

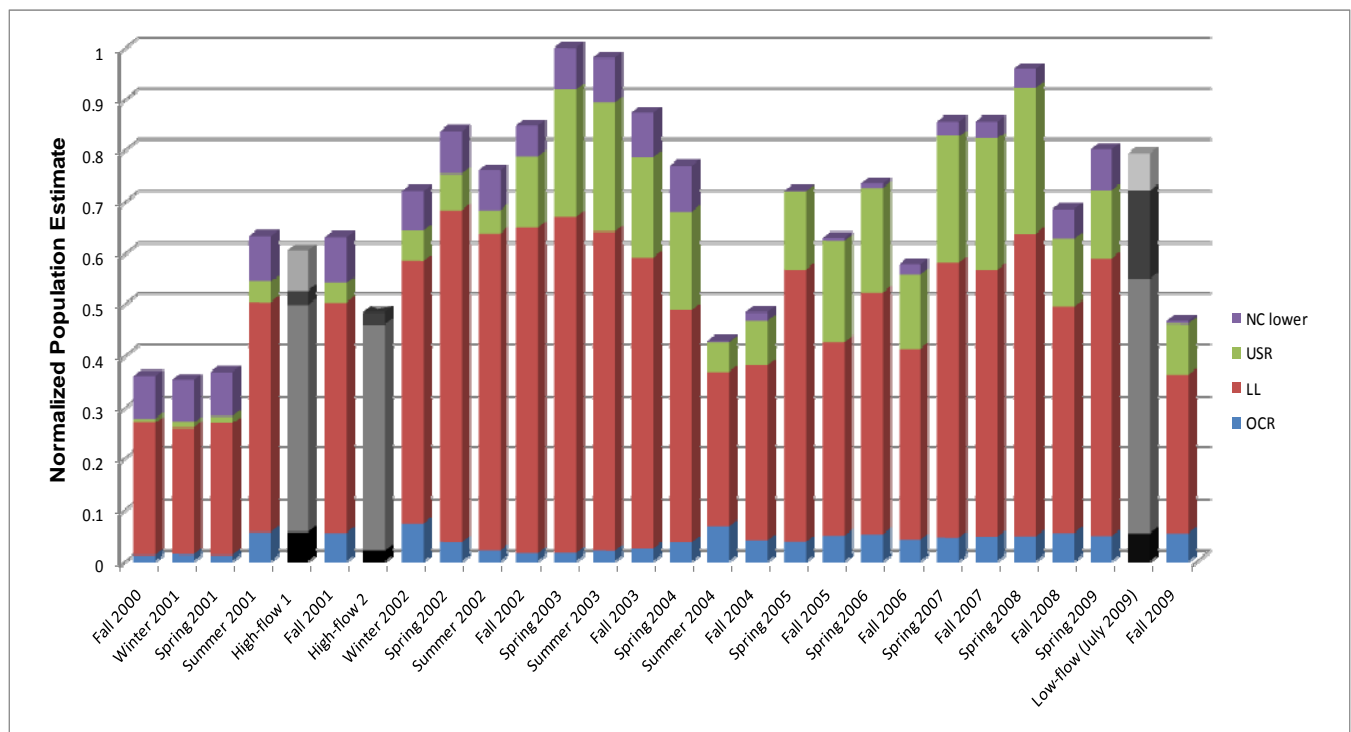


Figure 14. Population estimates of fountain darters in all four sample reaches combined (2000-2009); values are normalized to the maximum sample. Black and gray bars represent critical period sampling events.

Drop netting efforts in 2009 resulted in collection of 1,692 fountain darters in the Comal River/Springs ecosystem. The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem during spring and fall 2009 is presented in Figure 15 (all data collected in previous years are 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.

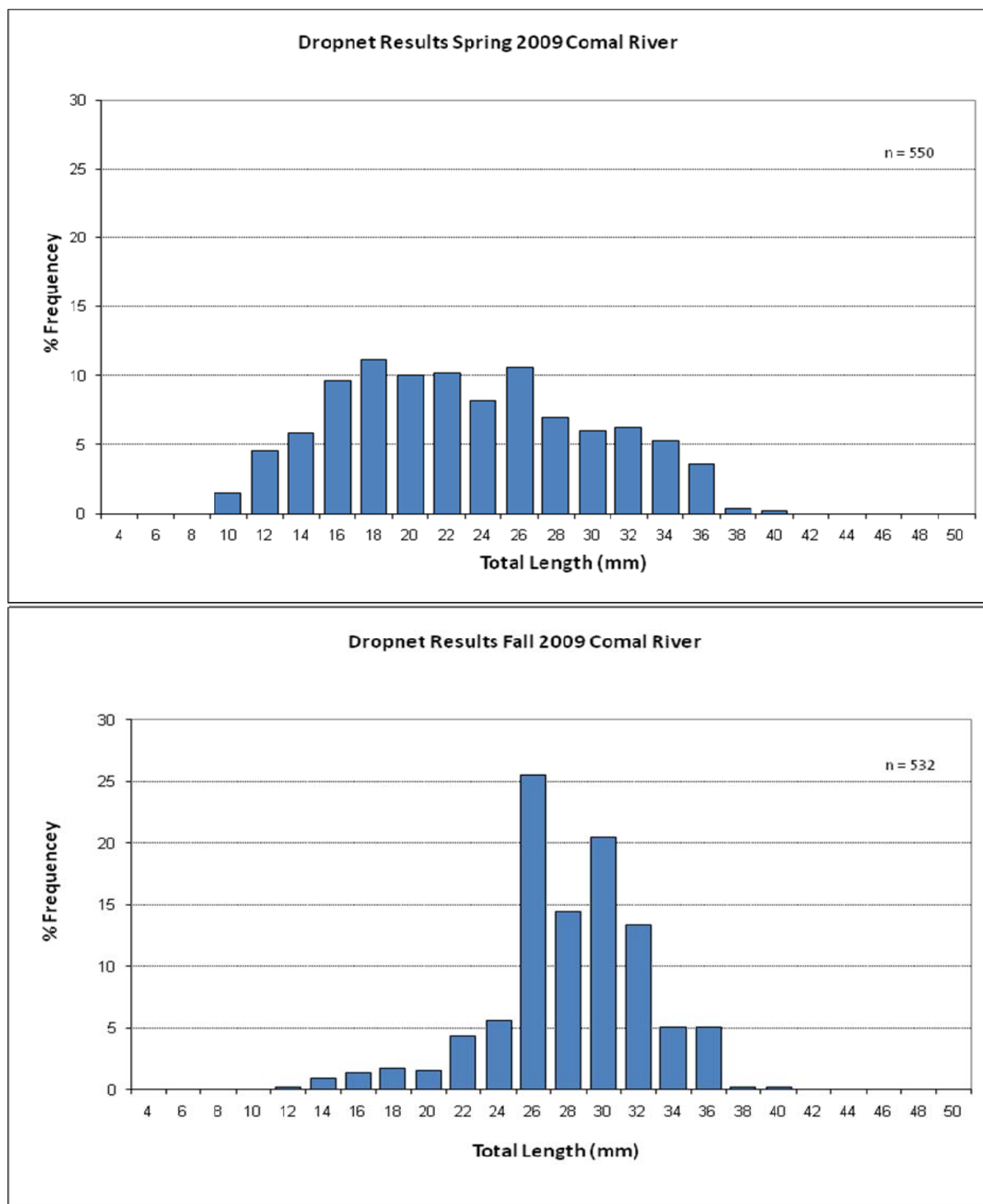


Figure 15. Length frequency distribution of fountain darters collected from the Comal River by drop-netting in spring and fall 2009.

In addition to fountain darters, 116,105 specimens representing at least 25 other fish taxa have been collected by drop netting from the Comal Springs/River ecosystem during the study period. Of these, seven are considered exotic or introduced (Table 9). 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 may be further evaluated under extended periods of low discharge.

Other potential impacts of exotic fish species include negative effects of herbivorous species such as the armadillo del río or suckermouth catfish (*Hypostomus plecostomus*) on algae and vegetation communities that serve as critical 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.

Table 9. Fish taxa and the number of each collected during drop-net sampling.

Family	Scientific Name	Common Name	Status	2009	2000-2009
Cyprinidae	<i>Campostoma anomalum</i>	Central stoneroller	Native	0	1
	<i>Dionda nigrotaeniata</i>	Guadalupe roundnose minnow	Native	61	504
	<i>Notropis amabilis</i>	Texas shiner	Native	53	211
	<i>Notropis volucellus</i>	Mimic shiner	Native	2	31
	<i>Pimephales vigilax</i>	Bullhead minnow	Native	0	4
Characidae	<i>Astyanax mexicanus</i>	Mexican tetra	Introduced	2	373
Ictaluridae	<i>Ameiurus melas</i>	Black bullhead	Native	0	1
	<i>Ameiurus natalis</i>	Yellow bullhead	Native	7	99
Loricariidae	<i>Hypostomus plecostomus</i>	Armadillo del rio	Introduced	11	56
Poeciliidae	<i>Gambusia</i> sp.	Mosquitofish	Native	18681	106744
	<i>Poecilia latipinna</i>	Sailfin molly	Introduced	424	4429
Centrarchidae	<i>Ambloplites rupestris</i>	Rock bass	Introduced	0	24
	<i>Lepomis auritus</i>	Redbreast sunfish	Introduced	0	136
	<i>Lepomis cyanellus</i>	Green sunfish	Native	0	10
	<i>Lepomis gulosus</i>	Warmouth	Native	0	32
	<i>Lepomis macrochirus</i>	Bluegill	Native	0	213
	<i>Lepomis megalotis</i>	Longear sunfish	Native	0	250
	<i>Lepomis microlophus</i>	Redear sunfish	Native	0	2
	<i>Lepomis miniatus</i>	Redspotted sunfish	Native	302	1499
	<i>Lepomis</i> sp.	Sunfish	Native/Introduced	50	717
	<i>Micropterus punctulatus</i>	Spotted bass	Native	0	3
	<i>Micropterus salmoides</i>	Largemouth bass	Native	6	128
Percidae	<i>Etheostoma fonticola</i>	Fountain darter	Native	1692	13596
	<i>Etheostoma lepidum</i>	Greenthroat darter	Native	5	35
Cichlidae	<i>Cichlasoma cyanoguttatum</i>	Rio Grande cichlid	Introduced	43	554
	<i>Oreochromis aureus</i>	Blue tilapia	Introduced	27	49
Total				21366	129701

Another exotic species which has had considerable impact on the vegetation community in the Comal Springs/River ecosystem in the past is the giant ramshorn snail (*Marisa cornuarietis*). 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. Figure 16 shows the number of giant ramshorn snails collected during drop netting for each year. Since this exotic species can have considerable impacts at higher densities, close monitoring of their populations will continue.

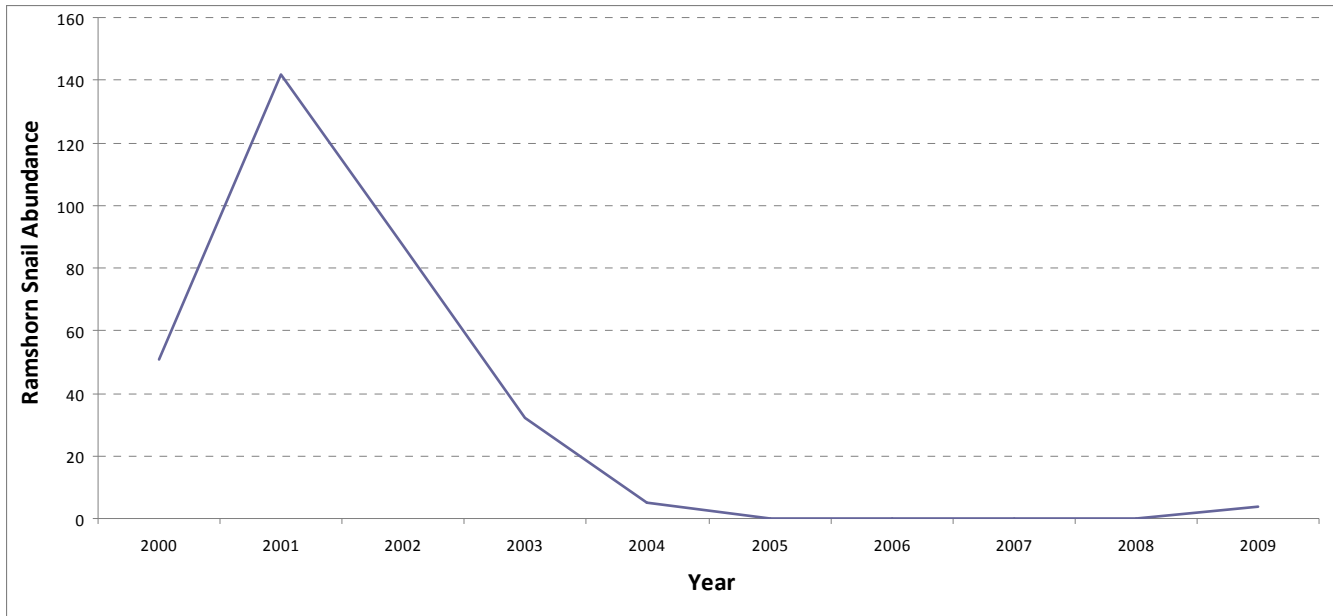


Figure 16. Abundance of giant ramshorn snails in drop net collections from the Comal Springs/River ecosystem during 2000-2009.

Dip Nets

Data gathered using dip nets are graphically represented in Figure 17 for the Old Channel Reach and in Appendix B for all other reaches. The boundaries for each section of the dip net collection efforts are depicted in Figure 18.

Figure 17 provides a good example of how changes in vegetation community can affect fountain darter population dynamics. In 2005, the vegetation community of the Old Channel Reach switched from being dominated by high-quality filamentous algae (native) to one dominated by *Hygrophila* (non-native). 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 are typically only 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.

Overall, size class distributions of fountain darters from dip netting correlate well with those of drop netting: small fountain darters most abundant in the spring, and larger darters dominating fall samples (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 filamentous algae and bryophytes, which provide high-quality fountain darter habitat according to drop net density estimates.

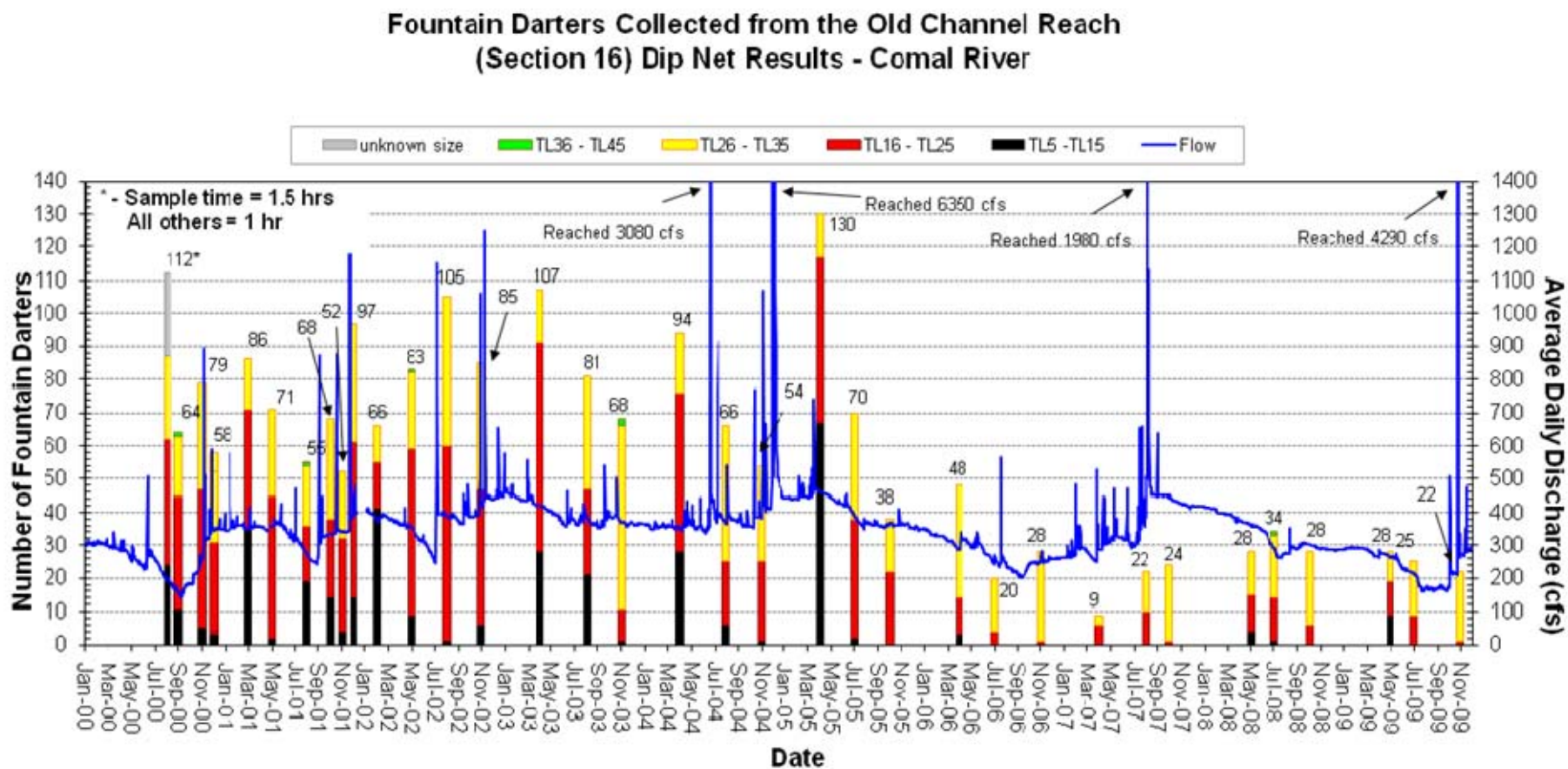


Figure 17. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

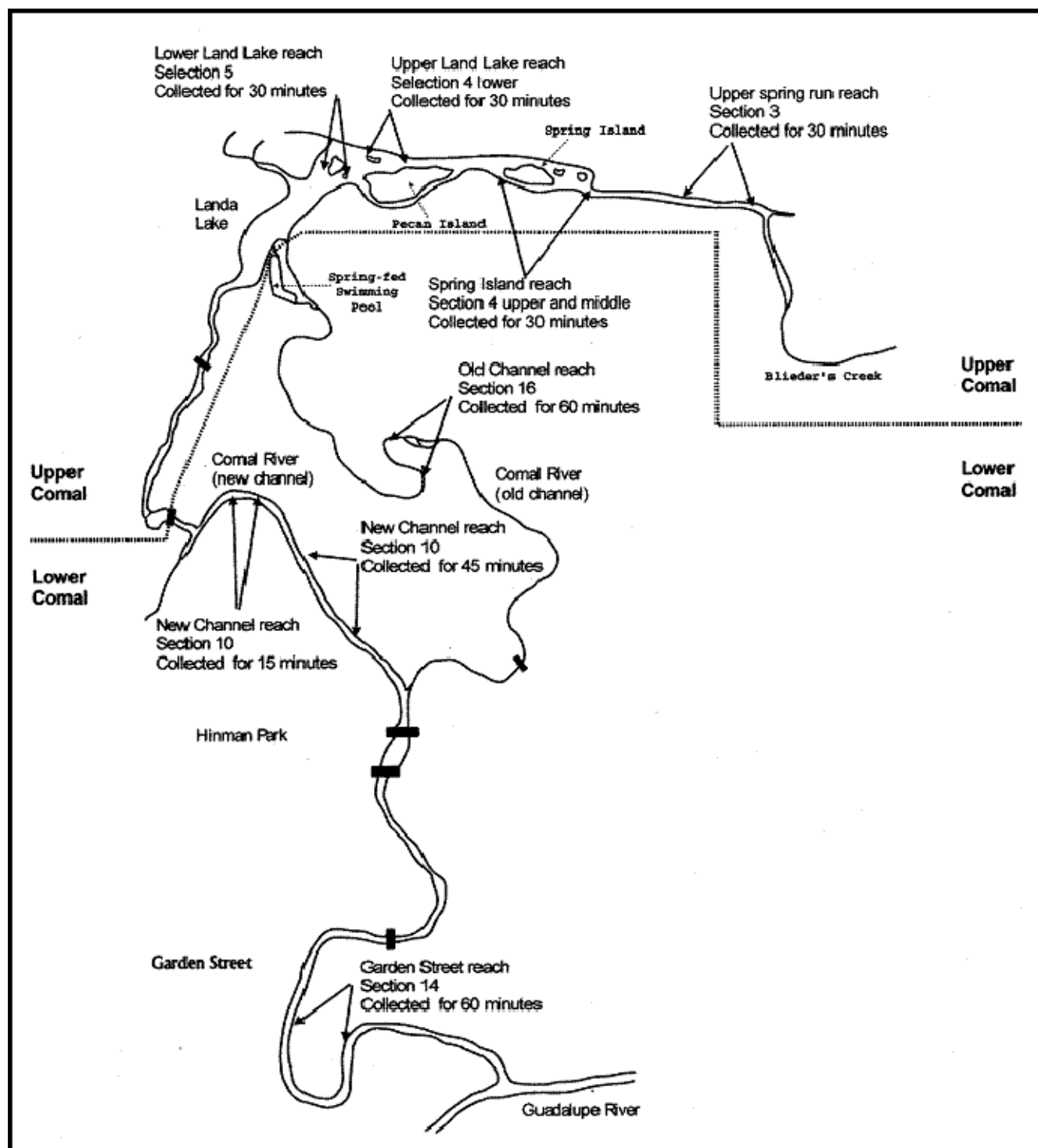


Figure 18. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

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 the 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

In 2009, presence/absence dip netting was conducted on the Comal River during the typical spring (May 7) and fall (October 20) sampling events, as well as one low-flow Critical Period event in the summer (July 3). The percentage of sites with fountain darters during 2009 varied from 58% in May and June to 52% in October (Figure 19). Results from fall 2008 through fall 2009 (52-58%) are somewhat lower than those observed from fall 2005 through spring 2008 (60-70%). However, this may simply represent the variation inherent in a random sampling routine.

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 provide a good baseline for comparison in future critical period events.

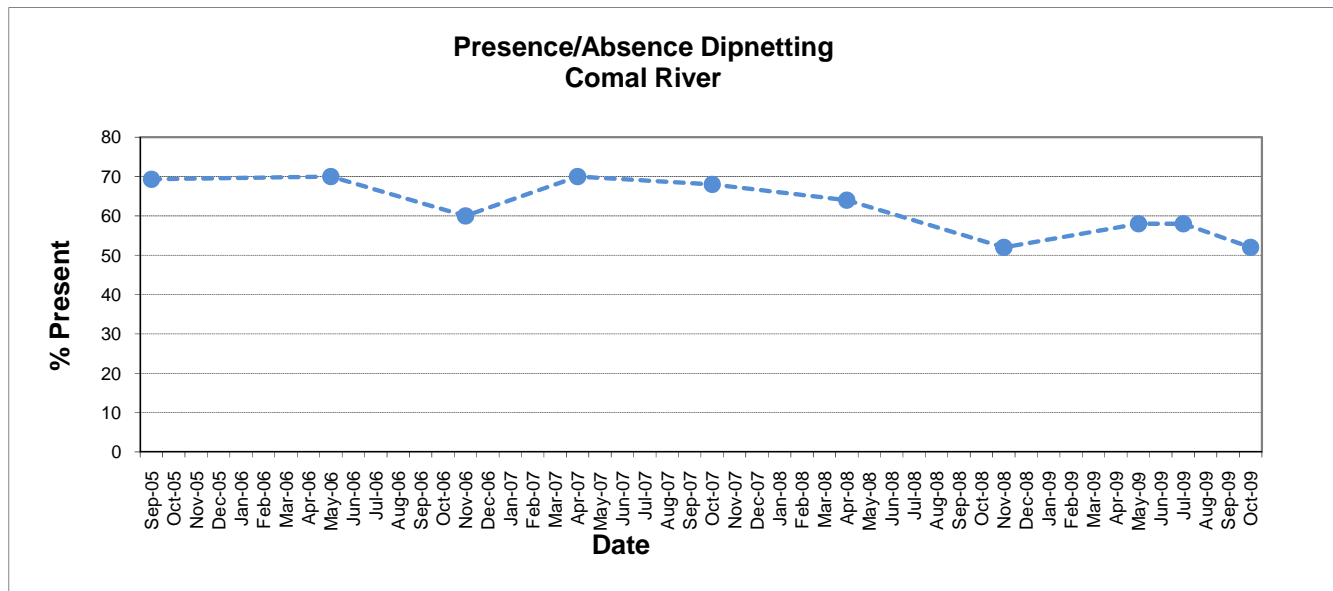


Figure 19. Percentage of sites (N = 50) in which fountain darters were present during 2005-2009.

Gill Parasite Evaluations

Bolick (2007) found that as distance increased from the head waters of the Comal River, the numbers of parasites increased. Average numbers of parasites per liter (L) of river water at the three sites were 1.3, 4.5, and 6.8. Bolick hypothesized that spring flow level had an effect on drifting parasite numbers but did not find this to be true. Between June 2006 and May 2007, the average flow from Comal Springs was 268 cfs with a range of 204 to 441 cfs. After Bolick completed her study, it was thought that the effect of spring flow on parasite numbers may not become a factor until spring flows dropped below 200 cfs. This current study was initiated because spring flows had dropped below 200 cfs and the drought was expected to continue.

During 2009, average parasite numbers per L of river water was 0.7 (site closest to head waters), 1.7, and 3.0 (site furthest from head waters). Over a 46% decrease in numbers of parasites collected at each site occurred between Bolick's study and the 2009 efforts. Similar to Bolick's findings, the 2009 sampling did not demonstrate that spring flow level had an effect on drifting parasite numbers at the sites evaluated. Two months after Bolick's last collection, Comal flow was 1,980 cfs. Flows at Comal Springs remained above 400 cfs for almost 6 months following the July 20, 2007 flood. *M. tuberculatus* is a large snail that requires a flow stable habitat, and high flow pulses as observed in 2007 wash snails downstream. It is assumed that the high flows of July 2007 caused a decrease in the *M. tuberculatus* population which caused a decrease in the parasite population. To understand parasite population dynamics, *M. tuberculatus* population dynamics may need to be monitored. Continued monitoring of the gill parasite will be important in determining how various factors such as current and past spring flow levels, season, turbulence, hosts numbers, and vegetation affect parasite numbers.

Comal Springs Salamander Visual Observations

Decreasing flows in the Comal River in 2009 did not appear to negatively affect the salamander populations in the Comal Springs Ecosystem (Table 10). The total number of salamanders observed during the spring (71) and Critical Period (71) sampling efforts were the highest since the inception of the study. Although total observations decreased in fall 2009 (50), they were still comparable to previous years. One salamander was observed at the Spring Island Spring Run during the spring 2009 observation. This represents the first salamander observed here since 2007. The abundant fist-sized rocks that were once common in this area have disappeared in recent years, possibly due to human disturbance. The largest decrease in observations during 2009 took place at Spring Run 3 between July and November when observations dropped from 26 to 9. The higher flows during November likely resulted in a less successful search effort. Salamander observations also decreased at the Spring Island East Outfall between spring (12) and fall (4) 2009. After several precipitation events, increased flows through this reach flushed out much of the bryophytes that cover the rocks. As a result, salamanders may have moved to more optimal habitat outside of the reach. Overall, salamander populations appear to be relatively stable.

Table 10. Total number of Comal Springs salamanders observed at each survey site during 2001 – 2009.

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
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
Spring 2009	32	26	1	12	71
Low-flow 2009	35	26	0	10	71
Fall 2009	37	9	0	4	50
Average	20.7	12.2	4.3	10.4	47.6

Macroinvertebrate Sampling

In 2009, drift net sampling around spring openings and regular monitoring of Comal Springs riffle beetles were conducted in several locations to assess habitat requirements and population dynamics of the federally listed invertebrate species.

Drift Net Sampling

At least 10 taxa were captured from 144 hours of sample time at the three drift net sites in Comal Springs during 2009 (Table 11). Table 12 displays the physico-chemical data collected at these sites during sampling. More Peck's cave amphipods (*Stygobromus pecki*) were caught in 2009 (92) compared to 2008 (81). Most were found in Spring Run 1 in 2009 compared to the majority observed at the upwelling site in Landa Lake in 2008. In 2009, it was observed that some seeps along the west side of Landa Lake had no measurable flow, and this may have contributed to the lower numbers caught in Landa Lake. One undescribed amphipod was captured in 2009 (*Ingolfiella* sp), bringing the total to 2 undescribed amphipod species that have been caught from Comal Springs during this study. *Ingolfiella* sp. was also recently recorded in springs along the Devil's River. 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*. In addition, a couple more specimens of the undescribed amphipod in the genus *Parabogidiella* were observed in 2009. Genetic analysis of these specimens and their relative *P. americana* would be beneficial because they have been collected together in a couple of sites (San Marcos and Comal Rivers), and *P. americana*'s range has recently been extended west to springs on the Devil and Pecos river.

As in 2008, a troglobitic (cave-dwelling) 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.

Table 11. Total numbers of troglobitic and endangered species collected in drift nets during June and December, 2009. 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				
<i>Stygobromus pecki</i> (E)	46	22	24	92
<i>Stygobromus russelli</i>	2	1	4	7
<i>Stygobromus</i> spp.	119	150	385	654
All <i>Stygobromus</i>	167	173	413	753
Hadziidae				
<i>Mexiweckelia hardeni</i>	8	13	3	24
Sebidae				
<i>Seborgia relict</i> a	4	2	5	11
Bogidiellidae				
<i>Artesia subterranea</i>	1			1
<i>Parabogidiella</i> n. sp		2		2
Ingolfiellidae				
<i>Ingolfiella</i> n. sp		1		1
Isopoda				
Asellidae				
<i>Lirceolus</i> (2spp.)	57	32	6	95
Cirolanidae				
<i>Cirolanides texensis</i>			3	3
Arachnids				
Hydrachnoidea				
Hydryphantidae				
<i>Almuerzothyas</i> n. sp	18			18
Insects				
Coleoptera				
Dytiscidae				
<i>Comaldessus stygius</i>		3 A		3
<i>Haideoporus texanus</i>				
Dryopidae				
<i>Stygoparnus comalensis</i>	4 L	1 L		5
Elmidae				
<i>Heterelmis comalensis</i>	2 (1L, 1A)			2

Table 12. Results of water quality measurements conducted in 2009 during drift net sampling efforts at Comal Springs.

	Spring Run 1		Spring Run 3		West Shore Upwelling	
	June	Nov	June	Nov	June	Nov
Date	23.1	23.1	23.3	23.2	23.8	23.7
Temperature (°C)	0.563	0.561	0.6	0.562	0.559	0.558
Conductivity (mS)	6.8	7.0	6.8	6.9	6.8	6.9
pH	5.6	5.8	3.1	5.7	5.4	5.6
Dissolved Oxygen (mg/L)	0.3	1.1	0.3	1.2	0.2	0.7
Current Velocity (m/s)						

As in previous years, water quality variables remained relatively constant at all sites in 2009, 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 number of riffle beetles captured in 2009 (762) was lower than in 2008 (954), but similar to that observed in 2007 (758) (Table 13). From June to December, the number of riffle beetles (both *Heterelmis* and *Microcyllloepus*) collected at Spring Run 3 and the West Shoreline of Landa Lake declined. However, the number of riffle beetles captured at Spring Island increased during this same time period. Most of the springs sampled in the Spring Island area were upwellings on the lake bottom, and likely less susceptible to the effects of drought than the seeps along the western margins of Landa Lake, some of which had no measurable flow during portions of 2009. The greatest number of riffle beetles captured in 2009 was at the West Shoreline site in June.

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 20). 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 13. 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
June 2009	136	226	74	436
December 2009	72	56	198	326
Total	2142	1844	1972	5958

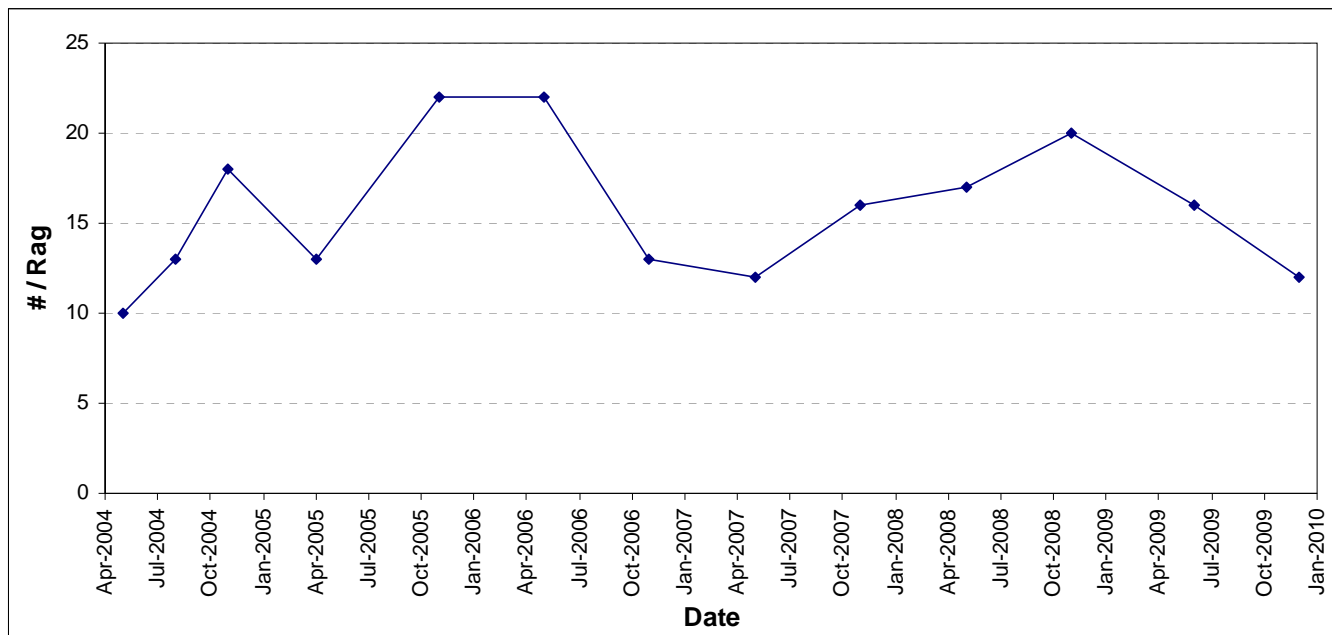


Figure 20. Combined density (#/cotton lure) of Comal Springs riffle beetles (*Heterelmis comalensis*) for each sampling date from 2004 – 2009.

REFERENCES

- BIO-WEST 2001a. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the Comal Springs / River aquatic ecosystem. Draft Report. Edwards Aquifer Authority, San Antonio, TX. 35p.
- BIO-WEST 2001b. Comprehensive and critical period monitoring program to evaluate the effects of variable flow on biological resources in the San Marcos Springs / River aquatic ecosystem. Draft Report. Edwards Aquifer Authority, San Antonio, TX. 33p.
- BIO-WEST 2003a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2002 Annual Report. Edwards Aquifer Authority. 45 p. plus Appendices.
- BIO-WEST 2003b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2002 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.
- BIO-WEST 2004a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2003 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.
- BIO-WEST 2004b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2003 Annual Report. Edwards Aquifer Authority. 30 p. plus Appendices.
- BIO-WEST 2005a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2004 Annual Report. Edwards Aquifer Authority. 70 p. plus Appendices.
- BIO-WEST 2005b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2004 Annual Report. Edwards Aquifer Authority. 57 p. plus Appendices.
- BIO-WEST 2006a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2005 Annual Report. Edwards Aquifer Authority. 43 p. plus Appendices.
- BIO-WEST 2006b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2005 Annual Report. Edwards Aquifer Authority. 33 p. plus Appendices.
- BIO-WEST 2007a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2006 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.
- BIO-WEST 2007b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2006 Annual Report. Edwards Aquifer Authority. 54 p. plus Appendices.
- BIO-WEST 2008a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2007 Annual Report. Edwards Aquifer Authority. 41 p. plus Appendices.

- BIO-WEST 2008b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2007 Annual Report. Edwards Aquifer Authority. 33 p. plus Appendices.
- BIO-WEST 2009a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2008 Annual Report. Edwards Aquifer Authority. 41 p. plus Appendices.
- BIO-WEST 2009b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2008 Annual Report. Edwards Aquifer Authority. 36 p. plus Appendices.
- Bolick, A. E. 2007. The Effects of Springflow on Heterophyd Cercariae in the Comal River, New Braunfels, TX. Master's Thesis. Texas State University.
- Bush, P.W., A.F. Ardis, L. Fahlquist, P.B. Ging, C.E. Hornig, and J.L. Lanning-Rush. 1998. Water Quality in South Central Texas, Texas 1996-98. U. S. Geological Survey, Circular 1212.
- U.S. Geological Survey (USGS). 12/2009. Provisional data for Texas. Location: <http://tx.waterdata.usgs.gov/niwis/help/provisional>.

APPENDIX A: AQUATIC VEGETATION MAPS

Upper Spring Run Reach

Comal River

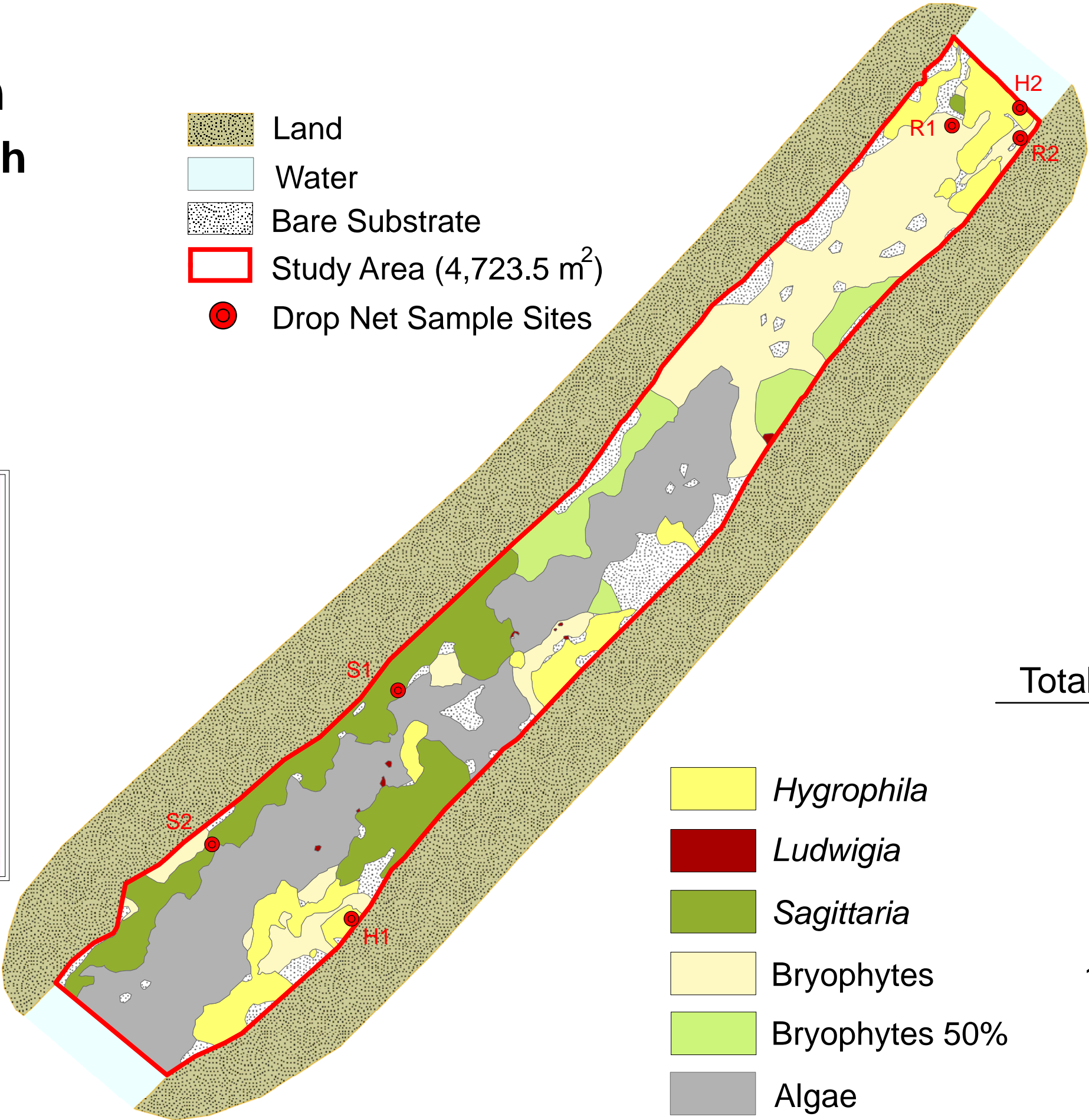
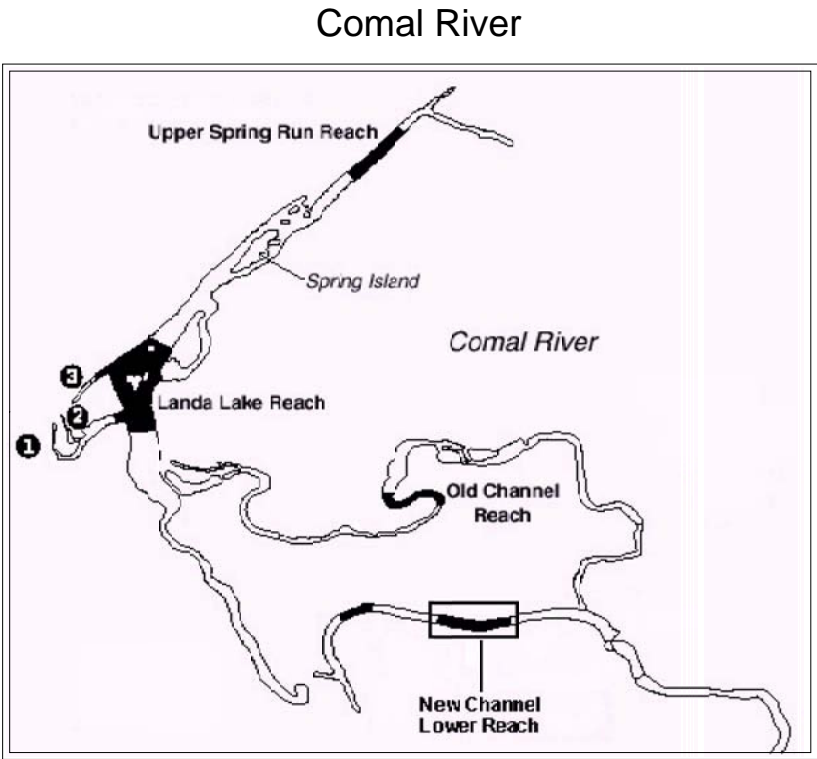
Aquatic Vegetation

Upper Spring Run Reach

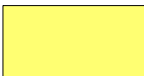





Spring

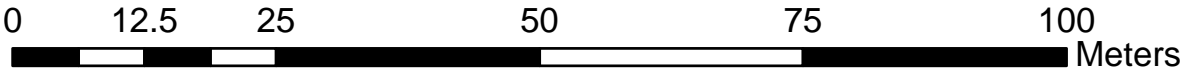
April 22, 2009

- Land
- Water
- Bare Substrate
- Study Area (4,723.5 m²)
- Drop Net Sample Sites



Total Area (m²)

	<i>Hygrophila</i>	529.0
	<i>Ludwigia</i>	7.0
	<i>Sagittaria</i>	734.9
	Bryophytes	1,067.8
	Bryophytes 50%	310.7
	Algae	1,568.4



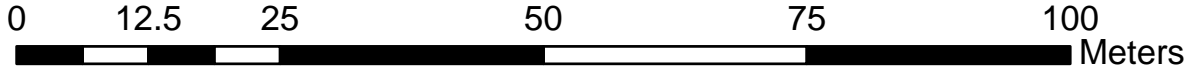
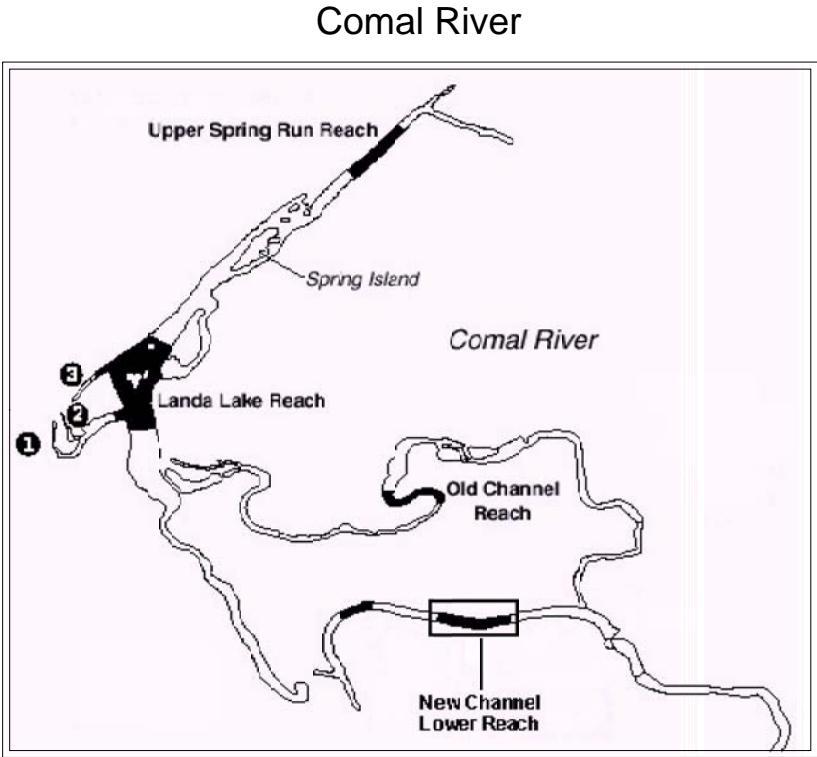
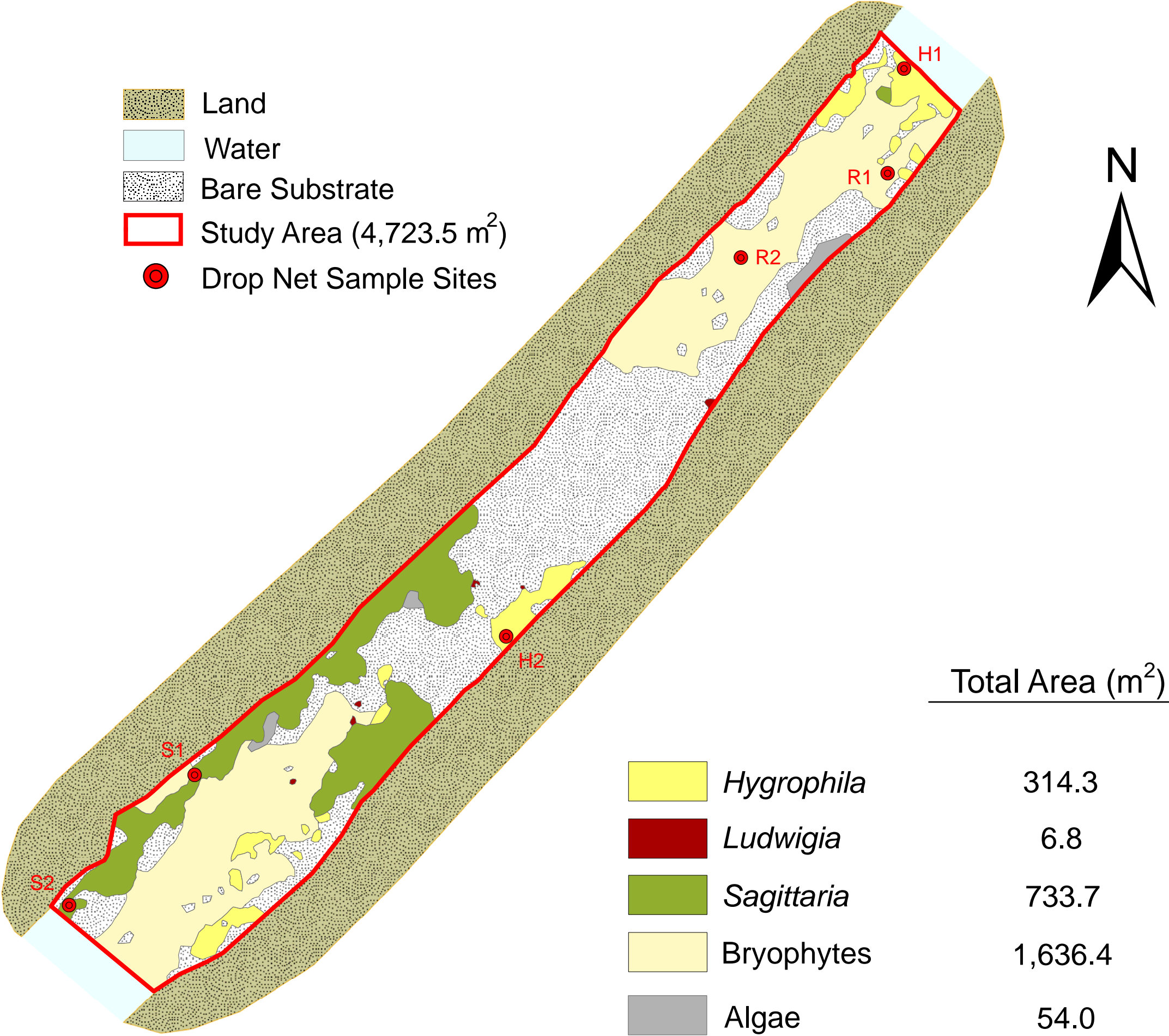
Comal River

Aquatic Vegetation

Upper Spring Run Reach

Critical Period 1

June 25, 2009



Comal River

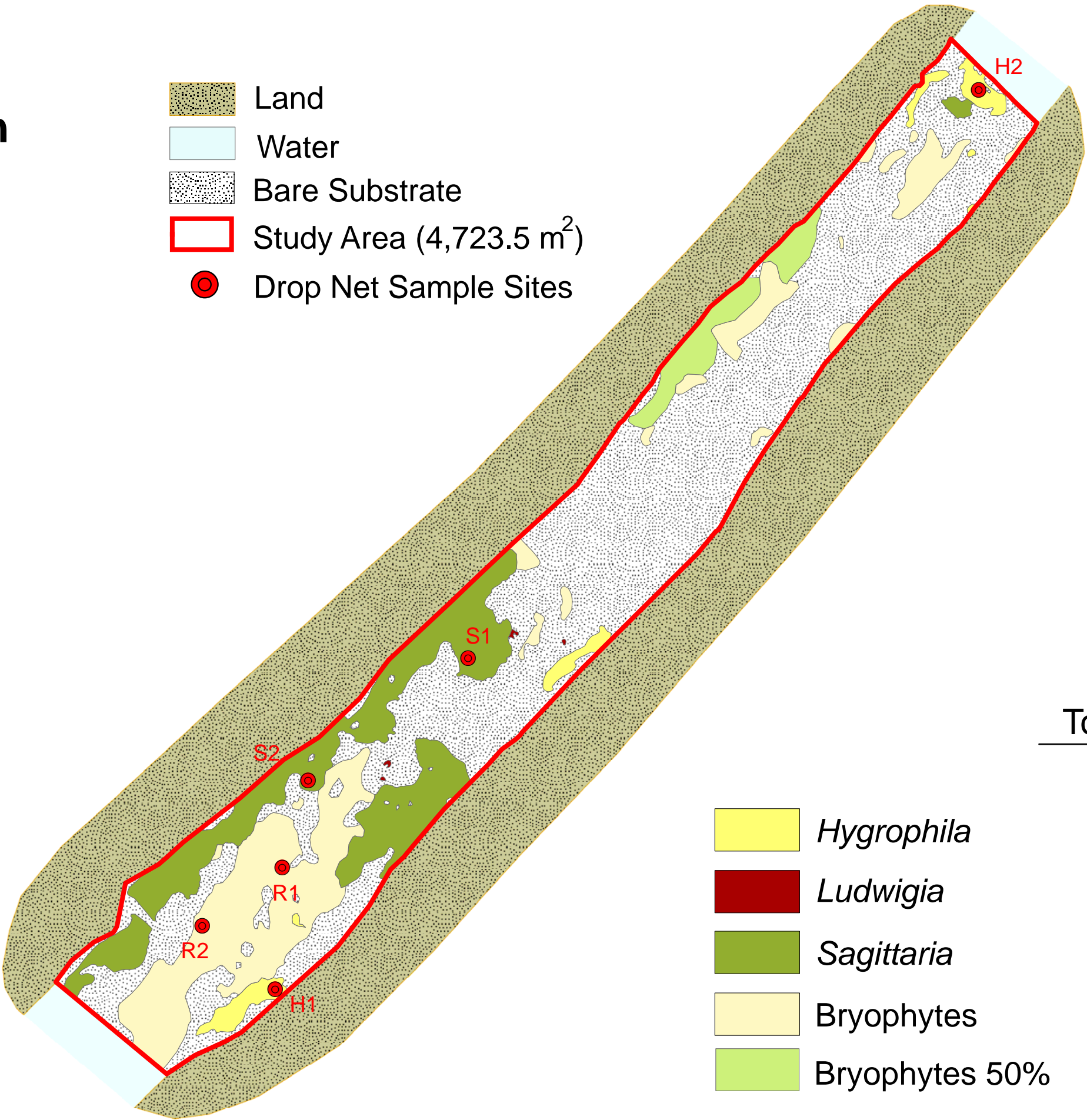
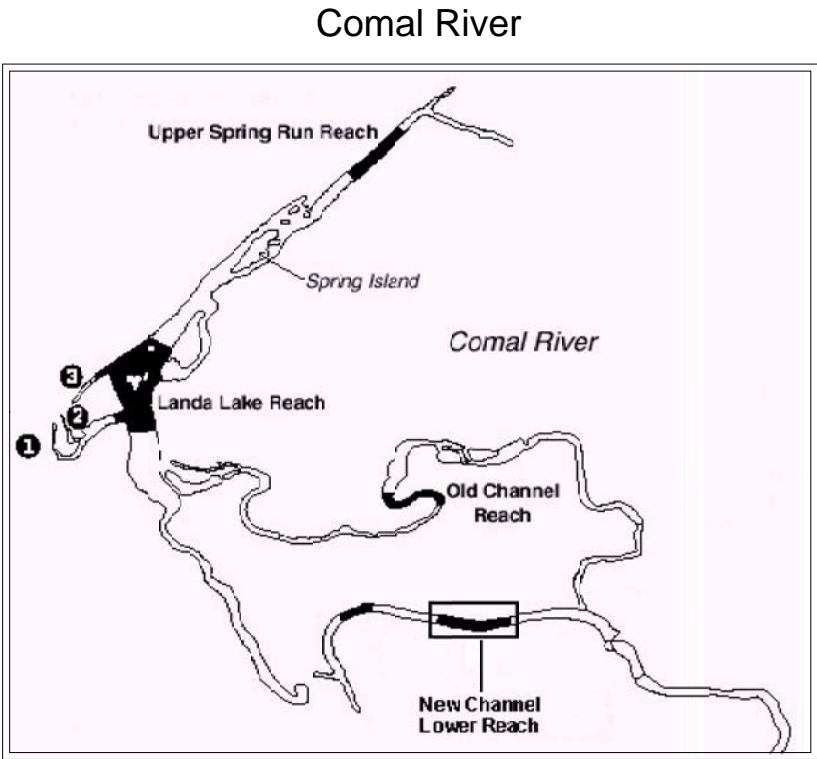
Aquatic Vegetation

Upper Spring Run Reach

Fall

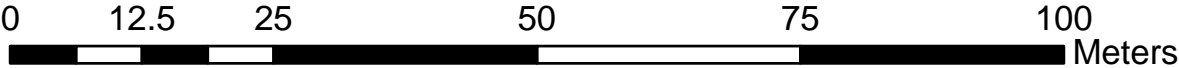
October 13, 2009

- Land
- Water
- Bare Substrate
- Study Area (4,723.5 m²)
- Drop Net Sample Sites



Total Area (m²)

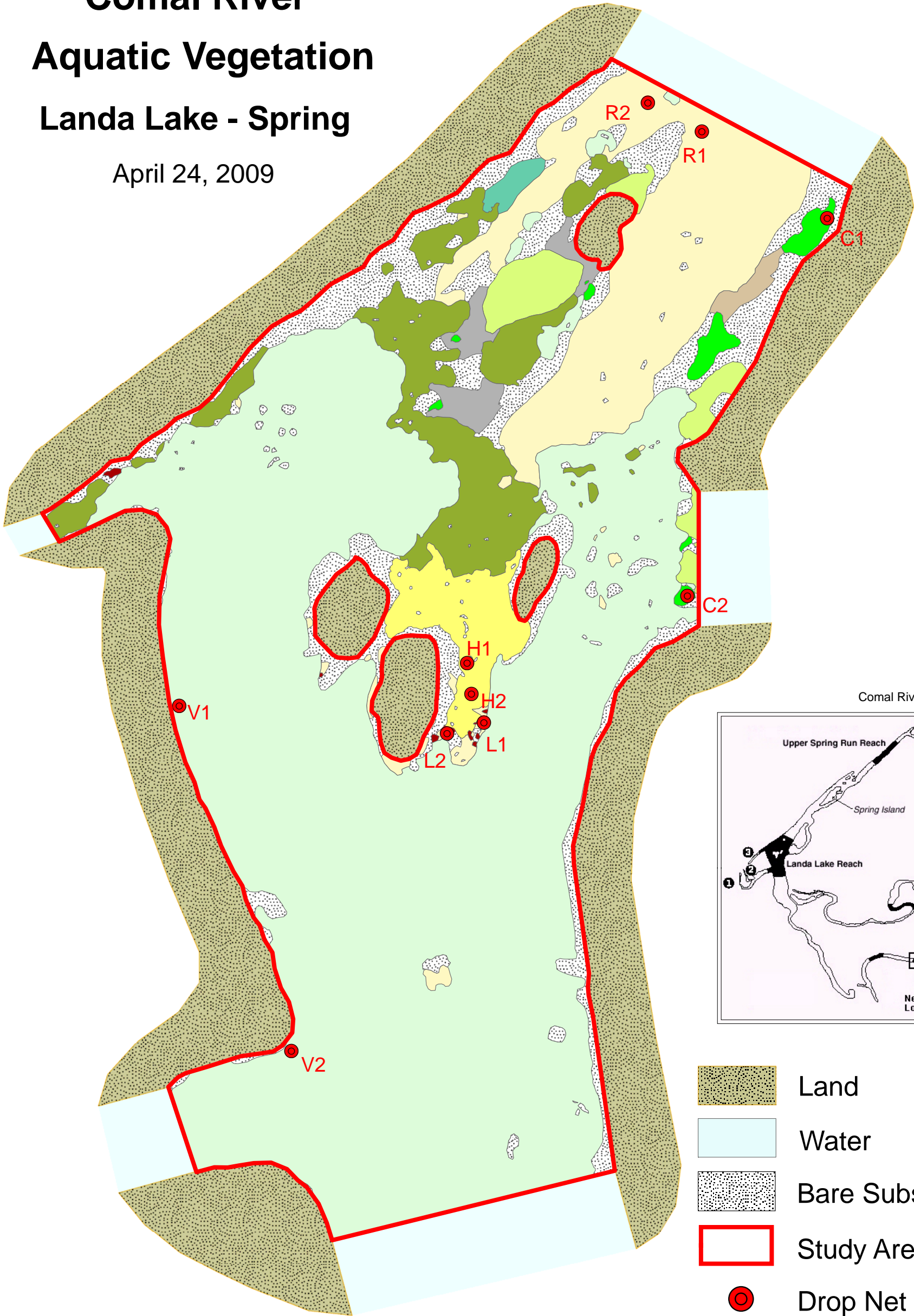
Hygrophila	131.0
Ludwigia	2.8
Sagittaria	747.9
Bryophytes	853.4
Bryophytes 50%	167.8



Landa Lake Reach

Comal River
Aquatic Vegetation
Landa Lake - Spring

April 24, 2009



Algae

266.5

Bryophytes

2,788.9

Cabomba

173.8

Hygrophila

605.3

Ludwigia

23.4

Nuphar

483.5

Sagittaria

1,552.1

Vallisneria

13,931.5

Bryophytes / Algae

81.9

Bryophytes 50%

81.0

01020406080

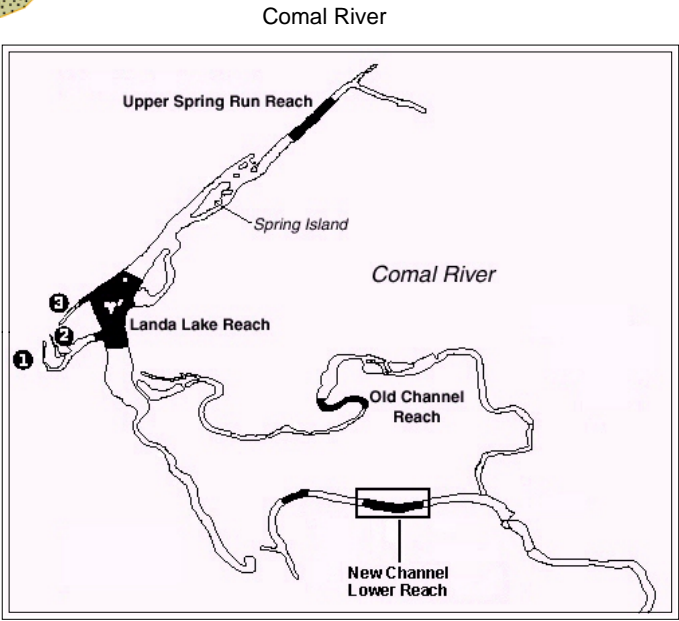
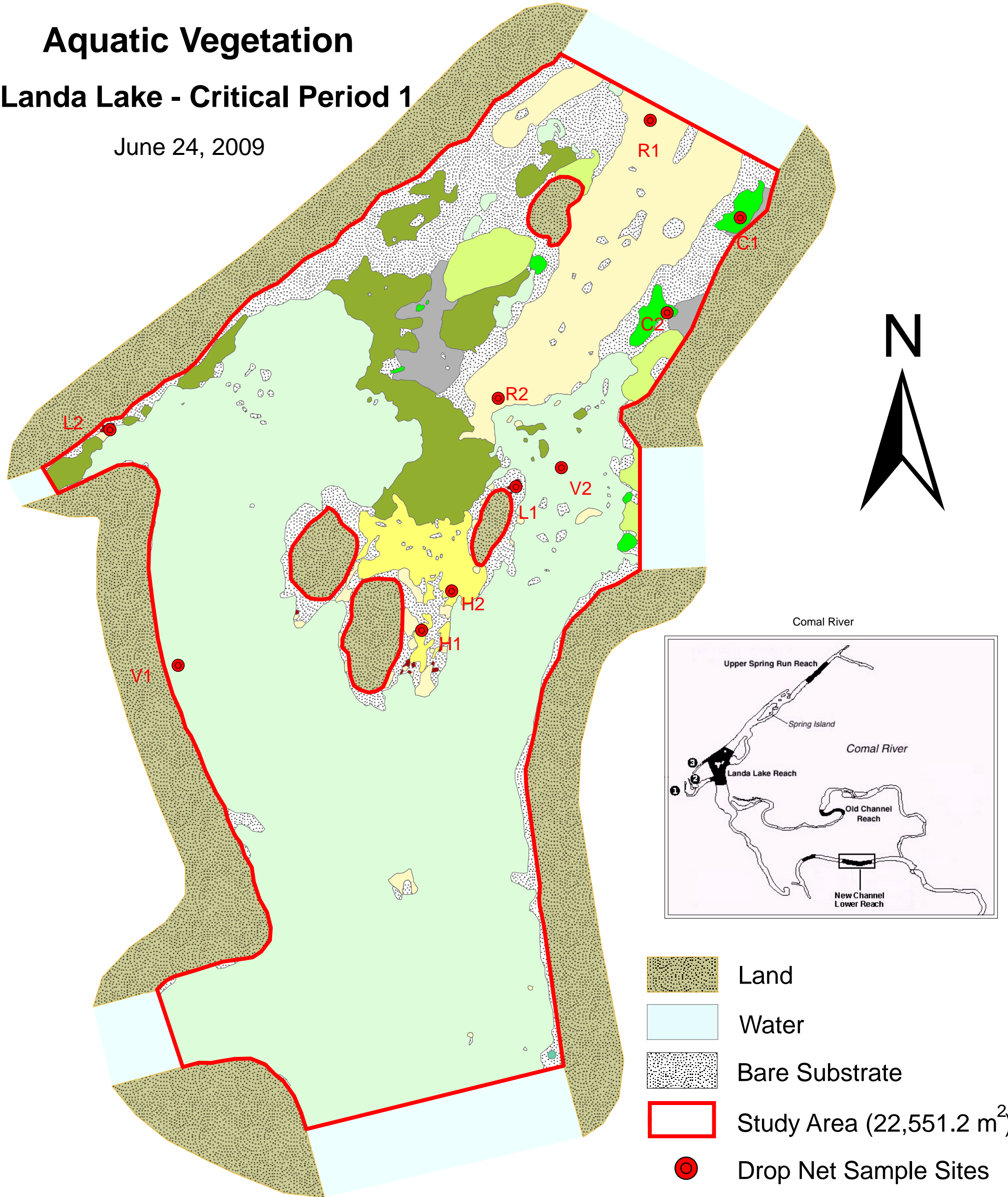
Meters

Comal River

Aquatic Vegetation

Landa Lake - Critical Period 1

June 24, 2009

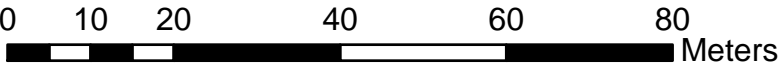


- Land
- Water
- Bare Substrate
- Study Area (22,551.2 m²)
- Drop Net Sample Sites

Total Area (m²)

Total Area (m²)

Algae	330.3	Nuphar	485.9
Bryophytes	2,347.3	Sagittaria	1,558.3
Cabomba	180.0	Vallisneria	13,941.9
Hygrophila	497.2	Bryophytes 50%	3.2
Ludwigia	17.1		

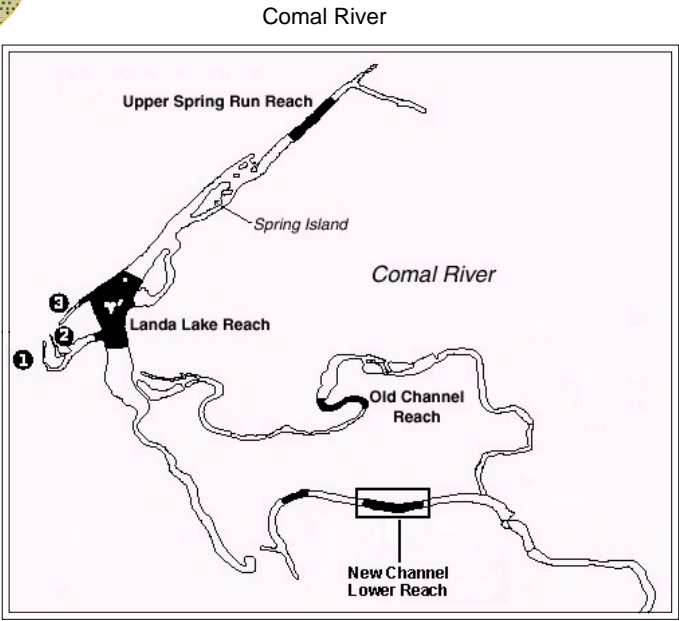
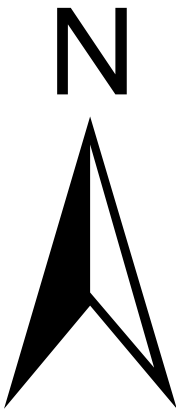
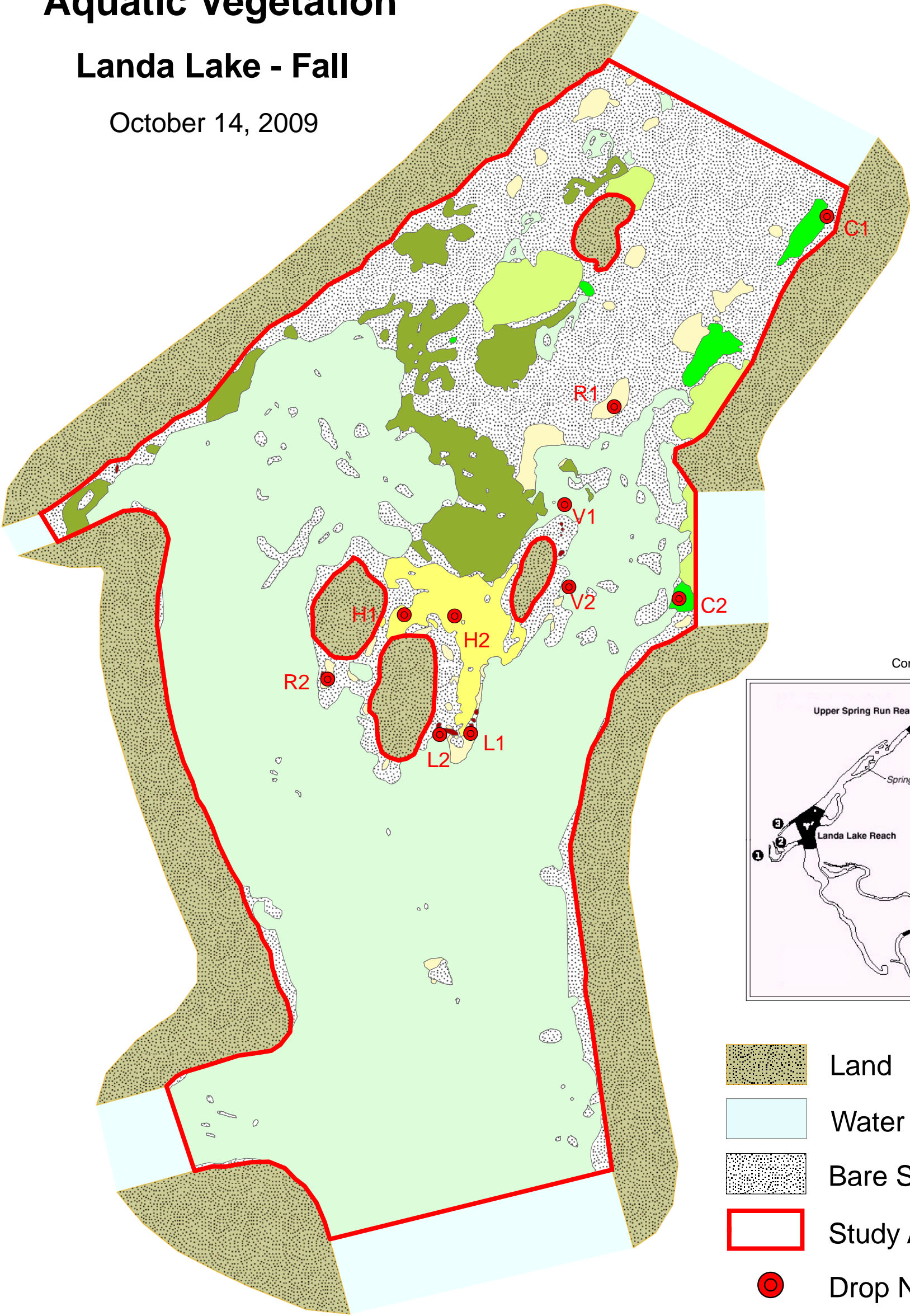


Comal River

Aquatic Vegetation

Landa Lake - Fall

October 14, 2009



- Land
- Water
- Bare Substrate
- Study Area (22,551.2 m²)
- Drop Net Sample Sites

Total Area (m²)

Bryophytes	385.6
Cabomba	181.0
Hygrophila	474.5
Ludwigia	17.8

Total Area (m²)

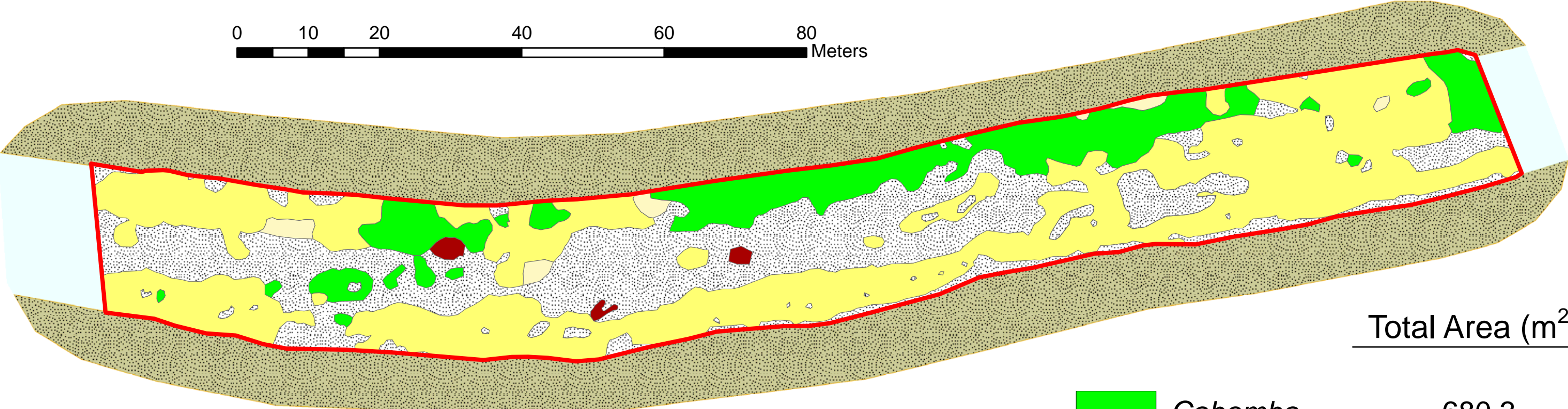
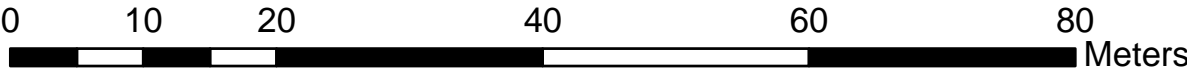
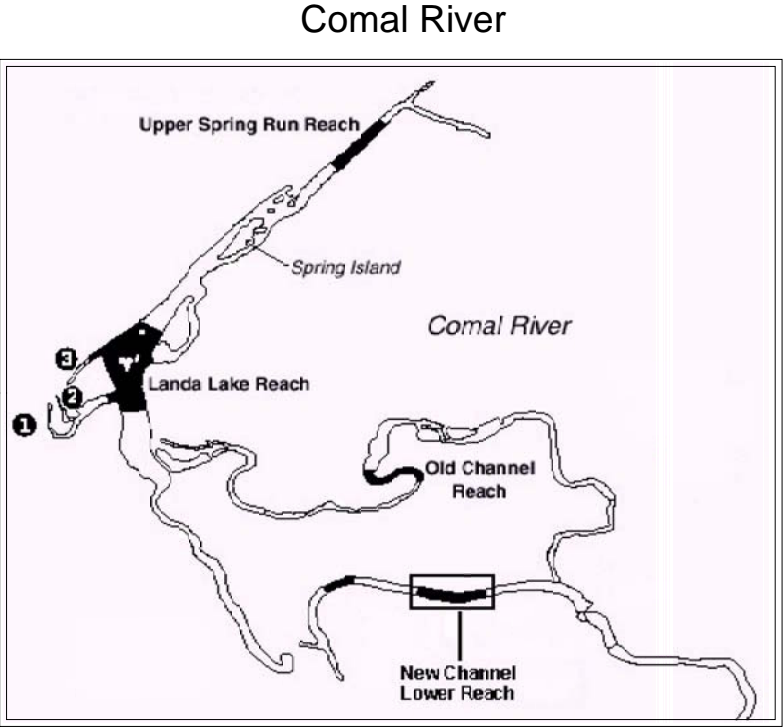
Nuphar	521.8
Sagittaria	1,296.9
Vallisneria	13,452.1

0 5 10 20 30 40 Meters

New Channel Reach

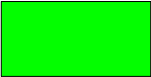


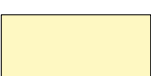
Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring

April 22, 2009



Total Area (m²)

- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m²)

	<i>Cabomba</i>	680.3
	<i>Hygrophila</i>	1,991.1
	<i>Ludwigia</i>	23.1
	Bryophytes	54.4

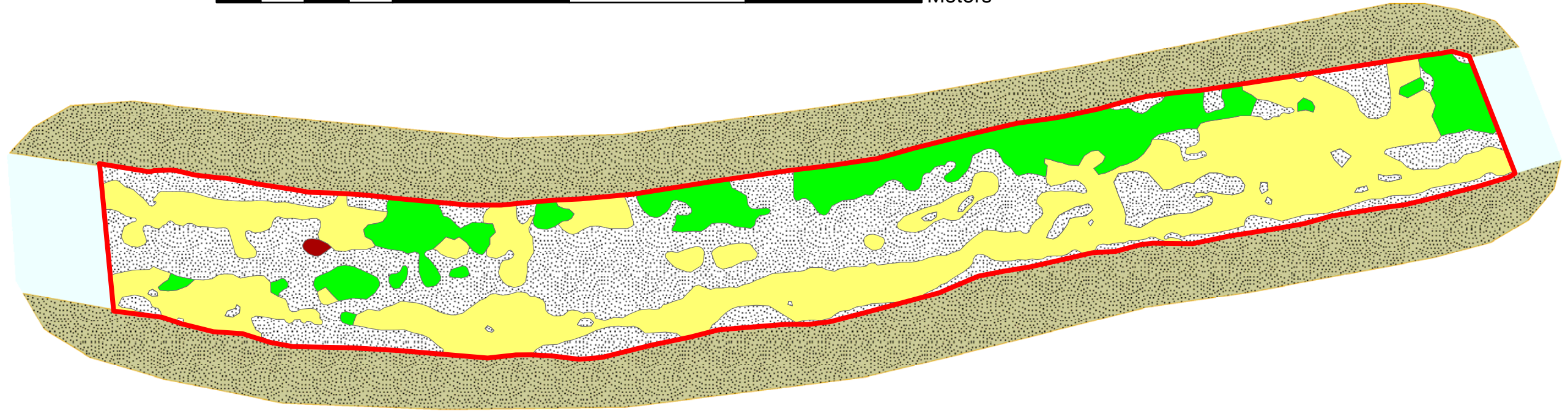
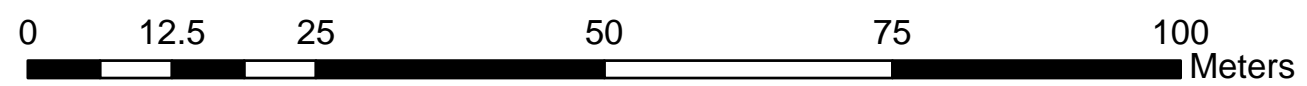
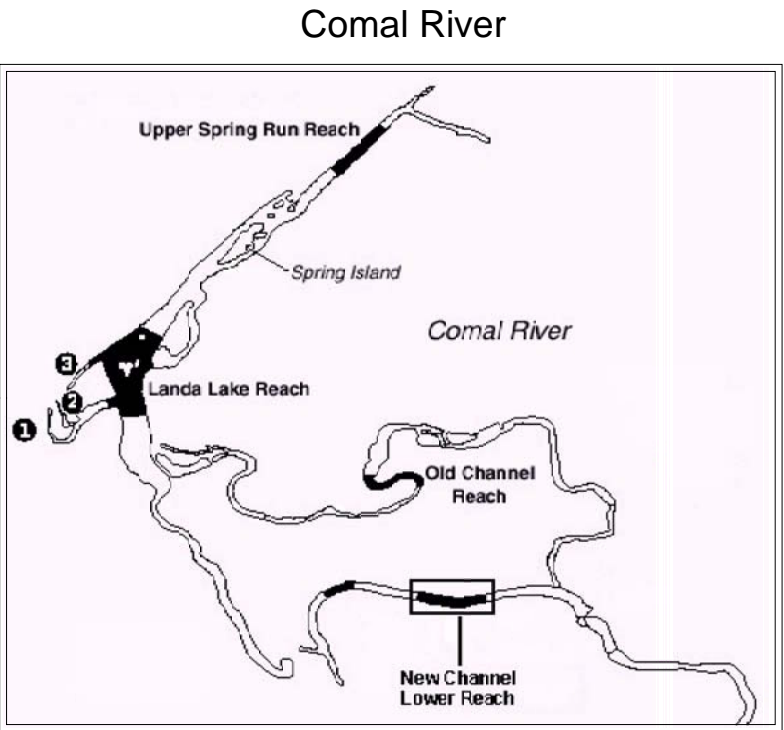
Comal River

Aquatic Vegetation




New Channel Lower Reach

Critical Period 1

July 3, 2009



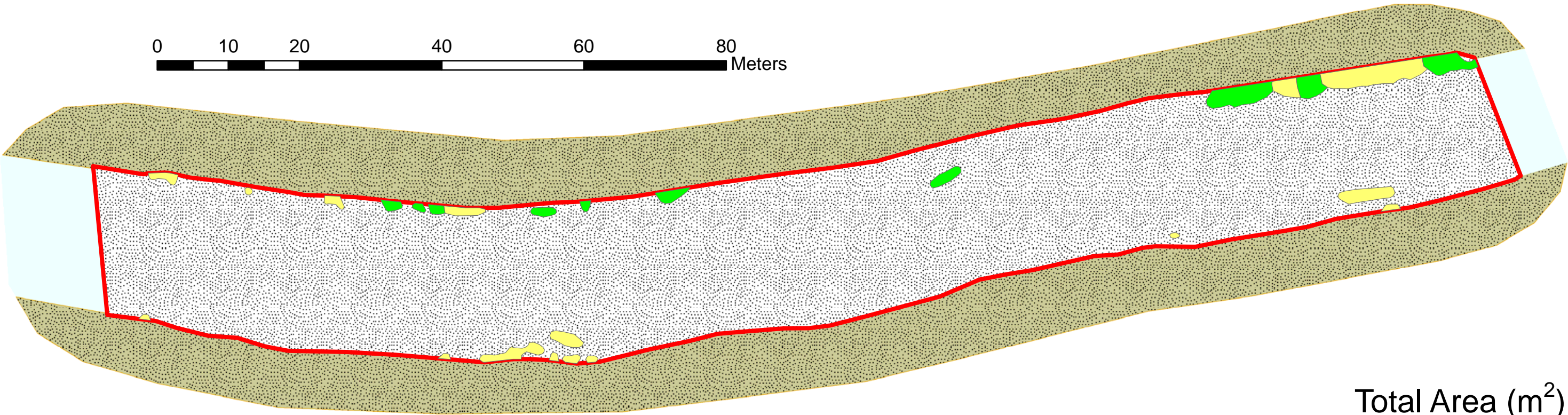
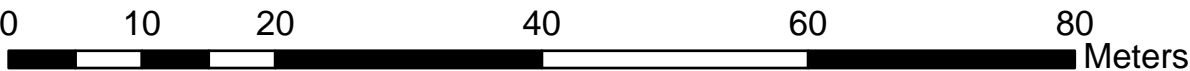
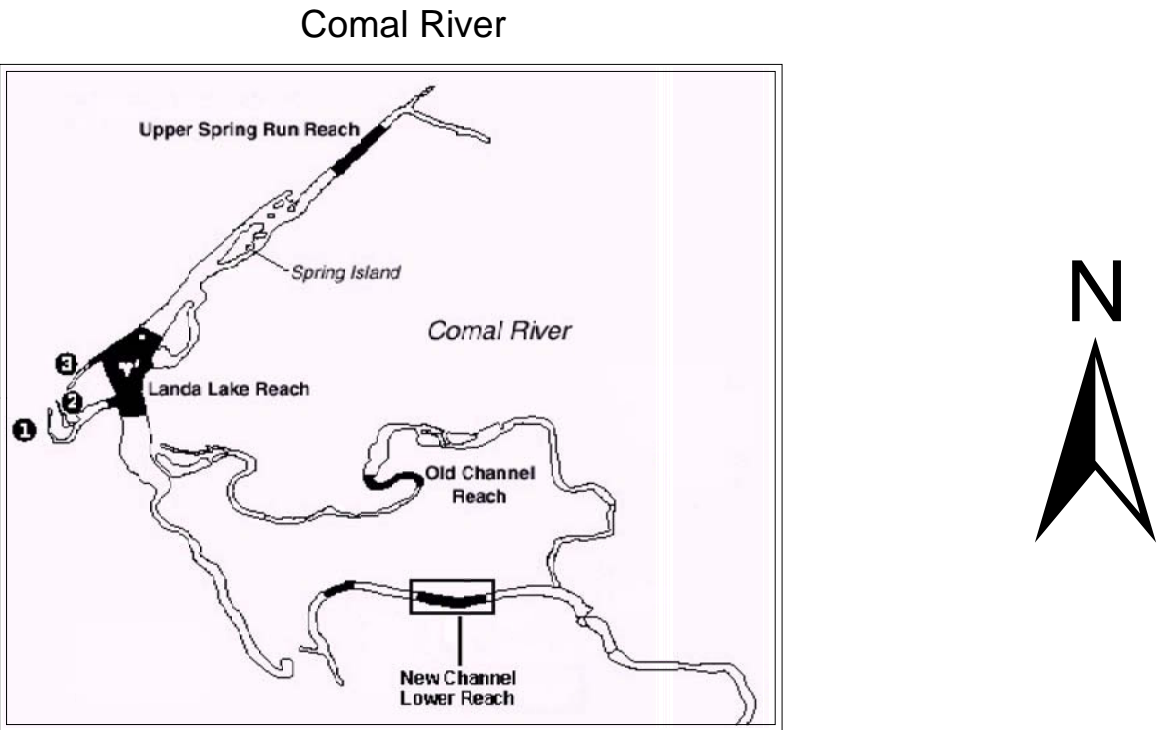
- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m²)

	<i>Cabomba</i>	682.8
	<i>Hygrophila</i>	1,722.1
	<i>Ludwigia</i>	6.9



Total Area (m²)

Comal River Aquatic Vegetation New Channel Lower Reach Fall

October 15, 2009



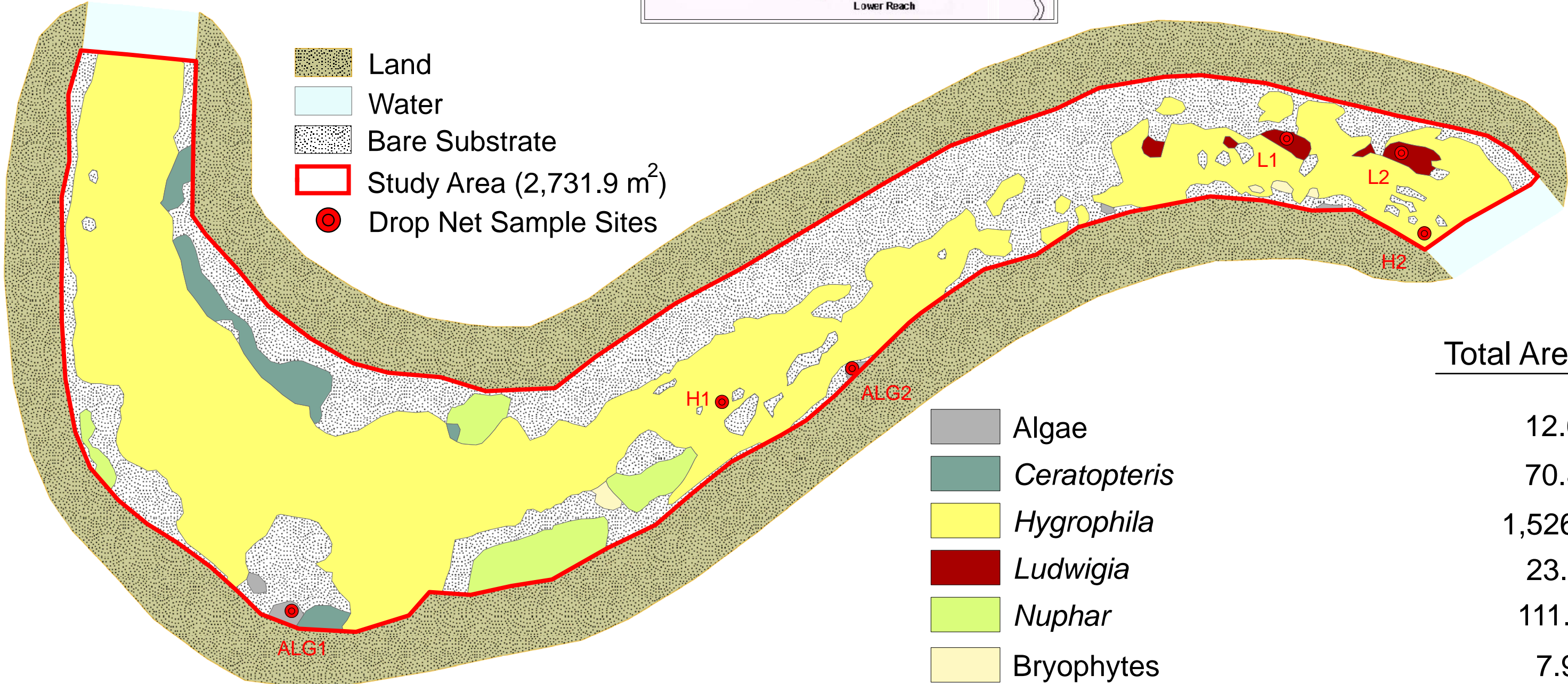
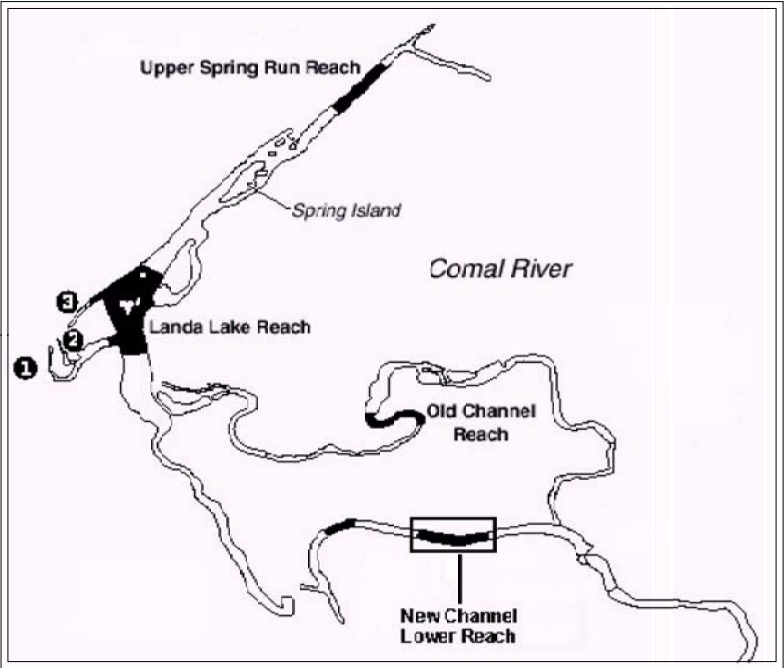
- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m²)

	<i>Cabomba</i>	73.0
	<i>Hygrophila</i>	100.0

Total Area (m²)

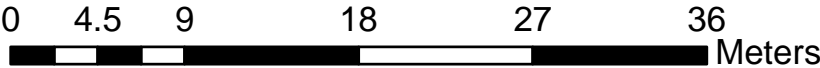
Old Channel Reach

Comal River
Aquatic Vegetation
Old Channel Reach
Spring
April 27, 2009



Total Area (m²)

Algae	12.6
<i>Ceratopteris</i>	70.8
<i>Hygrophila</i>	1,526.1
<i>Ludwigia</i>	23.3
<i>Nuphar</i>	111.2
Bryophytes	7.9



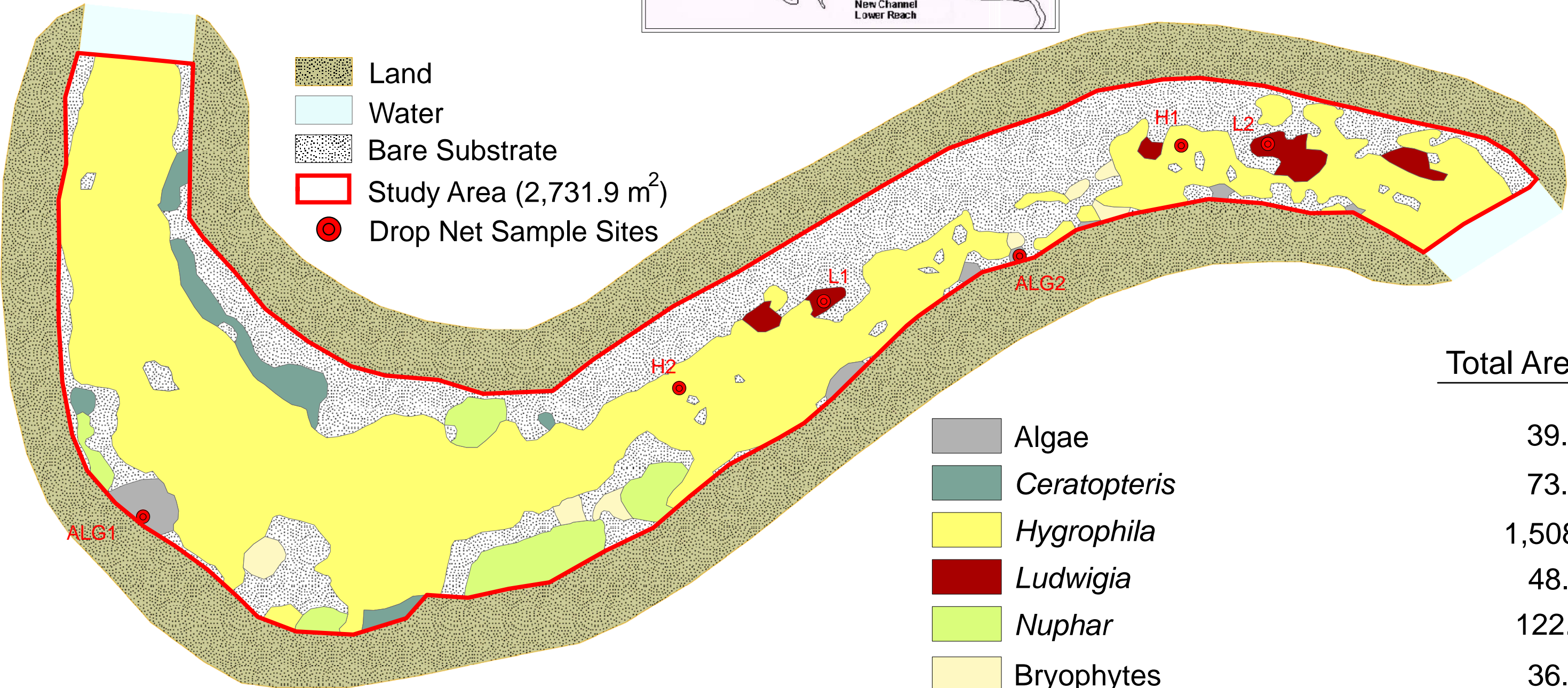
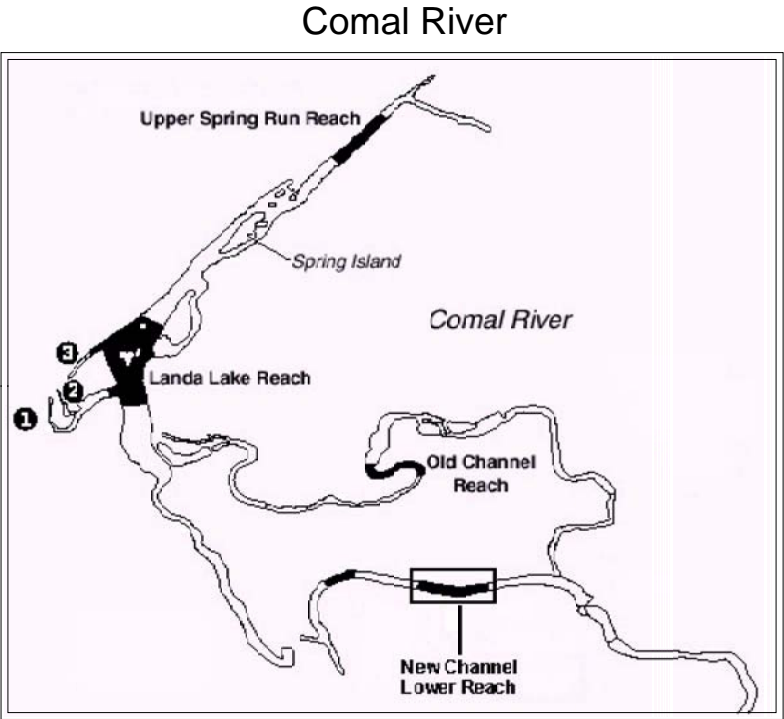
Comal River

Aquatic Vegetation

Old Channel Reach

Critical Period 1

June 26, 2009

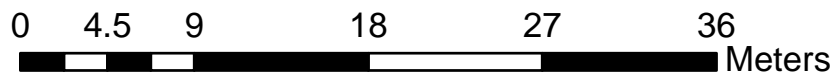


- Land
- Water
- Bare Substrate
- Study Area (2,731.9 m²)
- Drop Net Sample Sites

- Algae
- Ceratopteris*
- Hygrophila*
- Ludwigia*
- Nuphar*
- Bryophytes

Total Area (m²)

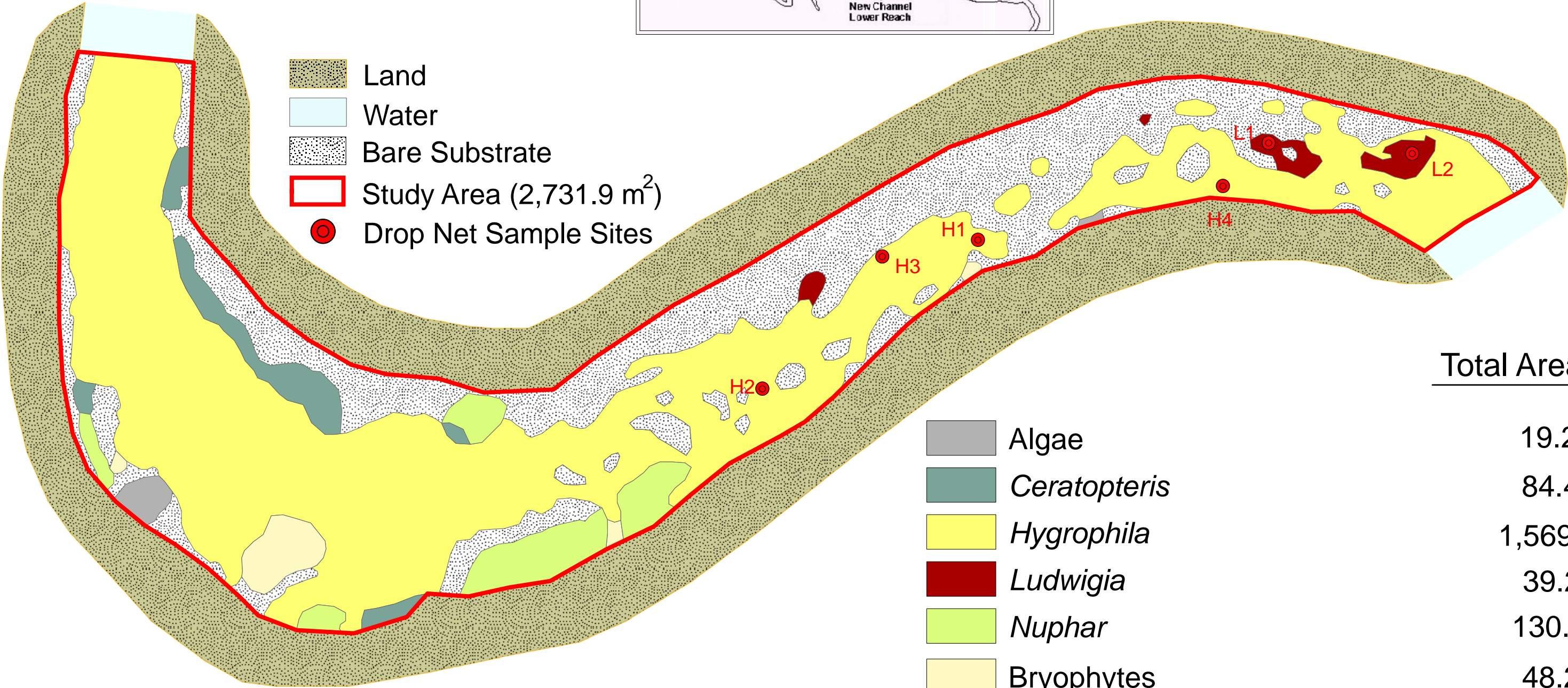
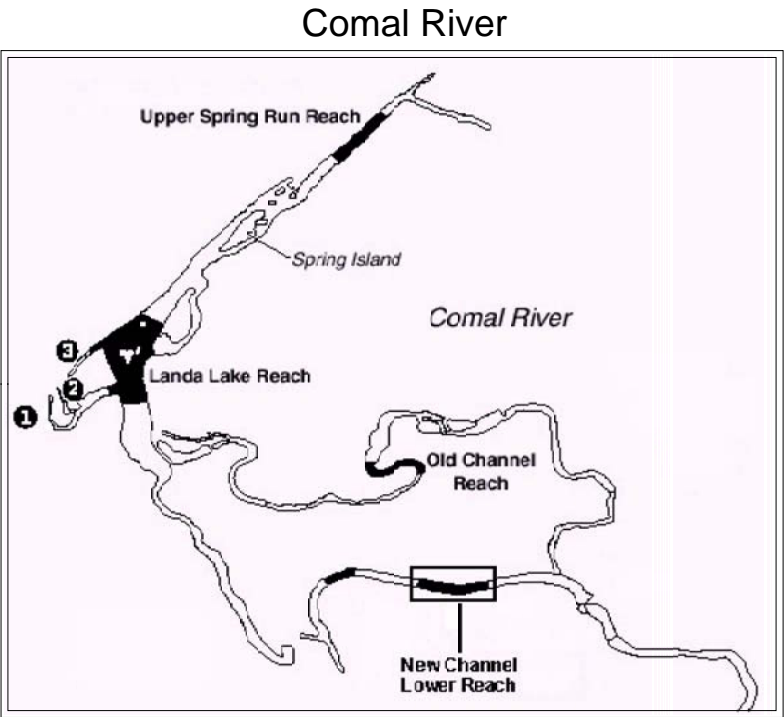
Algae	39.1
<i>Ceratopteris</i>	73.7
<i>Hygrophila</i>	1,508.7
<i>Ludwigia</i>	48.4
<i>Nuphar</i>	122.2
Bryophytes	36.2



Comal River Aquatic Vegetation Old Channel Reach

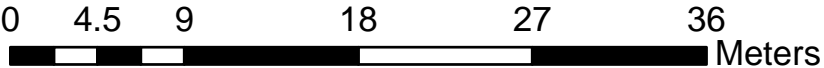
Fall

October 15, 2009



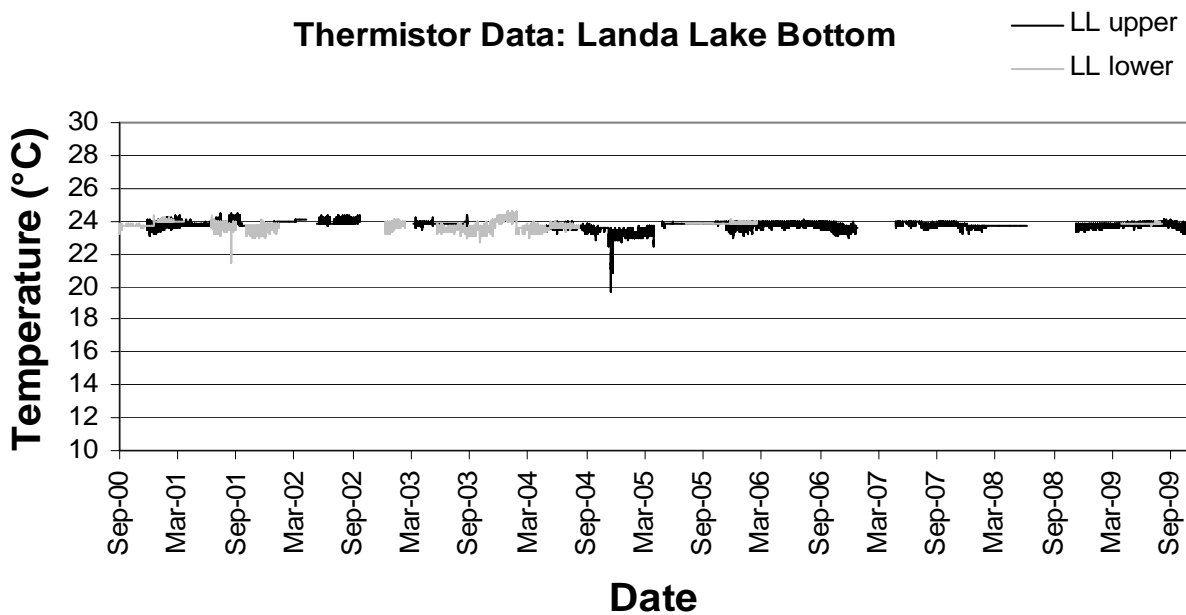
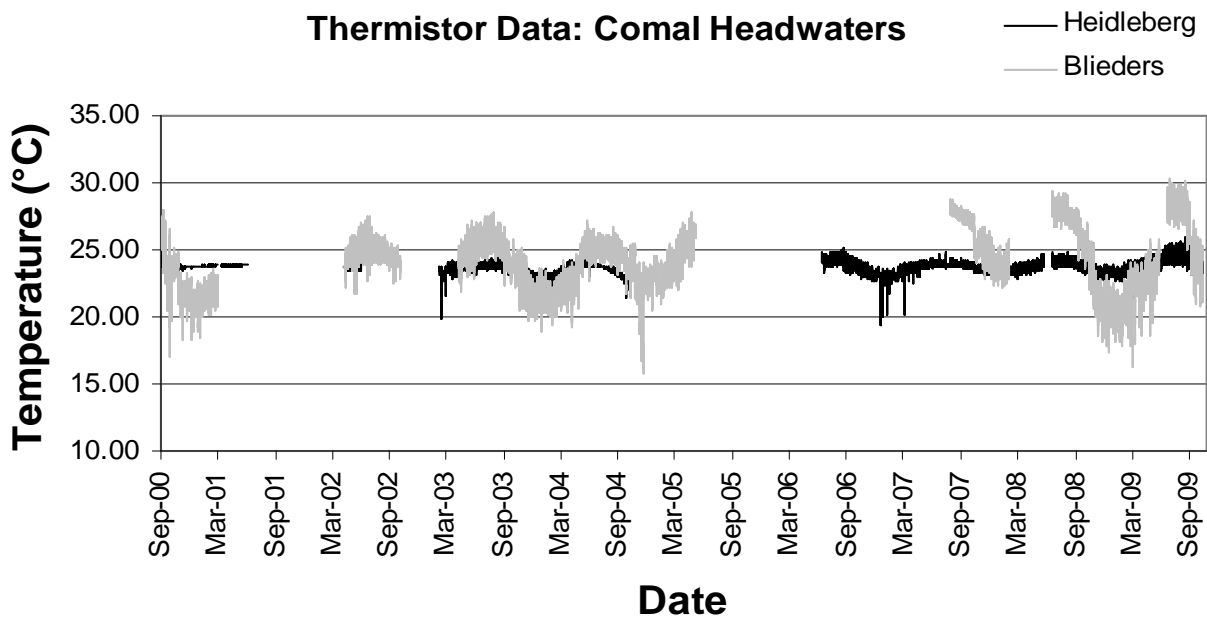
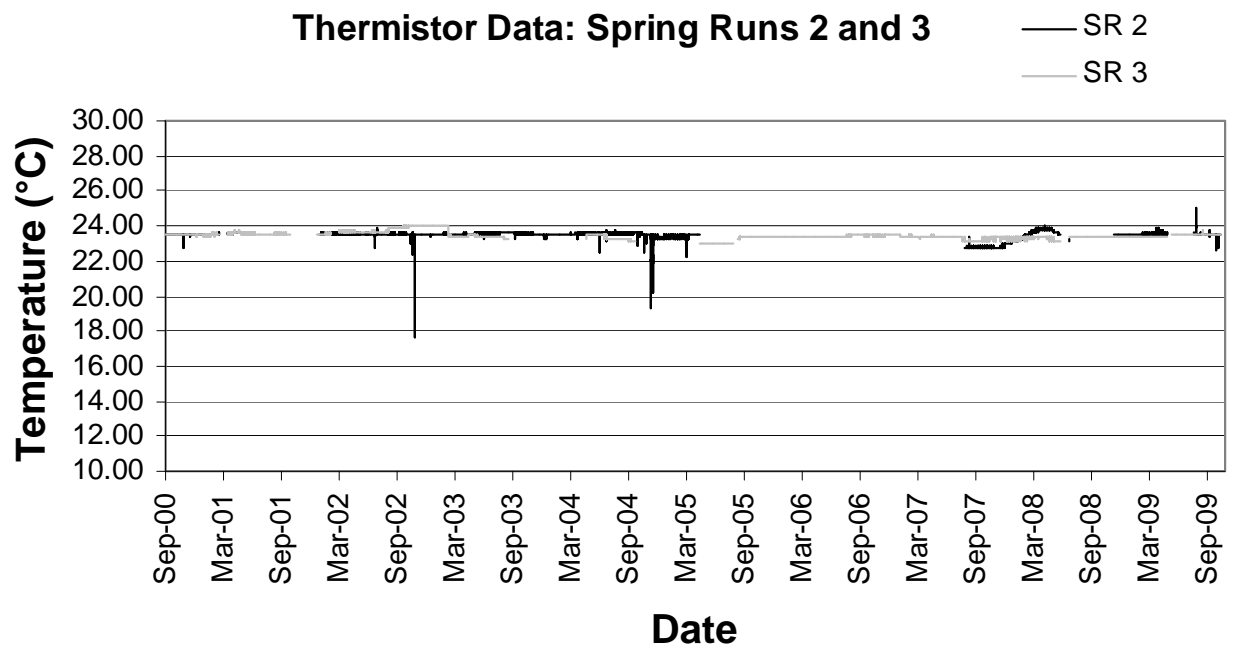
- Land
- Water
- Bare Substrate
- Study Area (2,731.9 m²)
- Drop Net Sample Sites

Total Area (m²)	
Algae	19.2
<i>Ceratopteris</i>	84.4
<i>Hygrophila</i>	1,569.1
<i>Ludwigia</i>	39.2
<i>Nuphar</i>	130.0
Bryophytes	48.2

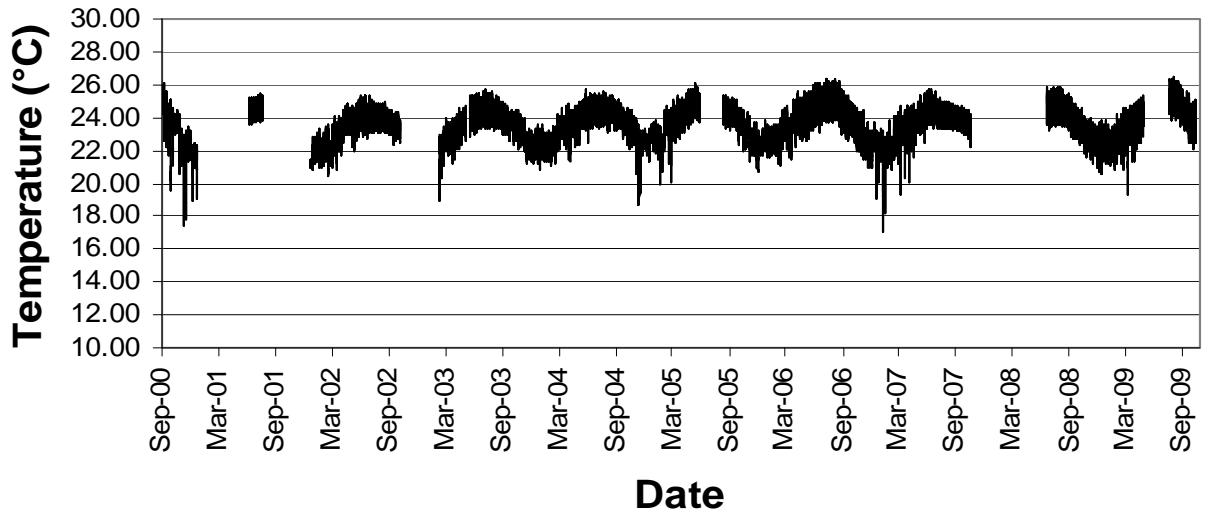


APPENDIX B: DATA AND GRAPHS

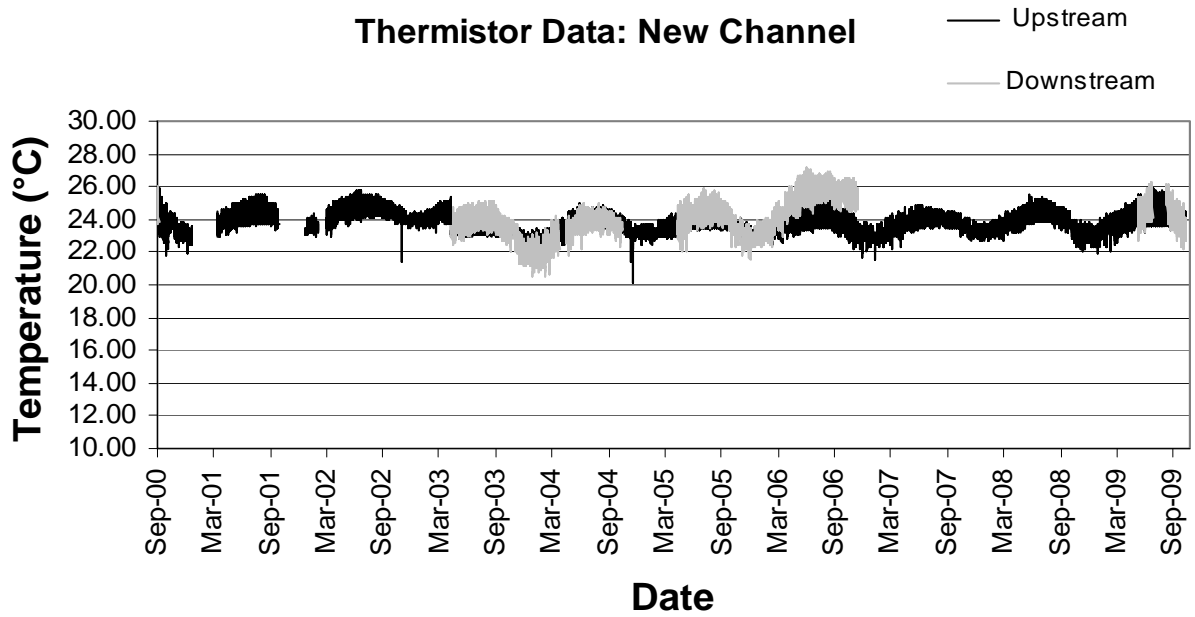
Water Quality Data and Thermistor Graphs



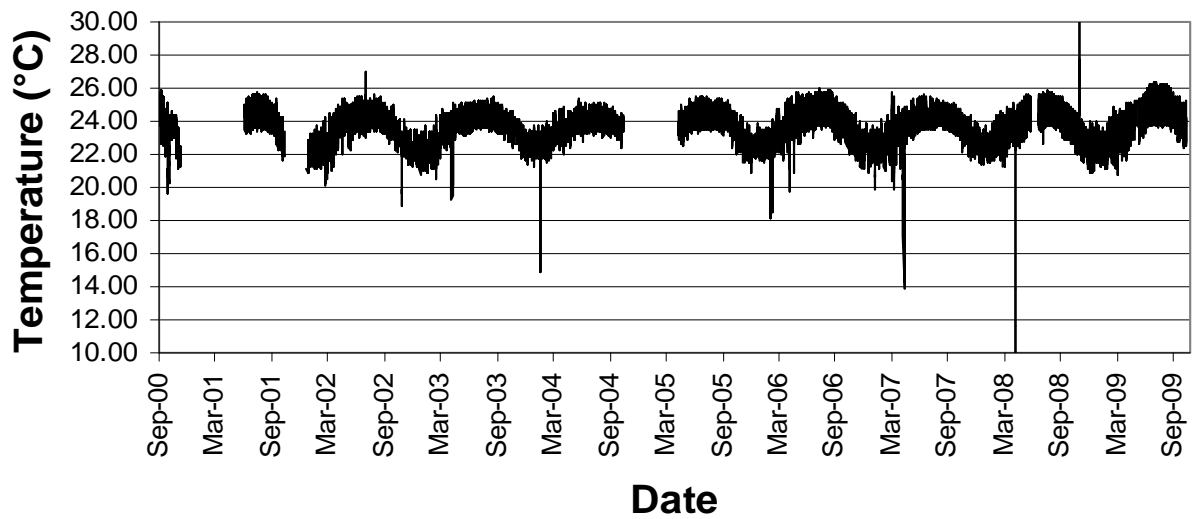
Thermistor Data: Other Place



Thermistor Data: New Channel

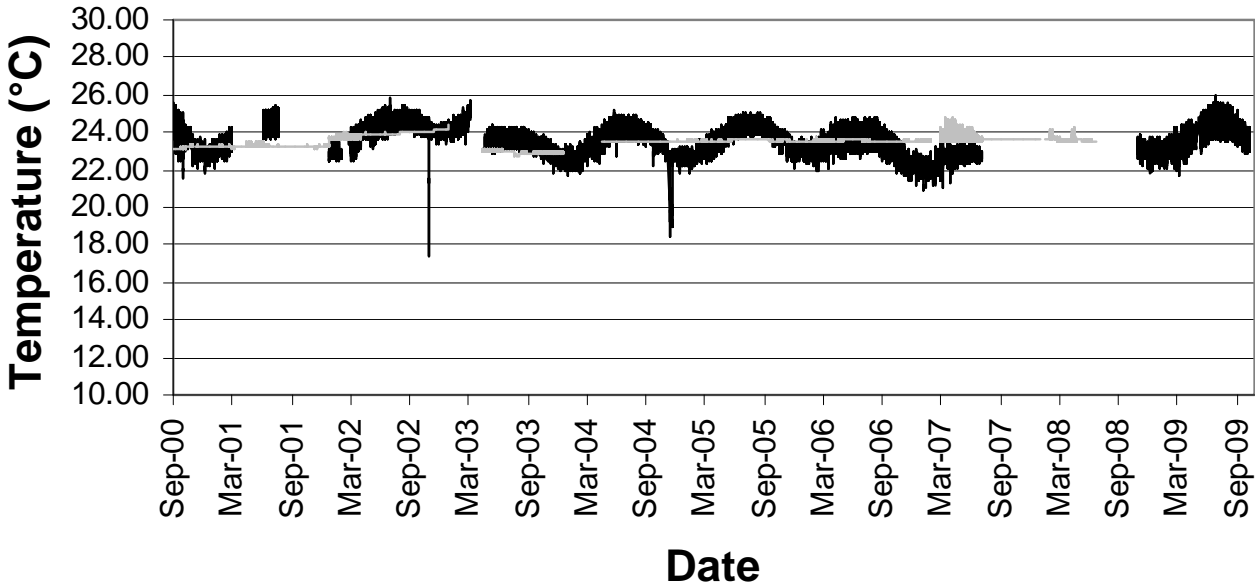


Thermistor Data: Old Channel



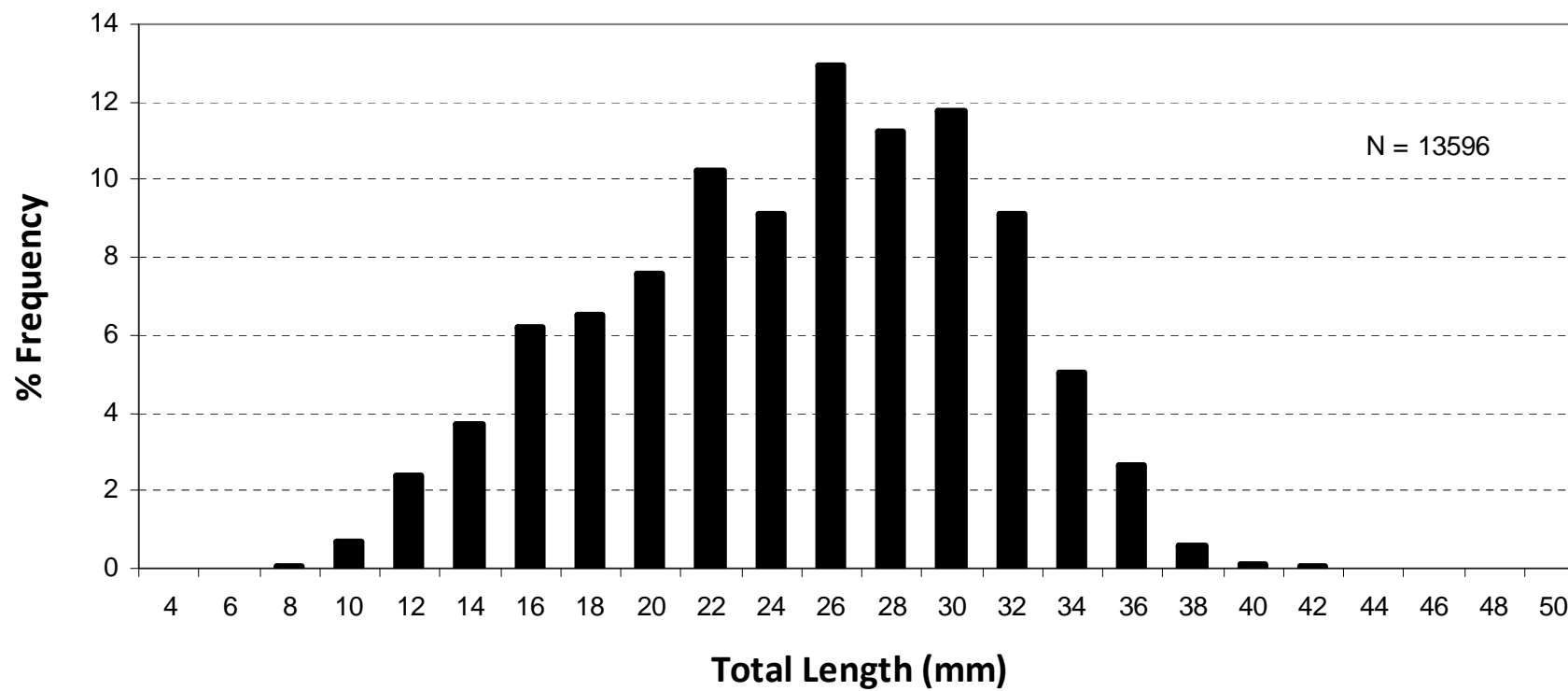
Thermistor Data: Spring Island

— West channel
— East channel



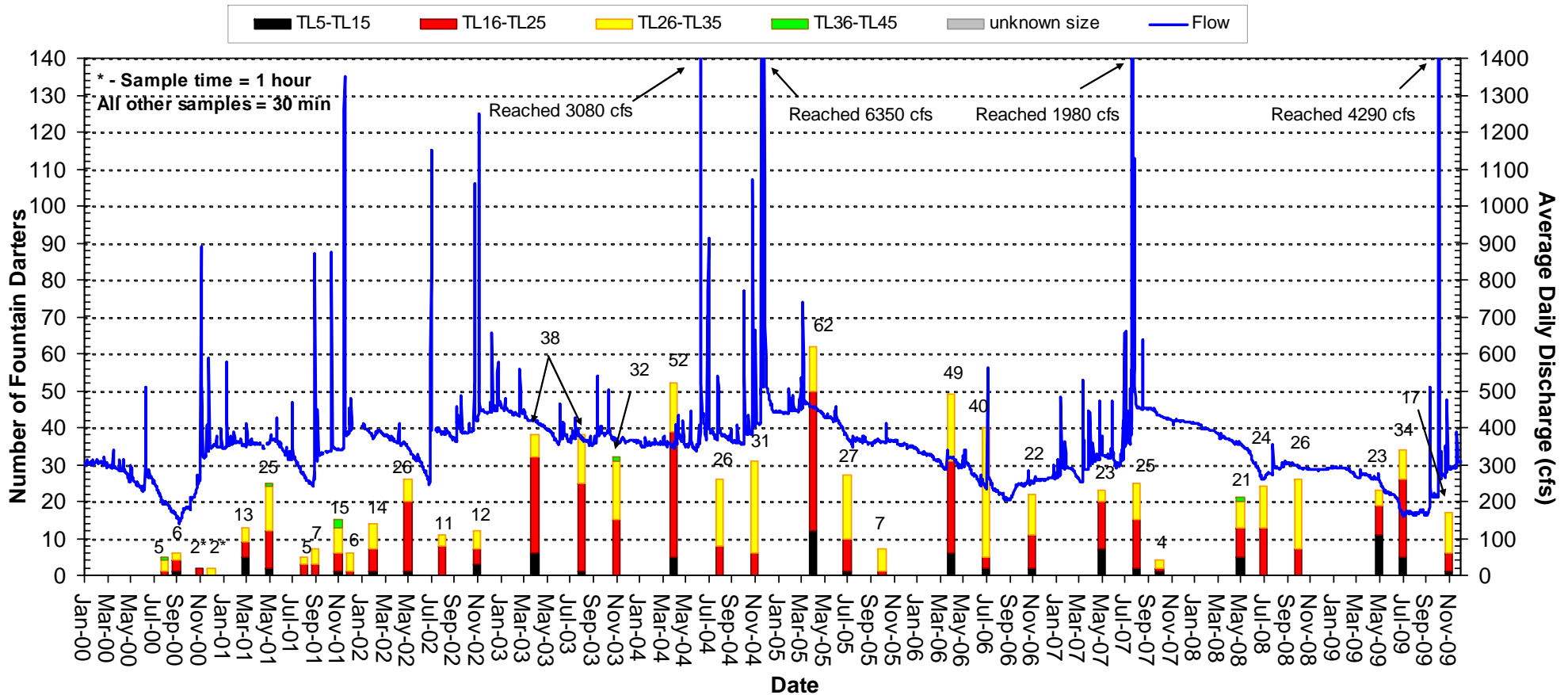
Drop Net Graph

Dropnet Results in the Comal River 2000-2009

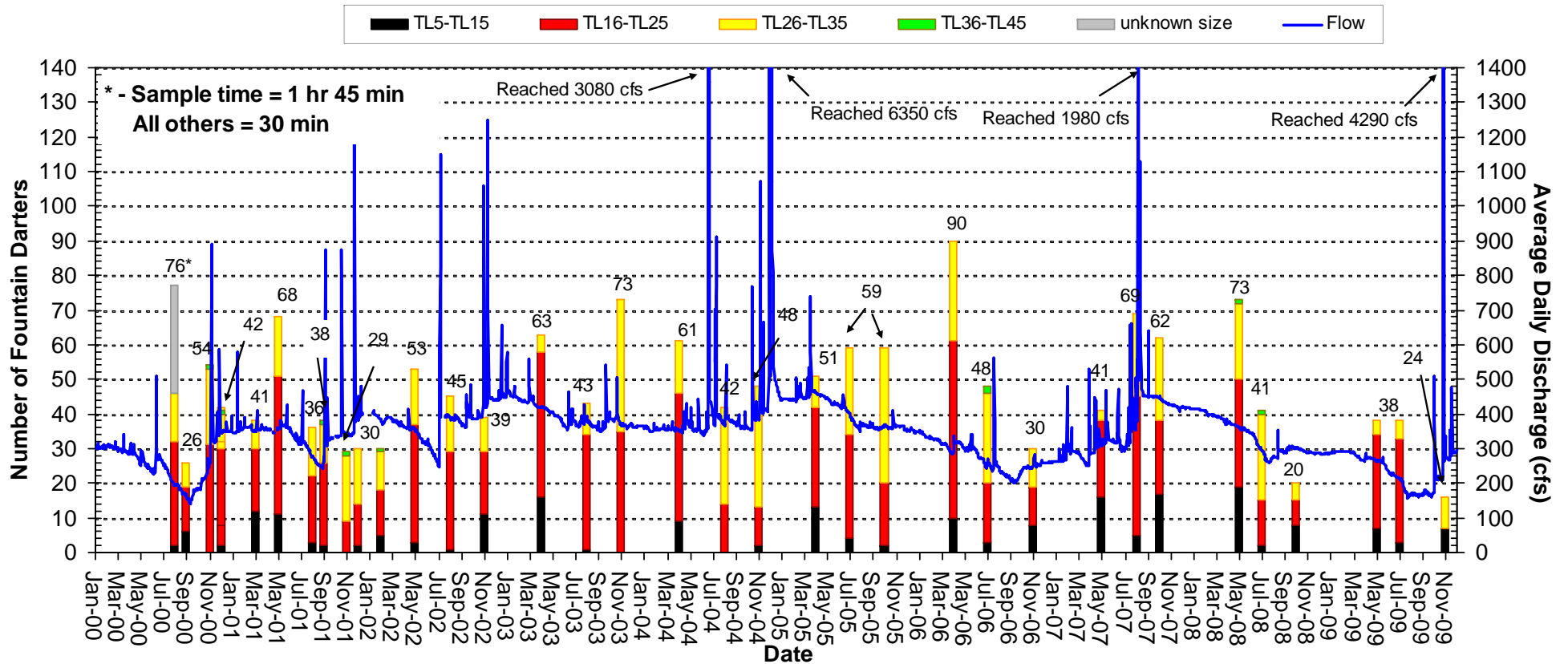


Dip Net Graphs

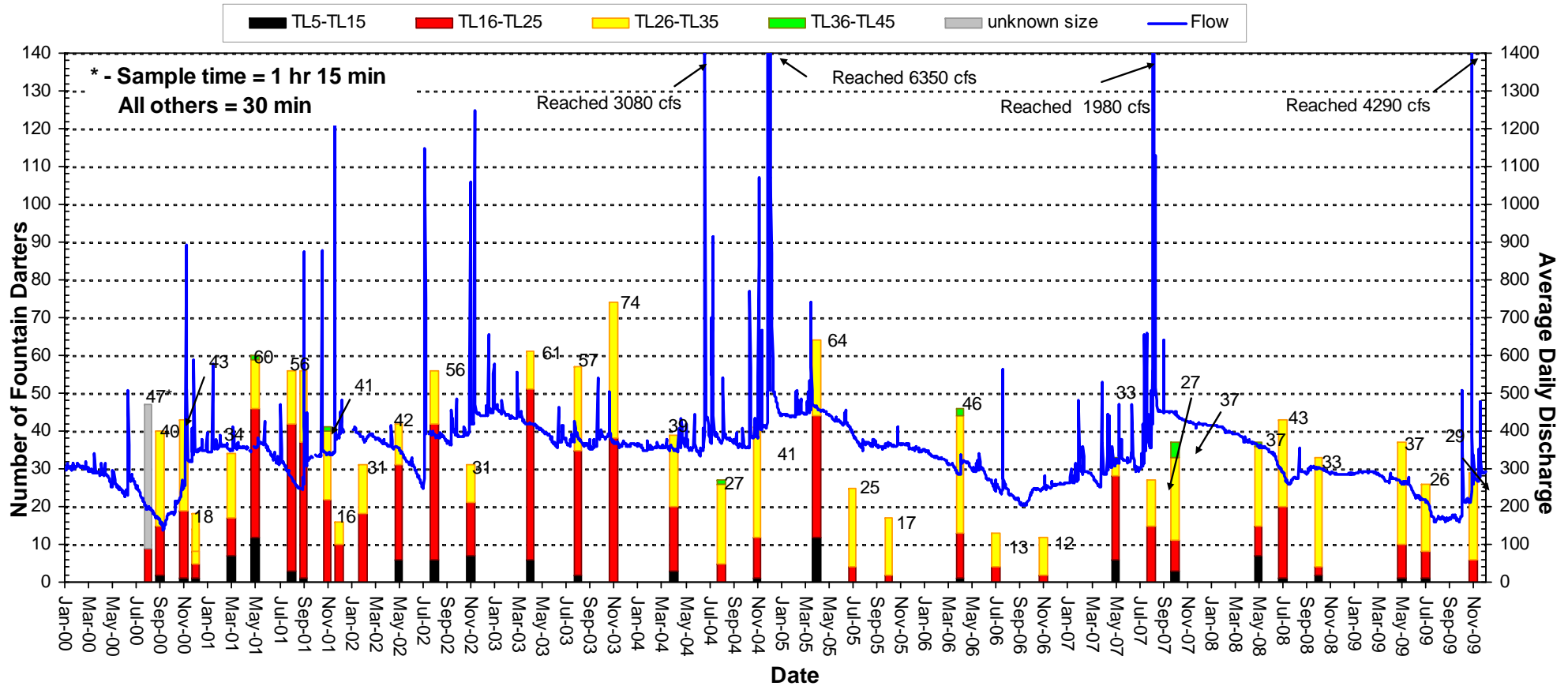
Fountain Darters Collected from the Upper Spring Run Reach (Section 3) Dip Net Results - Comal River



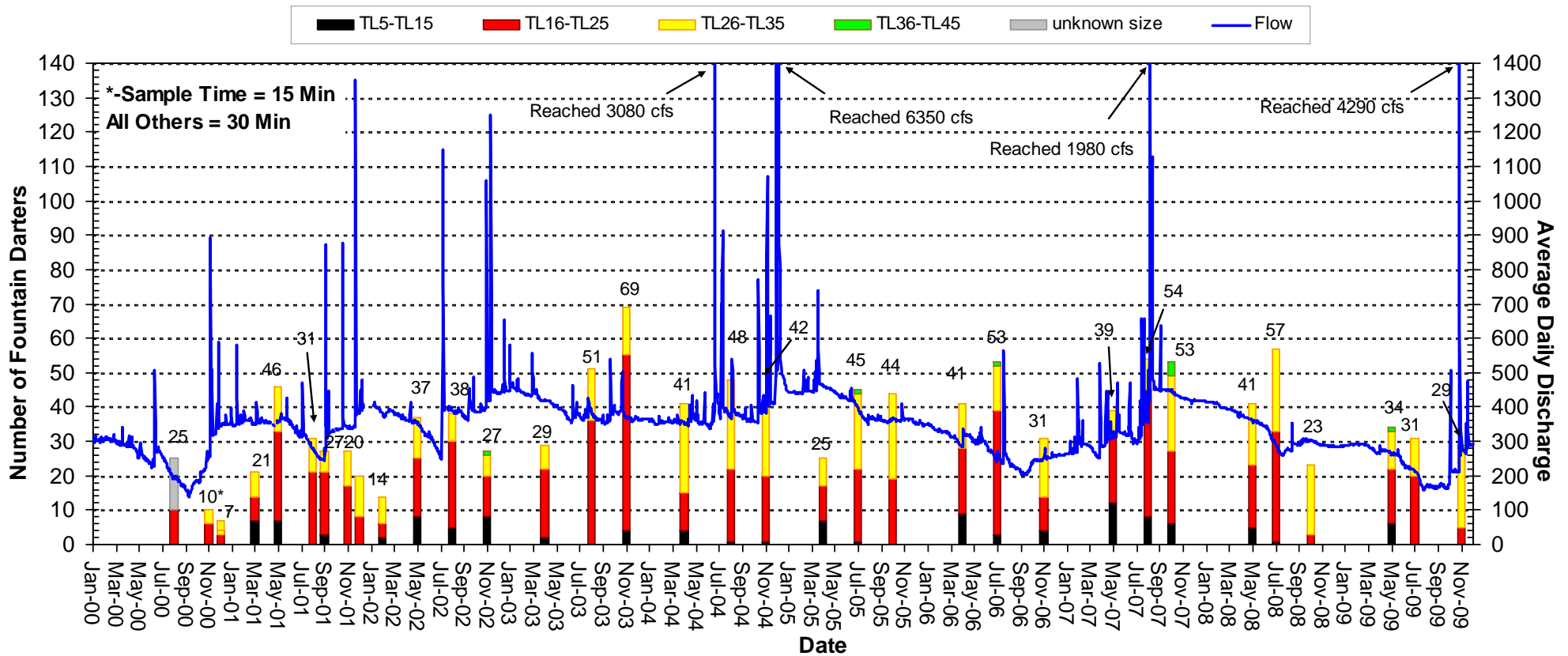
Fountain Darters Collected from the Spring Island Area (Section 4U-M) Dip Net Results - Comal River



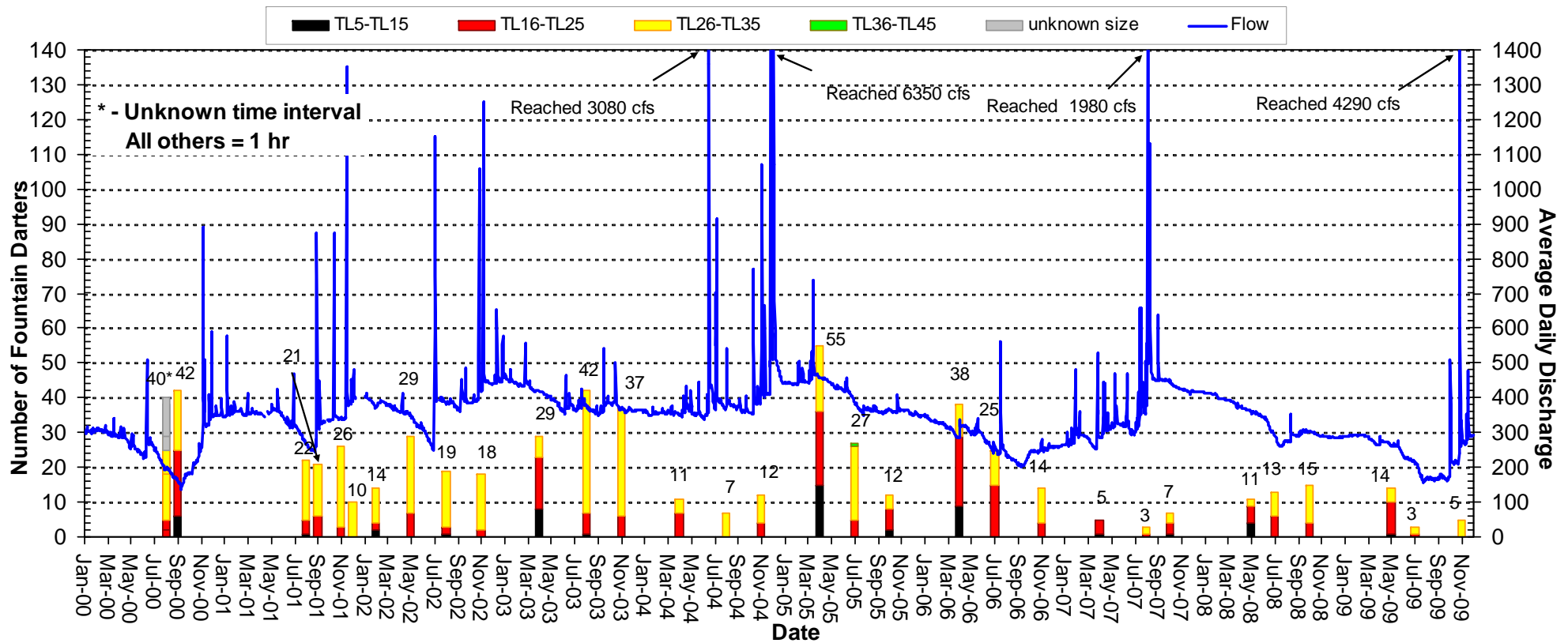
Fountain Darters Collected from the Landa Lake Reach (Section 4L) Dip Net Results - Comal River



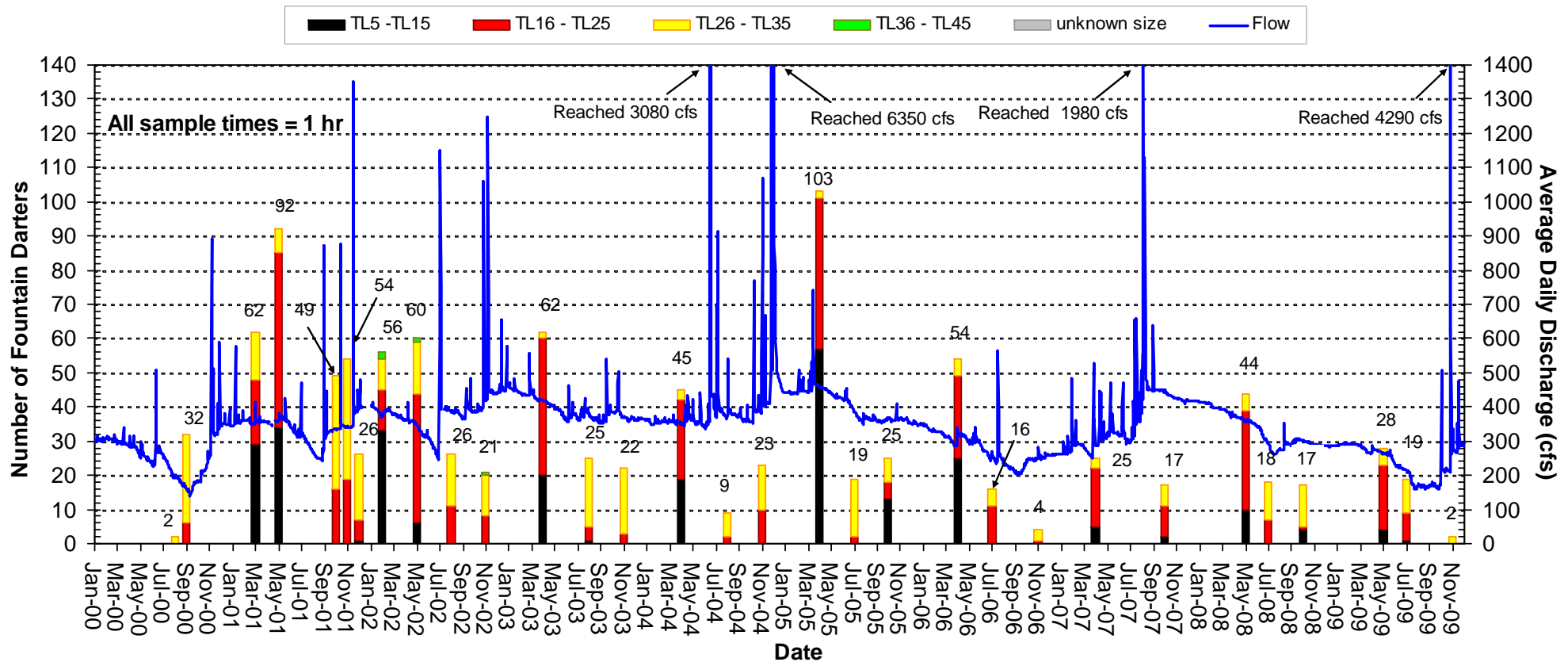
Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River



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



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



APPENDIX C:
DROP NET RAW DATA
(not available digitally)