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

Final 2011 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 four sampling events (two Comprehensive Monitoring efforts and two Critical Period low-flow efforts) on the Comal Springs/River ecosystem in 2011. For ease of comparison, the data are reported here in an annual report format similar to previous reports (BIO-WEST 2001-2011).

A prolonged drought in Central Texas that appeared to find relief in 2010 turned into an exceptional drought for a large portion of Texas in 2011. Severe lack of rainfall and record setting high temperatures during summer contributed to lower than average flows in the Comal River during almost the entirety of 2011. Flows quickly dropped below the historic average early in 2011, and culminated in the lowest average daily flow of 159 cubic feet per second (cfs) on September 14. This was only 1 cfs higher than 2009, and the third lowest single day flow since the inception of this study (the lowest of 138 cfs occurred in 2000). As a result, two Critical Period low-flow events were triggered. The initial event in June only included the Upper Spring Run Reach. During low flows, springs feeding the headwaters of the Comal River (Upper Spring Run Reach) are the first to cease flowing because they are higher in elevation than other spring openings downstream (Landa Lake, Spring Runs 1 – 3). Therefore, it is important to understand how the vegetation and fishes respond to these initial changes that affect this reach well before other reaches in the Comal River are impacted. In addition, a new discharge measurement was added just downstream of this reach to understand how decreased spring flows in this area contribute to the overall flow of the river. Continued lower than average flows over summer resulted in another Critical Period event in August, but this one included all of the reaches normally covered in this study.

Even though low-flows were prevalent in 2011, water temperatures varied little within the Comal River. High summer temperatures were reflected in elevated water temperatures at the Other Place and Blieder's Creek because these sites receive less spring influence. Although depths in the spring runs at Landa Park were decreased, water temperatures varied little. Grab samples of water were collected during Critical Period 2 (CP2) to examine water chemistry changes in lower than average flows. Dissolved oxygen readings at all sites met the water quality standard of 5.0 milligrams per liter (mg/L). As expected given the clarity of this system, total suspended solids were extremely low at all sites. Nitrate values exceeded water quality standards (1.0 mg/L) at all sites, but the levels observed are typical for Edwards Aquifer water and similar to those measured in previous years. Ammonium levels were well below these standards at all sites.

Aquatic vegetation is an important component of fountain darter habitat in the Comal River. Lower than average flows affected vegetation at all reaches, but changes were most apparent at the Landa Lake and Upper Spring Run Reaches. Changes in the composition of aquatic vegetation in the Comal River has differing effects on the fountain darter (*Etheostoma fonticola*) population because these fish prefer certain vegetation types. The highest densities of fountain darters are found in filamentous algae and bryophytes. However, only the latter has proven to be consistently found in multiple reaches in recent years. Within the Landa Lake reach, bryophytes cover much of the deeper portions of the lake, but are susceptible to being covered by green algae during lower flows. As the summer growing season commenced and flows continued to drop, large mats of green algae covered almost all of the bryophytes in Landa Lake. This resulted in the degradation of these mosses as they did not receive enough light to sustain themselves. This influences fountain darter populations because bryophytes offer more structure for both darters and their prey. As a result, fountain darter population estimates decreased from spring to CP2, but remained steady into fall. Similarly, bryophytes at the Upper Spring Run Reach decreased significantly from spring to summer as mats of green algae flourished. These mosses were absent by CP2, but recovered slightly by fall. As at Landa Lake, fountain darter population estimates in the Upper Spring Run Reach decreased from spring to summer due to the lack of bryophytes present at either reach.

Aquatic vegetation at the New Channel Reach is very dependent on the frequency of high-flow events due to its channelized nature (cement walls, little instream structure). After the flood event of 2010, only *Cabomba* and *Hygrophila* remained in the reach; however, the lower than average flows of 2011 allowed both plants to flourish. From spring to fall 2011 *Cabomba* increased by 81%, while *Hygrophila* expanded by 47%. The interaction between native and non-native plants in the Old Channel Reach is being closely monitored because of their importance as fountain darter habitat. *Ludwigia* (native) has higher densities of darters (13.7/ m^2) compared to non-native *Hygrophila* (7.1/ m^2), but by the end of 2011, *Hygrophila* covered 60 times as much area as *Ludwigia*. Filamentous algae, which has high densities of fountain darters and was previously abundant in this reach, was absent in the fall.

Higher densities of fountain darters are consistently found in native vegetation like bryophytes, filamentous algae, *Ludwigia*, and *Cabomba*. The flood event in 2010 scoured out much of these plants resulting in the lowest normalized population estimate since the inception of this study (2000). By spring this estimate had increased, but was still low compared to previous years. The continued low-flows during summer 2011 impacted aquatic vegetation (especially bryophytes) resulting in a decrease in population estimates by CP2. Length frequency distributions of fountain darters demonstrate a spring reproductive peak with subsequent year round reproduction in higher quality habitat like bryophytes in Landa Lake. Dip netting reflects these trends with most fountain darters captured in high quality habitat like *Cabomba* and bryophytes. Thus far, this study has exhibited the importance of native aquatic vegetation to fountain darter populations. Decreases in areal coverage of native plants results in a subsequent drop in fountain darter population estimates.

Comal Springs salamander (*Eurycea* sp.) populations were lower in spring 2011 than at any time during this study. The total number decreased by 75% from fall 2010 to spring 2011. Although it is unclear at this time why the numbers were low, it may be a combination of the high-flow event from 2010 depositing large amounts of fine sediment in the sampling areas, and the drought in Central Texas in 2011. Following the deposition of these fine sediments, there were no flushing flows to move these sediments downstream decreasing habitat quality. Observations at Spring Run 1 only totaled 6 in fall 2011, the lowest recorded in this study. In this spring run, lower flows led to decreased depths, and a large area covered by emergent vegetation (*Bacopa*, *Ludwigia*) both of which decreased sampling efficiency. In addition, a relative lack of bryophytes may have contributed to these low numbers in Spring Run 1. Salamanders at Spring Run 3 appeared to be less affected by the lower than average flows with numbers doubling by fall.

Macroinvertebrate collections in 2011 were highlighted by a new record for Comal Springs of *Tethysbaena texana*, which had only previously been found in Hueco Springs in Comal County. The highest amount of Comal Springs Riffle Beetles (*Heterelmis comalensis*) since the inception of the study was collected in 2011. Abundances were highest at Spring Run 3 and the Western Shoreline in spring, whereas abundances were highest at Spring Island in fall.

Following the major flood event in June 2010, the Comal River (and much of Central Texas) plunged into one of the worst droughts in history. As summer air temperatures increased and precipitation was sparse, flows continued to drop in the Comal River. These back to back events provided a unique opportunity to assess the aquatic community, evaluate stressors and compare observations to previous lower than average years. The 2011 Variable Flow study data indicated continued deleterious effects in the Upper Spring Run Reach on both aquatic vegetation and fountain darter populations as full recovery from the 2010 major flood event had not been accomplished in this reach prior to the extended period of drought. Less severe, but negative effects on aquatic vegetation and fountain darter populations were evident in Landa Lake during the majority of 2011. Aquatic vegetation and fountain darter conditions within the Old Channel remained consistent with post-culvert reconstruction conditions, while New Channel conditions improved during 2011. Neither water quality parameters measured (including water temperature) throughout the Comal Springs/River ecosystem, nor endangered macroinvertebrate habitat or population data posed concern during the extended drought witnessed in 2011. As such, the juxtaposition of high and low-flow events in 2010 and 2011 caused impacts (some severe) in the certain reaches of the ecosystem, but overall the aquatic communities presently remain intact. As the drought continues, or is broken it will be invaluable to continue to monitor the response and/or recovery of the Comal Springs/River ecosystem.

METHODS

Study Location

Comal Springs, which consists of numerous 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 approximately 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 (*Stygobromus pecki*). One additional species that is monitored during this study is the undescribed Comal Springs salamander (*Eurycea* sp.).

Two full comprehensive (spring and fall), one full comprehensive Critical Period low-flow (August) sampling effort, and one Critical Period low-flow (June) sampling event for the Upper Spring Run Reach were conducted in 2011. Additionally, Texas Master Naturalist 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:

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

<u>Aquatic Vegetation Mapping</u>

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

<u>Salamander Observations</u>

<u>Macroinvertebrate Sampling</u>
- Drift Nets
- Comal Springs Riffle Beetle Surveys

<u>Recreation Observations</u>
- Weekly Recreation Counts (Volunteer)

Comal Springflow

Total discharge data for the Comal River were acquired from United States Geological Survey (USGS) water resources division. The data are provisional as indicated in the disclaimer on the USGS website and, as such, may be subject to revision at a later date. According to the disclaimer, "recent data provided by the USGS in Texas – including stream discharge, water levels, precipitation, and components from water-quality monitors – are preliminary and have not received final approval" (USGS 2011). The discharge data for the Comal Springs ecosystem were taken from USGS gage 08169000 on the Comal River in 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. In 2011, a new site downstream of the Upper Spring Run Reach was added to assess the contribution of the springs in this section of the river. 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 2011 culminating in one full Critical Period event in August when initial flow data indicated that discharge dropped below 200 cfs in the river. Additionally one Critical Period event in June was conducted for the Upper Spring Run Reach only in order to examine drought effects where spring flow is likely to cease first.

High-Flow Sampling

There were no high-flow sampling events on the Comal Springs/River ecosystem in 2011.

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 2011. 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 YSI professional plus. Since 2003, one low-flow Critical Period event occurred in both 2006 and 2009, and two low-flow Critical Period events occurred in summer 2011. During the second low-flow Critical Period event in September 2011, 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/River 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.

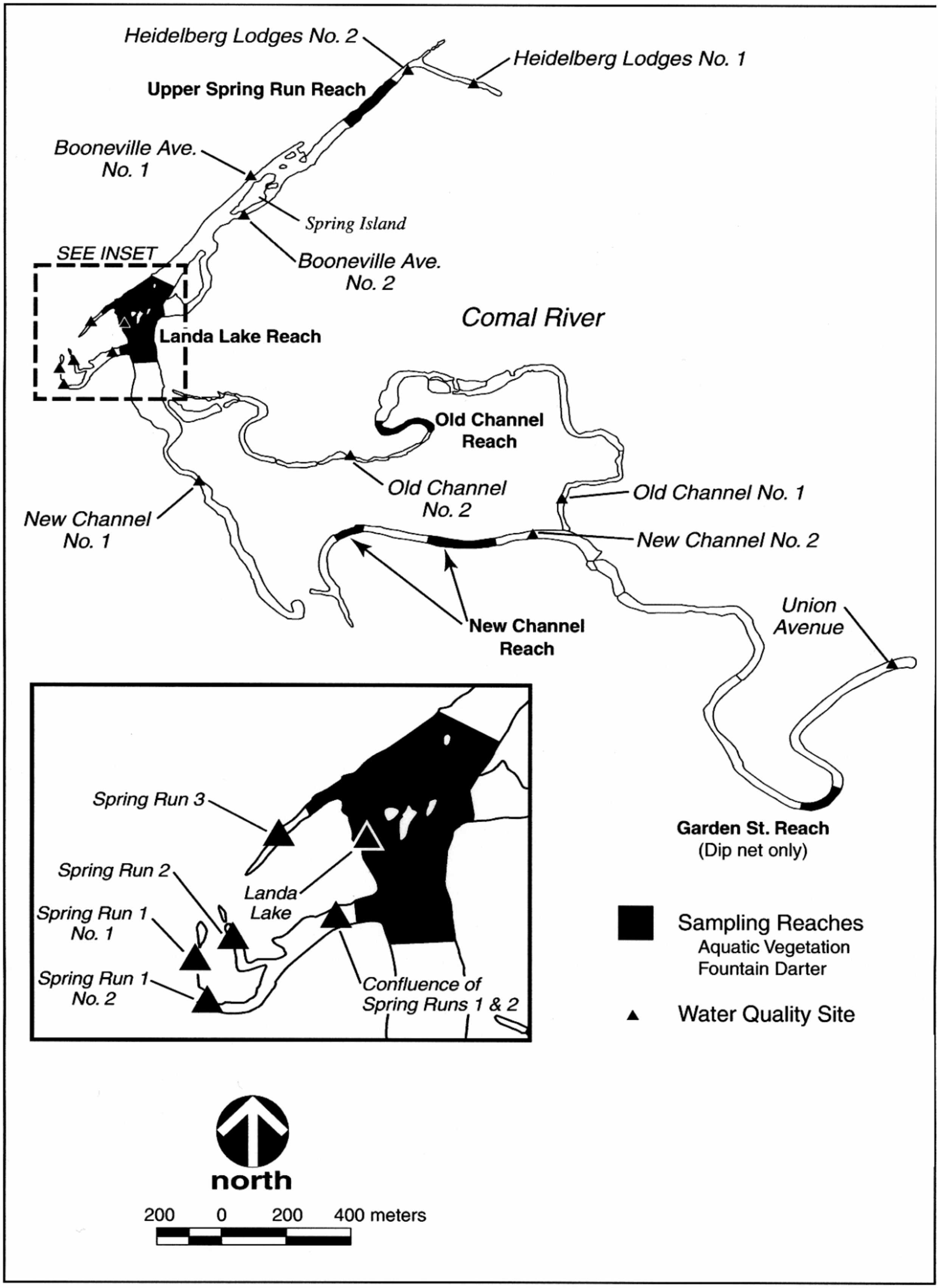


Figure 1. Comal River water quality and biological sampling areas.

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 comprehensive and Critical Period sampling events are described in the 2002 annual report (BIO-WEST 2003). Following the same protocols, water quality sample collection and analysis was conducted during one low-flow Critical Period sampling event in 2009 (BIO-WEST 2010) and September 2011 (low-flow CP2). During the 2011 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. Temperature, pH, conductivity, dissolved oxygen, and turbidity were measured *in situ* at each site using a handheld YSI 6920 datasonde in 2011. Chemical analyses of surface water samples for the 2011 CP2 sampling event were conducted by 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, Nitrite Nitrogen and Soluble Reactive Phosphorus: Following standard EPA Method 300.0, the concentrations of anions in a 10-µL sample are determined using an ion chromatography system equipped with a conductivity detector.

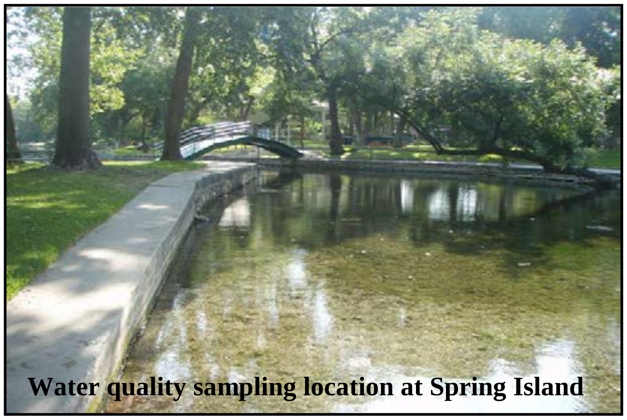
Ammonia: Following standard EPA Method 350.2, the sample is buffered at alkaline pH with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, distilled into a solution of boric acid and then determined by spectroscopy.

Total Kjeldahl Nitrogen and 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. Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate during the digestion. Total nitrogen is calculated as the sum of total Kjeldahl nitrogen, nitrate and nitrite.

Total Phosphorus: Following standard EPA Method SM4500-P E, 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 SM2540-D, 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 until residue weight is unchanged.



Water quality sampling location at Spring Island

Water quality sampling in Spring Run 3

Table 1. Water quality analyses performed on surface-water grab samples from 15 sites along the Comal River in 2011; along with the analytical method, technique, and minimum analytical detection levels of each analysis.

PARAMETER	EPA METHOD	TECHNIQUE (2009)	MINIMUM ANALYTIC LEVELS
Total Suspended Solids	SM4500-D	Gravimetric	4 mg
Alkalinity	310.1	Titration	20 mg
Nitrate Nitrogen	300.0	Ion Chromatography	0.05 mg[a]
Nitrite Nitrogen	300.0	Ion Chromatography	0.02 mg[a]
Ammonia	350.2	Spectroscopy	0.01 mg
Total Kjeldahl Nitrogen	351.2	Spectroscopy	0.1 mg
Total Nitrogen	351.2	Spectroscopy	0.1 mg
Soluble Reactive Phosphorous	300.0	Ion Chromatography	0.05 mg
Total Phosphorous	SM4500-P E	Spectroscopy	0.02 mg

[a] micrograms.

Master Naturalist Monitoring

Volunteers with the Texas Master Naturalist program continued their monitoring efforts in 2011 at select locations along the Comal Springs/River ecosystem. 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.

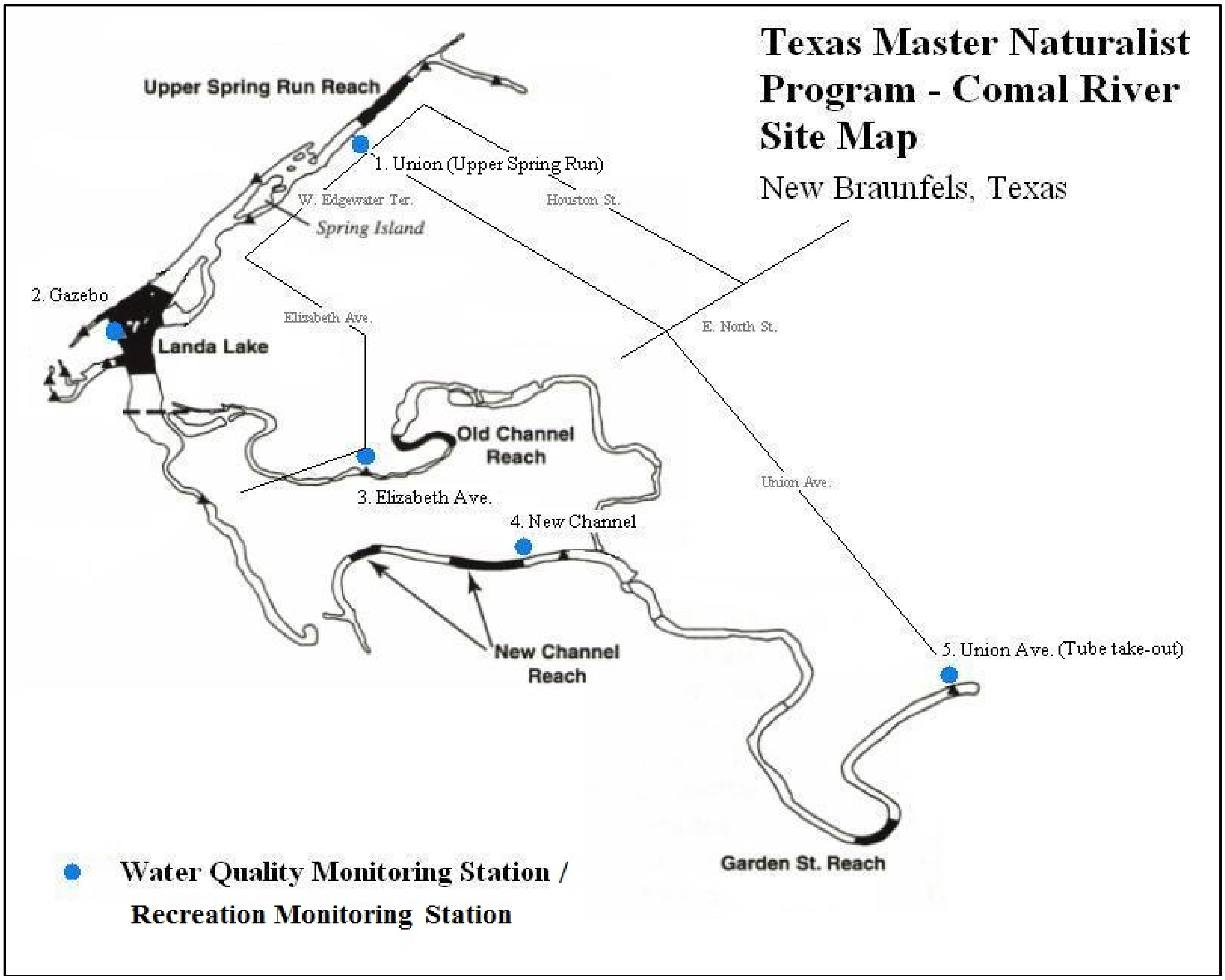


Figure 2. Weekly water quality / recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers.

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

10.6-foot (ft) Necky Rip kayak with the GPS antenna mounted on the bow. The aquatic vegetation was identified and mapped by gathering coordinates (creating polygons) 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 meter (m) in diameter were mapped by recording a single point. Vegetation stands less than 0.5 m in diameter were not mapped.

Hygrophila (top) and algae growth within *Hygrophila* (bottom) in the Old Channel Reach

Fountain Darter Sampling Methods

Drop Nets

A drop net is a sampling device 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 2000-2011 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 is due to changes in environmental conditions (scouring from 2011 flood, discharge, temperature, etc.) that had occurred since the last sample, but part is due to natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints), it is 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).

In addition to density and abundance calculations, drop net data were also used to generate length-frequency histograms for each season sampled. Analysis of these data, along with length-frequency data generated from dip netting, allows for inferences into reproductive seasonality.

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. 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 extreme 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.

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 2011 sampling events. These two-person surveys were conducted in Spring Run 1, Spring Run 3 (below), and the Spring Island area (Figure 1).

Spring Run 1

Spring Run 3

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, water depth, and presence/absence of vegetation species. 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 2011, 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 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 for Comal Springs riffle beetles were conducted in the two comprehensive sampling efforts in 2011 (June and November). These samples were conducted in Spring Run 3, along the western shoreline of Landa Lake, and near Spring Island in locations that were previously identified (BIO-WEST 2002a) to have high densities of Comal Springs riffle beetles. Samples were collected using the same "cotton lure" 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 lures were also noted. At each of the three study sites, 10 springs were sampled using this method.

OBSERVATIONS

The BIO-WEST project team conducted 2011 sampling on the dates shown in Table 2.

Table 2. Study components and sampling dates of the 2011 sampling events.

EVENT	DATES
Spring	
Vegetation Mapping	April 25 - 27
Fountain Darter Sampling	May 3 - 6, 16 - 19
Comal Salamander Observations	May 17
Macroinvertebrate Sampling	May 31 - June 2
Critical Period 1 (Upper Spring Run Only)	
Vegetation Mapping	June 20
Fountain Darter Sampling	June 20 - 21
Critical Period 2	
Vegetation Mapping	Aug. 15 - 17
Fountain Darter Sampling	Aug. 18 - 25
Comal Salamander Observations	Aug. 26
Fall	
Vegetation Mapping	Nov. 4 - 8
Fountain Darter Sampling	Nov. 14 - 21
Comal Salamander Observations	Nov. 17
Macroinvertebrate Sampling	Nov. 8 - 11

Comal Springflow

An exceptional drought in Central Texas contributed to lower than average discharge in the Comal River during much of 2011. This triggered two Critical Period events in June (Upper Spring Run Reach only) and August. The flows in the Comal River in 2011 contrasted sharply with 2010 when discharge remained above the historic average for the entire year (Figure 3). The lowest discharge occurred on September 14 (159 cfs), which is the second lowest discharge recorded since the beginning of the study (Table 3). While flows began the year above the historic average, discharge decreased below the average for the rest of the year (Figure 3). This trend was similar to 2009.

Table 3. Lowest discharge during each year of the study (2000-2011) 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
2010	305	Aug. 26, 30
2009	159	Sept. 14

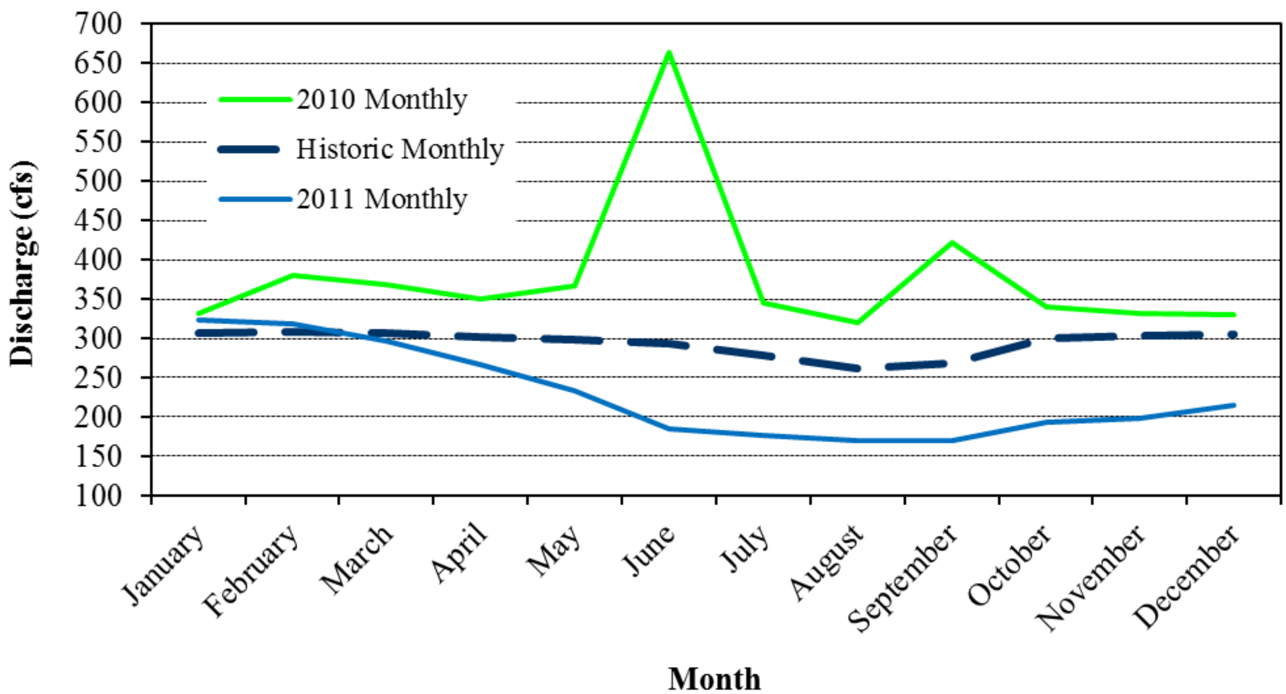


Figure 3. Mean monthly discharge in the Comal River during the 1934 – 2011 period of record.

For CP2, a new discharge location was added to represent the accumulation of springs near the top of the Upper Spring Run Reach. This location is located approximately 50 m downstream of the Upper Spring Run Reach, and will be included in all future sampling efforts.

As total discharge in the Comal River decreased through most of 2011, discharge in the Spring Runs generally decreased while discharge in the Old Channel stayed relatively steady (Table 4). Discharge in all spring runs decreased steadily from fall 2010 through the second Critical Period event in 2011 as the precipitation was sparse and summer air temperatures peaked. At Spring Run 1, discharge was cut by two thirds between the spring and full Critical Period event. Discharge at Spring Run 2 was reduced by half during this same period, and flows in Spring Run 3 exhibited a similar decrease (though not as severe). Although discharge decreased in the Old Channel Reach, it remained relatively steady because there is a culvert upstream that controls the flow through this section of the river. Discharge at all Spring Runs increased from the Critical Period to fall sampling event, but were still lower than spring at all sites except Spring Run 3 (downstream). Discharge at the Old Channel only increased slightly between the two events. This illustrates that the majority of flow in the Comal River is diverted down the New Channel. Discharge at the Upper Spring Run showed a similar increase from the Critical Period event to fall 2011.

While flows generally decreased over the whole system, the amount contributed from each Spring Run did not fit any particular trend (Table 5). As with most years the percentage of total discharge contributed by Spring Run 3 (downstream) varied very little during the year. In all events the Old Channel contributed between 20 and 25% of the total discharge for the Comal River. The Upper Spring Run made up between 12 and 17% of the total discharge in 2011.

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 2010 and all sampling efforts in 2011.

Location	Discharge (cfs)			
	Fall 2010	Spring 2011	Critical Period 2	Fall 2011
Total Discharge Comal River (USGS)	331	239	166	205
Spring Run 1	33.9	15.8	5.1	12.5
Spring Run 2	7.6	5.2	2.6	3.2
Spring Run 3 (upstream)	14.0	9.5	4.1	10.4
Spring Run 3 (downstream)	40.9	30.7	20.5	26.4
Old Channel	72.5	57.7	42.1	43.5
Upper Spring Run	n/a	n/a	12.9	16.5

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 2010 and all sampling efforts in 2011.

Location	Percentage of Total Discharge			
	Fall 2010	Spring 2011	Critical Period 2011	Fall 2011
Spring Run 1	10.2 %	6.6 %	3.1 %	6.1 %
Spring Run 2	2.3 %	2.2 %	1.6 %	1.6 %
Spring Run 3 (upstream)	4.2 %	4.0 %	2.4 %	5.1 %
Spring Run 3 (downstream)	12.4 %	12.8 %	12.4 %	12.9 %
Old Channel	21.9 %	24.2 %	25.3 %	21.2 %
Upper Spring Run	n/a	n/a	7.8 %	8.0 %

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/River ecosystem from 2000-2011. 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). Due to equipment malfunction and computer error, all temperature data from April 19 to October 29, 2010 were lost for all sites (this malfunction has since been corrected).

Thermistor data for the Comal Headwaters (Blieder's Creek and Heidleberg) are presented in Figure 4. This figure shows the contrast between a predominantly spring fed area (Heidleberg) and a stream relying on both spring inputs and runoff (Blieder's Creek). The constant temperatures of springflow remain relatively static throughout the year, whereas precipitation events and air temperatures during summer cause wide fluctuations in water temperatures within a flashy stream. Similarly, the downstream thermistor at the New Channel (Appendix B) has seasonal fluctuations because it is fed by both spring inputs from the Comal River, and runoff from Dry Comal Creek. This site also illustrates that as you move farther away from spring inputs, ambient air temperature has a much greater effect on water temperatures. In summer, temperatures often exceed 26.7 ºC (Texas Commission on Environmental Quality [TCEQ] water quality standard) in Blieder's Creek, but rarely at other sites. However, a temperature of 26.7 was reached on August 5 at the Other Place. This is not surprising as this site is the furthest downstream from spring inputs and therefore more prone to higher temperatures especially during an exceptional drought. Temperatures in the Spring Runs varied little as most of the water comes from the near constant temperatures of the Edward's Aquifer.

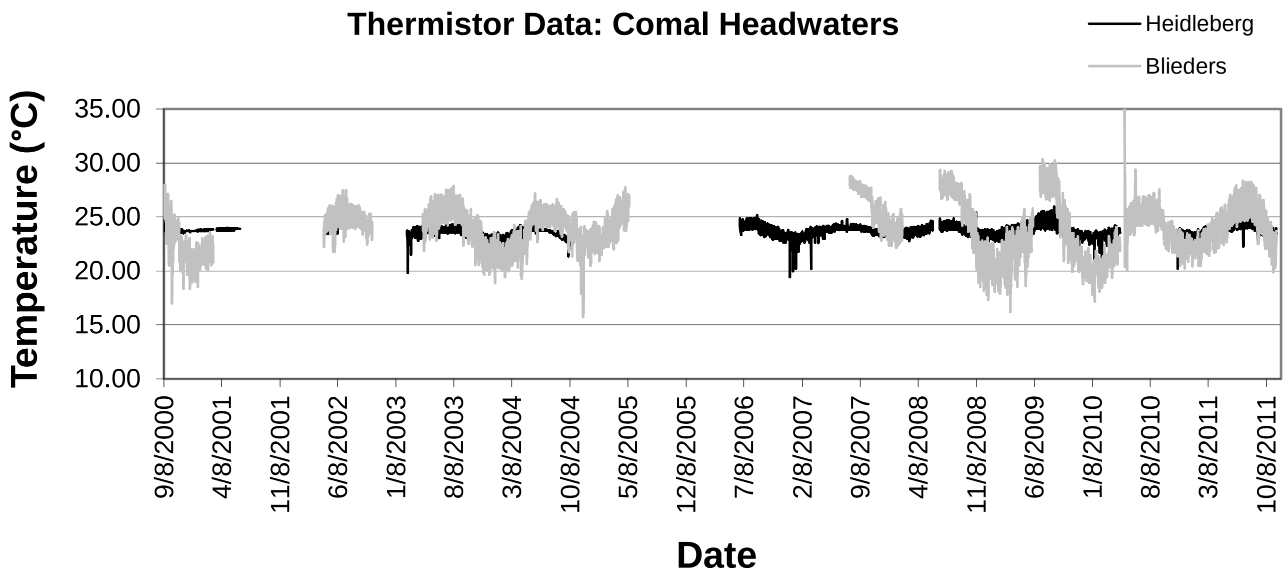


Figure 4. Temperature (ºC) data at Comal Headwaters from 2000 – 2011.

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), and a summary of results from the low-flow Critical Period event in 2009 is presented in BIO-WEST (2010). The same water quality sampling sites as in 2009 were sampled during the summertime CP2 low-flow sampling event in 2011 (Figure 1). The Comal River sampling site locations were as follows:

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

A summary of water quality data for the summer 2011 water quality sampling event is presented in Tables 6 and 7. 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 met the water quality standard of 5.0 milligrams per liter (mg/l) at all of the fifteen Comal River sample sites during the summer CP2 sampling event. Blieder's 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 Blieder's 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 spring run sites were lower than concentrations recorded at other sites (Table 6).

Table 6. Summary of Comal River ecosystem physical water quality measurements from CP2 sampling event.

Location	Temperature (°C)	pH	Conductivity (µS/cm)	Dissolved Oxygen (mg/l)
Blieder's Creek	24.31	7.57	542	6.23
Heidelberg, Main Channel	24.45	7.60	543	8.08
Island Park, Far Channel	23.87	7.58	544	6.88
Island Park, Near Channel	23.76	7.49	544	6.13
Spring Run 1, upstream	23.37	7.47	553	6.21
Spring Run 1, downstream	23.65	7.46	548	6.21
Spring Run 2	23.60	7.41	550	5.28
Confluence of Spring Runs 1 and 2	24.07	7.65	551	7.03
Spring Run 3	23.46	7.37	550	5.40
Landa Lake	23.78	7.47	550	6.40
New Channel, upstream	23.50	7.50	546	5.99
New Channel, downstream	23.29	7.85	545	8.30
Old Channel, upstream	23.37	7.73	548	6.20
Old Channel, downstream	23.43	7.69	544	7.15
Union Avenue (tube take-out)	23.37	8.02	546	7.65

Table 7. Summary of Comal River ecosystem water quality analytical results from CP2 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	240	0.094	1.55	2.31	<0.05	0.021
Heidelberg, Main Channel	<4	nd	0.087	1.49	0.78	<0.05	<0.02
Island Park, Far Channel	<4	240	0.099	1.72	2.40	<0.05	<0.02
Island Park, Near Channel	<4	230	0.103	1.80	2.32	<0.05	<0.02
Spring Run 1, upstream	<4	250	0.085	1.91	2.73	<0.05	<0.02
Spring Run 1, downstream	<4	260	0.234	1.86	2.94	<0.05	<0.02
Spring Run 2	<4	260	0.083	1.88	2.46	<0.05	<0.02
Confluence of Spring Runs 1 and 2	<4	240	0.073	1.84	2.78	<0.05	0.107
Spring Run 3	<4	240	0.370	1.92	3.37	<0.05	<0.02
Landa Lake	<4	250	0.071	1.88	2.65	<0.05	<0.02
New Channel, upstream	<4	240	0.073	1.77	2.35	<0.05	0.039
New Channel, downstream	<4	240	0.054	1.76	2.52	<0.05	<0.02
Old Channel, upstream	<4	250	0.096	1.69	2.39	<0.05	<0.02
Old Channel, downstream	<4	250	0.127	1.73	2.64	<0.05	<0.02
Union Avenue (tube take-out)	<4	nd	0.096	1.49	1.50	<0.05	0.0238

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 2011 sampling (Table 7), with values similar to those measured in 2000-2002 (BIO-WEST 2003). Alkalinity was not measured at two sites (Heidelberg and Union Avenue) due to a laboratory miscalculation of the sample processing volume required for the TSS measurements at these two locations.

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). None of the sites had a measurable concentration of SRP, and only four sites had measurable amounts of TP (ranging from 0.021 to 0.107 mg/l). Similar values of TP were measured in 2009 (0.023 to 0.026 mg/l; BIO-WEST 2010) and in 2000-2002 (0.027 to 0.030 mg/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 the Blieder's Creek, the confluence of Spring Runs 1 and 2, and the New Channel 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 higher nitrate values do not appear to be from anthropogenic inputs to the immediate surface waters, but rather a result of Edward's Aquifer spring water. 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 2011, and were similar to values in 2009 (1.0 to 1.64 mg/l; BIO-WEST 2010) and 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 (0.071 to 0.370 mg/l, Table 7), at levels well below the screening level although somewhat higher than values measured in 2009 (0.037 to 0.122 mg/l; BIO-WEST, 2010).

Master Naturalist Monitoring

Water quality data collected by Master Naturalist volunteers in 2011 showed that carbon dioxide (CO_2) concentrations continue to be highest near springs (Houston Street [Upper Spring Run Reach], Gazebo [Landa Lake/ Spring Run 3], Figure 5), while pH increased going downstream (Figure 6). At all sites, CO_2 concentrations were similar between years (2006-2011) but pH was higher at all downstream sites (Elizabeth Ave, New Channel, and Union Ave) than other years.

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). As in previous years, recreation use at Elizabeth Street was very low (Figure 7) because this area is not located within a city park. The annual summer increase in recreation at the Upper Spring Run is likely a result of more people staying at the Heidelberg Lodges (Figure 8). The Landa Lake park gazebo area is used for recreation regularly during all months of the year (Figure 9), and appeared to increase after this area was closed to vehicles following the 2010 flood. 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 an access point to the river. 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).

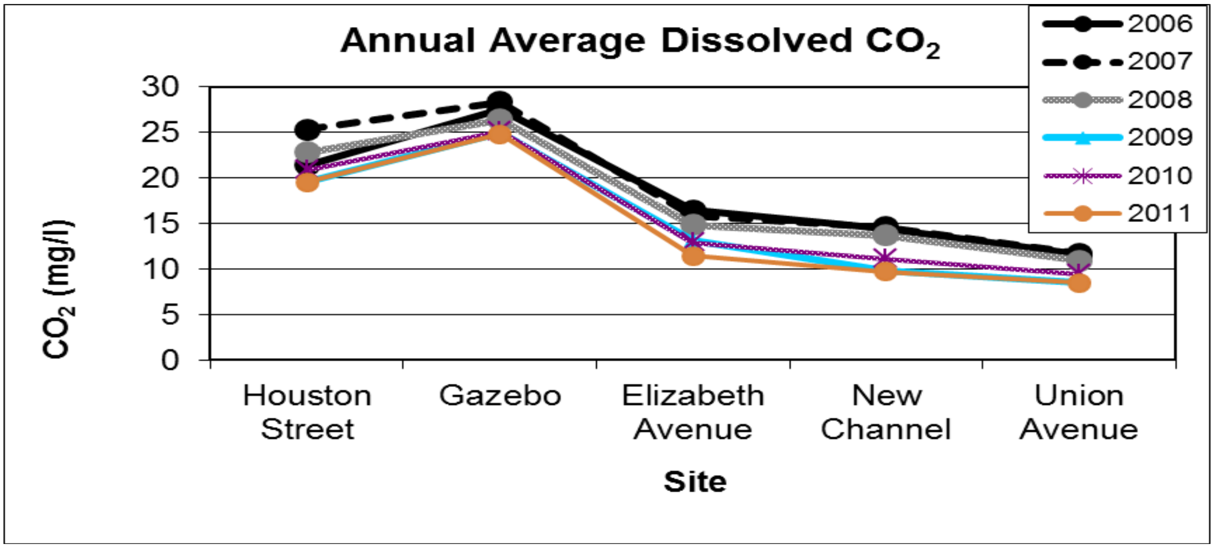


Figure 5. Annual average dissolved carbon dioxide (CO_2) concentrations at five sites on the Comal River system (2006-2011).

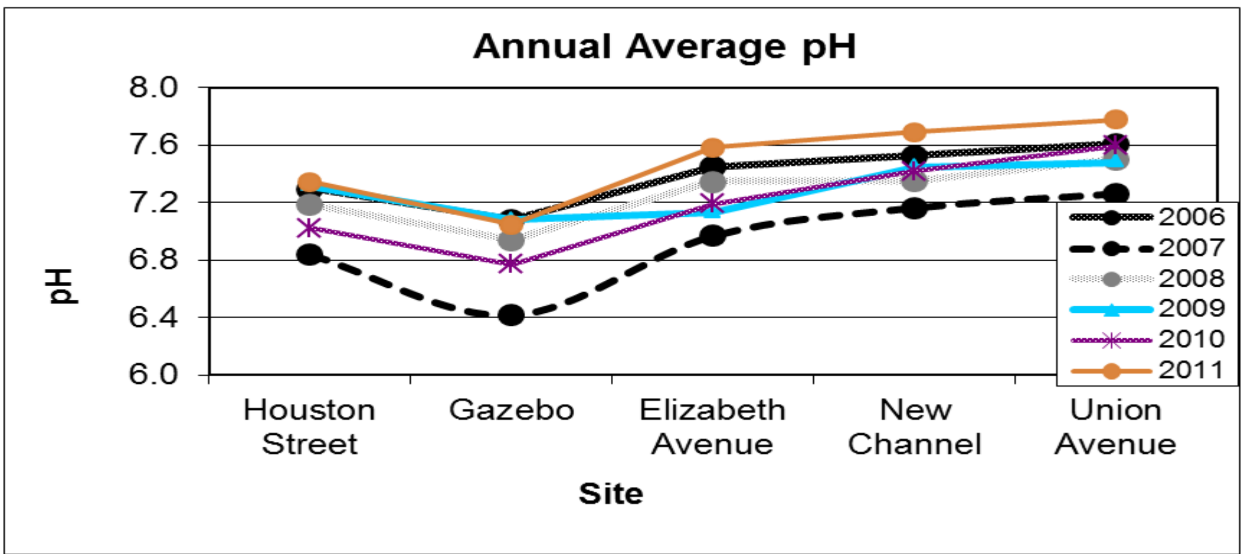


Figure 6. Annual average pH values at five sites on the Comal River system (2006-2011).

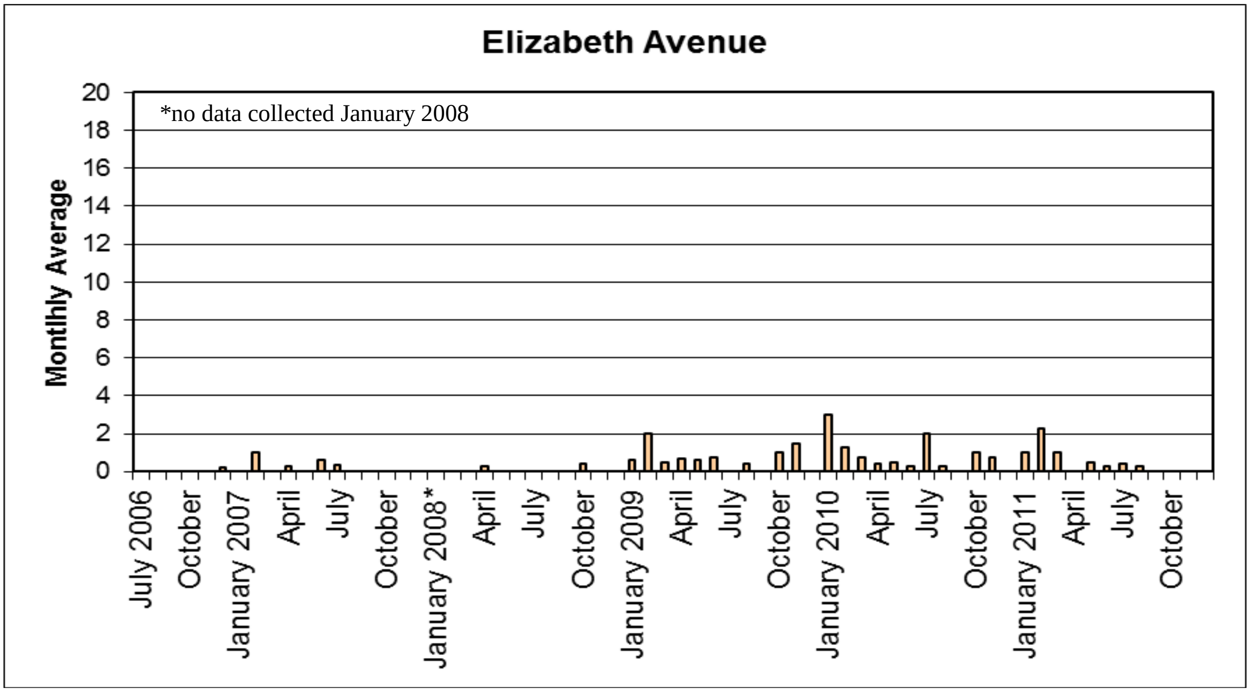


Figure 7. Average recreational use counts at the Elizabeth Avenue site (2006-2011).

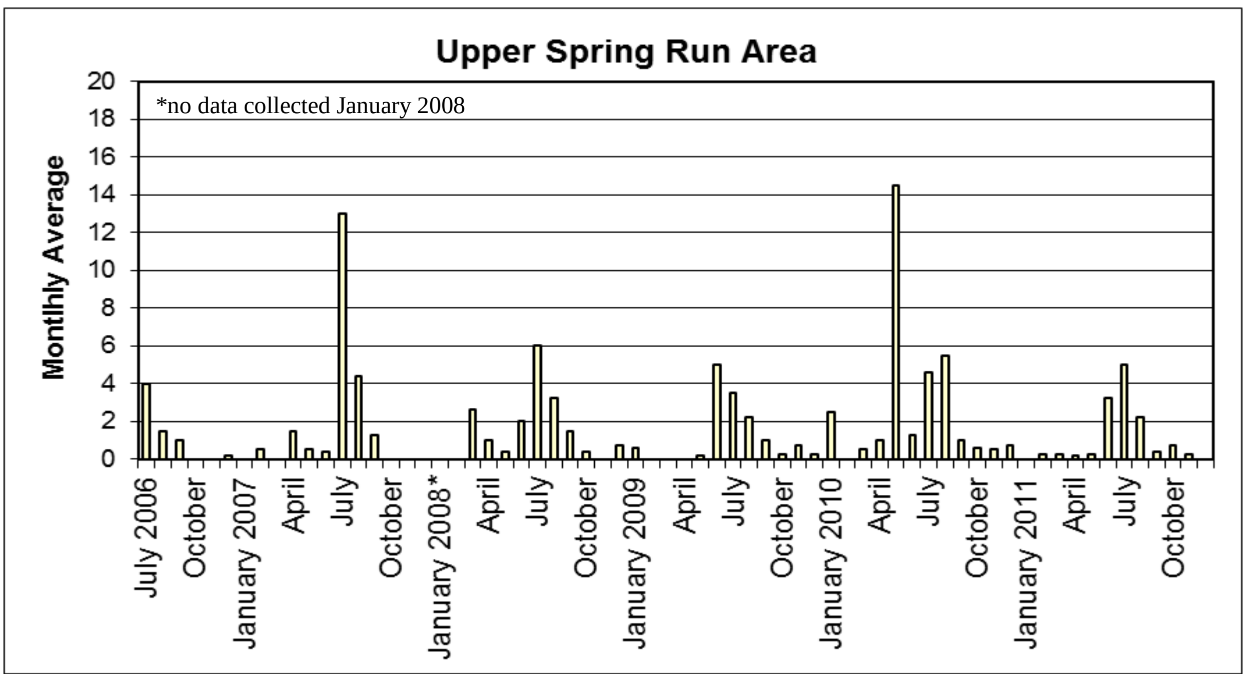


Figure 8. Average recreational use counts at the Upper Spring Run area (2006-2011).

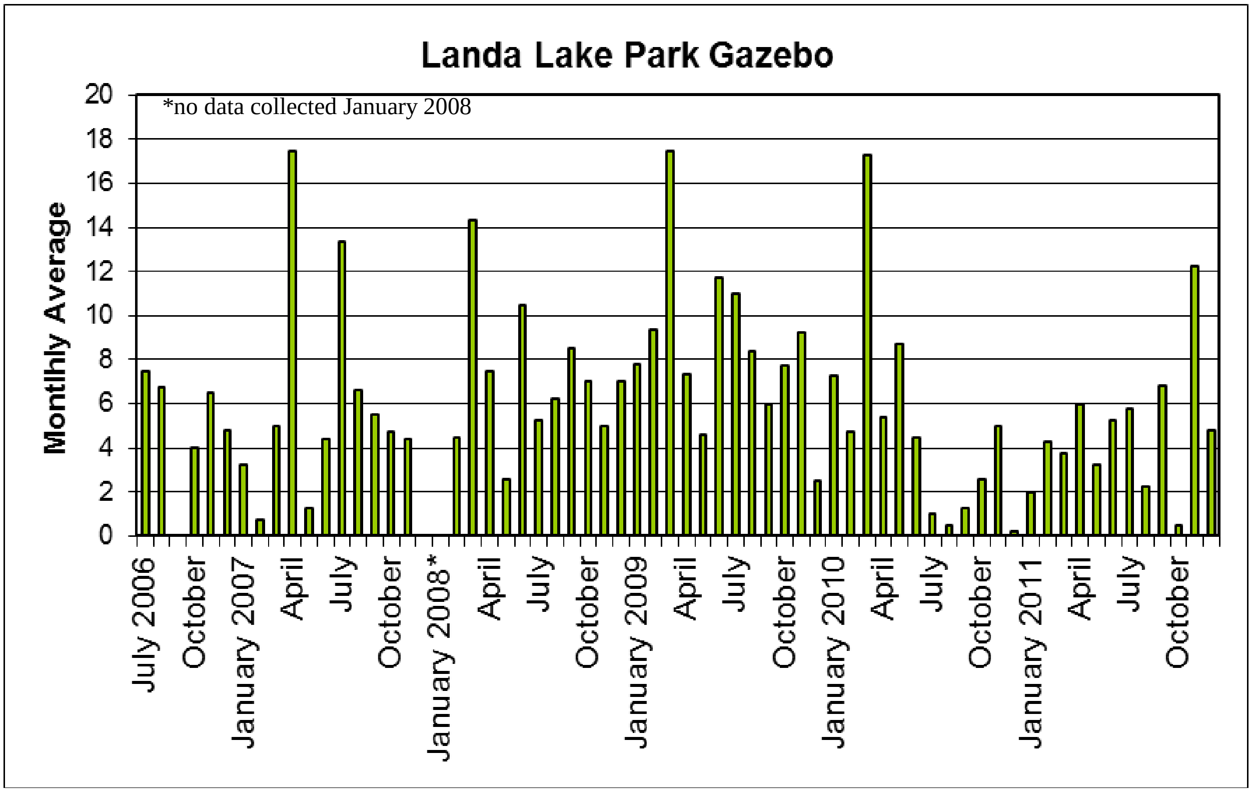


Figure 9. Average recreational use counts at the Landa Lake Park Gazebo site (2006-2011).

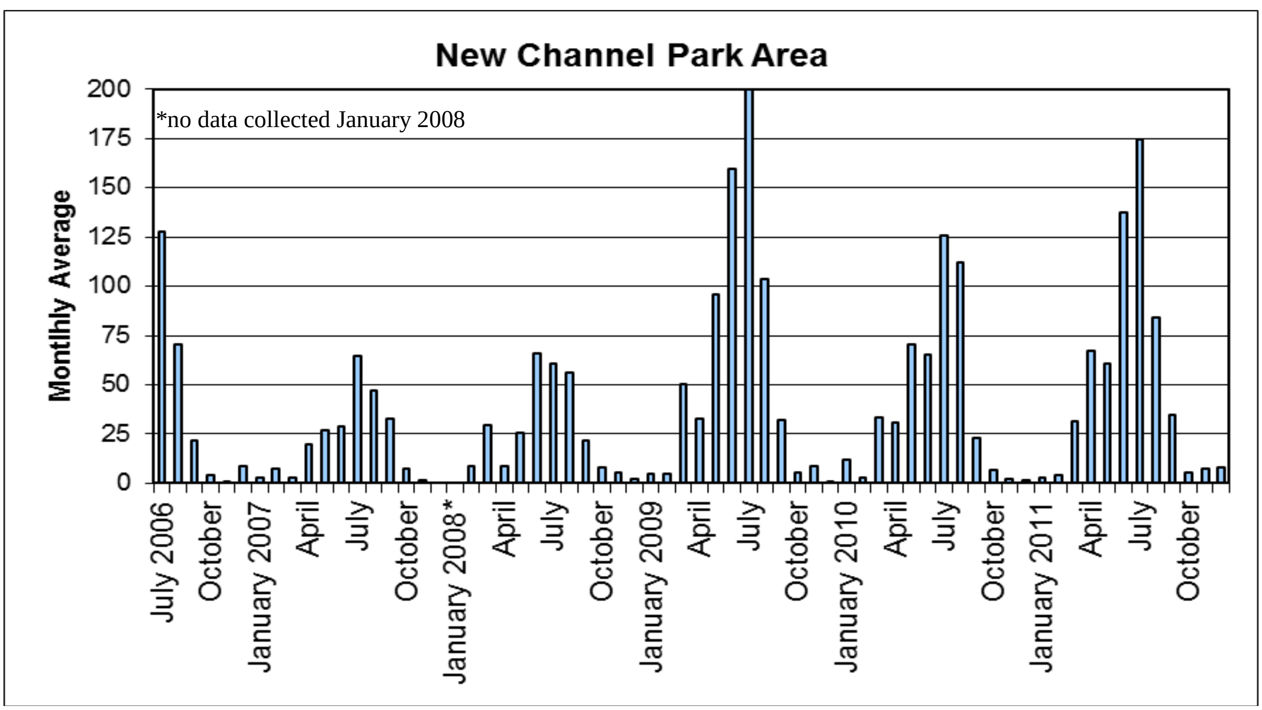


Figure 10. Average recreational use counts at the New Channel site (2006-2011) (note y-axis scale difference from previous recreation figures).

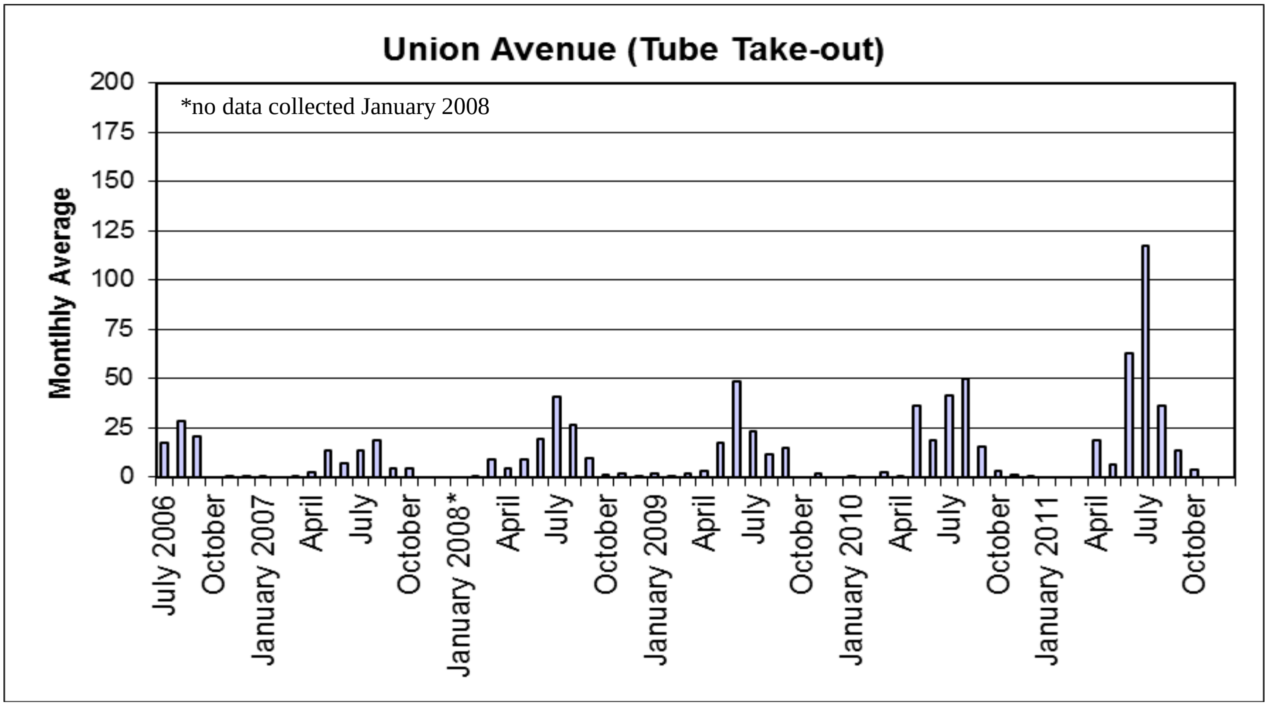


Figure 11. Recreational use counts at the Union Avenue site (2006-2011).

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 Upper Spring Run Reach is the most upstream reach of the Comal River in this study. In addition, the springs creating much of the flow here are higher in elevation than their downstream counterparts (Spring Island, Landa Lake complex). Therefore, these springs stop flowing first, and the aquatic vegetation in this reach exhibits negative effects before vegetation in other parts of the river. As a result an additional low-flow Critical Period (CP1) was conducted in June for this reach only.

Aquatic vegetation in the Upper Spring Run Reach continued its recovery after intensive scouring during the high-flow event of 2010. Shortly following that event, only *Sagittaria* remained in the reach, likely because it is more strongly rooted than other vegetation. This native vegetation continued re-establishing, growing to cover 648.8 m^2 of the river-left side of the reach. It continued to grow and fill in through both Critical Period events (688.4 m^2 and 704 m^2, respectively), but by fall decreased slightly in total area (697.4 m^2). This vegetation supports the least amount of darters (4.4/ m^2) for native plants sampled in this study. Like *Vallisneria* it has a simple leaf structure providing less cover for darters and their prey near the substrate. Unlike *Sagittaria*, bryophytes have more complex structure, and are responsible for the highest fountain darter densities found in the Comal River (27.6 darters/m^2). Unfortunately, high-flow events like the one in 2010 often completely remove bryophytes in this reach. With Blieder's Creek (non-springfed, flashy stream) situated just upstream of the reach, and the channelized nature of Upper Spring Run often leads to complete removal of these plants during high flow events. However, over winter they often grow quickly as was the case in 2011. By spring, these plants covered 666.7 m^2, increasing significantly from fall 2010 (15.6 m^2). But by CP1, large mats of green algae (1,041.6 m^2) began to cover bryophytes, thereby reducing bryophytes by 94%. While green algae continued to flourish in the lower than average flows, bryophytes were absent by the CP2 event, and only covered 30.6 m^2 during the fall sampling effort. This interaction between green algae and bryophytes in lower than average flows echoes that of these plants in the Landa Lake Reach.

Landa Lake Reach

The largest impact lower than average flows had on the Landa Lake Reach was in the amount of green algae and bryophytes. Traditionally these plants occupy the same areas of this reach and often exhibit a boom/bust cycle from year to year. Bryophytes hold the highest densities of fountain darters due to their structure and prey availability. Green algae don't root, but typically cover other plants as their numbers increase during the summer, especially under low flows. In this reach large areas of green algae can cover bryophytes and may result in a die-off like in 2011. In spring, bryophytes covered a total area of 1,114.8 m^2, an increase of over 700 m^2 from fall 2010 (411.7 m^2). By summer bryophytes were nearly absent from the reach after decreasing by 92%. At the same time green algae increased by almost the same amount (93%), and occupied the same areas previously used by bryophytes. Drop-netting and dip-netting efforts during the CP2 efforts indicated that bryophytes were still under the green algae, but decomposing quickly. By fall, bryophytes had increased slightly to 115.7 m^2.

Native *Ludwigia* exhibits the second highest densities of fountain darters in this reach even though they are present in low numbers and overall have been decreasing over the past several years. This plant decreased by 79% (from 48.4 m^2 to 10.2 m^2) from spring to CP2, and increased slightly by fall (11.8 m^2) in 2011. For the first time this plant ceased to exist in the middle part of the reach where *Hygrophila* has been expanding in recent years (though not in 2011). Similar in growth pattern, non-native *Hygrophila* also experienced die-off in the lower than average flows of 2011. It decreased by 30% from spring to CP2 fragmenting in several areas between the three islands located in the middle of the reach. The third highest densities of fountain darters in the Landa Lake Reach are found in the native plant *Cabomba*. This plant typically grows in lower velocity refuges along the river left section of the lake. It may be this ability to flourish in lower velocities that contributed to its growth over 2011 during lower than average flows. It increased by 18% from spring to CP2, and by 9% from CP2 to fall. This increase in coverage included an expansion of established plants, and new plants appearing within *Vallisneria* in the middle of the reach. *Vallisneria* continued to make up a majority (80% in fall 2011) of the aquatic vegetation within this reach because it flourishes within the deeper sections of the lake. Continued monitoring of this reach is important because it may prove to be an important refuge for many organisms if the exceptional drought in Central Texas continues into 2012 and beyond.

Old Channel Reach

Unlike other reaches, lower than average flows appeared to have little effect on aquatic vegetation in the Old Channel Reach in 2011. Native plant *Ludwigia*, recovered somewhat from fall 2010 contributing 26.7 m^2 to the reach in spring 2011. It increased slightly by CP1 (27.7 m^2), and covered 30.0 m^2 in fall. Although *Ludwigia* coverage increased over 3X in 2011, it still only covered about 2% as much area as *Hygrophila* by the end of 2011. Like every year of this study (including pre-culvert modification), *Hygrophila* was the dominant vegetation in the Old Channel Reach. *Hygrophila* increased by 5% from spring to fall 2011. The interaction and ebb and flow of *Ludwigia* and *Hygrophila* areas are important as they relate to fountain darter habitat. *Ludwigia* supports a higher density of darters (13.7/ m^2)

compared to *Hygrophila* (7.1/ m^2, see Fountain Darter Results). Close monitoring of this interaction will yield valuable knowledge in understanding this endangered species habitat requirements.

Other native vegetation types like filamentous algae and bryophytes are currently limited in the Old Channel Reach. Filamentous algae appeared to have gained a foothold in this reach in 2010 (7.0 m^2), but decreased by spring 2011 (4.3 m^2), and were absent from this reach for the rest of the year (Appendix A). Unlike in 2010 when they were scoured out, bryophytes increased in this reach in 2011. These plants are lightly rooted and susceptible to high flow events (like in 2010), but in average flows (absent of green algae) they have the potential to flourish. After being absent, a few plants established by spring (2.4 m^2), and by CP1 had increased to a high of 37.9 m^2, with a slight decrease by fall (28.4 m^2). Unlike *Ludwigia* and *Hygrophila*, these plants are habitat for higher densities of fountain darters. In the Comal River, bryophytes yield (on average) 27.6 darters/m^2, with filamentous algae contributing the second highest fountain darter density (22.4 m^2). Unfortunately, though several native plants with high fountain darter densities are present in this reach, they remain in very low amounts compared to less important (for fountain darters) non-native plants (*Hygrophila*).

New Channel Reach

The aquatic vegetation in the New Channel Reach continued its recovery after a flood in 2010 wiped out nearly all of it. Because this reach is channelized, only a single *Hygrophila* and *Cabomba* plant remained after the June 2010 flood. However, by spring 2011 total aquatic vegetation coverage was 538.1 m^2, an increase of over 500 m^2 from fall 2010. *Hygrophila* recovered fastest covering 392.1 m^2 by spring 2011. By fall, this plant type covered approximately 25% of the reach. Native *Cabomba* followed a similar trajectory in 2011. As lower than average flows continued, *Cabomba* flourished along the edges of the New Channel Reach. Although it covered less than half the area of *Hygrophila* in spring (143.2 m^2), by fall it expanded to 743.1 m^2, 10 m^2 more than *Hygrophila*. A few plants of *Ludwigia* also became established (high of 8.0 m^2 in fall) after disappearing in 2010.

Unlike other study reaches in the Comal River, the New Channel Reach is most susceptible to scouring of vegetation from high-flow events. At the same time, recovery of aquatic vegetation in this reach is often immediate. Large depths in this reach prevent drop-netting efforts, but dip-netting has shown that fountain darters are present in this reach. Therefore, the interaction between native (*Cabomba*, *Ludwigia*) and non-native (*Hygrophila*) vegetation will continue to drive fountain darter populations in this reach.

Fountain Darter Sampling Results

Drop Nets

A total of 72 drop net samples were conducted during 2011 in the Comal Springs/River ecosystem. Table 8 shows the number of drop net samples taken from each vegetation type in each reach during 2011 sampling events.

Table 8. Number of drop net samples collected in each vegetation type per reach during 2011 sampling events.

Vegetation Type	Spring (May 4-6)			Critical Period #1 (June 21)			Critical Period #2 (Aug. 22-24)			Fall (Nov. 14-16)		
	Upper Spring Run	*Landa Lake*	*Old Channel*	*Upper Spring Run*	*Landa Lake*	*Old Channel*	*Upper Spring Run*	*Landa Lake*	*Old Channel*	*Upper Spring Run*	*Landa Lake*	*Old Channel*
Filamentous Algae												
Bryophytes	2	2		2			2	2		2	2	
Ludwigia		2	3					2	3		2	3
Hygrophila	2	2	3	2			2	2	3	2	2	3
Sagittaria	2			2			2			2		
Vallisneria		2						2			2	
Cabomba		2						2			2	
Open												
TOTAL	**6**	**10**	**6**	**6**	**0**	**0**	**6**	**10**	**6**	**6**	**10**	**6**

A total of 1,616 fountain darters were collected during drop net sampling on the Comal Springs/River ecosystem in 2011. Five hundred and seventy-seven darters were collected during spring, 40 during the June Upper Spring Run Reach Critical Period (CP1) event, 442 during the August Critical Period Event (CP2), and 557 during the fall. Excluding collections from the New Channel Reach (since it is no longer sampled), the number captured during each full 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 overlaid on a hydrograph showing mean daily discharge. Due to the extremely variable nature of the data, discharge-abundance relationships are difficult to discern from this analysis. Additionally, it is important to remember that the number of drop net samples taken in each vegetation type has been modified slightly as vegetation communities have changed throughout the study. However, even across sampling events with exactly equal effort, Figure 12 shows that fountain darter abundance varies considerably. Data suggests a rather dynamic but stable population throughout the study period.

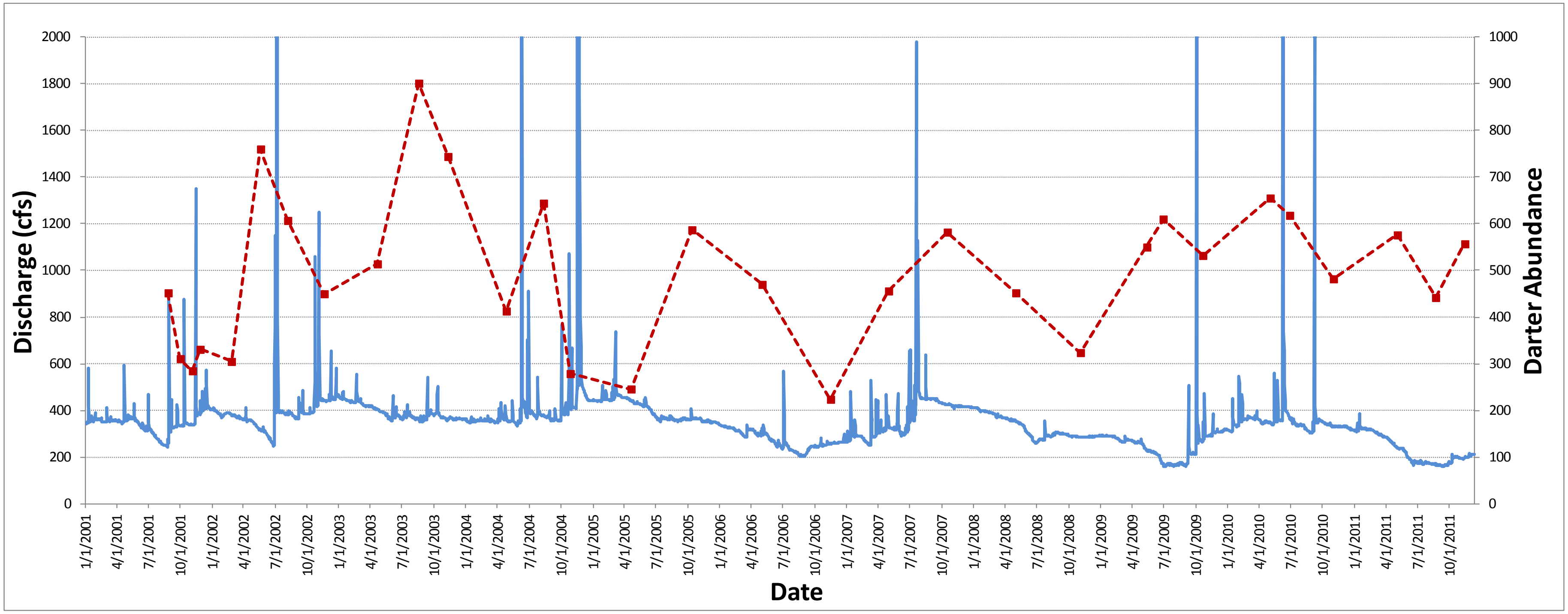


Figure 12. Abundance of fountain darters from each drop net sampling event (red dashed line) plotted over a hydrograph of mean daily discharge from the USGS gage on the Comal River at New Braunfels (blue line).

Drop net data collected from 2000-2011 show that average densities of fountain darters in the various vegetation types ranged from 3.6/m^2 in *Ceratopteris* to 27.6/m^2 in bryophytes (Figure 13). Open substrate with no aquatic vegetation contains few fountain darters (0.9/ m^2). Native vegetation types which provide thick cover at or near the substrate (i.e., bryophytes and filamentous algae [22.4/ m^2]) tend to have the highest fountain darter densities. Filamentous algae and bryophytes also contain high numbers of amphipods, a common food item for fountain darters. In contrast, exotic vegetation (*Ceratopteris* and *Hygrophila* [7.1/m^2]), and native vegetation with simple leaf structures (*Vallisneria* [4.7/m^2] and *Sagittaria* [4.4/m^2]) which provide little cover near the substrate tend to have fewer darters. In the Comal River, the native vegetation types *Cabomba* and *Ludwigia* exhibit intermediate fountain darter densities (10.0 and 13.7/ m^2, respectively).

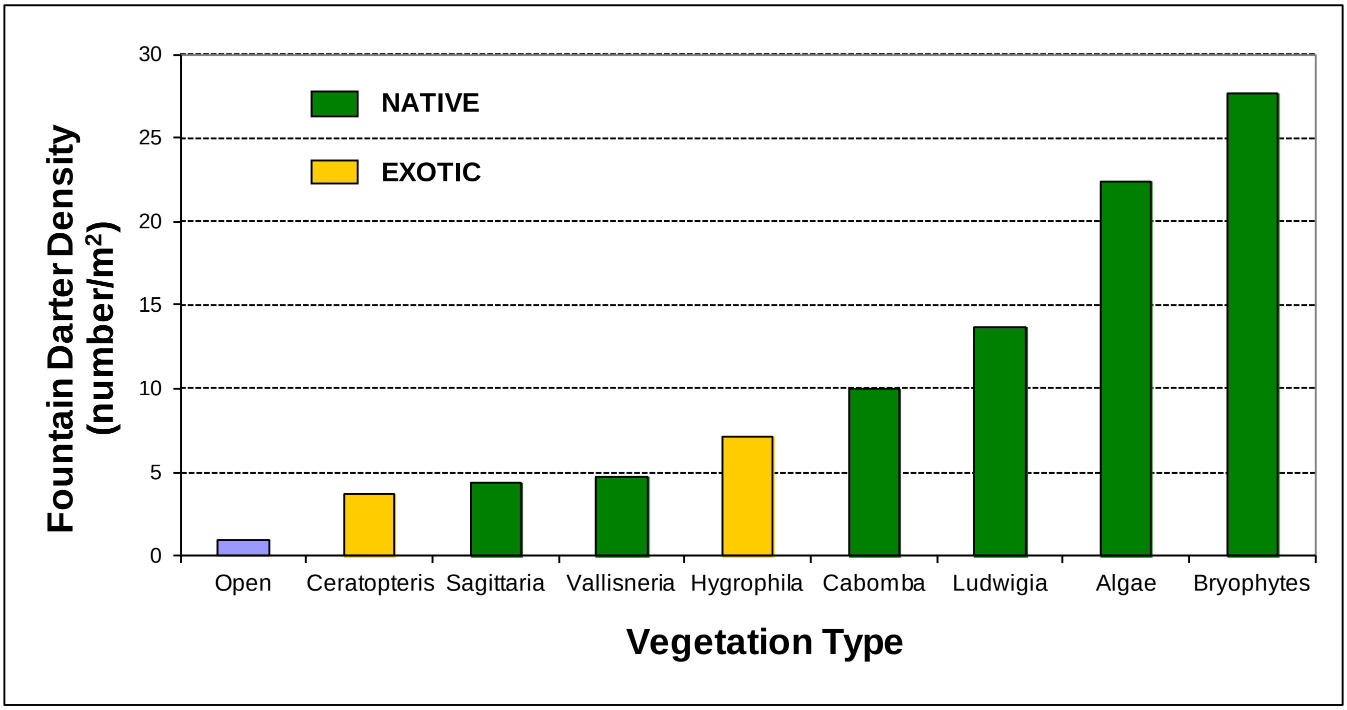


Figure 13. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem from 2000 - 2011.

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. These plants are not firmly rooted to the substrate, and can be easily uprooted in higher velocities. Filamentous algae was once the dominant vegetation type in the Old Channel Reach, however, it has been replaced in recent years by *Hygrophila* and *Ludwigia*. This has resulted in an overall decrease in the abundance of fountain darters in this reach (see dip net data). Bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake reaches, and thus make up a significant portion of the available habitat. *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 considered an important habitat component because it is abundant in all sample reaches.

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. This is due to the large 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. In fact, prior to summer 2001, bryophytes in the Landa Lake Reach were not sampled – leading to considerably lower population estimates. Estimates of population abundance were highest in spring 2003 when coverage of bryophytes peaked in Landa Lake (Figure 14).

Recently, population estimates have been low throughout the latter half of 2010 and 2011. A large localized flood event (the largest during the study) in June 2010 resulted in intensive scouring of the aquatic vegetation in the Upper Spring Run Reach and Old Channel Reach. Except for a few small patches of strongly-rooted *Sagittaria*, the Upper Spring Run Reach was void of vegetation after this event. The resulting loss of habitat displaced fountain darters which are typically abundant in the bryophytes of the Upper Spring Run Reach, causing population estimates to hit an all-time low. Previous high-flow events have led to less-intense scouring, and the system typically recovers quickly as large rainfall events often lead to increased springflow. However, sustained low flows since this event has resulted in a poor recovery of darters and vegetation within the Upper Spring Run Reach. Although conditions began to improve by spring 2011, low summer springflows led to a continued decline in the bryophytes of this reach. Low springflows during summer allowed for green algae to outcompete bryophytes, resulting in low fountain darter habitat quality. As a result, population estimates have remained low through summer and fall 2011.

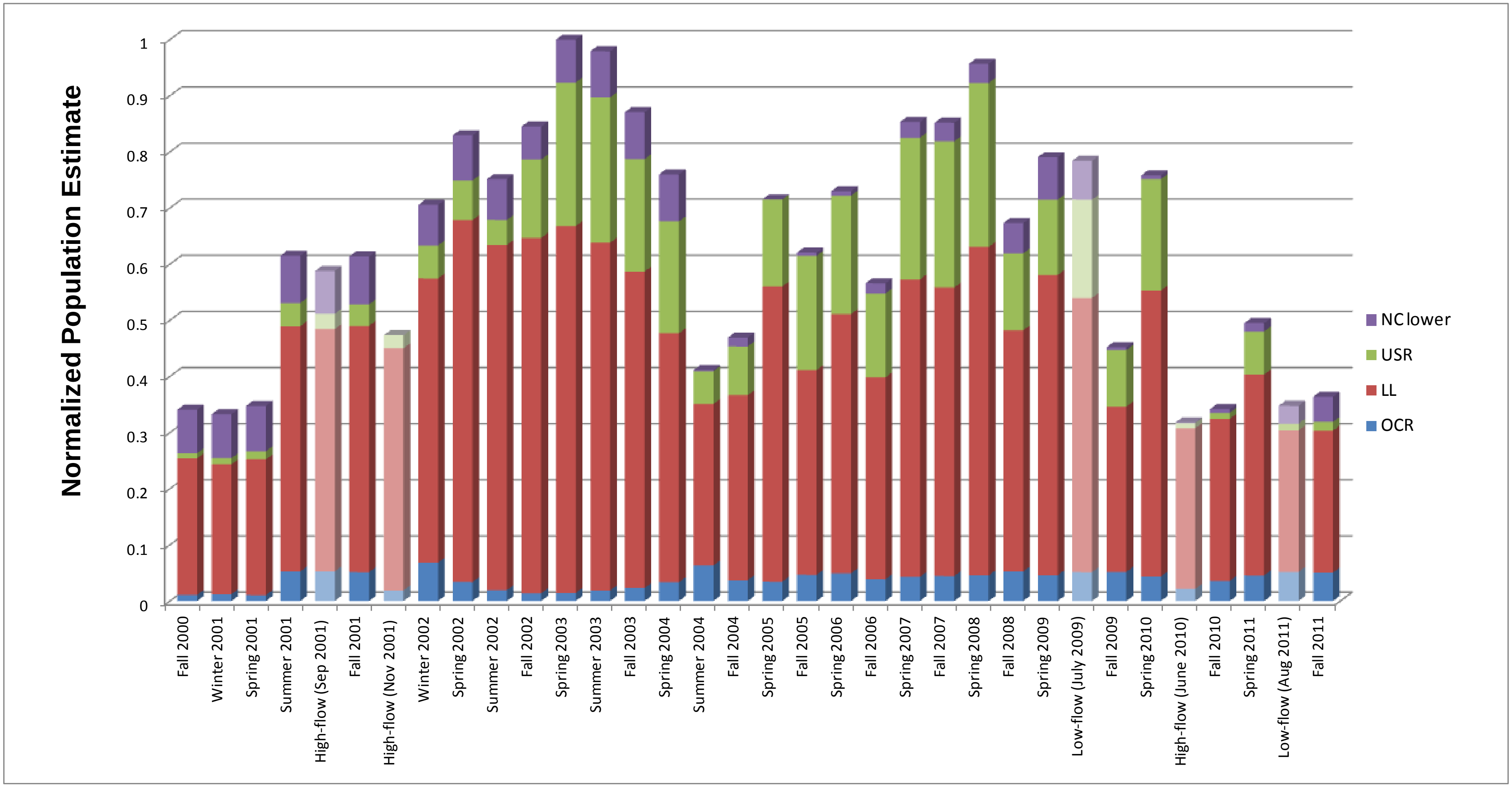


Figure 14. Population estimates of fountain darters in all four sample reaches combined (2000 – 2011). Values are normalized to the maximum sample. Lighter colors represent Critical Period sampling events.

Drop netting efforts in 2011 resulted in collection of 1,616 fountain darters in the Comal Springs/River ecosystem. The length frequency distribution for fountain darters collected by drop nets from the Comal ecosystem during each 2011 sampling event is presented in Figure 15 (data collected in previous years is presented in Appendix B). Analysis of length frequency data from 2011 and previous years suggests year-round reproduction with a spring time reproductive peak. Previous analyses has shown that year-round reproduction is typically limited to reaches with high-quality habitat such as Landa Lake, whereas reaches with lower-quality habitat such as the Old Channel Reach typically exhibit little reproduction during summer and fall months. Recent analysis shows that even within Landa Lake, darter reproductive activity is habitat dependent (Figure 16). In bryophytes, small darters are present during all samples, indicating year-round reproduction; whereas, small darters are only abundant during the spring season in *Hygrophila*. Although this could be a function of small darters simply selecting bryophyte habitats, this is doubtful given the distance between these habitat types in Landa Lake and considering that studies show little movement by fountain darters in such habitats (Dammeyer 2010). More likely, this represents continuous reproduction of fountain darters in bryophytes, and more limited seasonal reproduction in other areas. Recent studies on fountain darter egg deposition in the San Marcos River support this, finding significantly more eggs were deposited on filamentous algae (similar in growth form to bryophytes) than on common vegetation with other growth forms including *Hygrophila* (Phillips et al. 2011). An egg deposition study with a seasonal component, in which bryophytes were included, would likely confirm the trends discussed here.

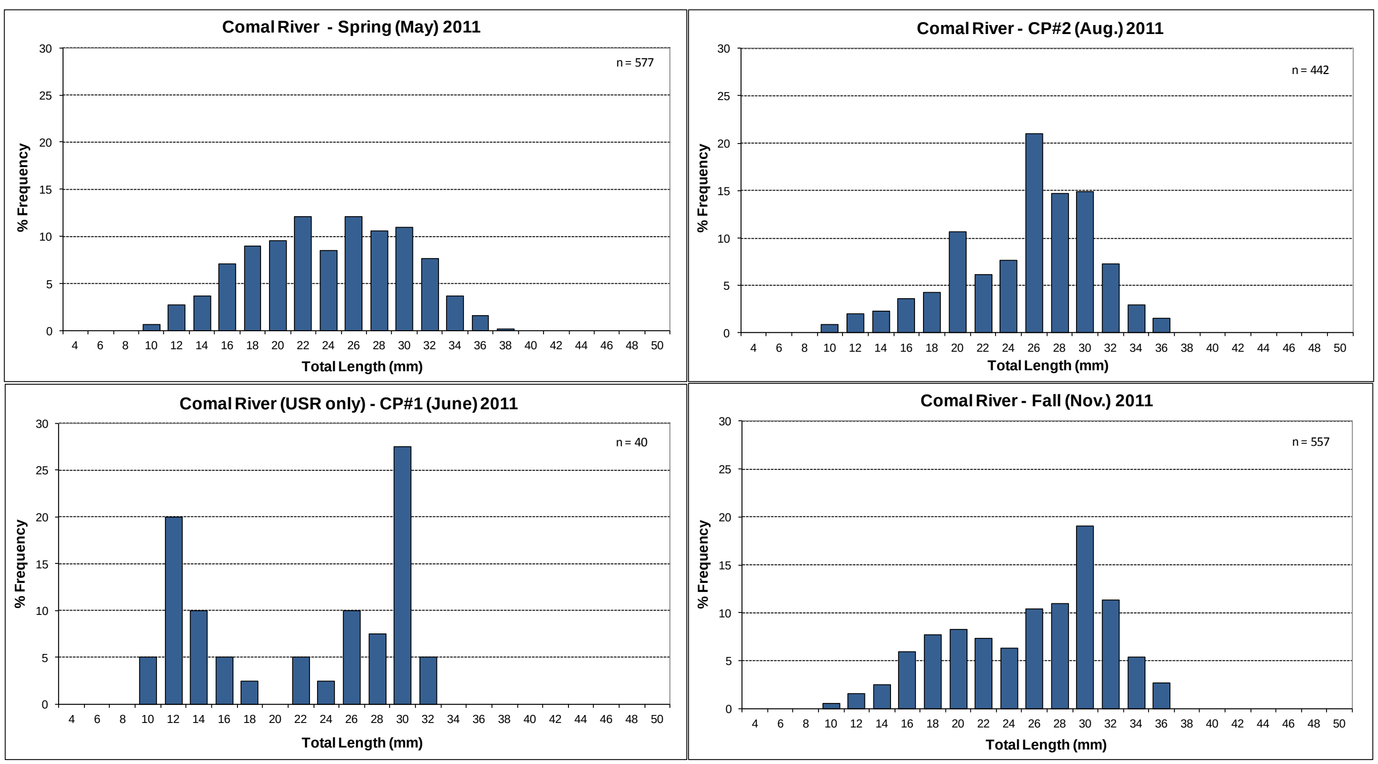


Figure 15. Length frequency distribution of fountain darters collected from the Comal River by drop netting in 2011.

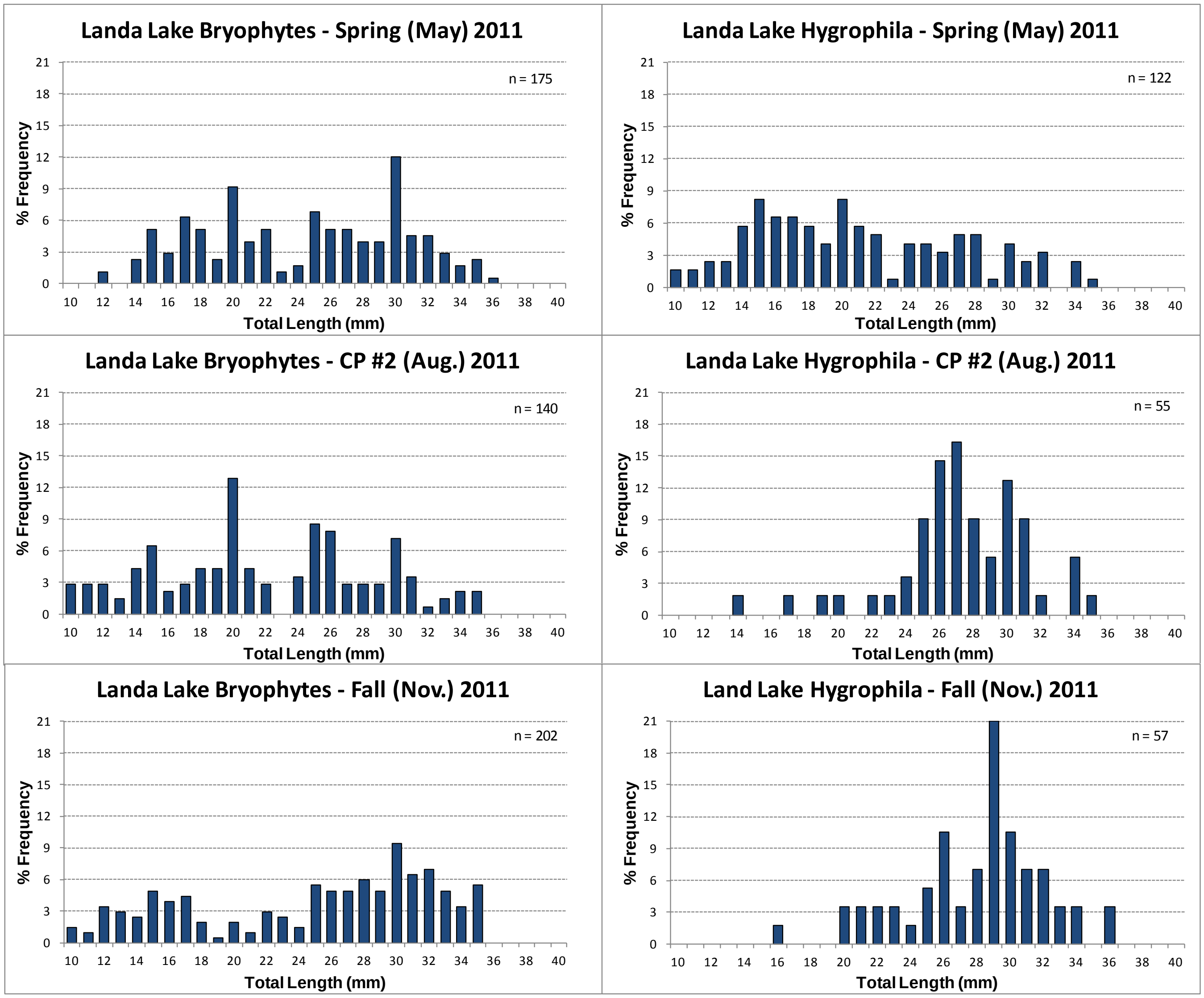


Figure 16. Length frequency distribution of fountain darters collected from bryophytes and *Hygrophila* in Landa Lake during each 2011 sampling event.

In addition to fountain darters, 133,371 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 is to be further evaluated under extremely low discharge.

Other potential impacts of exotic fish species include negative effects of herbivorous species such as the armadillo del rio (*Hypostomus plecostomus*) on algae and vegetation communities that serve as fountain darter habitat. Although these fish are rarely captured in drop nets, based on visual observations they are abundant in the system. This species has the potential to affect the vegetation community, and thus, impact important fountain darter habitats and food supplies. Therefore, close monitoring and management of the *H. plecostomus* population is crucial.

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

Family	Scientific Name	Common Name	Status	2011	2000-2011
Cyprinidae	*Campostoma anomalum*	Central stoneroller	Native	0	1
	Dionda nigrotaeniata	Guadalupe roundnose minnow	Native	204	1,046
	Notropis amabilis	Texas shiner	Native	10	227
	Notropis volucellus	Mimic shiner	Native	1	32
	Pimephales vigilax	Bullhead minnow	Native	0	4
Characidae	*Astyanax mexicanus*	Mexican tetra	Introduced	47	432
Ictaluridae	*Ameiurus melas*	Black bullhead	Native	0	1
	Ameiurus natalis	Yellow bullhead	Native	3	108
Loricariidae	*Hypostomus plecostomus*	Armadillo del rio	Introduced	14	71
Poeciliidae	*Gambusia* sp.	Mosquitofish	Native	7,818	122,423
	Poecilia latipinna	Sailfin molly	Introduced	112	4,673
Centrarchidae	*Ambloplites rupestris*	Rock bass	Introduced	0	24
	Lepomis auritus	Redbreast sunfish	Introduced	7	143
	Lepomis cyanellus	Green sunfish	Native	0	10
	Lepomis gulosus	Warmouth	Native	0	32
	Lepomis macrochirus	Bluegill	Native	0	213
	Lepomis megalotis	Longear sunfish	Native	8	258
	Lepomis microlophus	Redear sunfish	Native	0	2
	Lepomis miniatus	Redspotted sunfish	Native	219	1,914
	Lepomis sp.	Sunfish	Native/Introduced	56	782
	Micropterus punctulatus	Spotted bass	Native	0	3
	Micropterus salmoides	Largemouth bass	Native	50	190
Percidae	*Etheostoma fonticola*	Fountain darter	Native	1,616	16,967
	Etheostoma lepidum	Greenthroat darter	Native	15	51
Cichlidae	*Cichlasoma cyanoguttatum*	Rio Grande cichlid	Introduced	60	665
	Oreochromis aureus	Blue tilapia	Introduced	17	66
Total				10,257	150,338

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*). During the late 1980s and early 1990s, giant ramshorn snails were reported to be extremely abundant in the Comal System, and apparently denuded macrophyte beds in portions of Landa Lake (Horne et al. 1992). During this period, between 2 and 12 million ramshorn snails were believed to be present in the Comal Springs/Landa Lake area (Arsuffi 1993). However, numbers have since declined. Early in the study period giant ramshorn snails were relatively abundant - 142 snails were collected in 2001. However, from 2005 through 2008, no giant ramshorn snails were collected while drop netting in the Comal System. In 2009 and 2010, three snails were collected each year. In 2011, this number increased to 35. The reason for this sudden increase is currently unknown. Figure 17 shows the number of giant ramshorn snails collected per drop net sample during each year. Given the increase in numbers of giant ramshorn snails collected during 2011, close monitoring of the population will be necessary in the coming years.

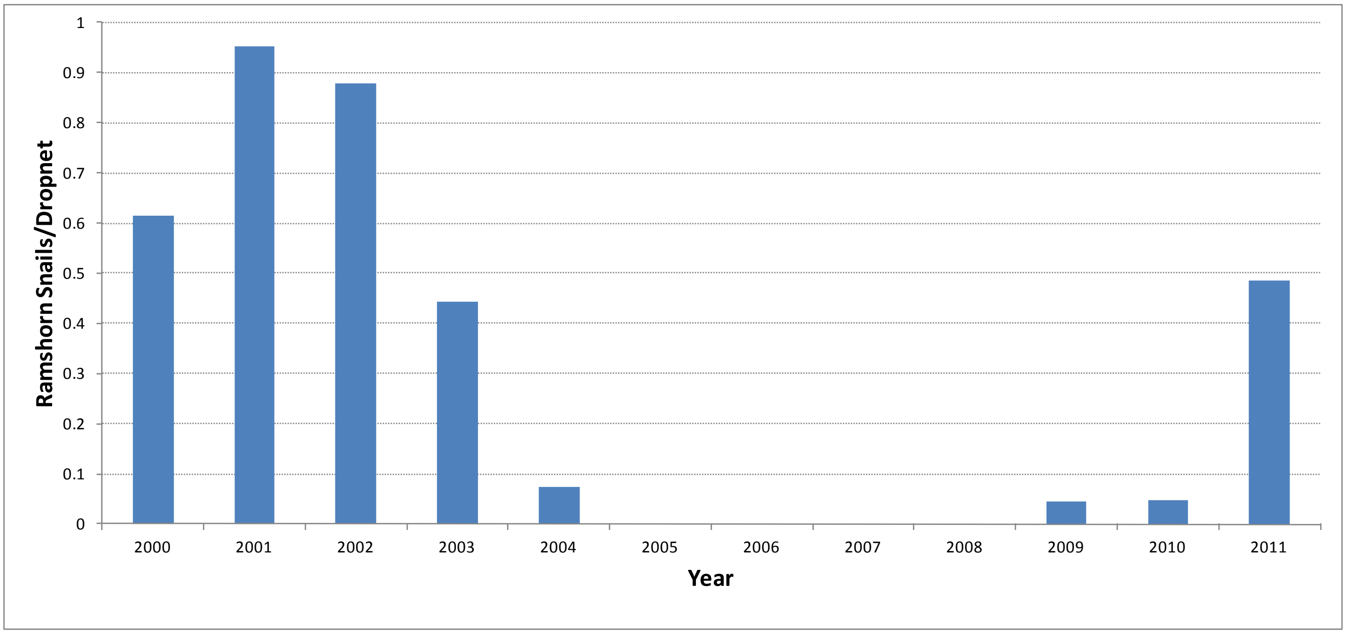


Figure 17. Abundance of giant ramshorn snails in drop net collections from the Comal Springs/River Ecosystem from 2000 – 2011.

<u>Dip Nets</u>

Data gathered using dip nets are graphically represented in Figure 18 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 19.

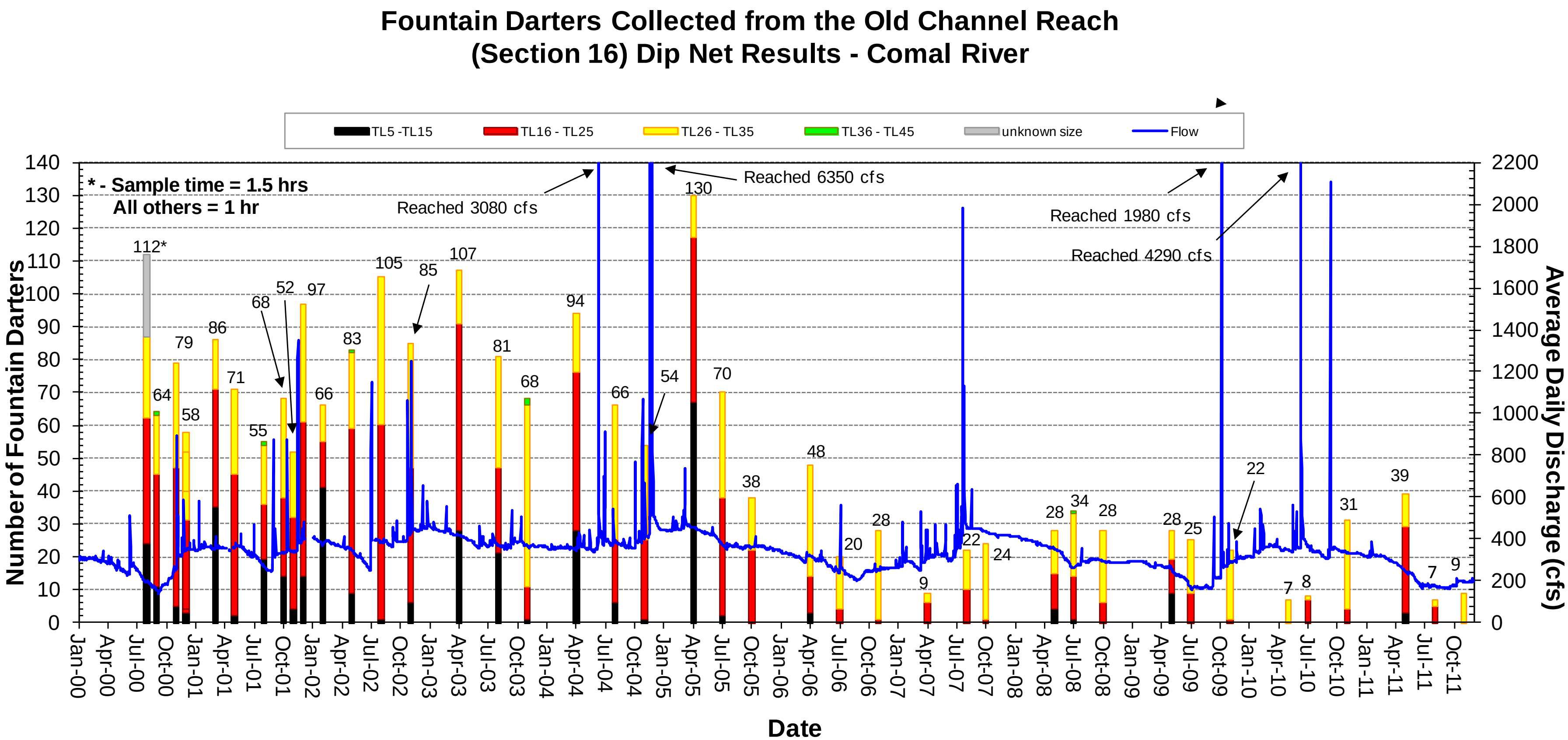


Figure 18. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

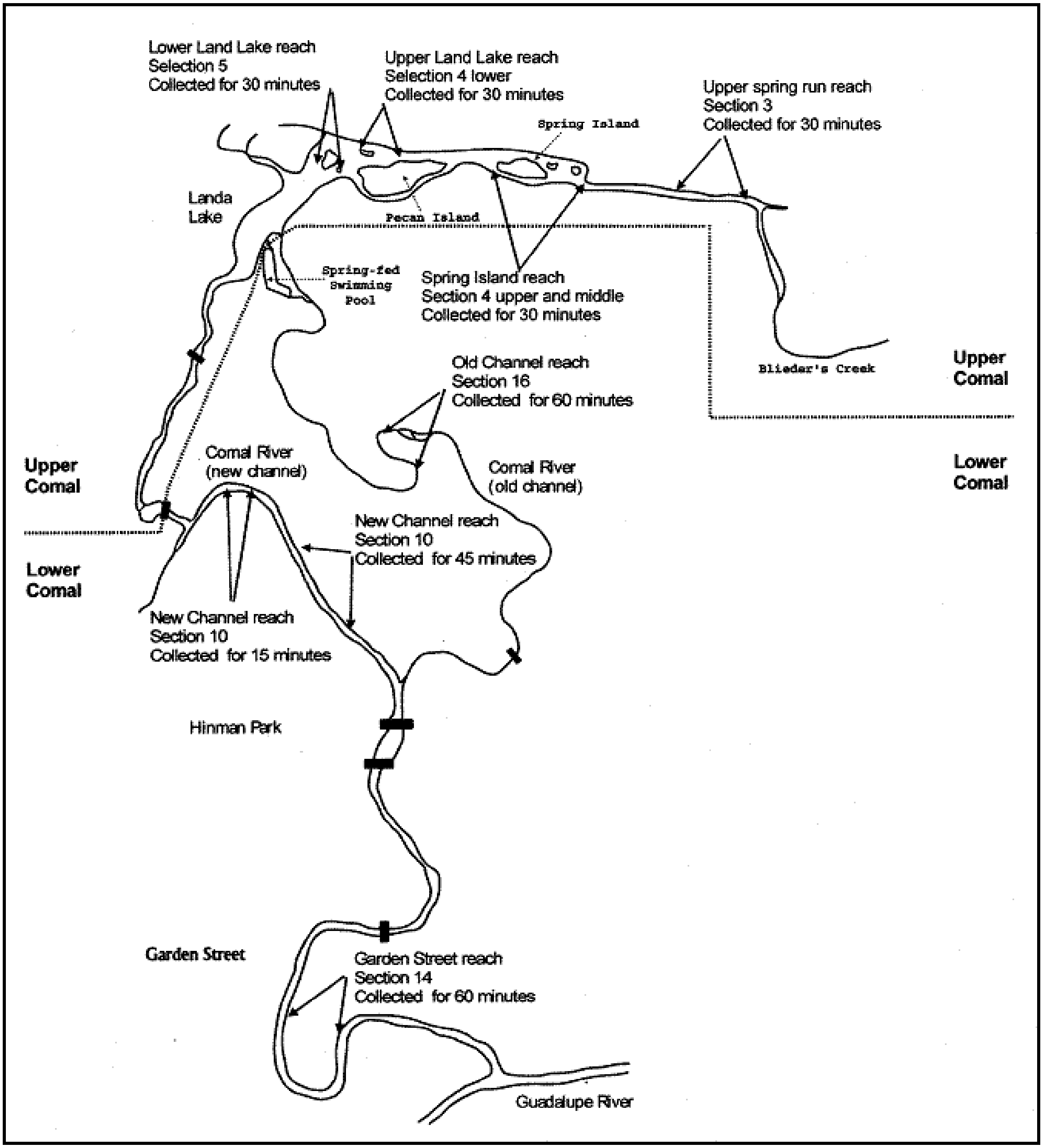


Figure 19. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

Figure 18 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 to one dominated by non-native *Hygrophila*. This switch resulted in a corresponding change in the fountain darter population. Before 2005, the number of darters collected per sample ranged from 54 to 130 and all samples contained small darters (<15 mm) indicating year-round reproduction. Since this change in vegetation, total number of darters per sample has ranged from 7 to 48 and small darters are typically only collected in spring months. However, prior to the June 2010 high-flow event, bryophytes had begun to establish 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 sampling 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.

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 2011, presence/absence dip netting was conducted on the Comal River during the comprehensive spring (May 16) and fall (November 17) sampling events, as well as two Critical Period events in the summer (June 20 [USR only] and Aug. 18 [all sites]). The percentage of sites with fountain darters started at 68% in spring and declined to 56% by fall (Figure 20). Although percentages declined throughout the year, values are similar to those observed in previous years.

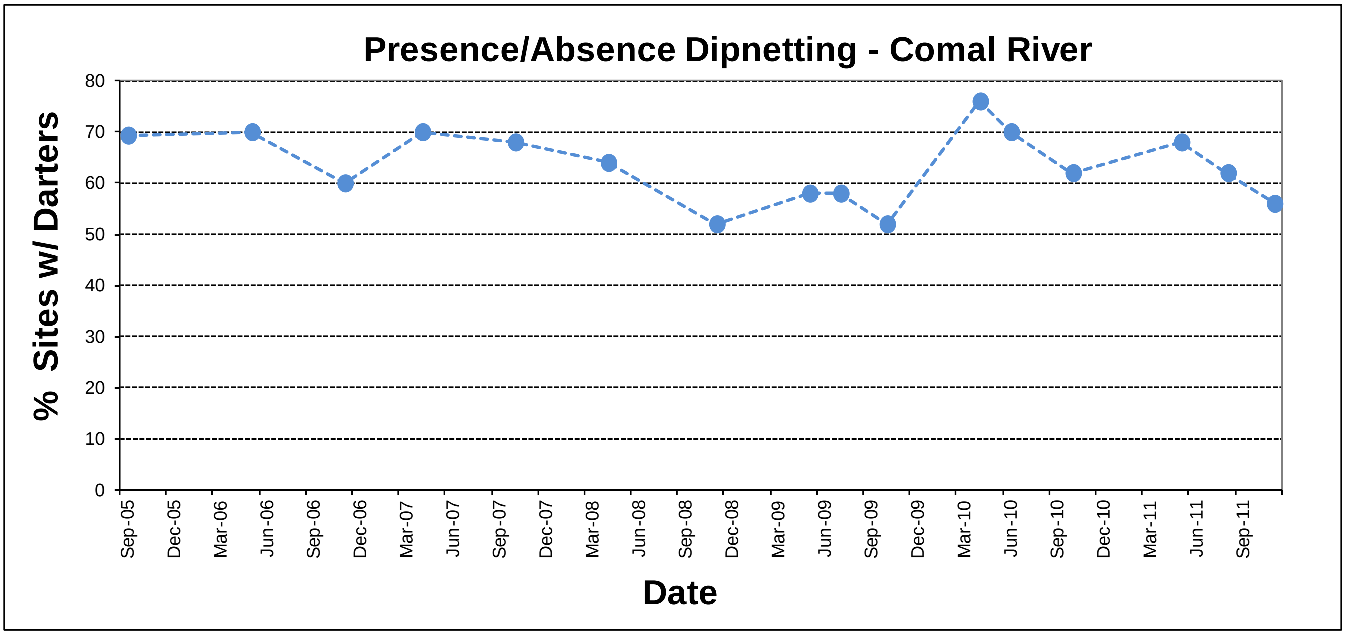


Figure 20. Percentage of sites (N = 50) in which fountain darters were present during 2005 - 2011.

Location specific trends in the fountain darter population are evident when these data are split by reach (Figure 21). It should be noted that greater fluctuations in data from the Upper Spring Run Reach (USR) are partly due to the fewer number of sites sampled in this reach (n=6) than in Landa Lake (LL; n=22). However, trends observed in this data confirm similar trends noted in drop net data. The percentage of sites with darters is rather consistent in the high-quality habitat found in Landa Lake, and fluctuates between 80 – 100%. In the Upper Spring Run Reach, a sharp drop is noted between May and June 2010 which corresponds to a large flood event which scoured most of the vegetation from this area (see BIO-WEST 2011a). This vegetation (mainly bryophytes) had still not returned by fall of 2010, and thus, numbers stayed low in this reach. By spring 2011, bryophytes were starting to recolonize this area, but continued low flows throughout the summer months led to proliferation of green algae in this reach resulting in desiccation of the bryophytes and another large decrease by late summer 2011. Although much of the green algae had disappeared by the fall 2011 sampling event, and numbers of darters seem to be responding, continued monitoring of the Upper Spring Run Reach is critical in coming months. This area is the first area to be affected by diminishing spring flows due to the higher elevation of the springs which feed this reach.

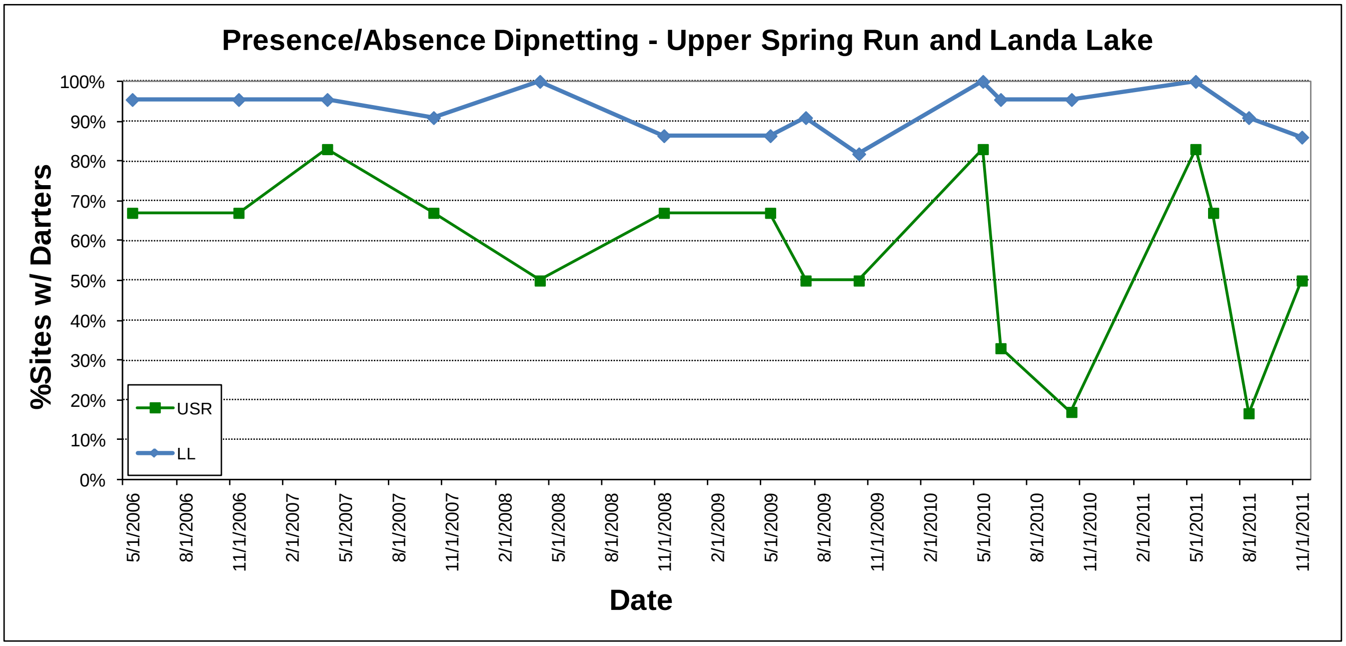


Figure 21. Percentage of sites from Landa Lake (LL, N = 22) and Upper Spring Run (USR, N = 6) in which darters were present from 2006 - 2011.

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

Visual Observations

Fountain darters were observed in the deepest portions of Landa Lake (depths greater than 2 m) during each of the three full sampling events (spring, fall, and CP2) in 2011. This is consistent with each SCUBA sampling effort in Landa Lake conducted since the adoption of this methodology in summer 2001 and documents the use of deeper water habitats within Landa Lake during all flow conditions observed to date. Excluding the 2002, 2004, and 2010 high-flow sampling events, average vegetation coverage in the fixed sample grid has been approximately 75% with an average of approximately 50 darters per sample. As documented in BIO-WEST (2011), the June 2010 flood event was the most severe disturbance observed in the SCUBA sampling area since the inception of the Variable Flow study. The June 2010 flood resulted in complete removal of aquatic vegetation and excessive siltation in the sample grid followed by the documentation of only 2 darters. Some recovery of that area was evident by fall 2010, when aquatic vegetation was at 20 percent and 22 darters were observed. By spring 2011, aquatic vegetation had rebounded to approximately 40 percent coverage which resulted in 46 darters, but both categories were still slightly below long-term averages. During the 2011 CP2 event, nearly 55 percent vegetation coverage was observed but consisted primarily of green algae covering decaying bryophytes and subsequently only 30 darters were observed. In contrast, only 20 percent coverage of vegetation was present during the fall 2011 sampling event, but it was back to mostly pure bryophtyes resulting in an increase to 39 darters observed. This sampling effort continues to confirm the importance of aquatic vegetation composition and coverage relative to the overall densities of fountain darters in the Comal Springs/River ecosystem.

Comal Springs Salamander Visual Observations

After the highest recorded total salamander count in 2010, spring 2011 saw the lowest total count in the spring runs since the inception of this study (Table 10). This count occurred less than a year after the highest daily average flow (7,280 cfs) since 2002, and in the midst of an exceptional drought in Central Texas. The high-flow event of 2010 deposited large amounts of sediment in all spring runs, but it was especially noticeable at Spring Island. Much of the area near the East Outfall of the Spring Island Spring Run was covered in fine sediments filling interstitial spaces after the event. Without any flushing flows in 2011, much of this sediment was still present. As a result, only 9 salamanders were observed at this site in all of 2011. Bryophytes usually common here were also noticeably absent being replaced in some areas by green algae (similar to the Upper Spring Run and Landa Lake Reaches, see Aquatic Vegetation section). One salamander was observed during each snorkel survey in the Spring Island Spring Run. This continues a trend of low numbers observed here since 2004.

Numbers of salamanders in Spring Run 1 were highly variable in 2011. Numbers dropped by 75% from fall 2010 to spring 2011 (44 and 11, respectively). As at other sites there were more fine sediments and depths were decreased, but aquatic vegetation and fist-sized rocks appeared to be in abundance making it difficult to ascertain the reasoning behind such a precipitous drop in observations. However, along the river-left bank much of the vegetation (*Bacopa*, *Ludwigia*) had become emergent due to the lower than average flows, and this may explain some of the variation. It should be noted though that this emergent vegetation directed much of the flow towards the other bank, conceivably creating more habitat there. By CP2, observations nearly doubled to 20, but by fall had decreased to only 6 salamanders, the lowest number observed in this study. Increased aquatic vegetation and decreased depths likely influenced sampling efficiency within this reach during the fall. These numbers are well-below the study average of 23.7. Salamander observations at Spring Run 3 in spring and CP1 (10 during each effort) were just below the study average, and observations doubled by the fall. The major differences in habitat between these two spring runs is the larger depths (comparatively) and relative lack of aquatic vegetation. Regardless, close monitoring of these variable populations should yield valuable information on how they respond to habitat changes as they continue through the exceptional drought plaguing Central Texas.

Table 10. Total number of Comal Springs salamanders observed at each survey site during 2001 – 2011.

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
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
Spring 2010	52	18	1	1	72
High-flow 2010	40	8	0	7	55
Fall 2010	44	7	1	3	55
Spring 2011	11	10	1	2	24
Low-flow 2011	20	10	1	4	35
Fall 2011	6	20	1	3	30
Average	**23.7**	**12.5**	**3.3**	**10.1**	**51.7**

Macroinvertebrate Sampling

To assess population dynamics and habitat requirements of the federally listed invertebrate species, two sampling techniques were used. Drift net sampling was conducted around spring openings as described in the Methods section. Results of this sampling are presented below. Additionally, continued regular monitoring of Comal Springs riffle beetles was conducted using the "cotton lure" methodology employed during previous years. Details of this methodology can be found in the Methods section, and results are presented below.

Drift Net Sampling

At least 13 taxa were captured from 144 hours of sample time at the three drift net sites in Comal Springs during 2011 (Table 11). Table 12 displays the physico-chemical data collected at these sites during sampling. A new record for Comal Springs was collected during 2011 drift net sampling - *Tethysbaena texana* is a primitive Crustacean found throughout the Edwards Aquifer. *T. texana* has also been collected at Hueco Springs in Comal County.

Table 11. Total numbers of troglobitic and endangered species collected in drift nets during June and November, 2011. Federally endangered species are designated with (E). A = adult beetles, L = larvae, P = probable pupae.

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	48	48	48	144
Crustaceans				
Amphipoda				
Crangonyctidae				
Stygobromus pecki (E)	3	9	7	19
Stygobromus russelli	1			1
Stygobromus bifurcatus				
Stygobromus flagellatus				
Stygobromus spp.	136	196	328	660
All *Stygobromus*	140	205	335	680
Hadziidae				
Mexiweckelia hardeni	7	15	5	27
Sebidae				
Seborgia relicta	1	4	2	7
Bogidiellidae				
Artesia subterranea		1		1
Parabogidiella americana				
Ingolfiellidae				
Ingolfiella n. sp				
Isopoda				
Asellidae				
Lirceolus (2spp.)	57	40	21	118
Cirolanidae				
Cirolanides texensis		1	1	2
Thermosbaenacea				
Monodellidae				
Tethysbaena texana		1		1
Arachnids				
Hydrachnoidea				
Hydryphantidae				
Almuerzothyas comalensis	5			5
Insects				
Coleoptera				
Dytiscidae				
Comaldessus stygius		10 A		10
Haideoporus texanus				
Dryopidae				
Stygoparnus comalensis (E)	1 L	1 L		2
Elmidae				
Heterelmis comalensis (E)	3 (1A, 2L)		2 L	5

Table 12. Results of water quality measurements conducted in 2011 during drift net sampling efforts at Comal Springs.

	Spring Run 1		Spring Run 3		West Shore Upwelling	
	June	Nov	June	Nov	June	Nov
Temperature (°C)	23.2	23.1	23.3	23.2	23.7	23.6
Conductivity (mS)	0.479	0.535	0.479	0.533	0.456	0.519
pH	6.78	6.65	6.78	6.64	6.80	6.65
Dissolved Oxygen (mg/L)	5.68	6.27	5.61	6.30	5.45	5.86
Current Velocity (m/s)	0.19	0.23	0.30	0.14	0.16	0.35

Comal Springs Riffle Beetle

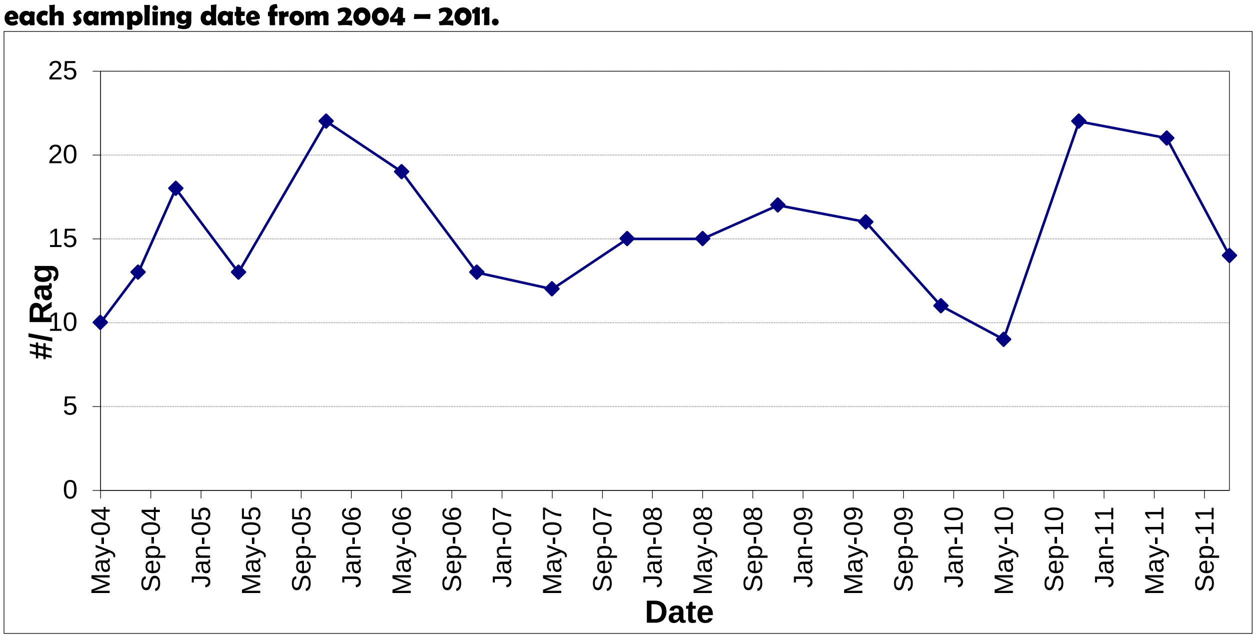
Comal Springs riffle beetle sampling conducted as part of this study provides basic information on the population dynamics and distribution of the species among sample sites. In 2011, 1,022 riffle beetles were collected – the highest number since twice-annual sampling began in 2005. When split by sampling event, 621 riffle beetles were captured in June, followed by 401 riffle beetles in November. Since sampling with cotton lures began, the number of Comal Springs riffle beetles has varied between 293 and 666 per sampling event. In June, riffle beetles were abundant at Spring Run 3 and the Western Shoreline, whereas abundance at Spring Island was relatively typical. In November, this trend reversed, with high abundance at Spring Island and relatively low to average abundance at Spring Run 3 and the Western Shoreline sites. The mechanisms behind these shifts in abundance are unclear at this time. However, it is encouraging to see abundant riffle beetle populations at Spring Island after the large gravel deposition caused by the June 2010 flood event.

As in previous years, riffle 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 27). 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 number of Comal Springs riffle beetles (*Heterelmis comalensis*) collected with cotton lures (adults and larvae) for each sampling date from 2004 – 2011.

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**
May 2010	53	110	20	**183**
November 2010	298	264	104	**666**
June 2011	255	245	121	**621**
November 2011	71	137	193	**401**
Total	**2819**	**2600**	**2410**	**7829**
Average	134.2	123.8	114.8	316.7

Figure 22. Combined density (#/cotton lure) of Comal Springs riffle beetles (*Heterelmis comalensis*) for each sampling date from 2004 – 2011.



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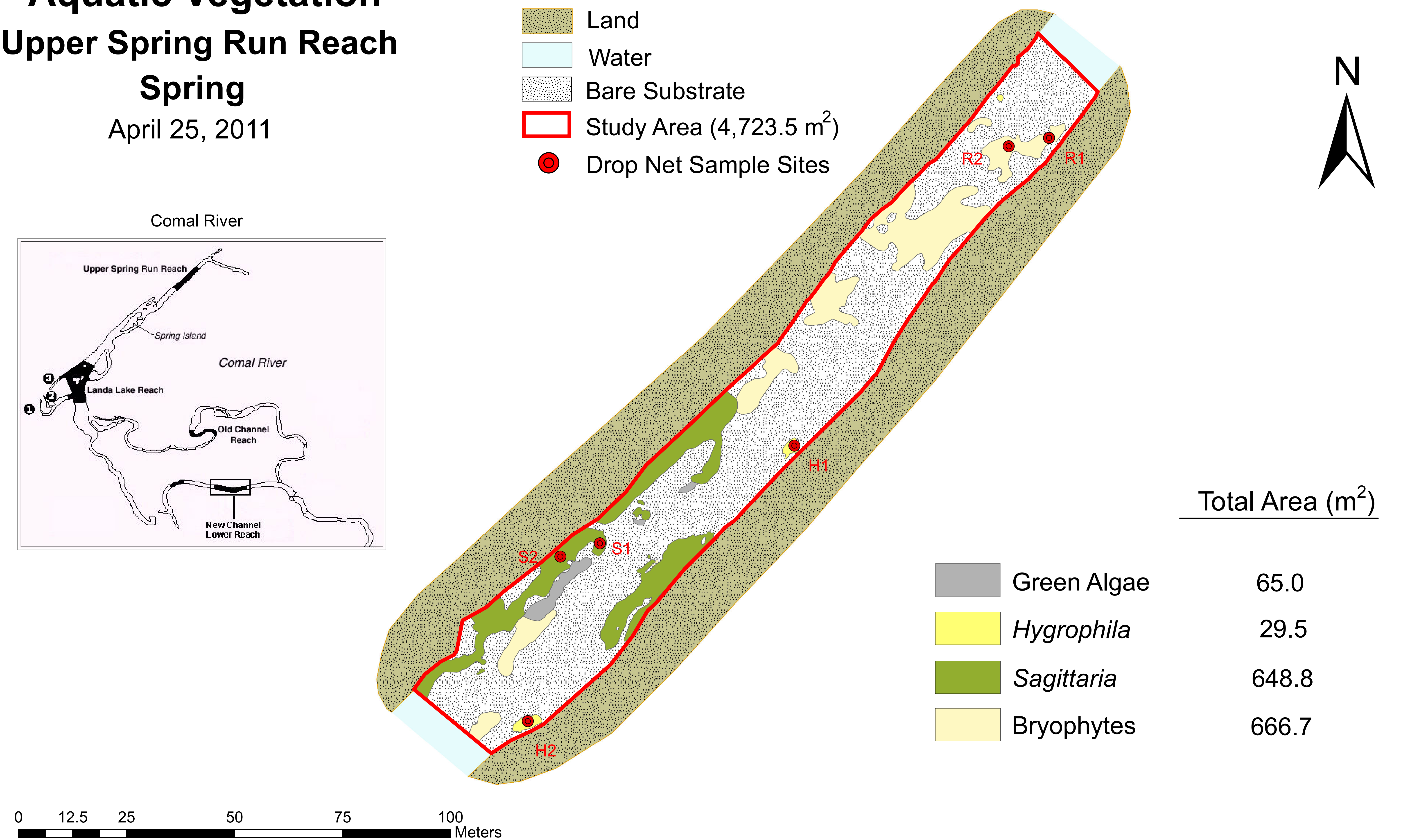
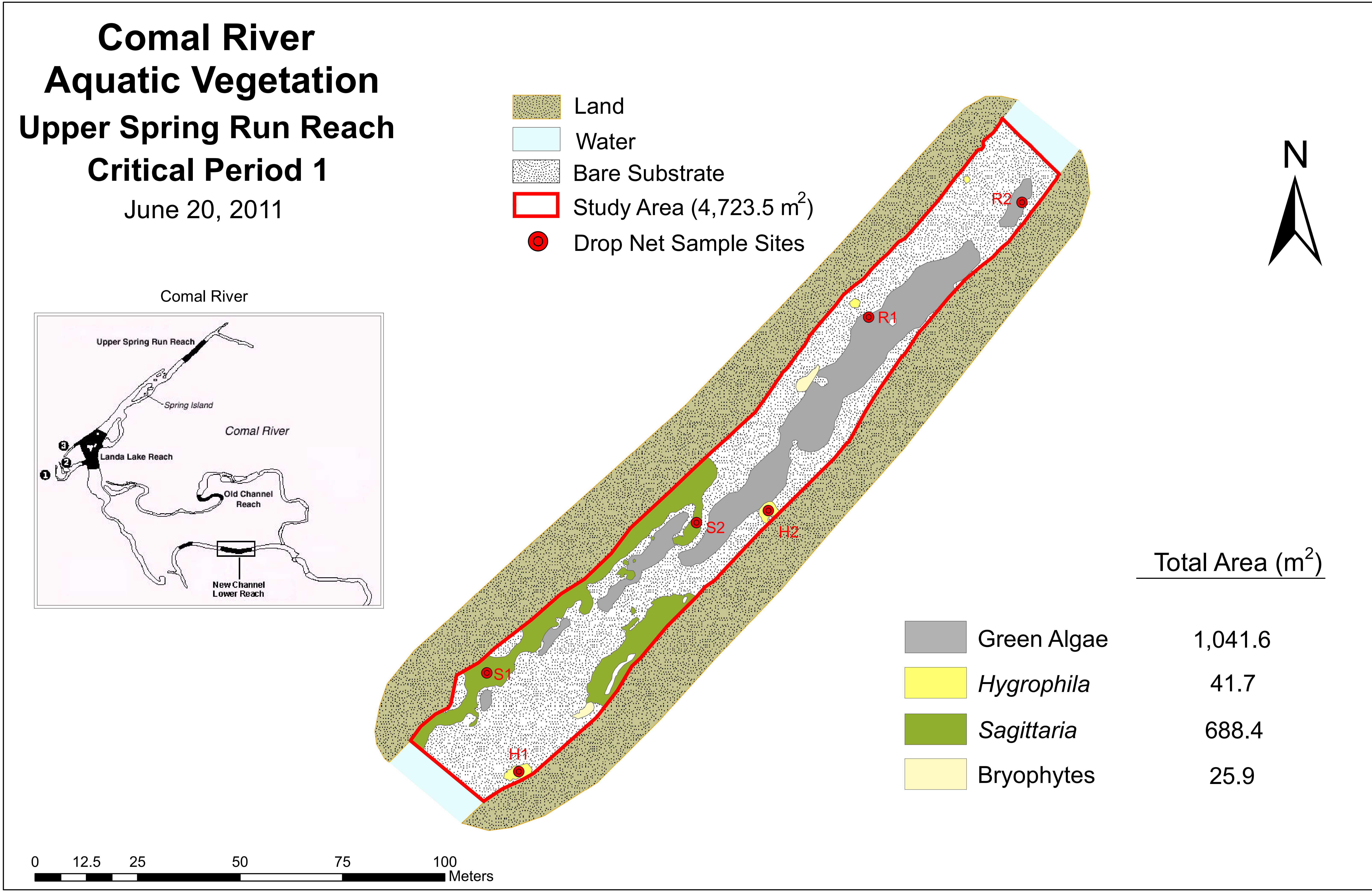
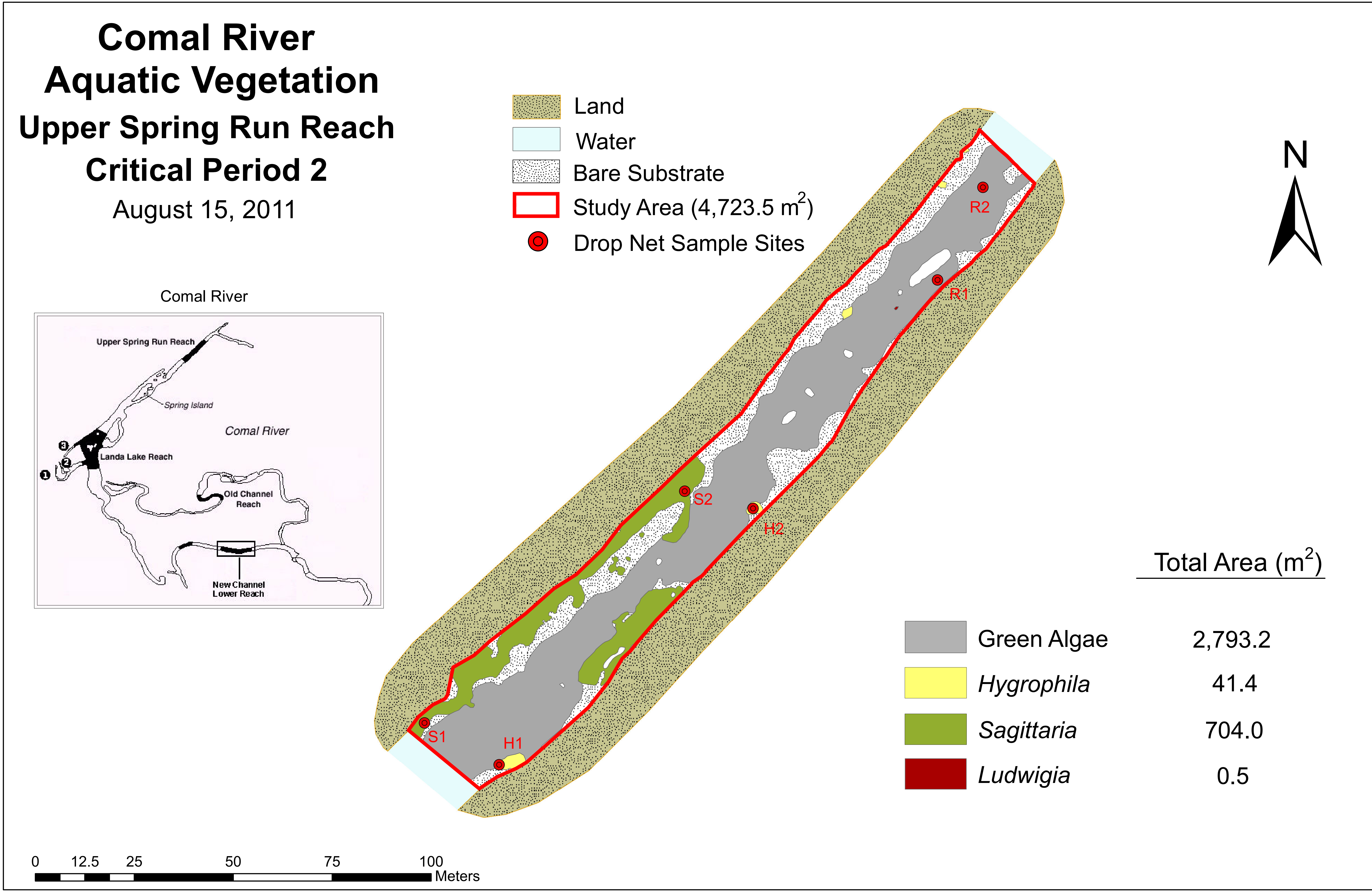
APPENDIX A:
AQUATIC VEGETATION MAPS

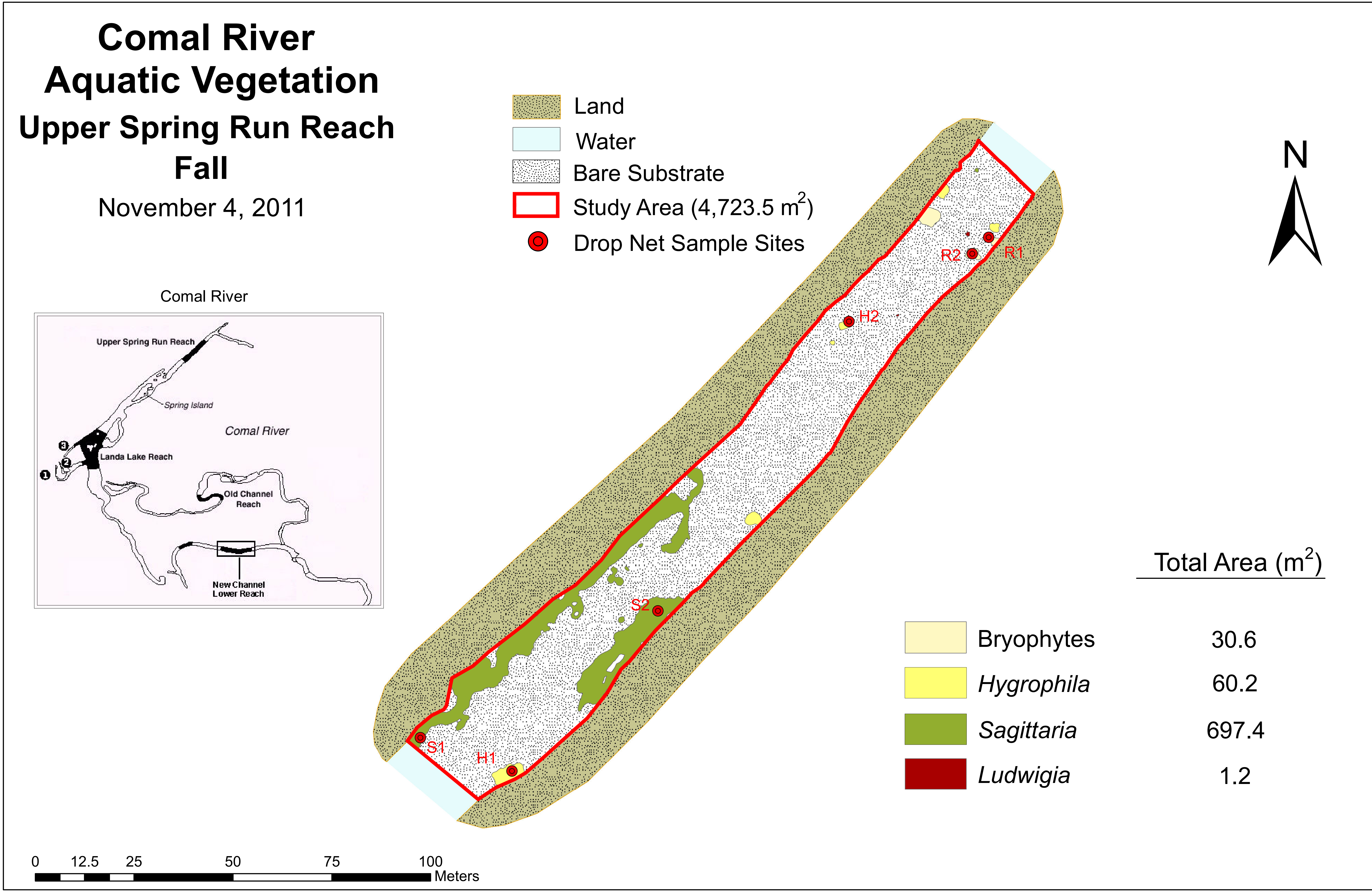
Upper Spring Run Reach

Comal River
Aquatic Vegetation
Upper Spring Run Reach
Spring
April 25, 2011




Comal River
Aquatic Vegetation
Upper Spring Run Reach
Critical Period 1
June 20, 2011
Land
Water
Bare Substrate
Study Area (4,723.5 m2)
Drop Net Sample Sites
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
R1
R2
S1
S2
H1
H2
N
Total Area (m2)
Green Algae 1,041.6
Hygrophila 41.7
Sagittaria 688.4
Bryophytes 25.9
0 12.5 25 50 75 100 Meters


Comal River
Aquatic Vegetation
Upper Spring Run Reach
Critical Period 2
August 15, 2011
Land
Water
Bare Substrate
Study Area (4,723.5 m²)
Drop Net Sample Sites
N
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
R2
R1
S2
H2
S1
H1
Total Area (m²)
Green Algae 2,793.2
Hygrophila 41.4
Sagittaria 704.0
Ludwigia 0.5
0 12.5 25 50 75 100 Meters

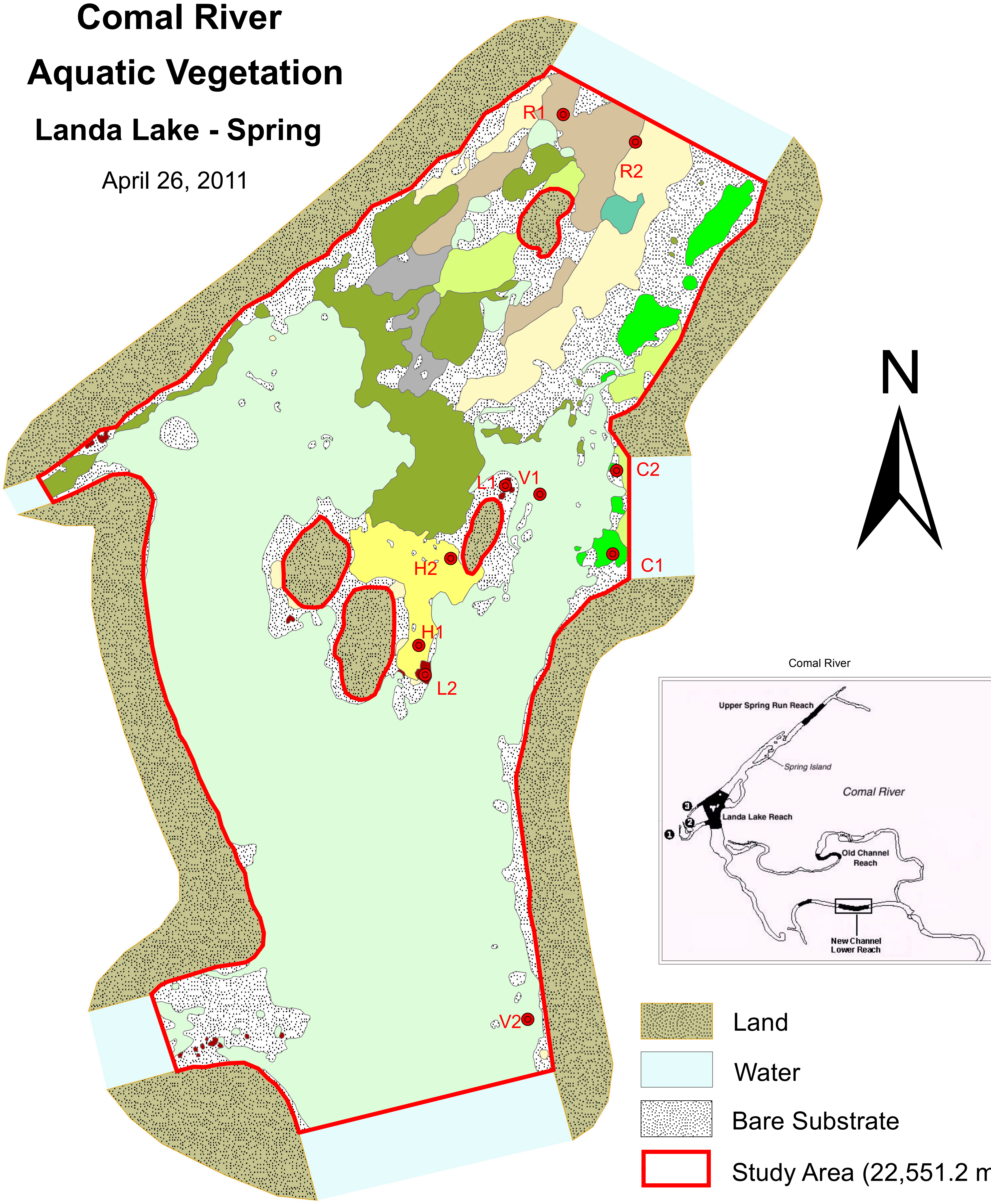

Comal River
Aquatic Vegetation
Upper Spring Run Reach
Fall
November 4, 2011
Land
Water
Bare Substrate
Study Area (4,723.5 m²)
Drop Net Sample Sites
N
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
R1
R2
H2
S2
S1
H1
Total Area (m²)
Bryophytes 30.6
Hygrophila 60.2
Sagittaria 697.4
Ludwigia 1.2
0
12.5
25
50
75
100
Meters

Landa Lake Reach

Comal River Aquatic Vegetation

Landa Lake - Spring

April 26, 2011



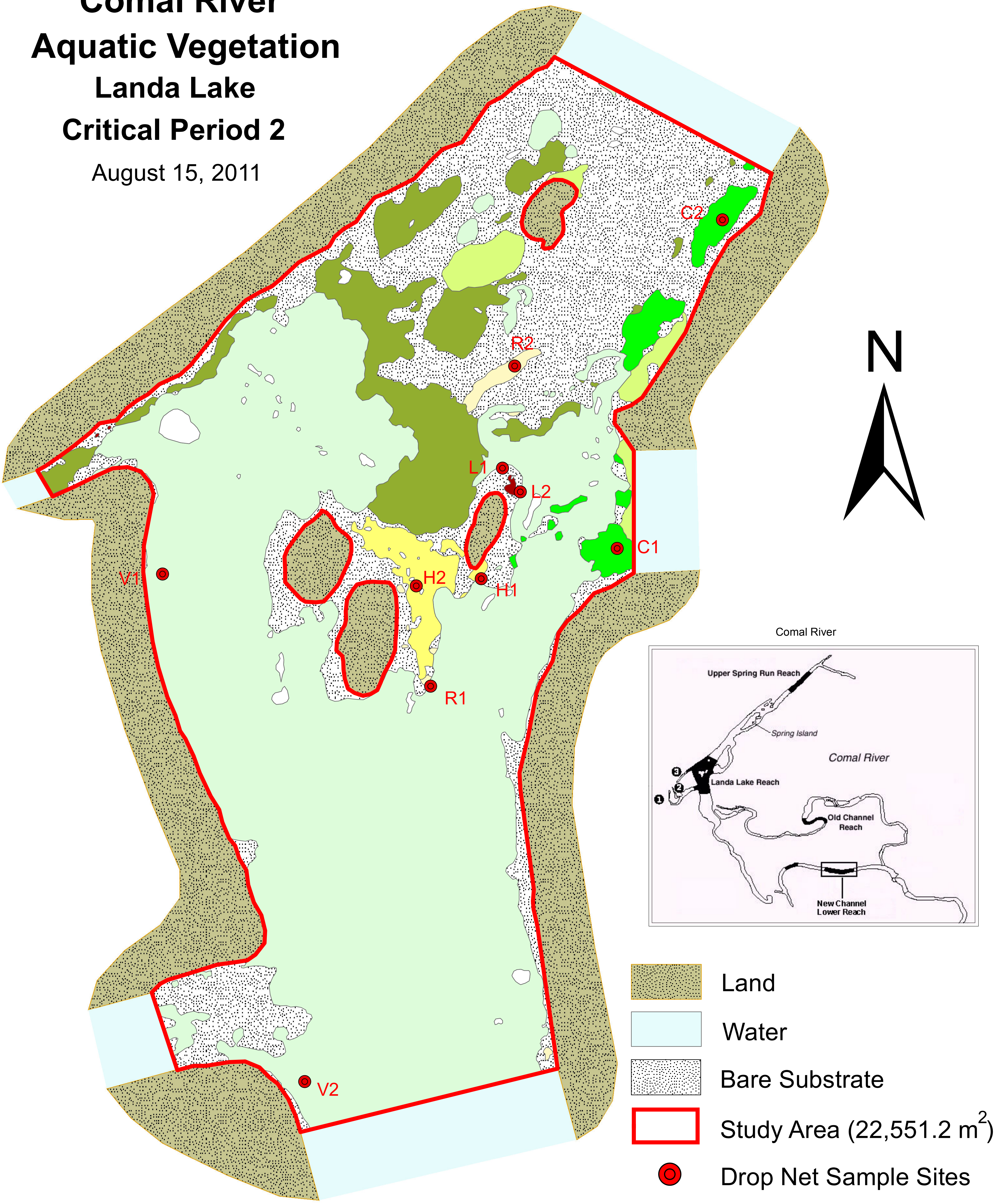
- Land
- Water
- Bare Substrate
- Study Area (22,551.2 m^2)
- Drop Net Sample Sites

	Total Area (m^2)		Total Area (m^2)
Algae	153.8	*Nuphar*	426.2
Bryophytes	1,114.8	*Sagittaria*	1,804.1
Cabomba	359.7	*Vallisneria*	13,013.0
Hygrophila	520.6	Algae / Bryophytes	767.9
Ludwigia	48.4	50% Bryophytes	64.5

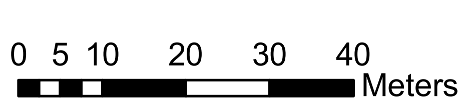
0 10 20 40 60 80 Meters

Comal River
Aquatic Vegetation
Landa Lake
Critical Period 2

August 15, 2011

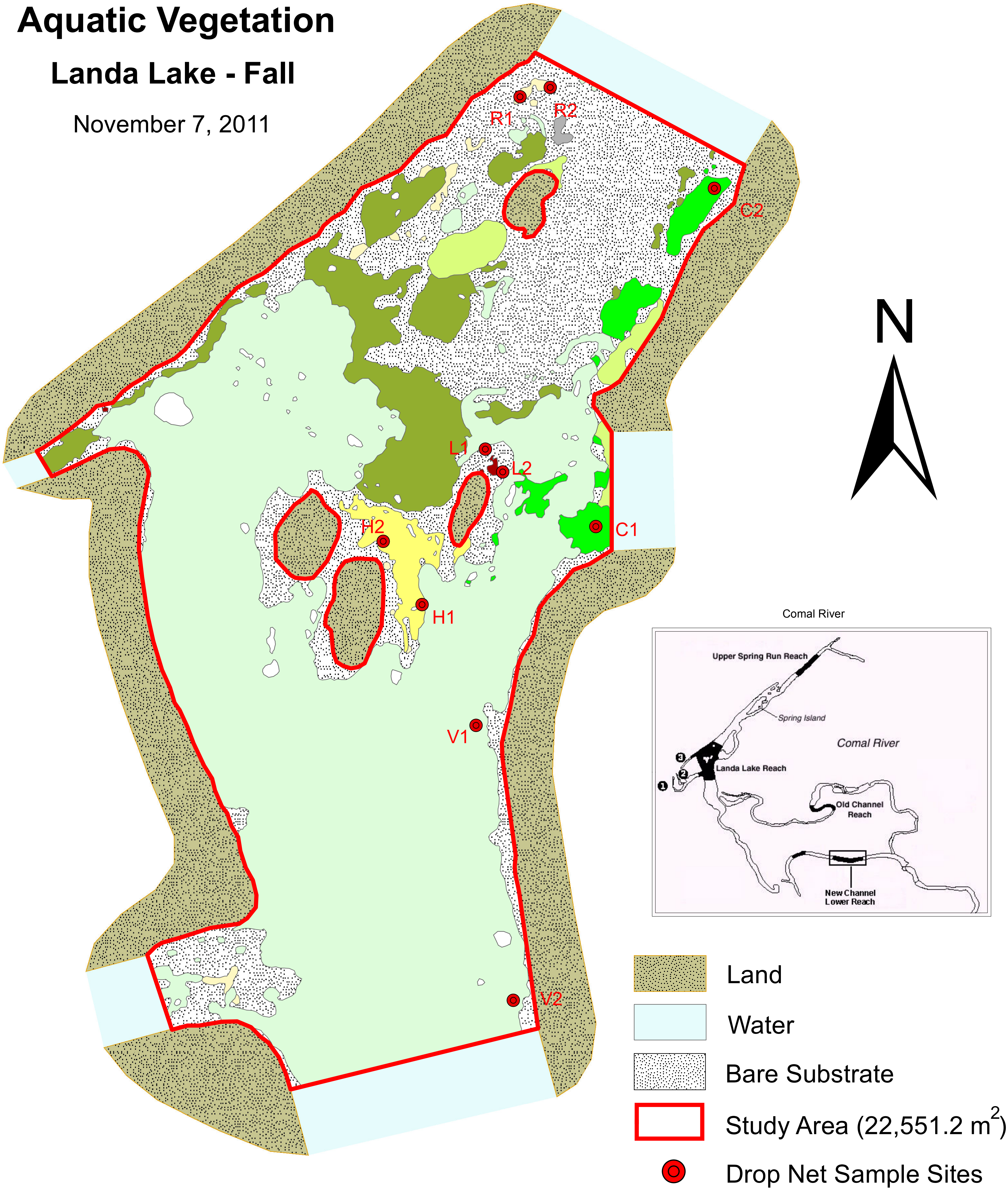


	Total Area (m^2)		Total Area (m^2)
Algae	2,166.4	*Ludwigia*	10.2
Bryophytes	93.2	*Nuphar*	373.3
Cabomba	439.6	*Sagittaria*	1,875.0
Hygrophila	362.8	*Vallisneria*	12,998.7

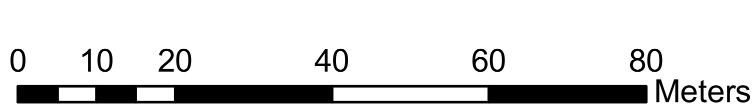


Comal River
Aquatic Vegetation
Landa Lake - Fall

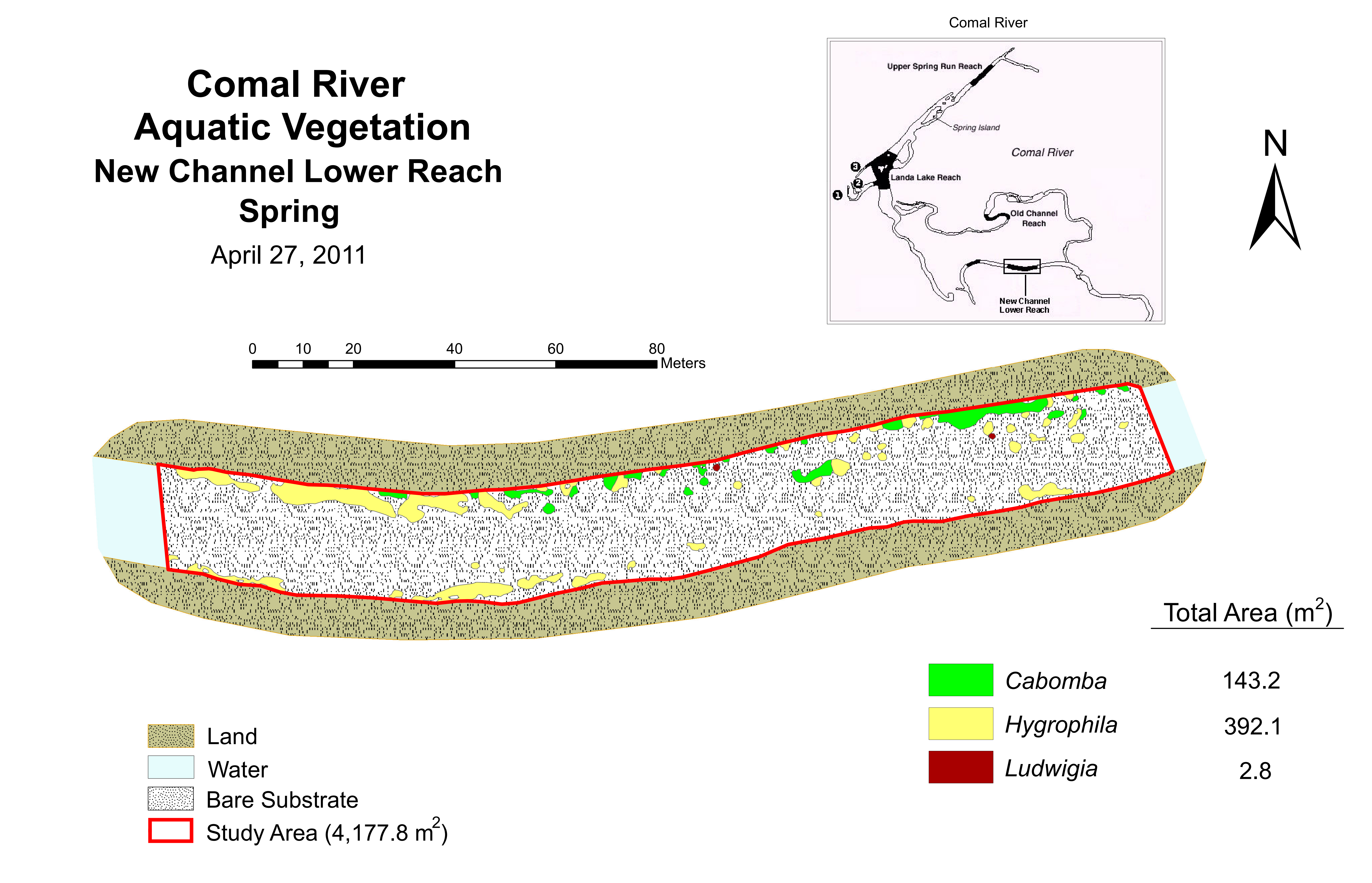
November 7, 2011



	Total Area (m^2)		Total Area (m^2)
Algae	21.3	*Ludwigia*	11.8
Bryophytes	115.7	*Nuphar*	362.8
Cabomba	481.4	*Sagittaria*	1,874.7
Hygrophila	346.8	*Vallisneria*	12,855.8

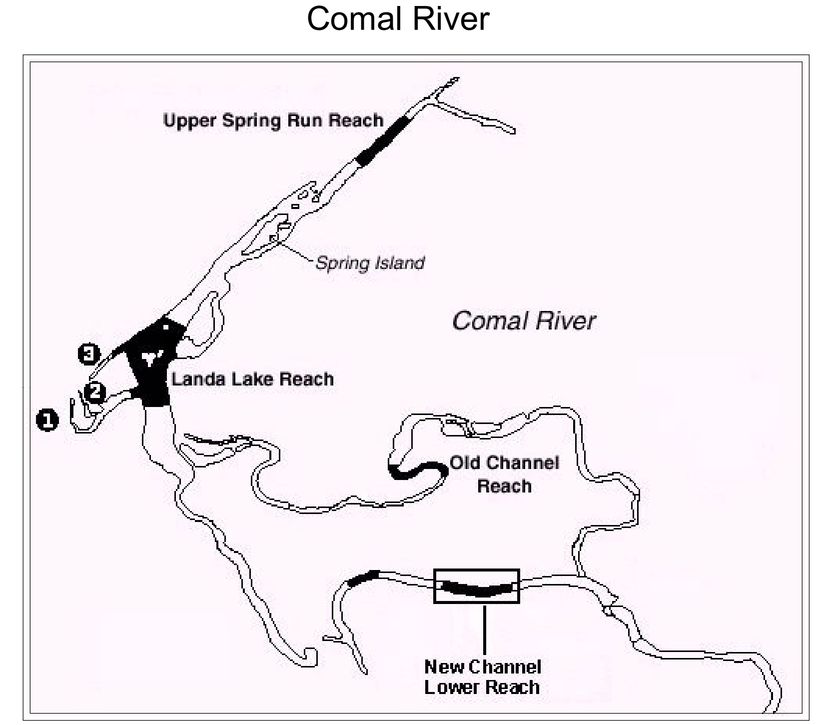


New Channel Reach

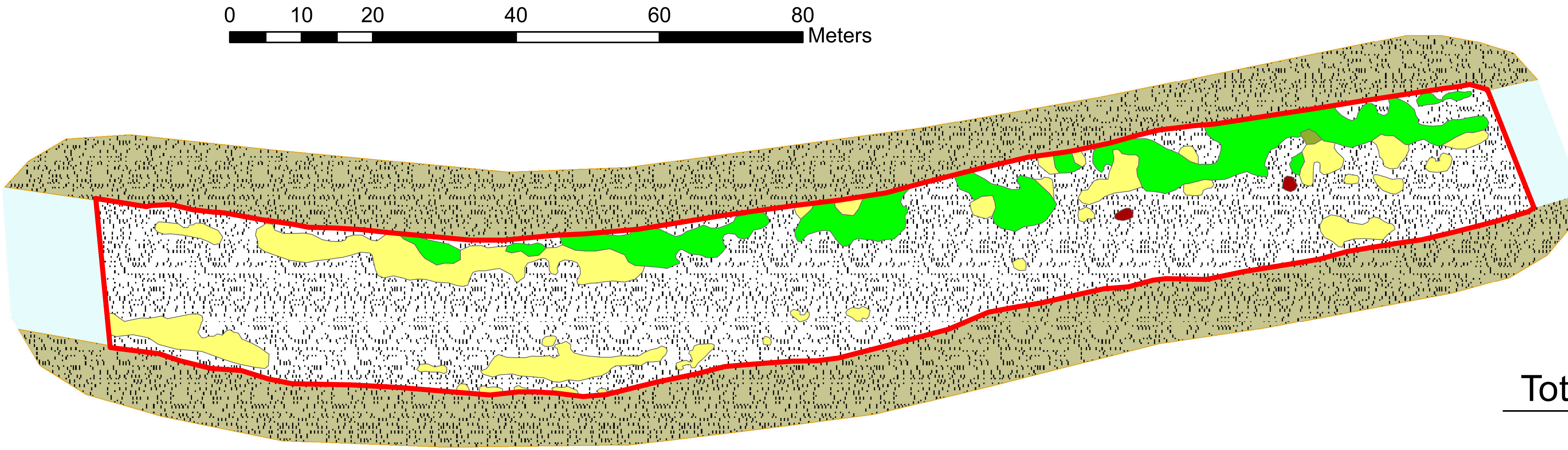

Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring
April 27, 2011
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
0 10 20 40 60 80 Meters
Total Area (m2)
Cabomba 143.2
Hygrophila 392.1
Ludwigia 2.8
Land
Water
Bare Substrate
Study Area (4,177.8 m2)

Comal River
Aquatic Vegetation
New Channel Lower Reach
Critical Period 2

August 17, 2011

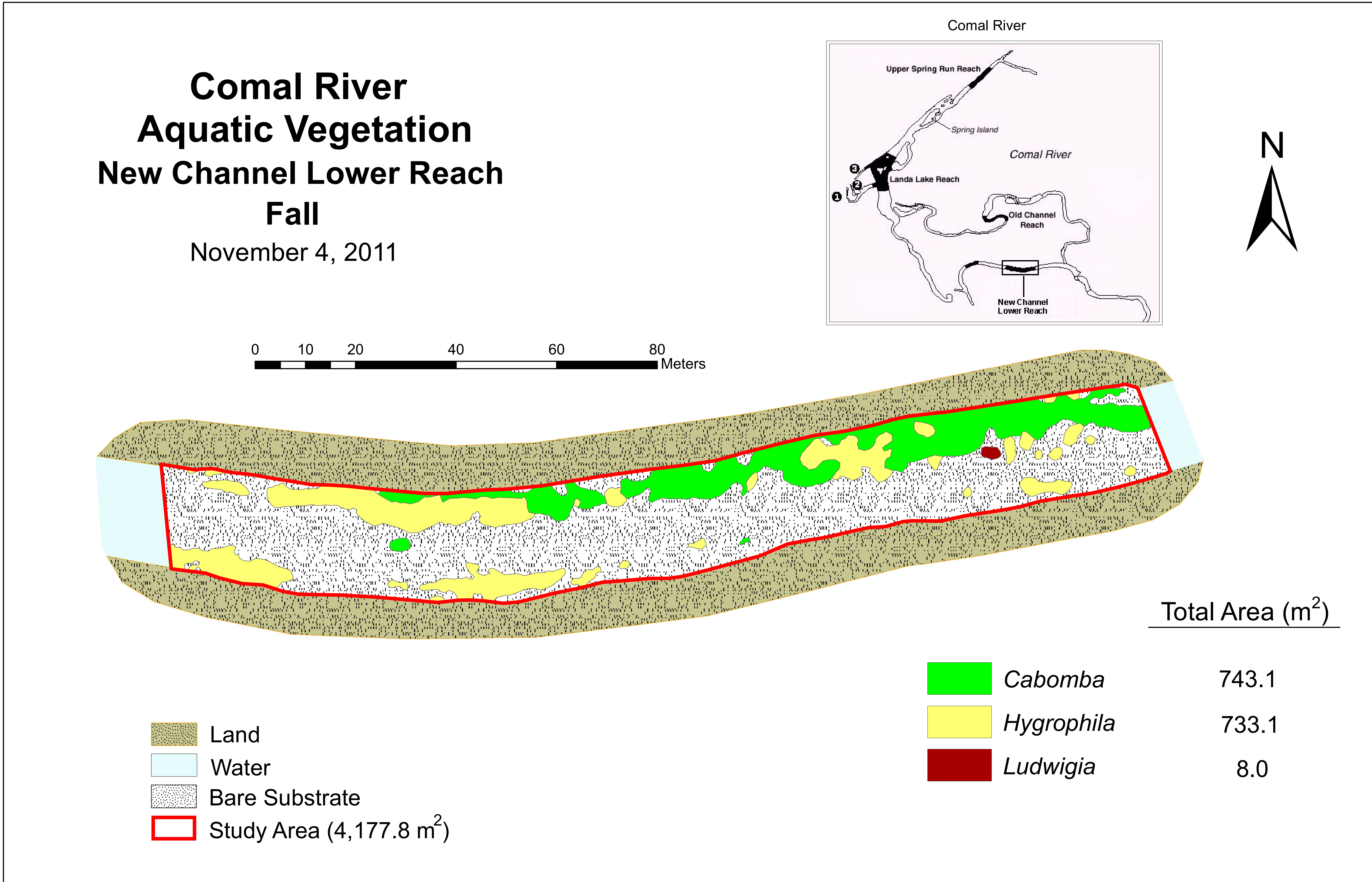


N

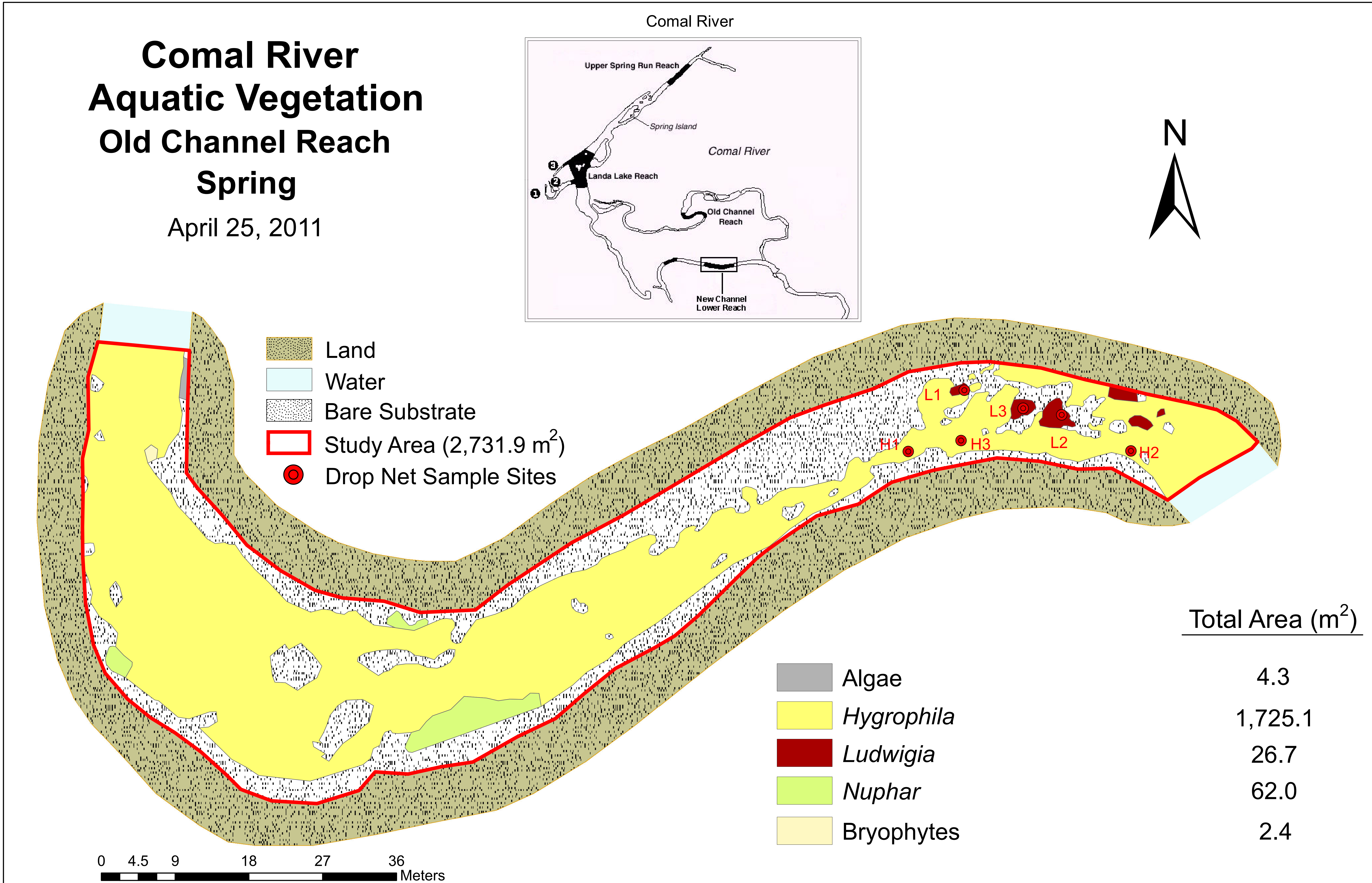


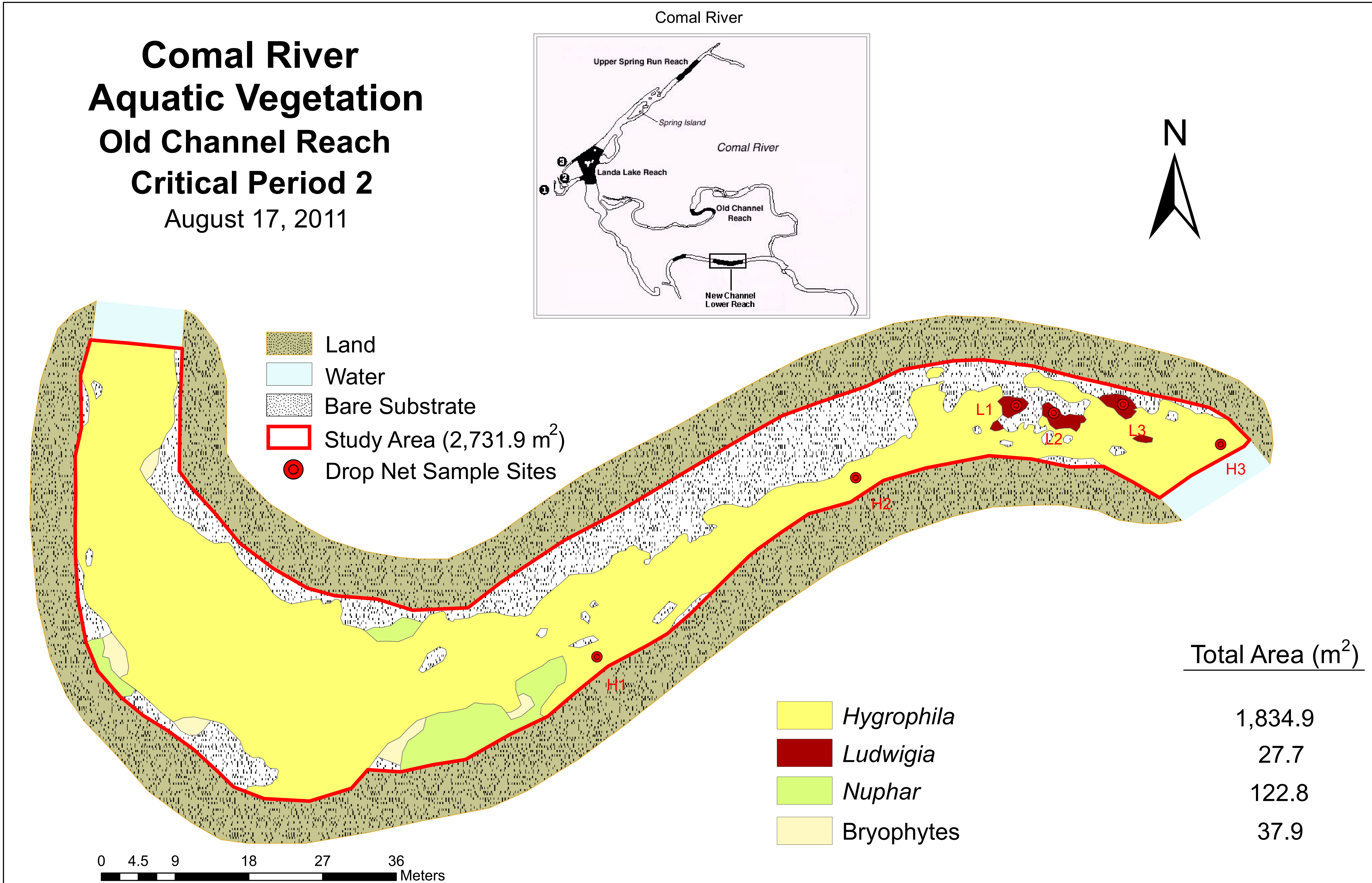
Land
Water
Bare Substrate
Study Area (4,177.8 m^2)

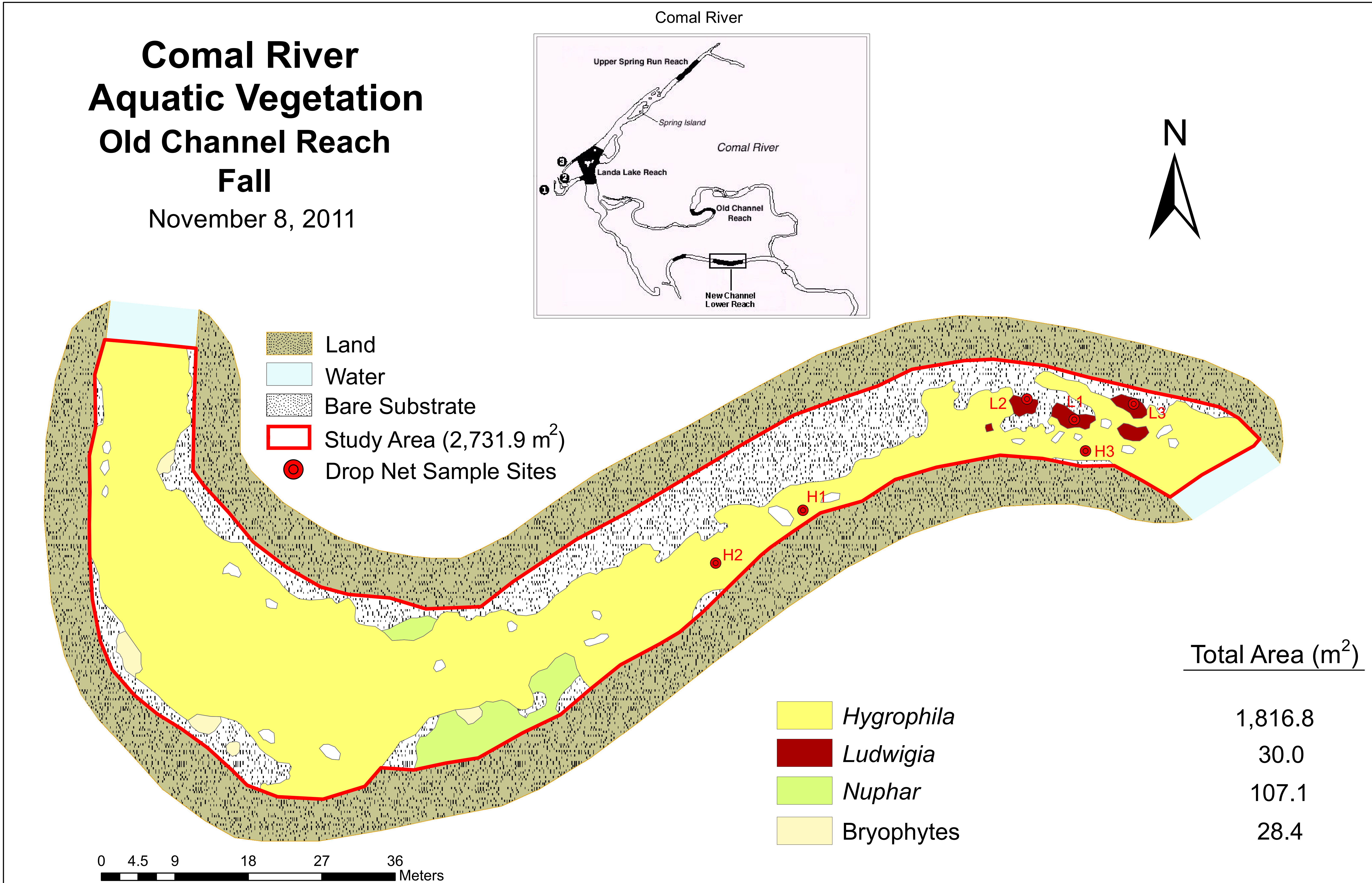
	Total Area (m^2)
Cabomba	510.0
Hygrophila	544.5
Ludwigia	6.6
Sagittaria	4.2


Comal River
Aquatic Vegetation
New Channel Lower Reach
Fall
November 4, 2011
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
0 10 20 40 60 80 Meters
Total Area (m2)
Cabomba 743.1
Hygrophila 733.1
Ludwigia 8.0
Land
Water
Bare Substrate
Study Area (4,177.8 m2)

Old Channel Reach

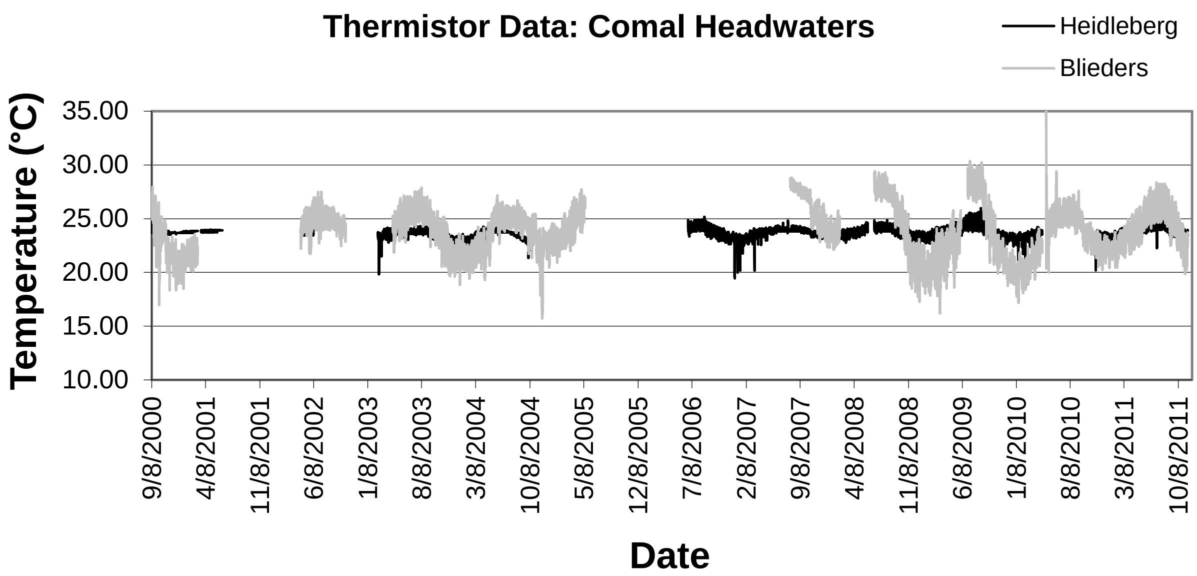

Comal River
Aquatic Vegetation
Old Channel Reach
Spring
April 25, 2011
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
Land
Water
Bare Substrate
Study Area (2,731.9 m2)
Drop Net Sample Sites
L1
L3
L2
H1
H3
H2
Total Area (m2)
Algae 4.3
Hygrophila 1,725.1
Ludwigia 26.7
Nuphar 62.0
Bryophytes 2.4
0 4.5 9 18 27 36
Meters

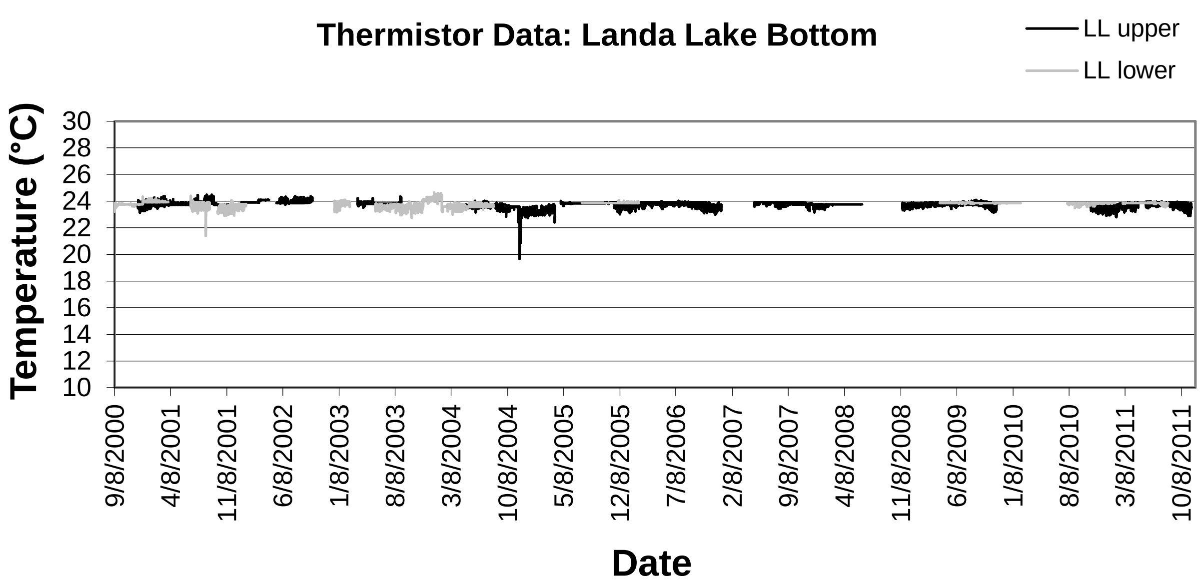

Comal River
Aquatic Vegetation
Old Channel Reach
Critical Period 2
August 17, 2011
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
Land
Water
Bare Substrate
Study Area (2,731.9 m2)
Drop Net Sample Sites
L1
L2
L3
H1
H2
H3
Total Area (m2)
Hygrophila 1,834.9
Ludwigia 27.7
Nuphar 122.8
Bryophytes 37.9
0 4.5 9 18 27 36
Meters

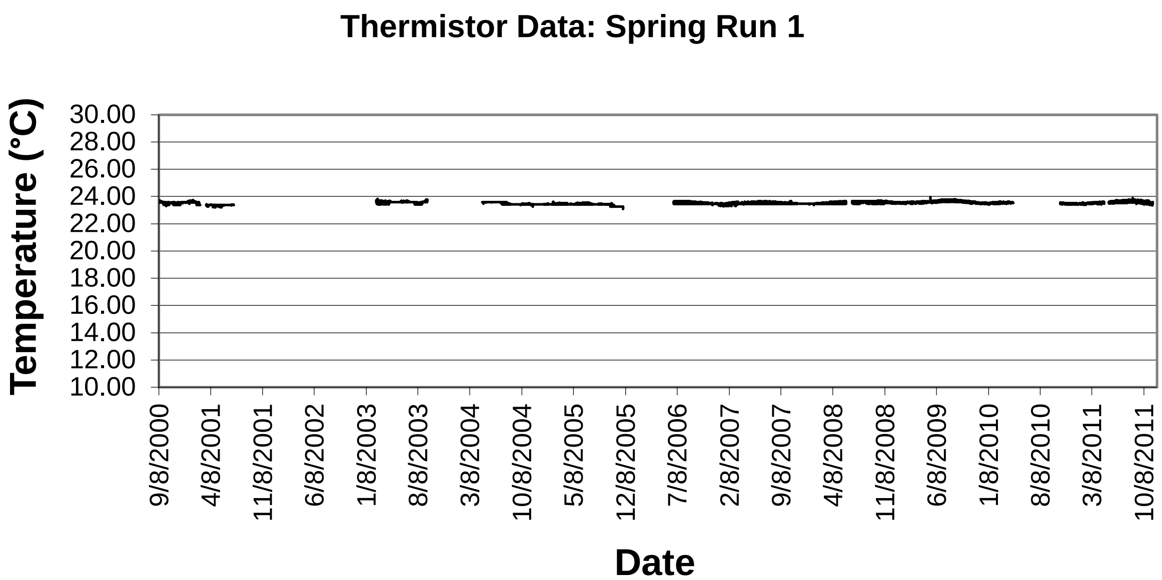

Comal River
Aquatic Vegetation
Old Channel Reach
Fall
November 8, 2011
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
Land
Water
Bare Substrate
Study Area (2,731.9 m2)
Drop Net Sample Sites
L1
L2
L3
H1
H2
H3
Total Area (m2)
Hygrophila 1,816.8
Ludwigia 30.0
Nuphar 107.1
Bryophytes 28.4
0 4.5 9 18 27 36
Meters

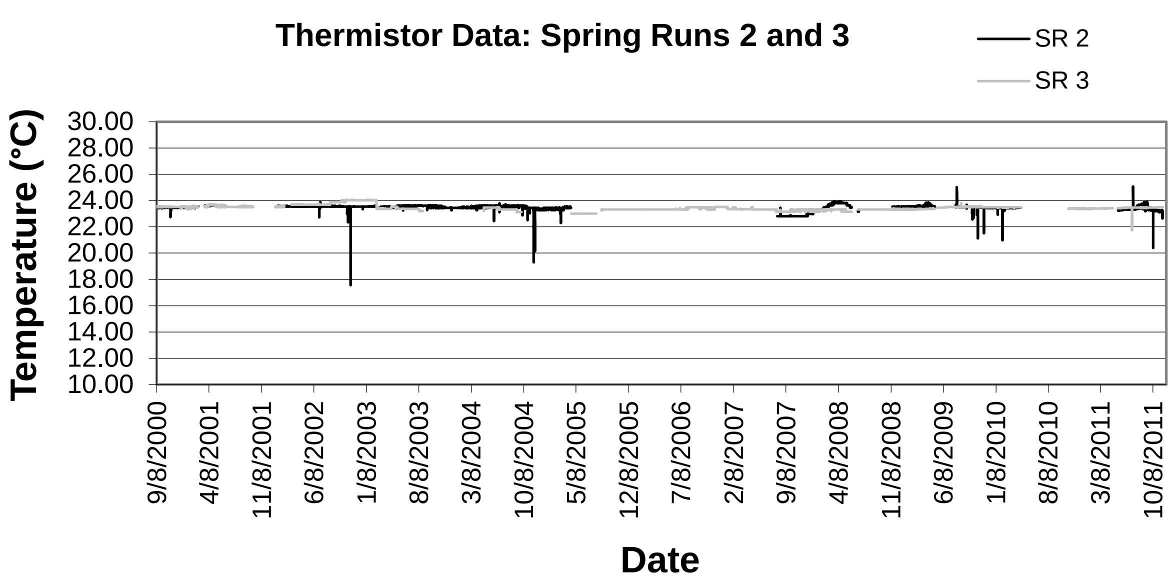
APPENDIX B:
DATA AND GRAPHS

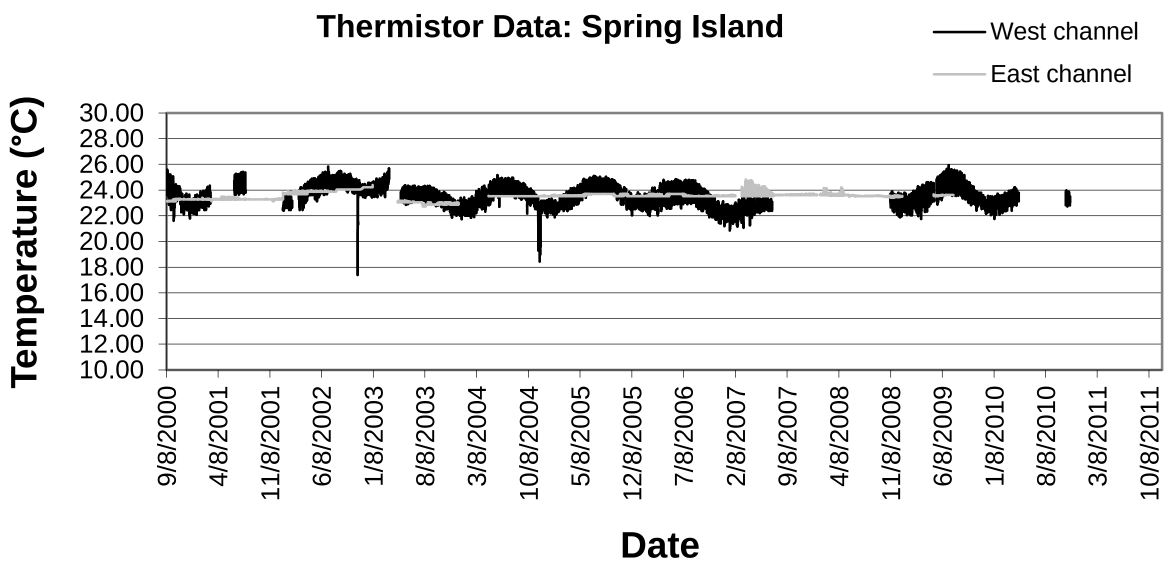
Water Quality Data
and
Thermistor Graphs

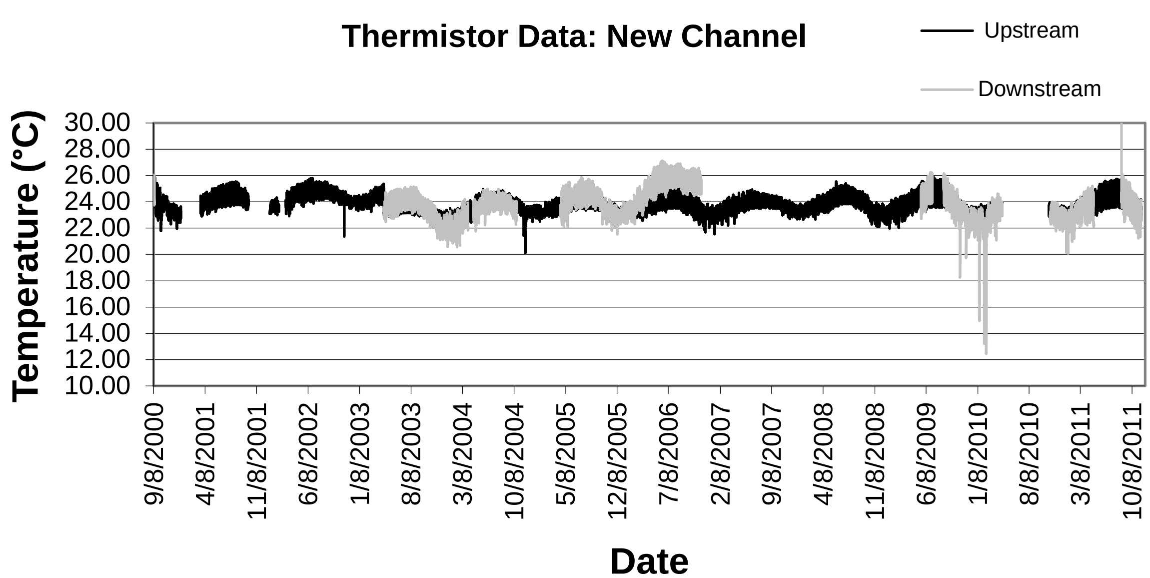

Thermistor Data: Comal Headwaters
Heidleberg
Blieders
Temperature (°C)
35.00
30.00
25.00
20.00
15.00
10.00
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date


Thermistor Data: Landa Lake Bottom
LL upper
LL lower
Temperature (°C)
30
28
26
24
22
20
18
16
14
12
10
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date

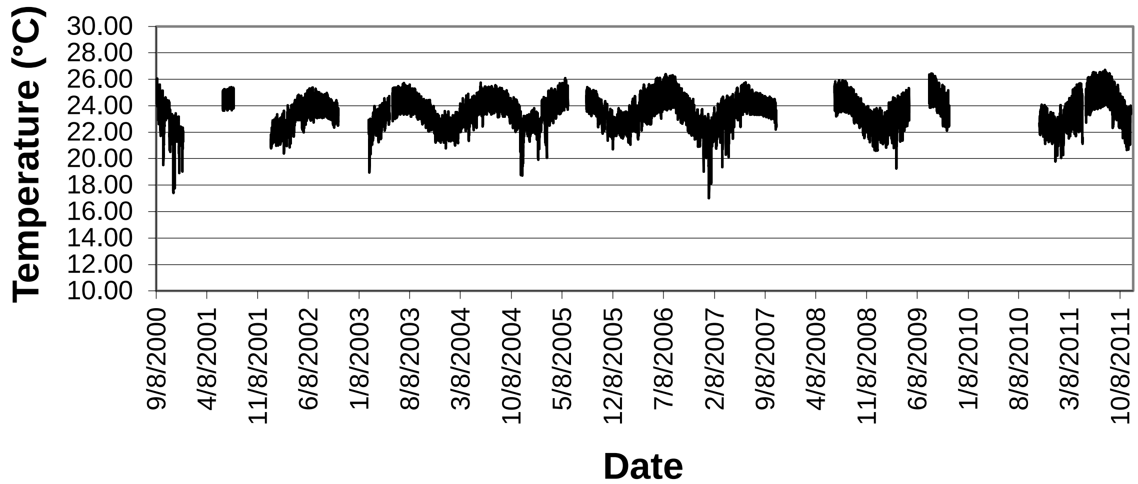

Thermistor Data: Spring Run 1
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date


Thermistor Data: Spring Runs 2 and 3
SR 2
SR 3
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date

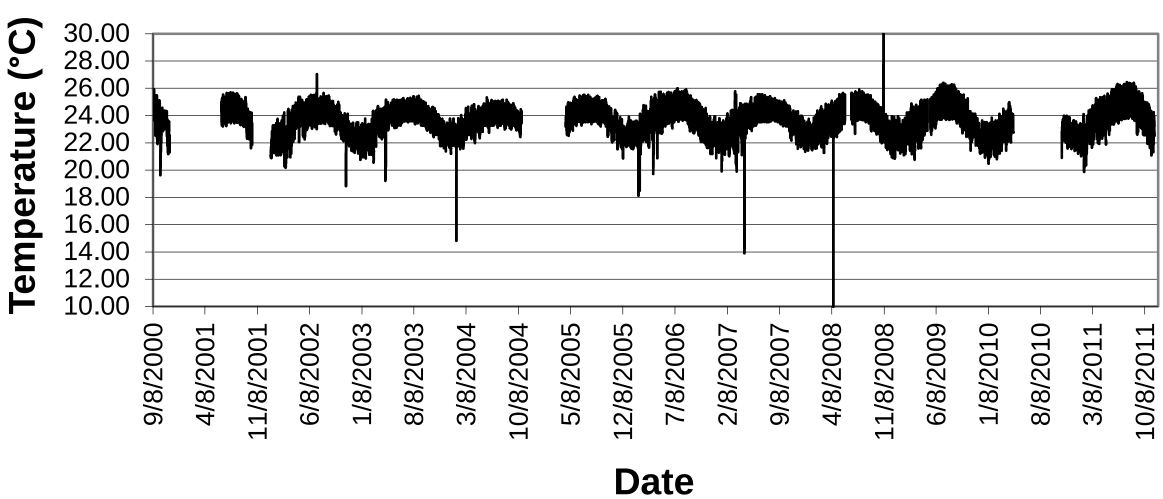

Thermistor Data: Spring Island
West channel
East channel
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date


Thermistor Data: New Channel
Upstream
Downstream
Temperature (°C)
30.00
28.00
26.00
24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
9/8/2000
4/8/2001
11/8/2001
6/8/2002
1/8/2003
8/8/2003
3/8/2004
10/8/2004
5/8/2005
12/8/2005
7/8/2006
2/8/2007
9/8/2007
4/8/2008
11/8/2008
6/8/2009
1/8/2010
8/8/2010
3/8/2011
10/8/2011
Date

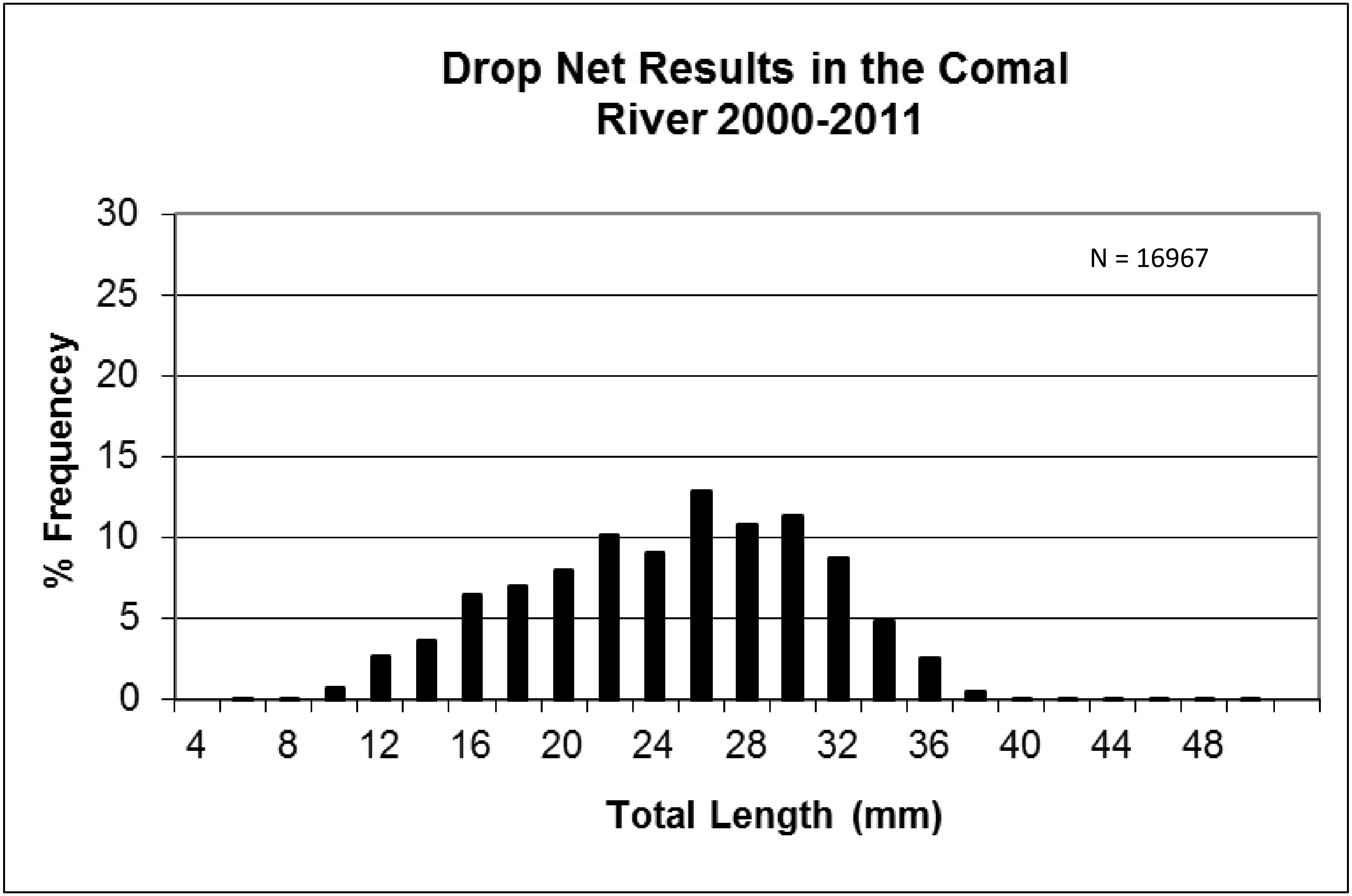
Thermistor Data: Other Place



Thermistor Data: Old Channel



Drop Net Graph

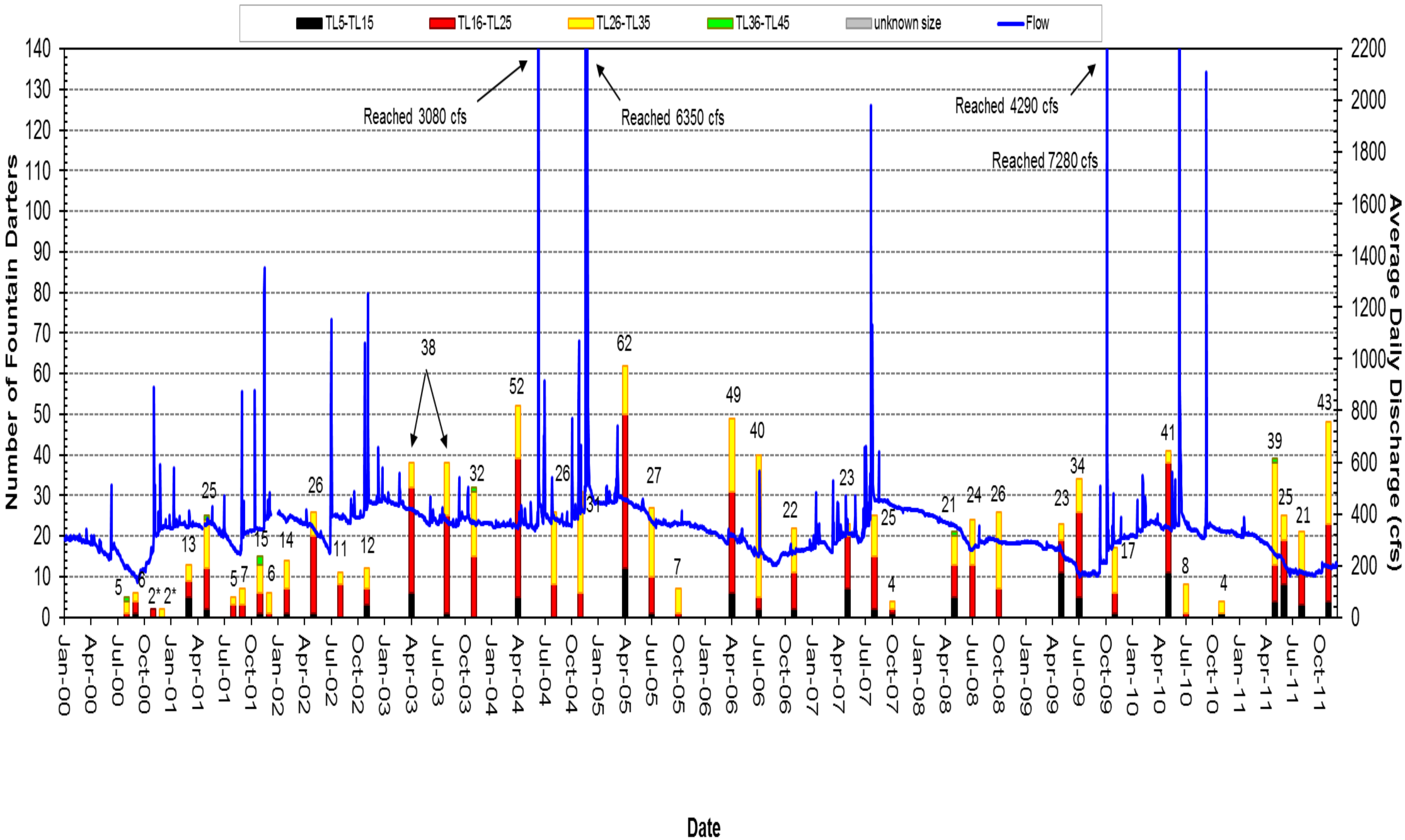


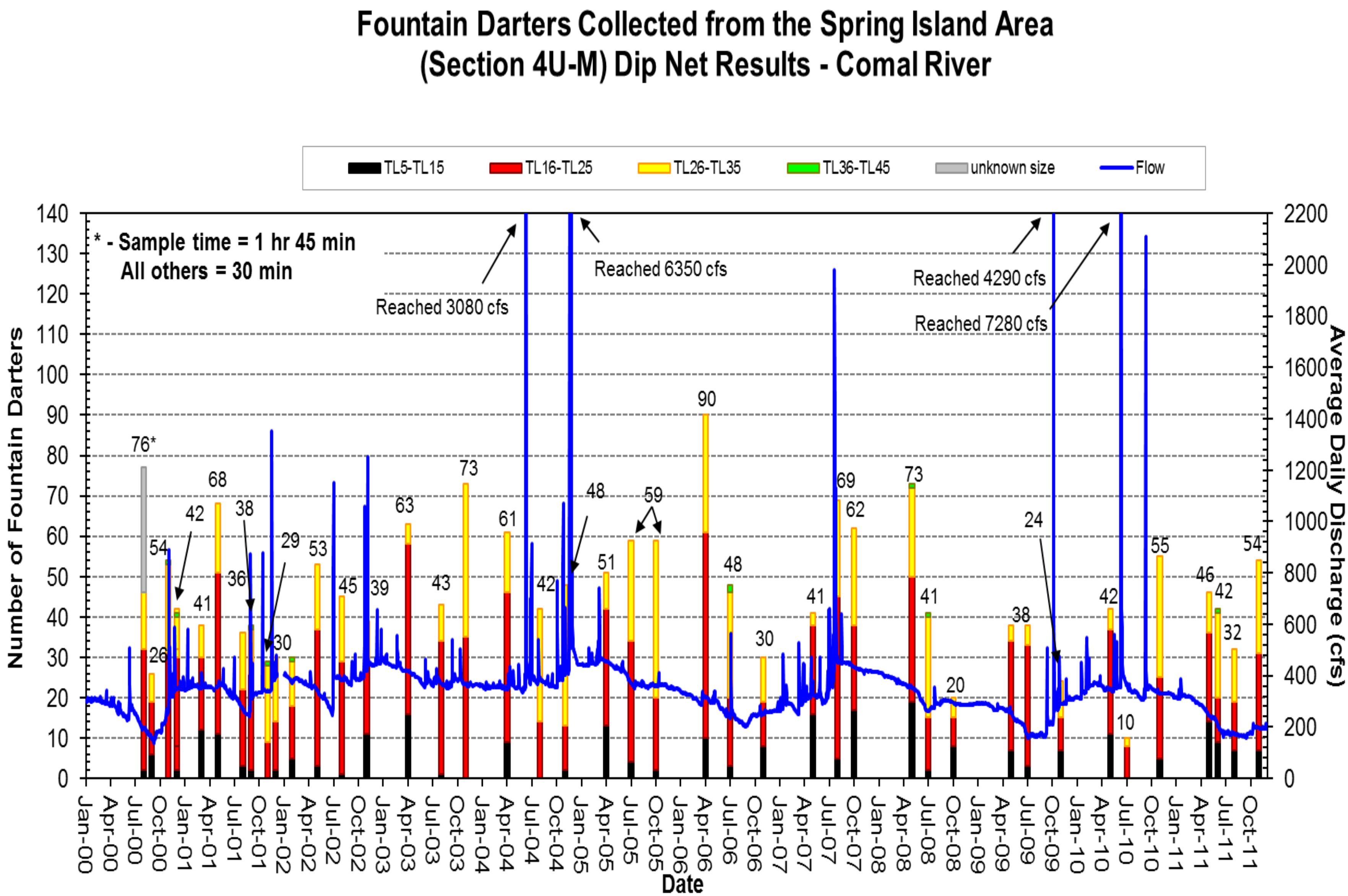
Drop Net Results in the Comal River 2000-2011
N = 16967
% Frequencey
30
25
20
15
10
5
0
4
8
12
16
20
24
28
32
36
40
44
48
Total Length (mm)

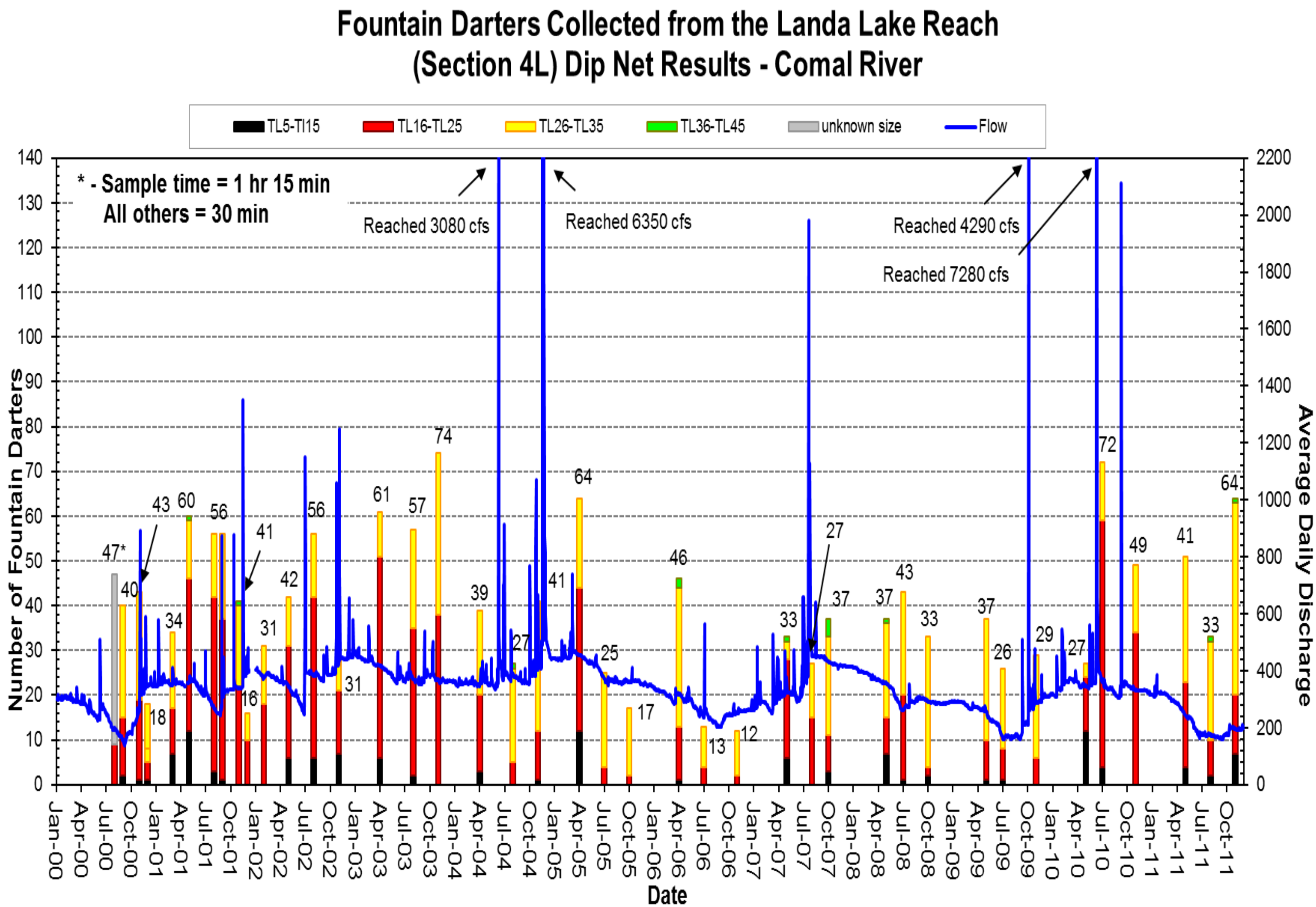
Dip Net Graphs

* - Sample time = 1 hour
All other samples = 30 min

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

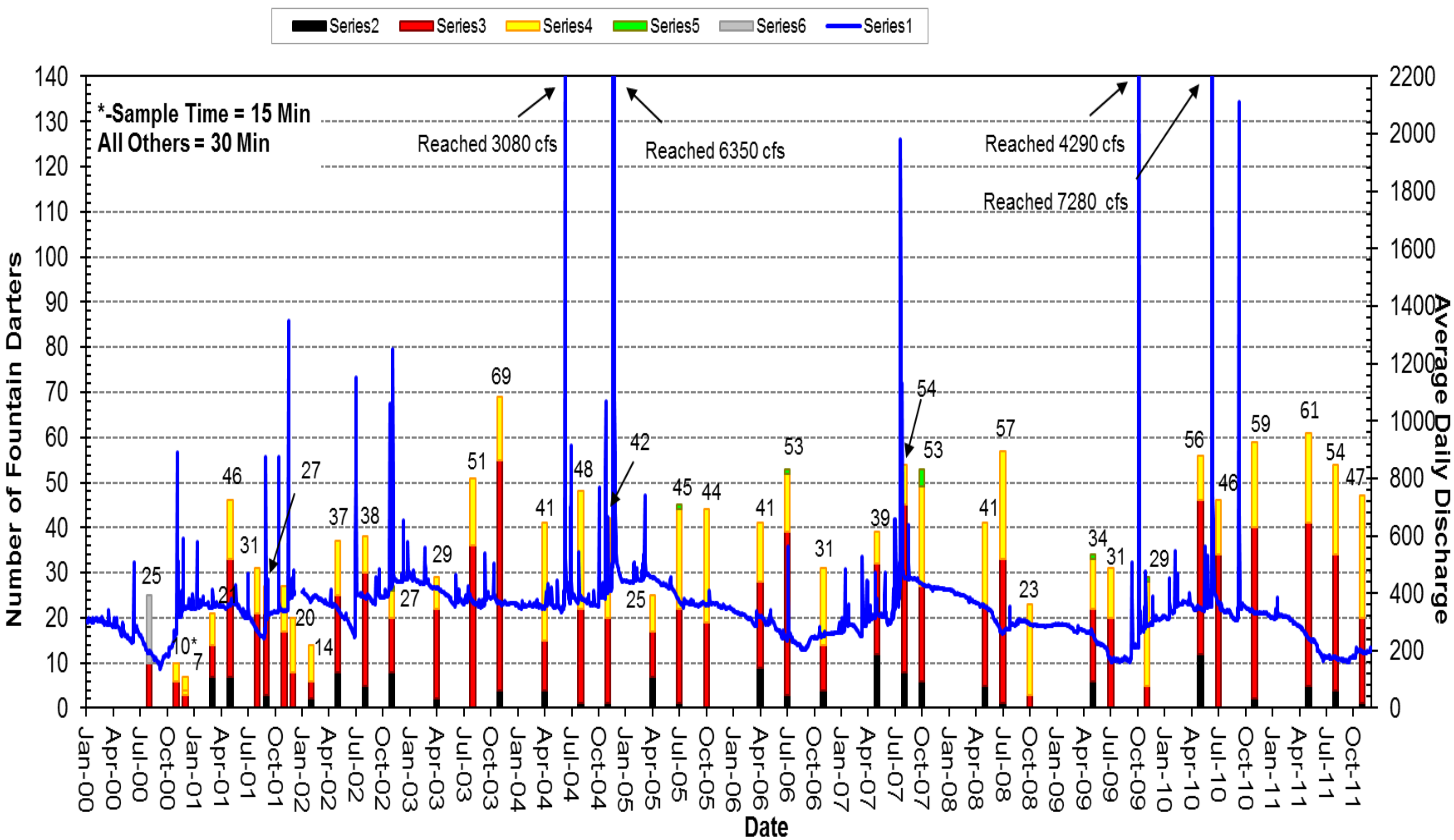


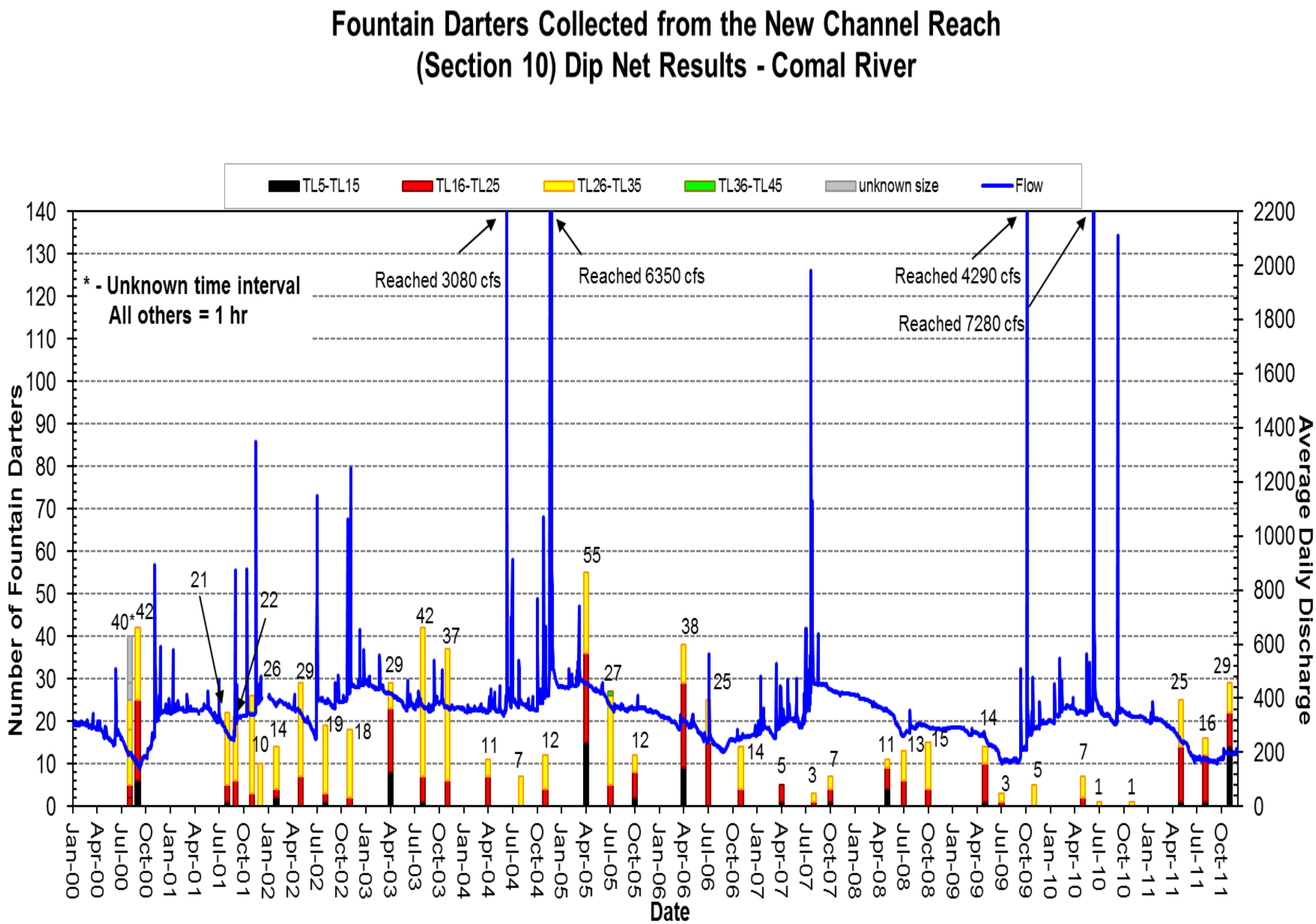

Fountain Darters Collected from the Spring Island Area
(Section 4U-M) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hr 45 min
All others = 30 min
Reached 3080 cfs
Reached 6350 cfs
Reached 4290 cfs
Reached 7280 cfs
Number of Fountain Darters
Average Daily Discharge (cfs)
Date



Fountain Darters Collected from the Landa Lake Reach
(Section 4L) Dip Net Results - Comal River
TL5-Tl15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hr 15 min
All others = 30 min
Reached 3080 cfs
Reached 6350 cfs
Reached 4290 cfs
Reached 7280 cfs
Number of Fountain Darters
Average Daily Discharge
Date

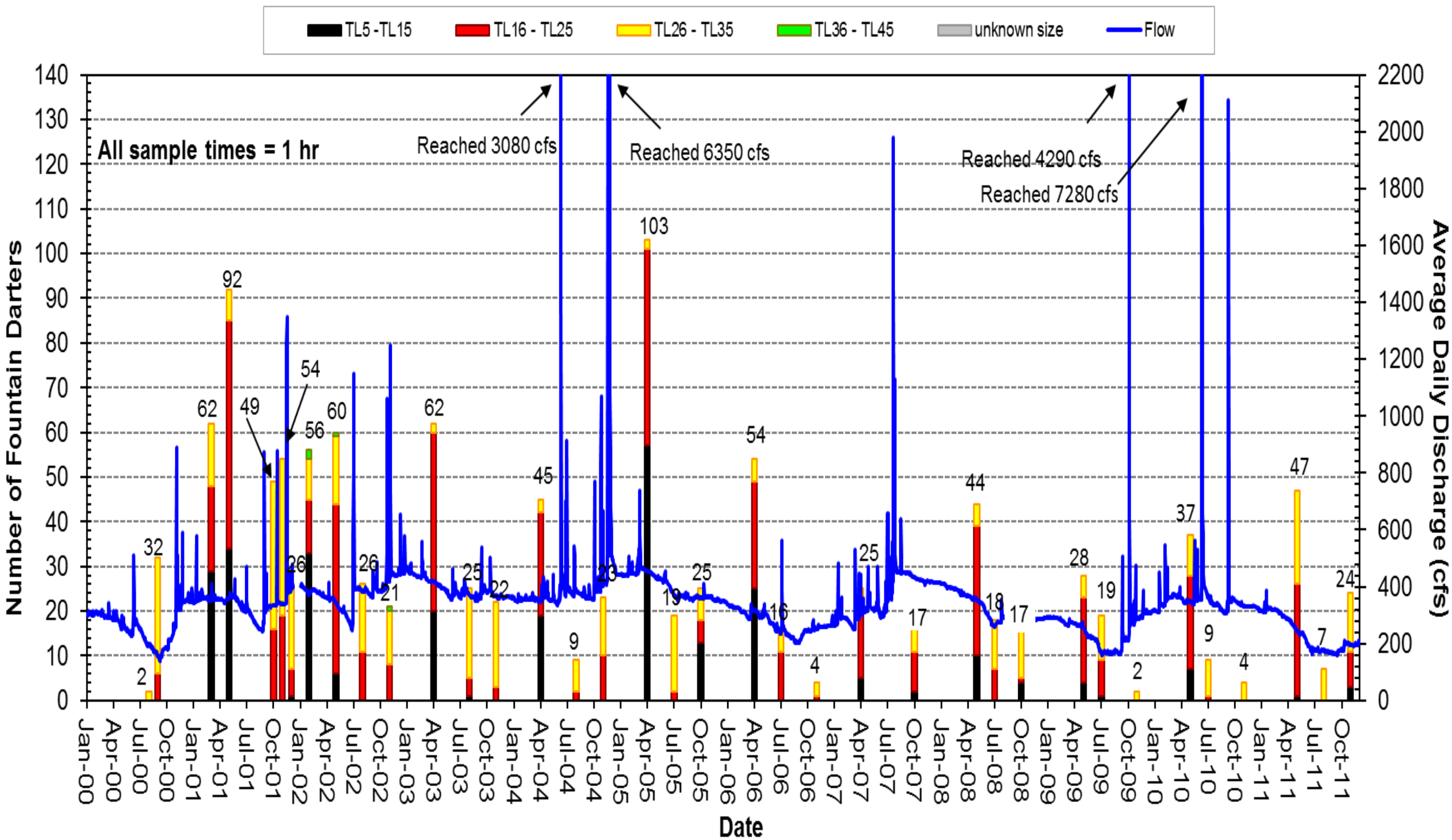
Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River

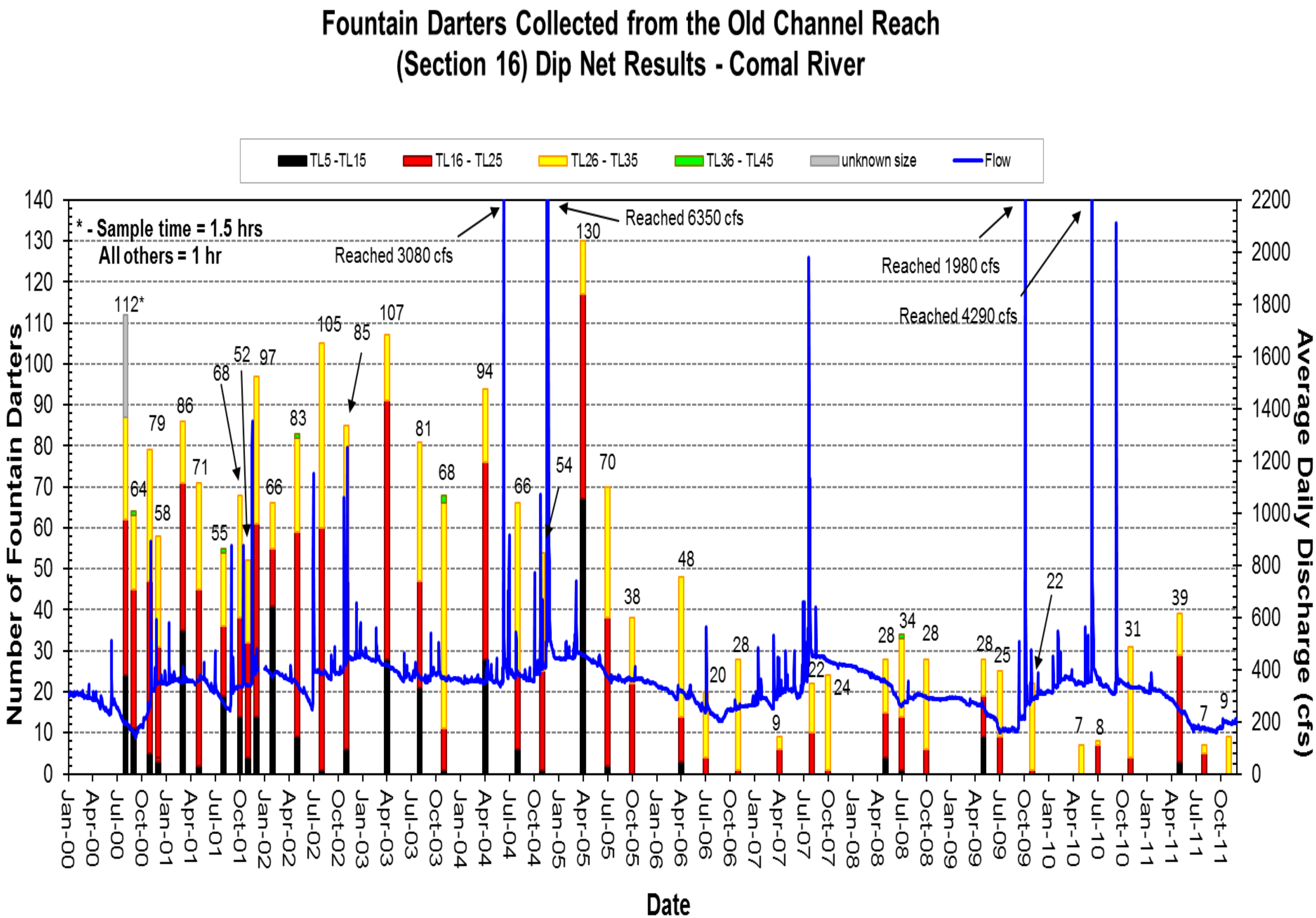




Fountain Darters Collected from the New Channel Reach
(Section 10) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Unknown time interval
All others = 1 hr
Reached 3080 cfs
Reached 6350 cfs
Reached 4290 cfs
Reached 7280 cfs
Number of Fountain Darters
Average Daily Discharge
Date
40*
42
21
22
26
10
14
29
19
18
29
42
37
11
7
12
55
27
12
38
25
14
5
3
7
11
13
15
14
3
5
7
1
1
25
16
29

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





Fountain Darters Collected from the Old Channel Reach
(Section 16) Dip Net Results - Comal River
TL5 -TL15
TL16 - TL25
TL26 - TL35
TL36 - TL45
unknown size
Flow
* - Sample time = 1.5 hrs
All others = 1 hr
Reached 3080 cfs
Reached 6350 cfs
Reached 1980 cfs
Reached 4290 cfs
Number of Fountain Darters
Average Daily Discharge (cfs)
Date

APPENDIX C:
DROP NET RAW DATA

(not available online)