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

FINAL 2007 ANNUAL REPORT

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TABLE OF CONTENTS

APPENDICES

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

This annual summary report presents a synopsis of methodology used and an account of sampling activities, including sample conditions, locations and raw data obtained during two sampling events (Comprehensive Monitoring Effort) conducted on the Comal Springs/River ecosystem in 2007. For ease of comparison, the data are reported here in an annual report format similar to previous reports (BIO-WEST 2001-2007).

Discharge in the Comal River in 2006 was below the historical average for most of the year. In 2007, precipitation events during the first half of the year contributed to flows above the historical average for three-quarters of the year. A considerable rain event occurred in July and the flow in the river peaked at 1,980 cfs. Except for one site (Spring Run 3), all of the locations where BIO-WEST measures discharge were higher than the previous year. As in past years, Spring Run 3 contributed the most flow to the system (of the locations measured). Higher flows following a below average discharge year had a myriad of effects on the biota in the Comal River/Springs Ecosystem.

A winter precipitation event in February caused water temperatures to drop sharply with the lowest reading recorded in the Old Channel Reach. Thermistors in the spring runs continued to display thermal uniformity (varied less than 1°C) throughout the year because these locations are less influenced by direct environmental changes. Blieder's Creek continued to display seasonal trends in water temperature because of the lack of spring influence. Water temperatures exceeded 30°C in August when air temperatures are typically highest. This high thermal variation in Blieder's Creek is likely a determining factor for the lack of any known fountain darter (*Etheostoma fonticola*) populations in this area. Thermistor data provide good baseline data for air temperature and precipitation effects within the study area, and will yield important information during any low-flow events.

Aquatic vegetation continued to flourish, but in some reaches non-native plants continued to out-compete native flora. The increased flows of 2007 appeared to stimulate growth in all reaches, and any scouring effects from flushing flows were either clouded by new growth or non-existent. In the Upper Spring Run Reach, bryophytes continued to dominate with many of the mosses forming tall columns reaching close to the water surface. These columns provided another dimension of cover for fountain darters that are more abundant in this vegetation. Bryophytes continued to provide habitat during the entire year, and did not get flushed out of the system by fall (as has occurred in previous years). *Hygrophila* flourished in 2007 increasing in area when large plants grew together in the extreme upstream portion of the reach. This non-native vegetation appears to be out-competing native vegetation like *Ludwigia*, which decreased in total area this year. This may affect fountain darter populations in the future as these fish prefer native vegetation. In addition, another native plant, *Cabomba*, was no longer observed in this reach in 2007.

Vallisneria was the dominant vegetation in the Landa Lake reach in 2007, though its importance as habitat to fountain darters continues to be minimal (compared to most vegetation types in the study area). The native plant, *Cabomba*, decreased early in 2007, but recovered by fall. This plant is getting more difficult to sample because there are only two stands that are shallow enough for efficient sampling. The number of fountain darters in these stands gives an indication to the importance of this habitat in shallow and deeper portions in the lake where it is found. The interplay between native and non-native vegetation was observed in this reach in 2007. From fall 2006 to spring 2007 *Hygrophila* stands in the central portion of the reach swelled, while *Ludwigia* in this area decreased. By fall 2007, *Ludwigia* surface area increased, while *Hygrophila* in the same region decreased. This trend has continued for several years and may have an important effect on fountain darter populations because sampling has shown that darters prefer *Ludwigia* to *Hygrophila*. Monitoring *Ludwigia* stands in Landa

Lake is necessary because there are few left, and most are located adjacent to *Hygrophila* stands in one portion of the lake. The interaction of these two plants also proved important in the Old Channel Reach in 2007. In spring 2007 a decrease in *Hygrophila* surface area was coupled with *Ludwigia* stands tripling in size. By fall however, *Ludwigia* decreased to a level less than it was in November 2006 with *Hygrophila* taking over many of the areas previously occupied by the native plant. In no other reach is the existence of native *Ludwigia* more important than in the Old Channel because another native plant, filamentous algae which was once abundant here is now nearly absent. Previously, the algae contained preferred habitat for darters, but with most of the algae gone few are found in these areas (though it must be noted that this vegetation is rarely sampled because there is not enough of it to cover the bottom of a drop net). This reach will continue to be closely monitored to observe the interaction between native and non-native plants, and the fountain darter populations that depend on them.

Vegetation continued to grow and colonize the New Channel Reach after having been nearly wiped out in 2005. *Hygrophila* continues to dominate much of the reach, but *Cabomba* and bryophytes have colonized and grew in several areas between *Hygrophila* plants. *Ludwigia* coverage changed little with only two plants present in the reach.

Drop-netting continues to be the best method for sampling fountain darters in varied vegetation types without harming the endangered fish. Native plants (filamentous algae, bryophytes, *Ludwigia*, *Cabomba*) yielded the highest densities of fountain darters in 2007 (as in other years). Unfortunately, *Ludwigia* and algae are patchily distributed in all reaches. Although flows were higher this year, bryophytes were present throughout much of the Upper Spring Run and Landa Lake reaches. This led to the highest population estimates in these reaches since 2003. As in previous years data reflected a spring reproduction peak of this species based on length frequency distributions showing the smallest fish present in spring. Dip netting of fountain darters in varied habitats reflected drop netting efforts with the percentage of darters present similar to previous years. Visual observation of fountain darters in the deepest portions of Landa Lake yielded the second highest number observed in this study in the fall. This corresponded to 95% coverage of bryophytes, one of their preferred habitats.

Snorkel surveys of salamanders in the spring runs exhibited a slight increase from 2006 with total numbers at or slightly above average. At Spring Island, numbers decreased by more than half in the east outfall. It is unclear why this occurred, but may be a result of increased flow or large patches of green algae present.

Fourteen different invertebrate taxa were observed at three drift net sites within Comal Springs in 2007. As in previous years, species of *Stygobromus* and *Lirceolus* were most abundant at all sites. In 2007, four different species of *Stygobromus* were collected within the Comal Springs making it (and the San Marcos Springs) the only locality in North America with four co-occurring *Stygobromus* species. Another specimen of a rare subterranean amphipod *Parabogidiella* sp. was collected in November 2007, after only two were collected last year. Fewer individuals of the Comal Springs riffle beetle (*Heterelmis comalensis*) were collected in 2007, which was likely related to mechanical disturbance to several of the cotton rags used in capturing them. Beetles were patchily distributed with wide ranges of abundance between sites and seasons.

In 2007, Anne Bolick completed her Master's thesis studying the abundance of the gill parasite *Heterophyid cercaria*. The study's objective was to determine the effect of springflow on the parasites abundance in the Comal River. This is of importance to the fountain darters because previous studies have documented that the abundance of cercariae in the water is positively associated with infection rates of caged fountain darters. Her findings did not show any relationship between flow and cercarial abundance, but season and distance downstream did have some effect. It must be noted that the lack of relationships between abundance and discharge can only be applied over the range of flows present in 2007. As a result further study is needed to understand the effects of this parasite at low-flows.

Water quality data collected by Master Naturalist volunteers in 2007 showed that carbon dioxide (CO_2) concentrations were highest near springs, while pH increased as you go downstream. At all sites, CO_2 concentrations were higher and pH lower in 2007 compared to 2006. Recreational use data indicated that the New Channel was most heavily used by tubers.

Overall, the higher discharge in 2007 positively affected fountain darter and plant growth. However, it appeared that in some reaches non-native vegetation continues to outcompete the native flora. This may have serious effects on fountain darter populations in certain reaches, like the Old Channel where non-native vegetation (*Hygrophila*) dominates and seems to be pushing preferred habitat out of the reach. The ubiquitousness of bryophytes in the Landa Lake and Upper Spring Run reaches will continue to provide important habitat for fountain darters. Unfortunately, the inability of these mosses to firmly attach to the substrate makes it vulnerable to high flow events. If flushing flows continue over extended periods of time, fountain darter populations may be adversely affected if there are no other preferred habitats present (like *Cabomba* and *Ludwigia*).

Though the comprehensive portion of the study has been reduced to two annual samples (plus a limited summer effort), it is still adequate to maintain a continuous record of conditions. Maintaining this continuous record is vital since antecedent conditions influence community-level response to reduced discharge conditions. Sampling only during a low-flow event simply does not provide the necessary context to adequately assess changes that occur during such conditions. As such, comprehensive monitoring will continue in the Comal Springs/River ecosystem in 2008.

METHODS

As in 2005 and 2006, two full comprehensive sampling efforts were conducted (spring and fall) in 2007 with additional summertime dipnetting by the project team, and some volunteer assistance (initiated in 2006) on the Comal system. A full comprehensive event includes the following sampling components and volunteer activities:

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

Aquatic Vegetation Mapping

Fountain Darter Sampling
- Drop Nets
- Dip Nets
- Visual Observations

Salamander Observations

Macroinvertebrate Sampling
- Drift Nets
- Comal Springs Riffle Beetle Surveys

Recreation Observations
- Weekly Recreation Counts

Gill Parasite Evaluation

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 2007). The discharge data for the Comal ecosystem was taken from USGS gage 08169000 from the Comal River at New Braunfels. This site represents the cumulative discharge of the springs that form this river system.

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

Low-Flow Sampling

There were no low-flow events in 2007.

High-Flow Sampling

There were no high-flow events in 2007.

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. The water quality component of this study was reduced in 2003, but the two components necessary for maintenance of long-term baseline data, temperature loggers (thermistors) and fixed station photography, were continued in 2007. In addition, conventional physico-chemical parameters (water temperature, conductivity compensated to 25°C, pH, dissolved oxygen, water depth at sampling point, and observations of local conditions) were taken at the surface and near the bottom in all drop-net sampling sites using a Hydrolab Quanta. When conditions trigger Critical Period events in the future, the full range of water quality sampling parameters will be employed, including water quality grab samples and standard parameters from each of the water quality sites in the Comal Springs ecosystem (Figure 1).

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

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

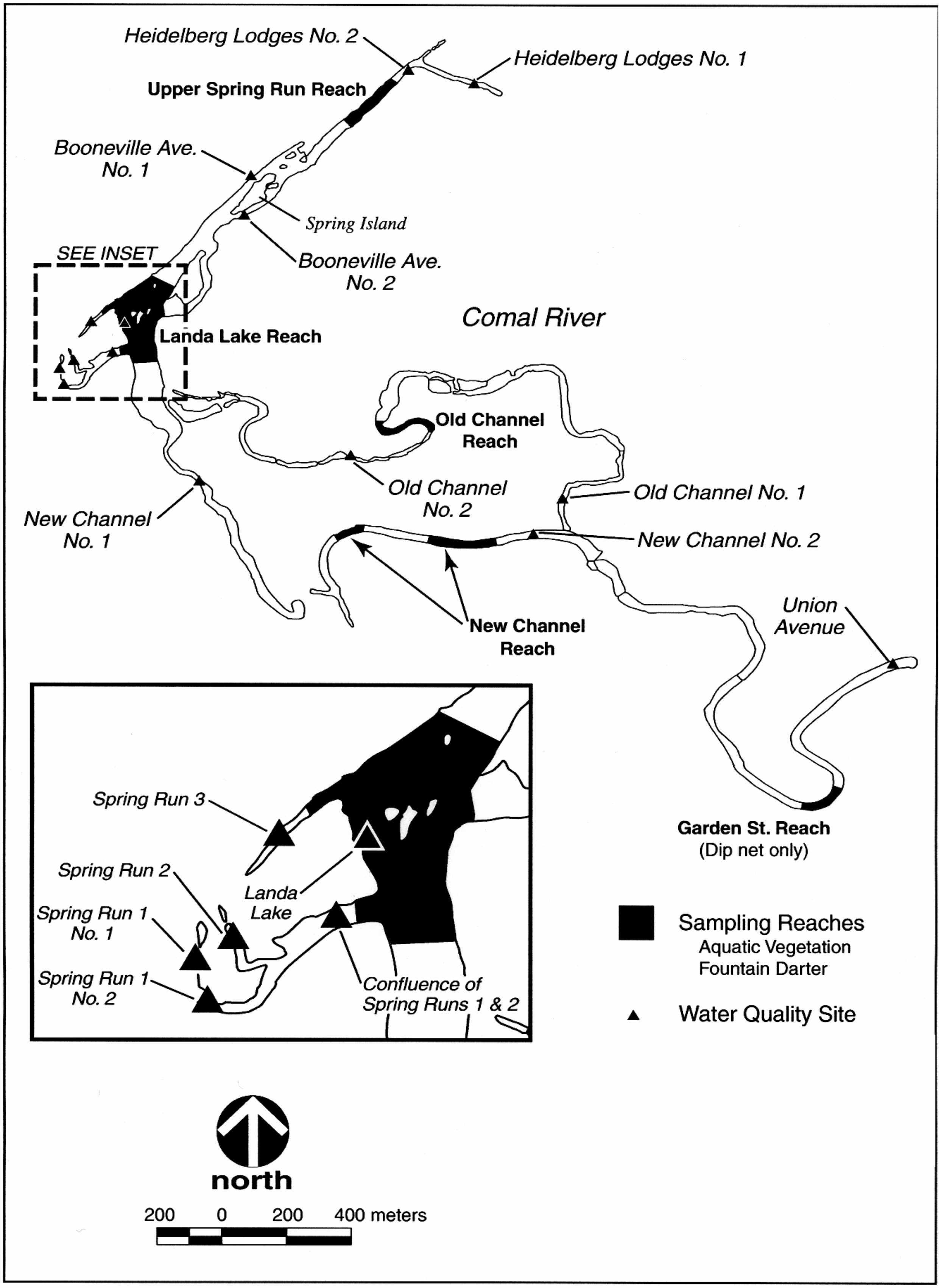


Figure 1. Comal River water quality and biological sampling areas.

Aquatic Vegetation Mapping

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

Hygrophila in the Old Channel Reach

Filamentous algae (in the Old Channel) and bryophytes (*Riccia* and *Amblystegium*; primarily in the Upper Spring Run and Landa Lake) were included in all 2007 sampling events. Difficulties with mapping these vegetation types (patchiness, bryophytes were easily obscured by filamentous algae, etc.) precluded them from early samples; however, these vegetation types were clearly important fountain darter habitat and were included in all sample events beginning in the summer of 2001.

Fountain Darter Sampling

Drop Net Methods

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

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

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 2000-2007 provide valuable data on species/habitat relationships. These average density values were also used with aquatic vegetation mapping data on total coverage of each vegetation type by sampling effort to create estimates of the population abundance in each reach (fountain darter density within a vegetation type x total coverage of that vegetation type in the given reach). Because there were generally only two drop net samples in each vegetation type within each reach, density estimates between sampling efforts had great variation and population estimates based on those densities would be greatly influenced by this variation. Part of the variation would be due to changes in environmental conditions (discharge, temperature, etc.) that had occurred since the last sample, but part would be due to natural variation between samples. Without adding samples (the total number is limited by federal permit and time constraints) it is impossible to tell how much of the variation is attributed to each source within a given sampling effort. Using the average density of fountain darters across all samples for a given vegetation type does not account for changes in density across samples (differences associated with changes in environmental conditions), but the increased sample size substantially reduces the high natural variability. This type of comparison between samples, where density values are held constant across all samples, is based entirely upon changes in vegetation composition and abundance between sampling efforts. Because these abundance estimates use the same density values across sites and seasons, and do not include estimates of fountain darters found in vegetation types that are not sampled with drop nets, the absolute numbers generated with this method have some uncertainty associated with them. Thus, the estimates are presented as relative comparisons by normalizing the data to the maximum estimate (the absolute value of all samples are converted to a percentage of the maximum value).

Dip Net Methods

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

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

Dip Net Data Analysis

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

Dip Net Techniques Evaluation

In 2007, presence/absence dip netting was conducted on the Comal River during the spring (April 27) and fall (October 25) sampling events. During each sample, fifty sites were distributed among the 4 sample reaches based on total area, diversity of vegetation, previous fountain darter abundance estimates, and overall biological importance of each reach. The distribution of sample sites was the same as in previous years except that one filamentous algae site was substituted with a bryophyte site in the Old Channel Reach during the fall sample (Table 1). This more appropriately represents the vegetation communities currently found in this reach. In most cases, sites were randomly selected from a grid overlain on the most recent vegetation map of that reach. However, occasionally, where certain vegetation types exhibited limited coverage, sites were chosen to fall within the proper vegetation type. Four dips were conducted at each site. After each dip, presence or absence of fountain darters was noted and the entire contents of the net were placed into a plastic tub filled with river water to avoid recapturing organisms. After all dips were completed at a site, all organisms were released and time of day was recorded.

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

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

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Hygrophila (3)	*Cabomba* (3)	*Hygrophila* (6)	Filamentous Algae (1)
Bryophytes (3)	*Hygrophila* (8)		*Hygrophila* (8)
	Ludwigia (3)		*Ludwigia* (6)
	Bryophytes (8)		Bryophytes (1)
Total (6)	**Total (22)**	**Total (6)**	**Total (16)**

Visual Observations

Visual surveys were conducted using SCUBA in Landa Lake to verify the continued fountain darter and Comal Springs salamander (*Eurycea sp.*) use of habitat in deeper portions of the lake. The locations of these time-constrained surveys were deeper than drop net or dip net methods than efficient sampling for the darters would allow. Observations were conducted in the early afternoon for each effort. An additional component to these surveys was a grid (0.6 m x 13.0 m) added in summer 2001, and subsequent sampling. The grid was used to quantify the number of fountain darters using these deeper habitats. To sample the area, all fountain darters within the grid were counted. Time constraints limited the sampling to just one grid. A much more labor-intensive effort would have been required to develop an estimate of the true population size in the sample area, but the data were useful in providing an indication of the relative abundance of the fountain darters that are found in areas similar to those sampled. This method also allowed some insight into trends in population dynamics that may occur over time.

Gill Parasite Evaluation

A special study was conducted in 2007 titled "The effects of spring flow on the abundance of Heterophyid cercariae in the Comal River, New Braunfels, Texas." This study was conducted by Ms. Anne Bolick, a Master's student at Texas State University – San Marcos, with the assistance of her committee members Dr. David Huffman (Texas State University), Dr. Tom Brandt (USFWS), and Dr. Alan Groeger (Texas State University). The purpose of this study was to examine the effects of varying spring flows on the concentration and abundance of *C. formosanus cercariae* in the Comal River.

Previous studies have documented that the abundance of cercariae in the water is positively associated with infection rates of caged fountain darters (Cantu 2003). Given that infection pressure on fountain darters is a function of cercarial density, decreases in discharge would likely result in increases in infection pressure. Assuming a constant emergence rate of *C. formosanus* cercariae, a decrease in water volume stemming from reduced spring flows would result in a subsequent increase in cercarial densities. Additionally, there was anecdotal evidence that emergence rates of cercariae may actually increase as flows decrease, thus exacerbating this phenomenon. Regardless, increased cercarial densities potentially compound impacts to the fountain darter under low flow situations.

To examine cercarial abundance a battery-powered pump was used to pump water from specific locations within the water column at three transect locations (Houston Street, Liberty Avenue, and Elizabeth Avenue) in the Comal River. Two 5-liter samples were collected from six points within each transect every two weeks for 12 months. This water was then treated with formalin to fix cercariae and filtered through a three-step filter apparatus with a 30 µm final filter (Figure 2). Filters with cercariae were then removed, stained with Rose Bengal, and preserved with 10% formalin. Cercarial counts were conducted in the laboratory with the help of a dissecting microscope at 100X magnification. For a more detailed description of methods please see Appendix D for a copy of Ms. Bolick's thesis.

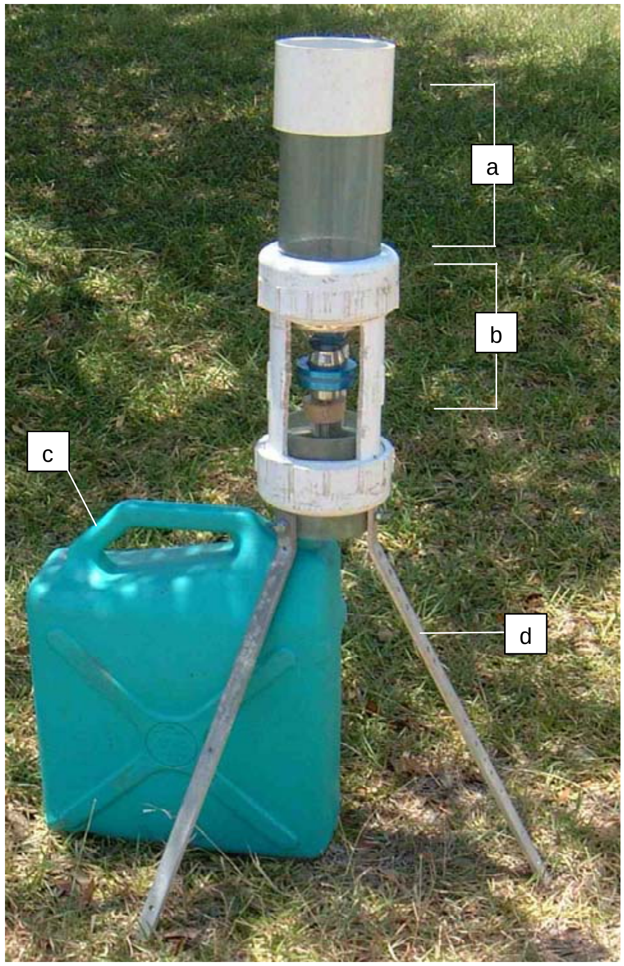


Figure 2. Modified filtration apparatus. a) prefilters (220 μm and 86 μm). b) sample filter mount with 30 μm monofilament filter. c) 22.7 L formalin collection container. d) filter apparatus support.

Comal Springs Salamander Visual Observations

In addition to the visual observations made in the deeper portions of Landa Lake for fountain darters and Comal Springs salamanders, the BIO-WEST project team performed presence/absence surveys for the Comal Springs salamanders in the spring reaches located at the head of the Comal River during both 2007 sampling events. Surveys were conducted in Spring Run 1, Spring Run 3, and the Spring Island area (Figure 1) and performed by two people in each spring reach. Each survey began at the downstream-most edge of the sampling area and involved turning over rocks located on the substrate surface within the Spring Run while moving upstream toward the main spring orifice. A dive mask and snorkel were utilized when depth permitted. The Comal Springs salamander locations were noted, along with time and water depth. In order to maintain consistency between samples, all surveys were initiated in the morning and terminated by early afternoon.

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

Salamander survey in Spring Run 1

Macroinvertebrate Sampling

In 2007, drift nets were placed in spring openings during the spring and fall comprehensive sampling efforts. Drift nets were placed over the openings of Comal Spring Runs 1 and 3 and a moderate-sized spring upwelling along the western shoreline of Landa Lake. The nets were anchored into the substrate directly over the spring opening, with the net face perpendicular to the direction of flow of water. The nets had a 0.45-m by 0.30-m rectangular opening and mesh size of 350 micrometers (µm). The tail of the net was connected to a detachable 0.28-m long cylindrical bucket (300-µm mesh). The buckets were removed at 4-hour intervals, and the cup contents were sorted in the field. Except for voucher specimens of Comal Springs riffle beetle, Peck's cave amphipod, and Comal Springs dryopid beetle, all organisms of these three species were identified and returned to their spring of origin. Voucher specimens included fewer than the 20 living specimens (identifiable in the field) of each species. All other invertebrates were preserved in 70% ethanol for later identification. Water quality measurements (temperature, pH, conductivity, dissolved oxygen, and current velocity) were taken at each drift net site using a Hydrolab multiprobe and DataSonde (model 2) and a Marsh McBirny portable water current meter (model 201D).

In addition to drift nets placed over spring openings, surveys of the endangered Comal Springs riffle beetle, were conducted in the two comprehensive sampling efforts in 2007 (May and November). These samples were conducted in three disjunct areas of Landa Lake on the Comal River, in locations that were previously identified (BIO-WEST 2002) to have the highest densities of Comal Springs riffle beetles. The three sites included Spring Run 3, the western shoreline of the lake, and upstream of Spring Island. Samples were collected using the same methodology as in previous years. Bed sheets (60% cotton, 40% polyester) were cut into 15-cm x 15-cm squares. At each of the three study sites, 10 springs found in potential habitat were selected and sampled using this method. Depth (ft), current velocity (m/s), and landmark distance measurements were taken at each spring. Each square had the corners folded inward and placed in the spring with rocks loosely stacked over top to keep it in place.

Approximately four weeks later, squares were located and removed followed by depth and current velocity measurements. Beetles were identified, counted, and returned to their spring of origin. Other spring invertebrates collected on the squares were also noted.

Master Naturalist Monitoring

Volunteers with the Texas Master Naturalist program continued their monitoring efforts in 2007 at select locations along the Comal Springs/River system. The Texas Master Naturalist Program is a partnership among the Texas Cooperative Extension, Texas Parks and Wildlife Department, and numerous local partners designed to provide natural resource education, outreach, and other services through volunteer efforts. To become a Master Naturalist, an individual must complete an approved training course and complete at least 40 hours of volunteer service per year. The program currently supports over 2,750 volunteers across the state of Texas (http://masternaturalist.tamu.edu).

In 2007, Master Naturalist volunteers assisted BIO-WEST by collecting weekly water quality and recreation data on the Comal Springs/River ecosystem. Volunteers collected data at five sites (Figure 3) on a weekly basis (typically on a Friday afternoon) from January through December. 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.

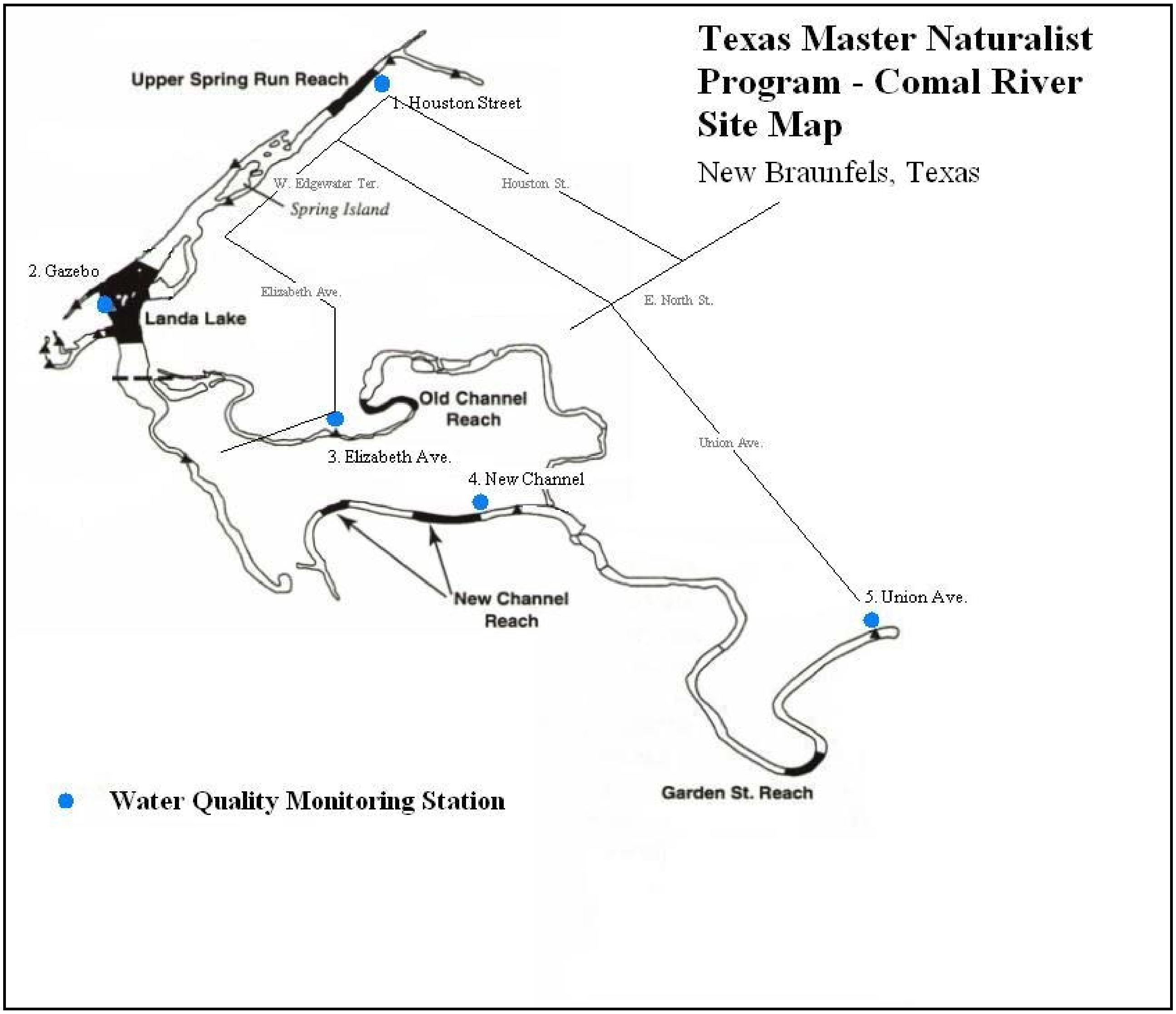


Figure 3. Weekly water quality / recreation monitoring sites on the Comal River used by Texas Master Naturalist volunteers.

Exotics / Predation Study

This sampling component was not included in 2007 as no critical period sampling efforts were triggered.

OBSERVATIONS

The BIO-WEST project team conducted the 2007 sampling events as shown in Table 2.

Table 2. Components of 2007 sampling events.

EVENT	DATES	EVENT	DATES
Spring Sampling		**Fall Sampling**	
Vegetation Mapping	Apr 23 - 27	Vegetation Mapping	Oct 11 - 18
Fountain Darter Sampling	Apr 30 May 1 - 3	Fountain Darter Sampling	Oct 17 – 19, 23
Comal Salamander Observations	Apr 26	Comal Salamander Observations	Oct 24
Macroinvertebrate Sampling	May 2 - 4	Macroinvertebrate Sampling	Nov 6 - 9
Summer Sampling			
Fountain Darter Sampling	Aug 1		

Comal Springflow

Discharge remained fairly constant from late 2006 into early 2007 (Figure 4). Discharge was close to the historical average during the first half of 2007 and increased to greater than average conditions during the latter part of the year (Figure 5). The minimum flow during 2007 was recorded in March and was higher than the minimum flow recorded in 2006 (Table 3). A significant amount of rain fell over the Edward's Aquifer during the summer, and a peak discharge for the year of 1,980 cfs was recorded on July 20 (Figure 4). In mid-August, discharge began to slowly decline for the rest of the year, but remained at higher than average discharge levels (Figure 5).

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

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

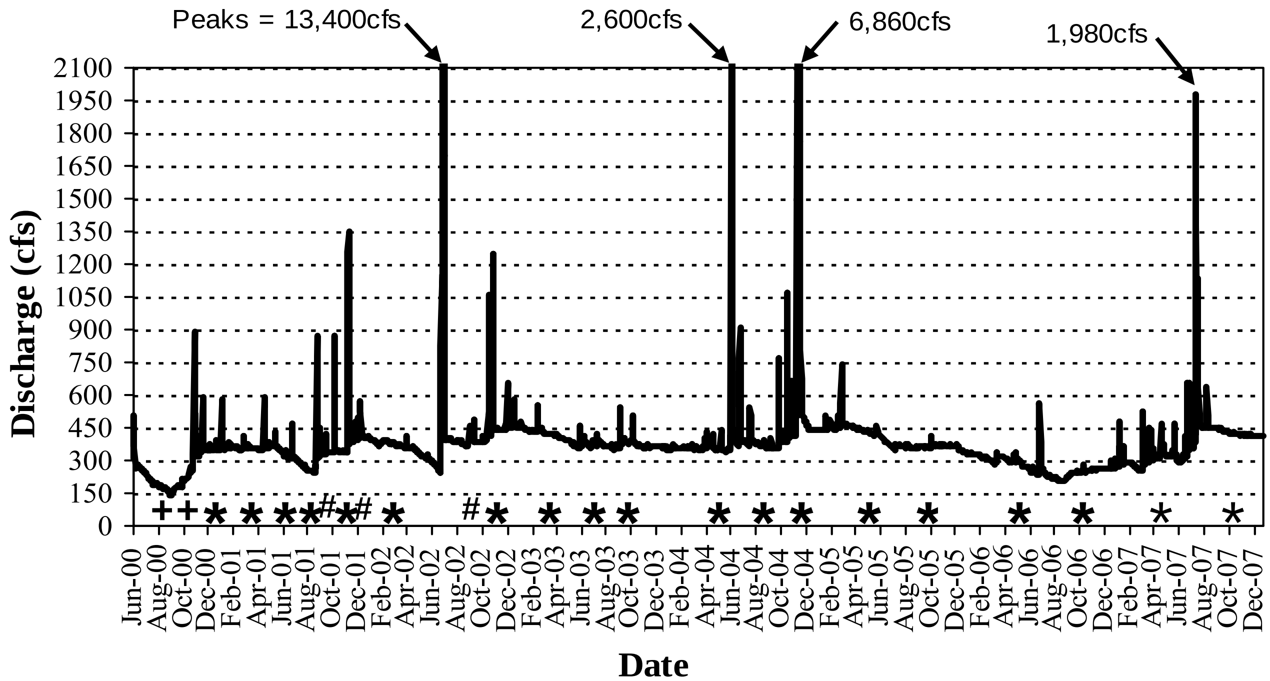


Figure 4. Mean daily discharge in the Comal River during the study period; approximate dates for quarterly (*), low (+), and high-flow (#) sampling events are indicated.

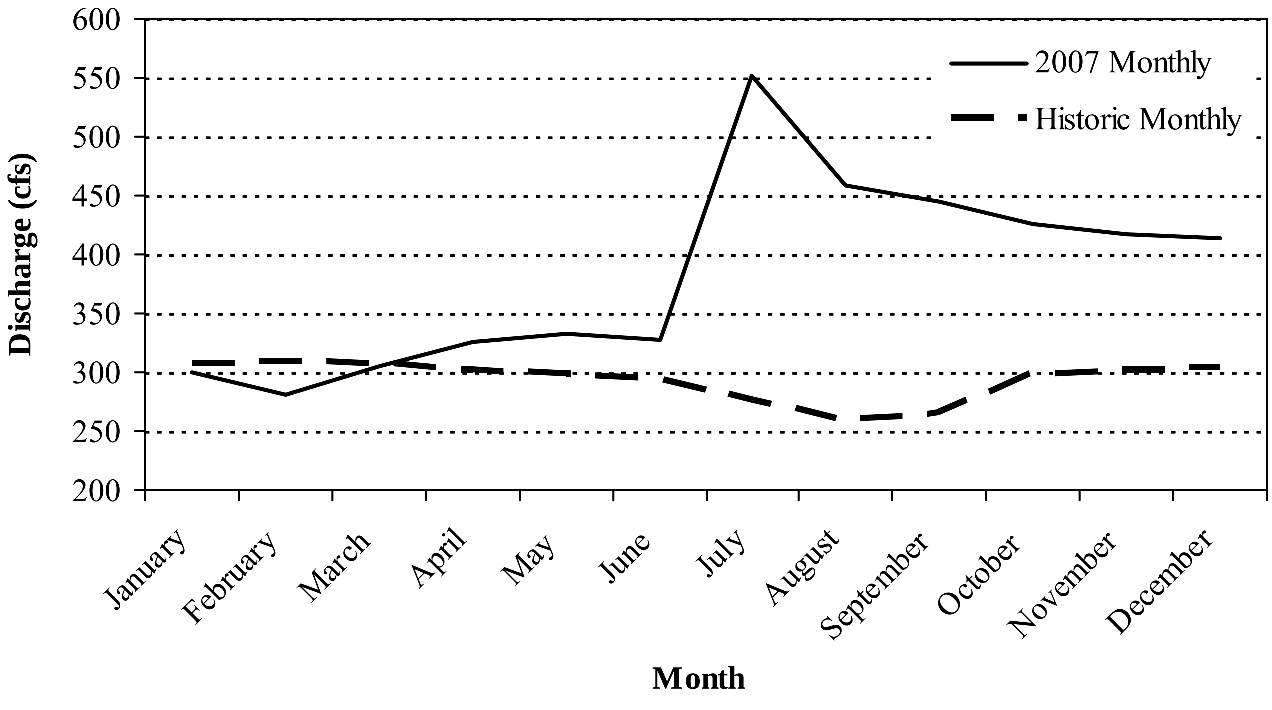


Figure 5. Mean monthly discharge in the Comal River during the 1934-2007 period of record.

Table 4 shows the discharge measured in each of the Spring Runs (including one upstream and one downstream site in Spring Run 3) and the Old Channel. Table 5 shows the proportion that each spring contributed to the total Comal River discharge and the proportion of total discharge that traveled down the Old Channel during each sample effort. Discharge was well above 2006 levels for all sites and dates in 2007. Discharge increased at all Spring Runs from fall 2006 to spring 2007. Total discharge exceeded 300 cfs in spring, and with the summer rains increased to 424 cfs by fall 2007. The Old Channel exhibited the largest increase in discharge from fall 2006 to spring 2007, but decreased slightly by fall. Spring Run 3 was the only site that decreased in proportion of total discharge from 2006 to 2007. Although flows were significantly higher in 2007, the proportion of total discharge of each spring remained fairly constant.

Table 4. Total discharge in the Comal River (USGS data) and discharge estimates for Spring Runs 1, 2, and 3 and Old Channel reach during each sample effort in 2006 - 2007.

	Discharge (cfs)			
Location	**Spring 2006**	**Fall 2006**	**Spring 2007**	**Fall 2007**
Total Discharge Comal River (USGS)	295	259	330	424
Spring Run 1	27.4	24.5	31.9	42.2
Spring Run 2	3.7	3.3	5.1	6.4
Spring Run 3 (upstream)	28.2	14.6	16.0	14.9
Spring Run 3 (downstream)	36.4	33.6	40.0	52.1
Old Channel	51.6	42.5	57.3	54.6

Table 5. Proportion of total discharge in the Comal River (USGS data) that each Spring Run contributed and proportion that traveled down the Old Channel during each sample effort in 2006 - 2007.

	Proportion of Total Discharge			
Location	**Spring 2006**	**Fall 2006**	**Spring 2007**	**Fall 2007**
Spring Run 1	9.3%	9.5%	9.7 %	10.0 %
Spring Run 2	1.3%	1.3%	1.5 %	1.5 %
Spring Run 3 (upstream)	9.6%	5.6%	4.8 %	3.5 %
Spring Run 3 (downstream)	12.3%	13.0%	12.1 %	12.3 %
Old Channel	17.5%	16.4%	17.4 %	12.9 %

Water Quality

The continuously sampled water temperature data have provided a good view of the thermal conditions experienced by fountain darters and other species throughout the Comal Springs ecosystem from 2000 - 2007. Water temperatures are most constant at or near the spring inputs, and become more variable downstream as other inputs (streams, runoff, and precipitation) become more influential. At times, precipitation can have acute impacts (typically very cold rainfall) in some locations, but these are generally short-lived and the overall relationship at these sites is more directly associated with air temperature (air temperatures also strongly influence precipitation temperatures).

A representative graph of thermistor data for the Comal Springs/River ecosystem in 2007 is presented in Figure 6; additional graphs for all years can be found in Appendix B. Gaps in readings present on some graphs are indications of thermistor failure, and these readings were excluded because they may not be accurate. All thermistors were immediately replaced when inaccurate readings were identified. The greatest contrast in variable versus stable readings is exhibited in the Comal headwaters. At this location Blieder's Creek (non-springfed) converges with water emanating from several springheads upstream of the confluence. The thermistor located near these springs varied little (19 – 24.5 °C), while the thermistor located in Blieder's Creek varied widely (21.1 – 31.2 °C) in 2007 (Appendix B). These temperatures reflect the fact that Blieder's Creek is prone to these variable temperatures because much of the water comes from runoff during precipitation events that can drop the water temperature quickly. Without spring inputs, the creek can become stagnant when flows (and precipitation events) are reduced causing the temperatures to rise. With these fluctuations in temperature it is unlikely that fountain darters would find suitable habitat in Blieder's Creek.

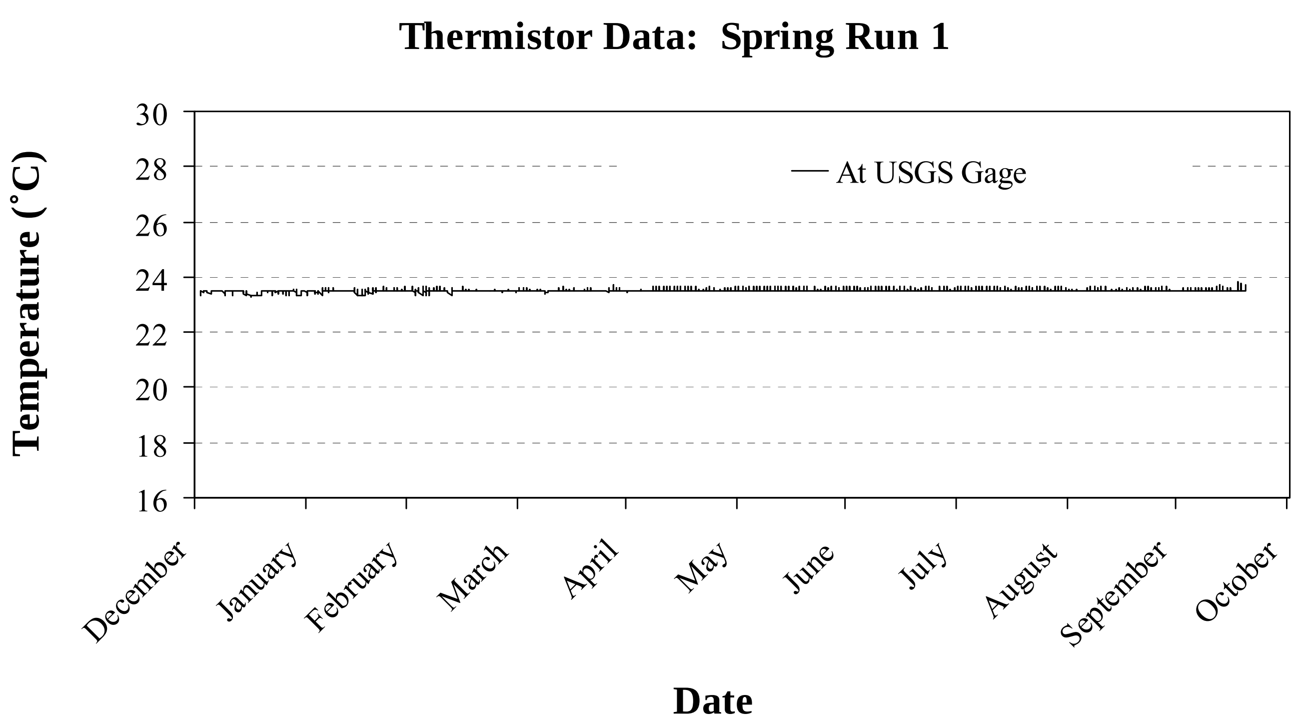


Figure 6. Thermistor data from Spring Run 1 (December 2006 – November 2007).

Water temperatures at the three Spring Runs varied less than 1 °C, which is similar to previous years (Figure 6, Appendix B). The Spring Island (west channel) exhibited more variable temperatures because the area where the thermistor is located is more influenced by upstream inputs including runoff, whereas the east channel shows less variable temperatures because it is nearer to spring inputs. As water travels downstream to Landa Lake it receives more water from spring inputs including several along the western shore of the lake. As a result, water temperatures vary slightly in the lake (23.4 – 23.9 °C). The Old Channel reach receives water from Landa Lake through a culvert that regulates flow in this section. Temperatures still vary widely here because it is far enough downstream of spring inputs that environmental influences (i.e. precipitation) are more of a controlling factor. In 2007, water temperatures here ranged from 13.4 °C (the lowest recorded temperature at all sites in 2007) to 26.5 °C (Appendix B). The New Channel is also affected by runoff and precipitation, but temperatures here (or at any of the thermistor locations excluding Blieder's Creek) did not exceed the Texas Commission on Environmental Quality (TCEQ) water quality standards value of 26.67 °C. The thermistor located farthest downstream from spring inputs (The Other Place) recorded a low temperature of 16.9 °C shortly after a cold precipitation event in February 2007.

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

By April 2007, discharge in the Comal River began to increase due to several precipitation events over the Edward's Aquifer, and average monthly flows were above the historical average. These increased flows appeared to stimulate growth in the Upper Spring Run Reach. Total vegetation area increased substantially from fall 2006 (2,772.5 m^2) to spring 2007 (3,767.0 m^2). *Hygrophila* exhibited a large increase in 2007 with many of the larger stands in the lower portion of the reach growing together to form a single unit. In addition, *Hygrophila* stands in the upper most portion of the reach nearly extended from bank to bank. *Hygrophila* continued its expansion into fall where it increased to 713.9 m^2. While this non-native vegetation expanded its range, the native vegetation decreased in the presence of higher discharge. *Ludwigia* area shrank by nearly half from 42.0 m^2 in fall 2006 to 26.7 m^2 in April 2007, and decreased slightly more by fall 2007 (25.7 m^2). Similarly, *Sagittaria* decreased by 52 m^2 from 2006 to 2007. By October 2007, *Sagittaria* had recovered with plants filling in spaces previously occupied by bryophytes. The number of *Cabomba* plants had been decreasing steadily in recent years (including losing its hold in the upper part of the reach), and in 2007 no plants were observed in the Upper Spring Run.

The increased flows of 2007 stimulated bryophyte growth throughout the entire reach. These mosses also appeared to better attach to the substrate because they covered large areas of this reach despite high discharges that usually flush them out of the reach. It must be noted, however, that bryophyte area in this reach was not measured immediately after flushing events and the fall 2007 growth may be a result of quick recovery of these mosses. From 2006 to April 2007 bryophyte area nearly doubled (1,250.8 m^2 to 2,357.8 m^2). Bryophyte area increased slightly by October 2007 (2,407.2 m^2). Although native plants appear to be losing a foothold in this reach (*Cabomba*, *Ludwigia*), bryophytes continue to provide vital fountain darter habitat because of the structure they offer throughout the reach.

Landa Lake Reach

Vallisneria continued to dominate the central and lower portions of the Landa Lake Reach where depths tend to be greatest. It increased slightly from 2006 to April 2007 (13,243.7 m^2 to 13,412.6 m^2) because the plants filled in several areas that were previously bare substrate. This increasing trend continued into the fall as more gaps were filled with these plants (13,601.9 m^2). *Hygrophila* continued to fill in spaces in the central portion of the reach between the islands. *Hygrophila* increased from November 2006 (520.3 m^2) to April 2007 (620.4 m^2) by filling in gaps between *Ludwigia* plants in the center of the lake. By October, however, bare spots in the *Hygrophila* formed decreasing the total surface area (549.4 m^2). This fragmentation may be due to mechanical damage from paddle boats because this area is shallower than other parts of the lake.

Several native plants in the lake declined in overall area. *Ludwigia* decreased by nearly half from November 2006 (41.0 m^2) to April 2007 (21.9 m^2). Most of these plants are located in the central part of the reach between *Hygrophila* and *Vallisneria* stands. For several years a trend has developed where the *Hygrophila* mixes in with these *Ludwigia* plants leading to fewer of the native plant (though the reasons why this occur are unclear). Therefore, an increase in *Hygrophila* in this part of the reach often coincides with a decrease in *Ludwigia* (exemplified by the change in proportion of these plants from 2006 to 2007). This is further observed by the change in these plants in 2007. From April to October 2007, *Ludwigia* increased (21.9 m^2 to 37.1 m^2) while *Hygrophila* decreased. These changes can be observed in the small area in the central part of the reach where most of the *Ludwigia* is located. Another native plant, *Cabomba* also decreased from 2006 (332.4 m^2 to 181.0 m^2) when a large plant near the golf course side of the lake fragmented substantially. It is unclear if flows were a mechanism by which this plant lost much of its surface area. This plant (and others) did recover by October 2007 increasing the surface area of *Cabomba* to 272.5 m^2. After several years of data collection, it has become clear that *Cabomba* is an important plant for fountain darter habitat. However, only two plants in Landa Lake are shallow enough to assess this trend making it difficult to pinpoint distinct population changes in fountain darters in more of the *Cabomba* plants in the lake. Unlike some of the other native vegetation, *Sagittaria* still thrives in the Landa Lake Reach. In spring it increased to 1,217.4 m^2, but by fall it appeared to decrease dramatically (651.1 m^2). This can be attributed to a layer of green algae that covered much of the lake in October 2007. As a result half of the *Sagittaria* was classified as a *Sagittaria*/algae mix (697.1 m^2).

Bryophytes continued to dominate large expanses in the upstream portion of the lake. In spring the surface area swelled to 2,778.5 m^2, and decreased only slightly by fall (2,601.0 m^2). In previous years, bryophytes decreased substantially by the fall sampling period likely due to rain events scouring them out of the system. In 2007, large rain events occurred in July and August, but bryophytes remained a large presence in the lake by October. It is unclear if these mosses were scoured out and regrew, or if they managed to attach to the substrate during these flushing events. This growth could also be due to a BIO-WEST hypothesis that higher CO_2 levels resulting from greater discharge stimulate bryophyte growth. In addition, a lab study confirmed that growth of the two bryophytes (*Riccia* and *Amblystegium*) was clearly related to CO_2 concentration (BIO-WEST 2004). Therefore, CO_2 concentration is likely a key factor in the spatial and temporal bryophyte abundance in the Comal system. The bryophytes remain an important vegetation type in Landa Lake because they support the greatest densities of fountain darters in that reach (of the vegetation types sampled).

Old Channel Reach

Until 2003, the Old Channel Reach maintained the most stable aquatic vegetation community with a structure (culvert) that regulates flow through this section. Later, the USFWS reconstructed this culvert to increase its capacity which resulted in increased and more variable discharge that has affected aquatic communities downstream.

In recent years, non-native vegetation (*Hygrophila*, *Ceratopteris*) have begun to out-compete and displace native vegetation (*Ludwigia*, filamentous algae) in the Old Channel Reach. However, at the beginning of 2007 it looked like this trend may be starting to change. *Hygrophila* decreased by April 2007 (1,292.3 m^2 to 1,260.3 m^2), while *Ludwigia* surface area tripled (35.5 m^2 to 108.0 m^2). It appeared that increased flow in this reach over winter stimulated *Ludwigia* growth. In addition, filamentous algae (considered high quality fountain darter habitat) surface area expanded to 12.2 m^2 with two new patches appearing along the inner bend of the river in the middle of the reach. By fall, however, *Ludwigia* decreased to 18.6 m^2, which was less than what was measured in November 2006. *Hygrophila* replaced many of these patches that *Ludwigia* formerly occupied and increased to 1,518.7 m^2 in surface area. *Ludwigia* was relegated to several patches near the upstream end of the reach.

The interaction between these plants in this reach is very important to fountain darter populations. With *Hygrophila* dominating more of this reach, fountain darter populations may decrease because this vegetation type provides lower quality habitat than the native vegetation it replaced. This ebb and flow of non-native versus native vegetation areas will likely have lasting implications on fountain darter populations in this unique reach.

New Channel Reach

Vegetation in the New Channel Reach continued to increase after much of it was wiped out in early 2005. This recolonization began with *Hygrophila* (which was the only plant present after this event), and has continued with *Ludwigia*, *Cabomba*, and bryophytes. Flushing events did occur several times in 2007, but the vegetation that has gained a foothold in this highly channelized reach continued to flourish. *Hygrophila* increased from November 2006 (715.4 m^2) to April 2007 (1,107.6 m^2), and into October 2007 (1,300.0 m^2), where it reached its greatest surface area since this studies inception. Native flora like *Ludwigia* and *Cabomba* also gained footholds in this reach. *Ludwigia* procured two small patches near the upper end of the reach in April, but by October one patch disappeared and another formed in the lower end of the reach. *Cabomba* faired much better with several patches growing along the north wall in spring (106.9 m^2), which grew together to over 5X the surface area by fall (535.5 m^2). Since this reach is too deep for drop net sampling it is difficult to assess fountain darter numbers in native versus non-native vegetation. However, if other reaches are any indication, the growth of native plants may be an important factor in establishing fountain darter populations in the New Channel.

Higher than average discharge was prevalent in 2007 in the Comal River/Ecosystem. Increased flows were especially important because 2006 was a below average year for discharge. These flows appeared to stimulate growth in all reaches, however, with the exception of bryophytes, non-native vegetation (especially *Hygrophila*) seemed to benefit most. If these trends continue, bryophytes will continue to be the most important vegetation for fountain darter populations in the Comal River.

Fountain Darter Sampling

Drop Net Sampling

A total of 584 drop net samples were conducted during 2000-2007 in the Comal Springs/River ecosystem. Forty-four of these samples (22 in spring and 22 in fall) were conducted in 2007. The number of drop net sites and vegetation types sampled per reach are presented in Table 6. Drop net site locations are depicted on the aquatic vegetation maps (Appendix A) for the respective reaches per sample event, and data sheets for the drop net sampling are presented in Appendix C by reach and specific site, respectively. There were some changes over the course of the study including a shift from sampling two bare substrate sites during each sampling event in the Upper Spring Run and Landa Lake in 2000-2001 to sampling two bryophytes sites in those reaches beginning in the summer of 2001. In 2004, there was a change in the sample design for the Old Channel Reach in response to the dramatic shift from a vegetation community dominated by filamentous algae and *Ceratopteris* to one dominated by *Hygrophila* and *Ludwigia*. Also, in 2005 the New Channel Reach was removed from the drop net sampling effort as vegetated areas were often too deep to sample.

Table 6. Drop net sites and vegetation types sampled per reach in 2007.

UPPER SPRING RUN REACH	LANDA LAKE REACH	NEW CHANNEL REACH	OLD CHANNEL REACH
Bryophytes [a] (2)	Bryophytes [a] (2)	None [b]	*Ludwigia* (2)
Sagittaria (2)	*Hygrophila* (2)		*Hygrophila* (3) [c]
Hygrophila (2)	*Cabomba* (2)		Filamentous Algae (1) [d]
	Vallisneria (2)		
	Ludwigia (2)		
Total (6)	**Total (10)**	**Total (0)**	**Total (6)**

[a] Switched from Open to Bryophytes, summer 2001.
[b] Areas with vegetation were too deep to sample in spring and fall 2007.
[c] Three Hygrophila sites were sampled in 2007 sample to make up for one filamentous algae site.
[d] Only one filamentous algae site was sampled in 2007 due to limited coverage.

The diversity of aquatic vegetation found in the Comal Springs/River ecosystem provides a range of available habitat for fountain darters covering a wide level of suitability. Data collected from 2000-2007 show that average densities of fountain darters in the various vegetation types ranged from 3.6 per 1.0 m^2 in *Ceratopteris* to 26.3 per 1.0 m^2 in filamentous algae (Figure 7). Native vegetation types which provide thick cover at or near the substrate (i.e., filamentous algae, and bryophytes [23.3/m^2]) tend to have the highest fountain darter densities. Filamentous algae and bryophytes also contain high numbers of amphipods, a common food item for fountain darters. In contrast, exotic vegetation (*Ceratopteris* and *Hygrophila* [6.9/ m^2]), and native vegetation with simple leaf structures (*Vallisneria* [4.2/ m^2] and *Sagittaria* [5.4/ 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.

Filamentous algae and bryophytes, which provide the best fountain darter habitat, are also the most susceptible to scouring during high-flow events and have shown considerable fluctuation in coverage over the study period. Filamentous algae was once the dominant vegetation type in the Old Channel Reach, however, it has been replaced in recent years by *Hygrophila* and *Ludwigia*. This has resulted in an overall decrease in the abundance of fountain darters in this reach (see dipnet data). Although densities are slightly less in bryophytes than in filamentous algae, bryophytes are a key habitat component because they occupy large areas of the Upper Spring Run and Landa Lake reaches, and thus make up a significant portion of the available habitat. Bryophytes were also starting to appear in the Old Channel Reach in fall 2007. *Cabomba* and *Ludwigia* are also relatively common and therefore provide substantial amounts of fountain darter habitat. Although fountain darter densities are relatively low in *Hygrophila*, it is an important habitat component because it is abundant in all sample reaches.

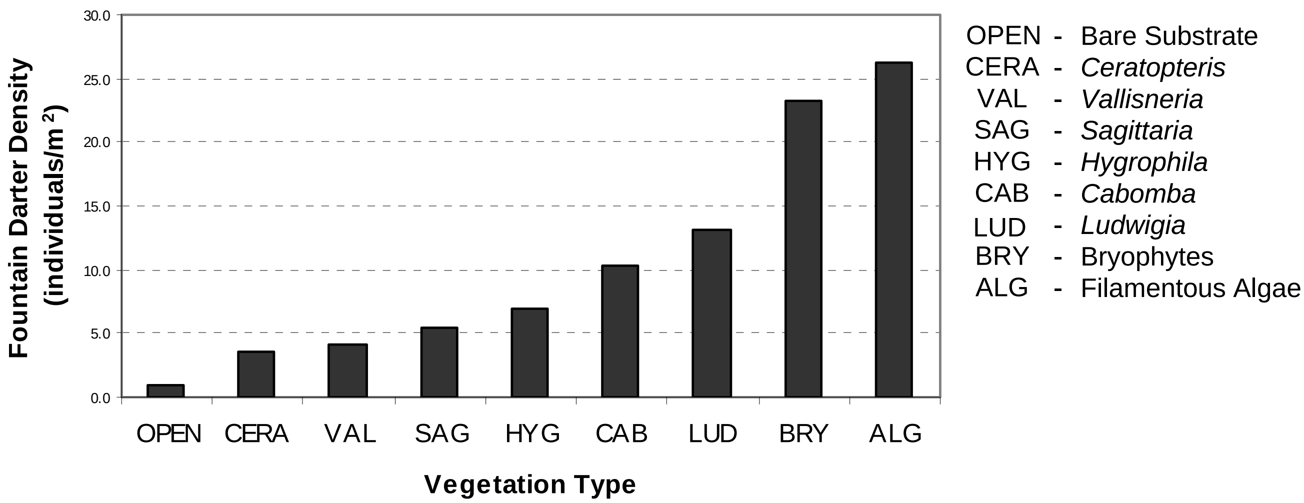


Figure 7. Density of fountain darters collected by vegetation type in the Comal Springs/River ecosystem from 2000-2007.

Estimates of fountain darter population abundance in all reaches (Figure 8) were based on the changes in vegetation composition and abundance and the average density of fountain darters found in each, as described in the methods section. The vegetation type that had the greatest influence on these estimates was the bryophytes because of the size of the Landa Lake Reach (where most of the bryophytes were mapped) and the density of fountain darters found there. Thus, as coverage of bryophytes in this reach fluctuate, so do fountain darter population estimates. Estimates of population abundance were highest in spring 2003 when coverage of bryophytes peaked in Landa Lake. Population estimates for spring and fall 2007 were considerably higher than those from 2004-2006. Although they did not approach the highest estimates observed in spring 2003, they fell within the range of variation observed during the study period. Population estimates in fall 2000, winter 2001, and spring 2001 are low because mapping at the time did not include algae in the Old Channel Reach or bryophytes in the Landa Lake Reach. All four high-flow Critical Period samples during the study period showed a decrease in the population estimate relative to the previous sample, although there was an increase in the subsequent sample each time. This is most likely related to scouring of important vegetation types resulting in fountain darters becoming more scattered at high flows.

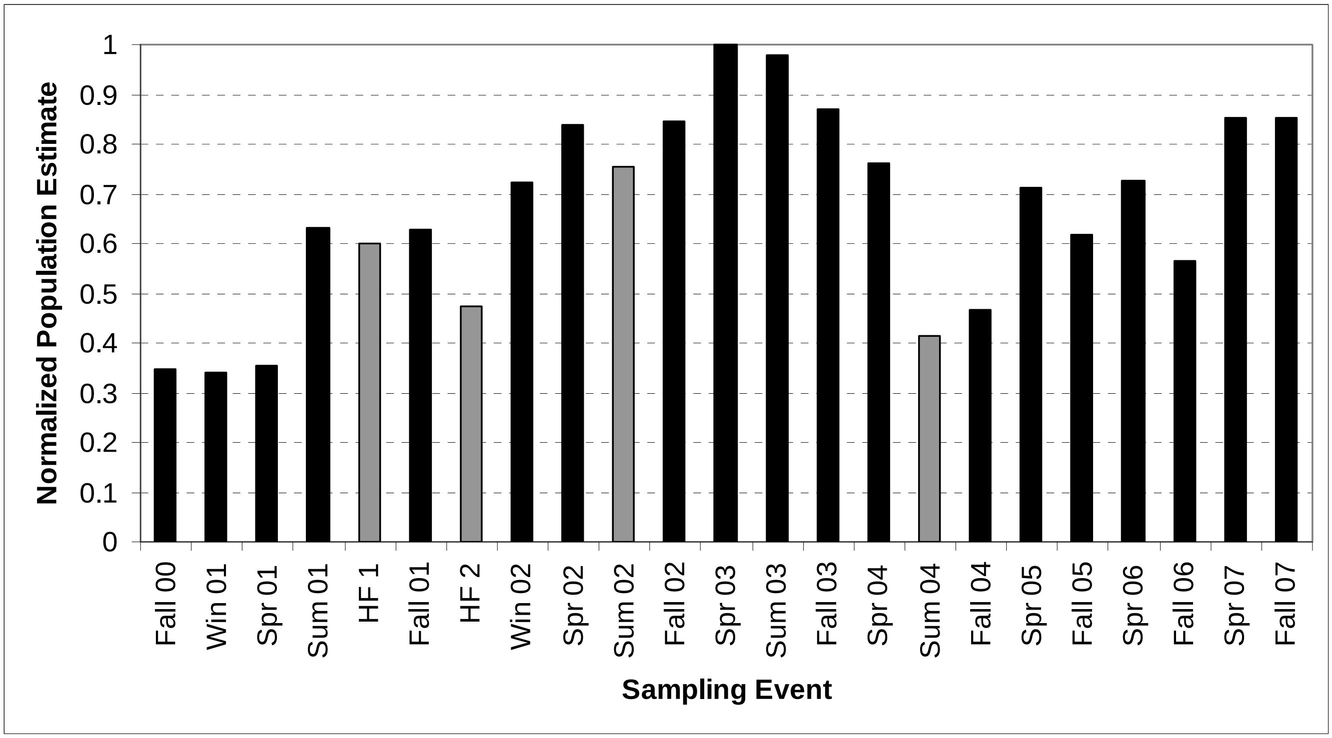
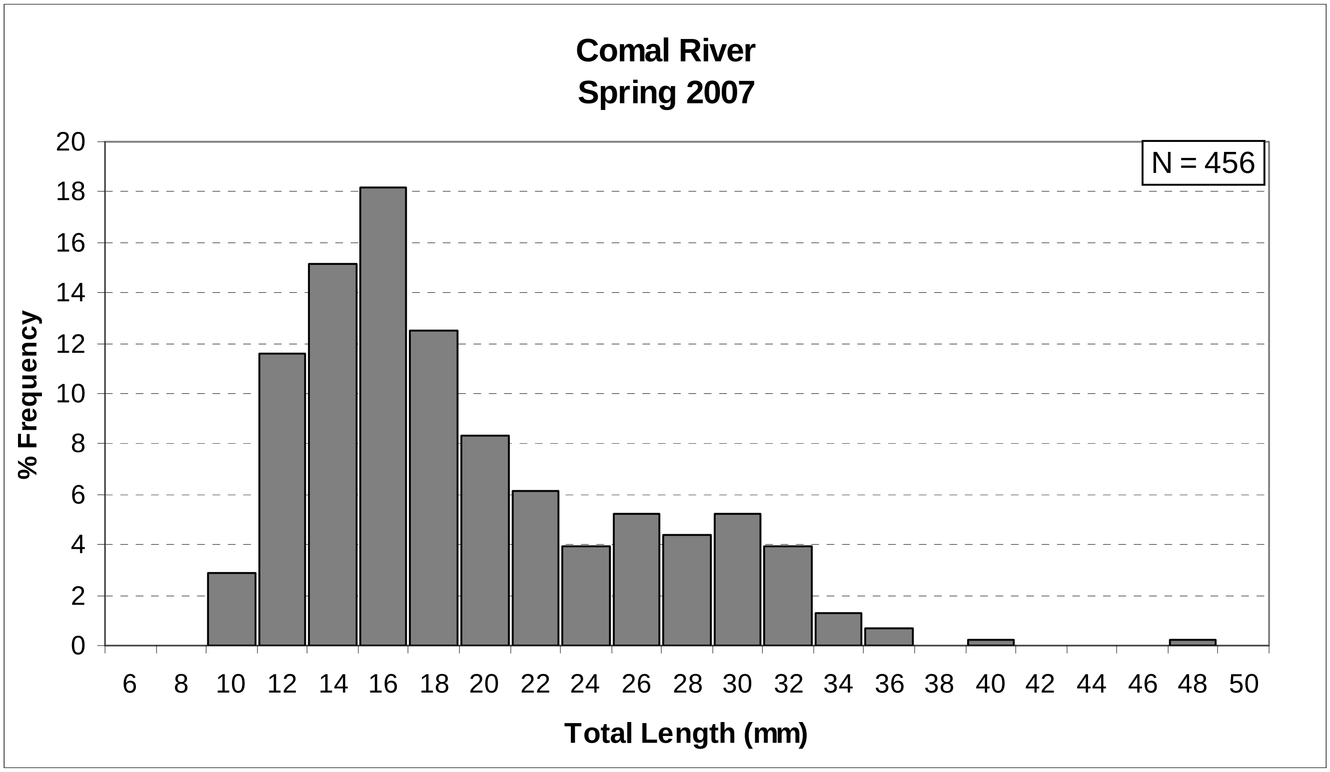


Figure 8. Population estimates of fountain darters in all four sample reaches combined (2000-2007); values are normalized to the maximum sample. Light-colored bars represent high-flow Critical Period sampling events.

Drop netting efforts in 2007 resulted in collection of 1,045 fountain darters in the Comal River/Springs ecosystem. The size-class distribution for fountain darters collected by drop nets from the Comal ecosystem during each sample period in 2007 is presented in Figure 9 (all data collected in previous years is presented in Appendix B). As in previous years, small fountain darters are more abundant in the spring sample suggesting a peak in reproduction during this time. However, in some reaches reproduction seems to occur year-round. Small darters were present in both spring and fall samples in the Upper Spring Run Reach suggesting that year-round reproduction is occurring in this higher quality habitat dominated by bryophytes (Figure 10). In the Old Channel Reach, length frequency histograms suggest little, if any, reproduction in the fall months.



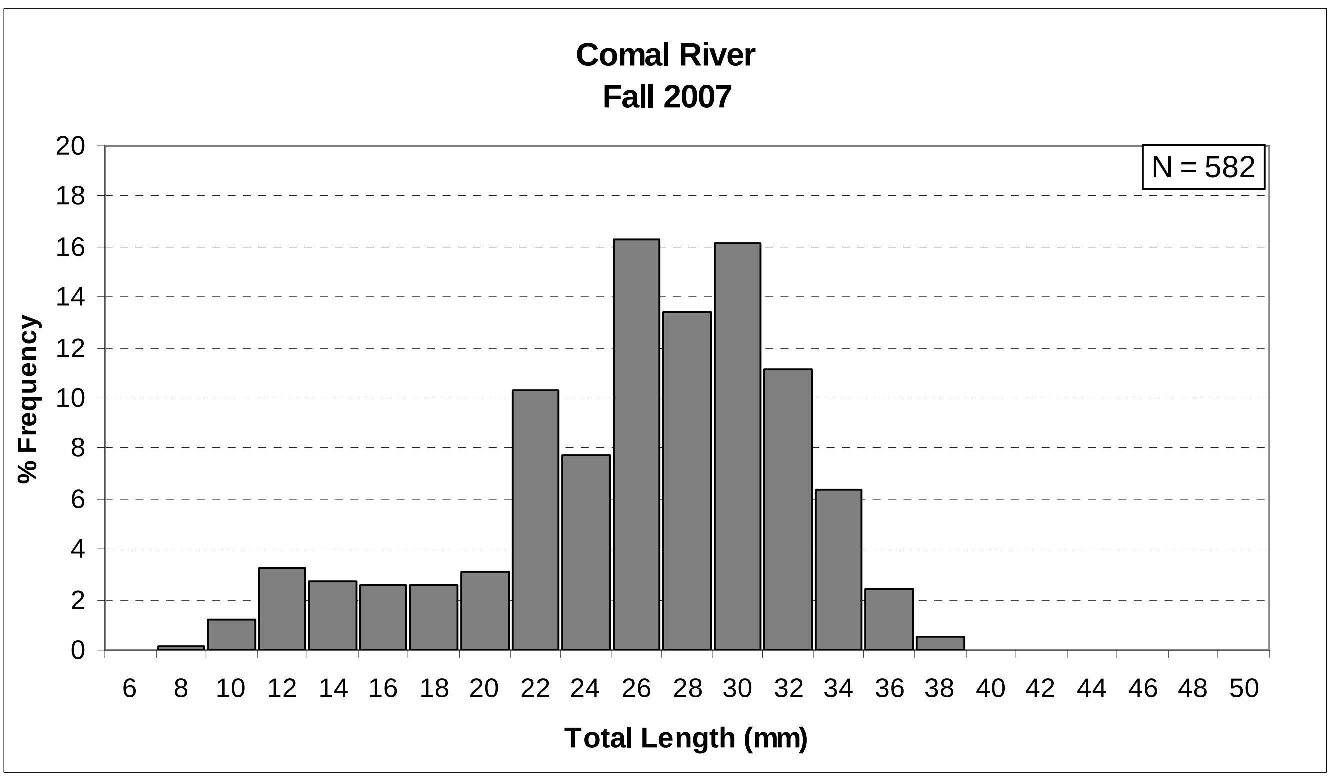


Figure 9. Length frequency distribution of fountain darters collected from the Comal River by drop-netting in spring and fall 2007.

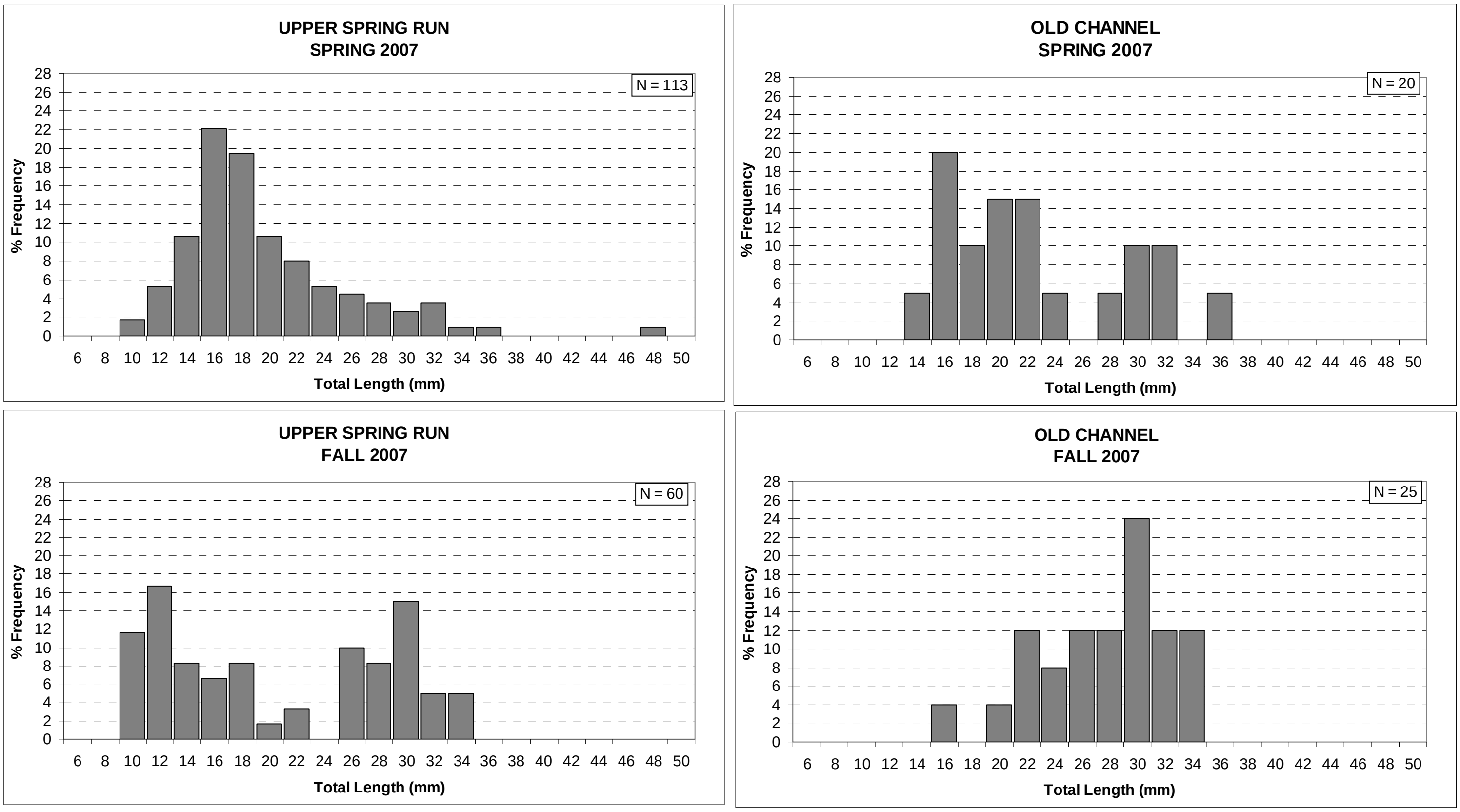


Figure 10. Length frequency distributions of fountain darters collected in spring and fall 2007 in the Old Channel and Upper Spring Run Reaches.

In addition to fountain darters, 79,182 specimens representing at least 24 other fish taxa have been collected by drop netting from the Comal Springs/River ecosystem during the study period; of these, seven are considered exotic or introduced (Table 7). Although several of these species are potential predators of fountain darters, previous data collected during this study suggested that predation by both native and introduced predators is minimal during average discharge conditions. The impact of predation is to be further evaluated under low discharge.

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

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

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

COMMON NAME	SCIENTIFIC NAME	STATUS	NUMBER COLLECTED 2007	2001-2007
Rock bass	*Ambloplites rupestris*	Introduced	0	18
Black bullhead	*Ameiurus melas*	Native	0	1
Yellow bullhead	*Ameiurus natalis*	Native	4	85
Mexican tetra	*Astyanax mexicanus*	Introduced	34	285
Central stoneroller	*Campostoma anomalum*	Native	0	1
Rio Grande cichlid	*Cichlasoma cyanoguttatum*	Introduced	24	355
Guadalupe roundnose minnow	*Dionda nigrotaeniata*	Native	1	260
Fountain darter	*Etheostoma fonticola*	Native	1045	10466
Greenthroat darter	*Etheostoma lepidum*	Native	2	55
Gambusia	*Gambusia sp.*	Native	5403	72233
Suckermouth catfish	*Hypostomus plecostomus*	Exotic	1	60
Redbreast sunfish	*Lepomis auritus*	Introduced	13	132
Green sunfish	*Lepomis cyanellus*	Native	0	10
Warmouth	*Lepomis gulosus*	Native	0	24
Bluegill	*Lepomis macrochirus*	Native	0	30
Longear sunfish	*Lepomis megalotis*	Native	2	38
Redear sunfish	*Lepomis microlophus*	Native	1	1
Redspotted sunfish	*Lepomis miniatus*	Native	97	1075
Sunfish	*Lepomis sp.*	Native/Introduced	32	663
Spotted bass	*Micropterus punctulatus*	Native	0	1
Largemouth bass	*Micropterus salmoides*	Native	4	86
Texas shiner	*Notropis amabilis*	Native	1	34
Mimic shiner	*Notropis volucellus*	Native	27	28
Sailfin molly	*Poecilia latipinna*	Introduced	221	3689
Blue tilapia	*Oreochromis aurea*	Exotic	2	18

Dip Net Sampling

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

Figure 11 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 *Hygrophila*. This switch resulted in a corresponding change in the fountain darter population. Before 2005, the number of darters collected per sample ranged from 54 to 130 and all samples contained small darters (<15 mm) indicating year-round reproduction. Since this change in vegetation, total number of darters per sample has ranged from 9 to 48 and small darters have only been collected in spring months. However, bryophytes were noted in the Old Channel in Fall 2007. If bryophytes become established in the Old Channel, it will likely lead to a rebound in the number of fountain darters collected in this reach.

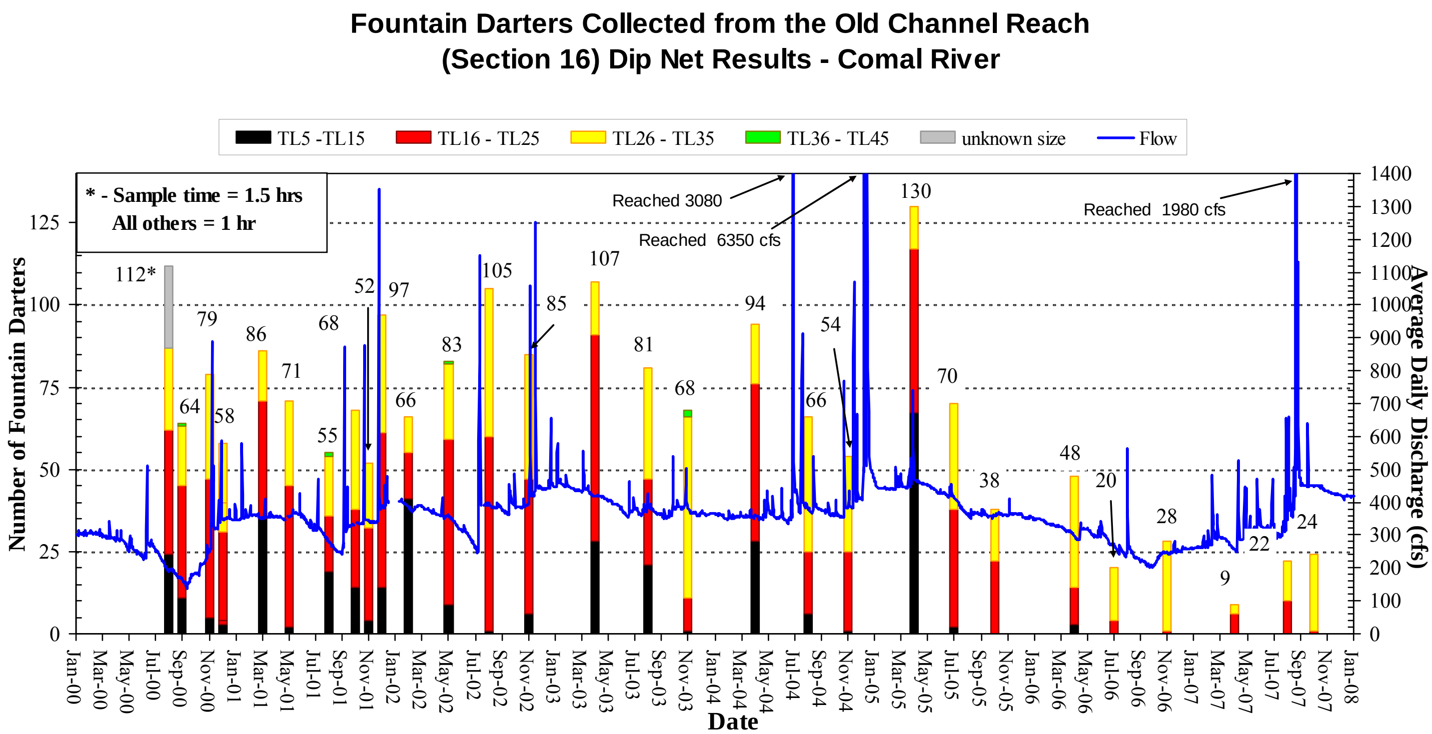


Figure 11. Number of fountain darters, by sample date and size class, collected from the Old Channel Reach (section 16) using dip nets.

Overall, size class distributions of fountain darters from dip netting correlate well with those of drop netting: small fountain darters most abundant in the spring, and larger darters dominating fall samples (Appendix B). However, small fountain darters are occasionally captured in summer, winter, and fall sample periods as well. This indicates that there is some reproduction occurring year-round, although perhaps on a limited basis and only in certain areas. In 2007, areas exhibiting year-round reproduction were relatively close to spring upwellings and contained large amounts of bryophytes, which provided high-quality fountain darter habitat according to drop net density estimates.

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

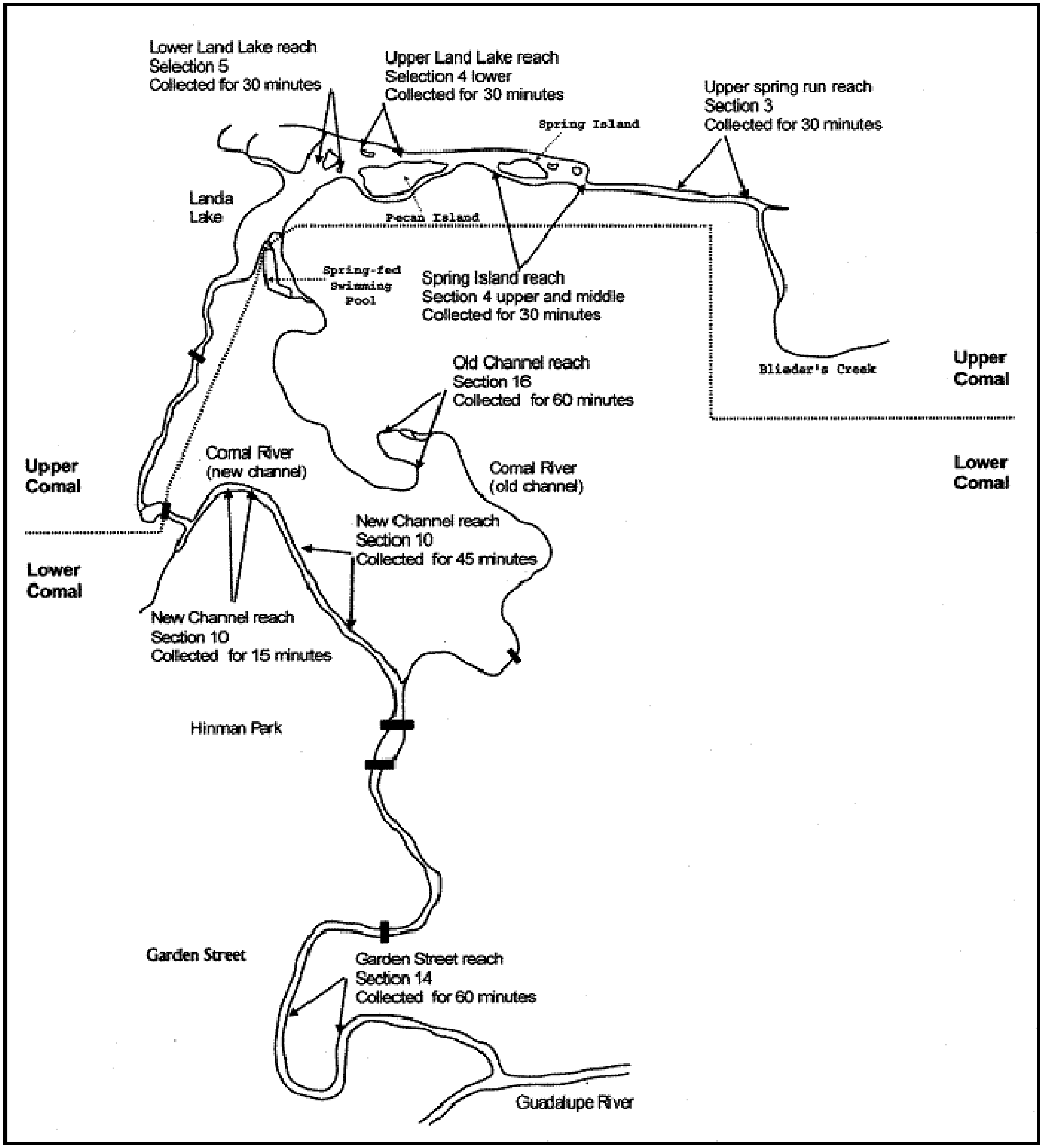


Figure 12. Areas where fountain darters were collected with dip nets, measured, and released in the Comal River.

Dip Net Techniques Evaluation

The overall percentage of sites and percentage of dips in which fountain darters were present has remained relatively consistent since initiation of this technique in Fall 2005 (Figure 13). The percentage of sites (N = 50) containing darters has varied from 60-70% across five sample periods, and the percentage of dips (N = 4 @ each site = 50*4 = 200) containing darters has varied from 35-45%.

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

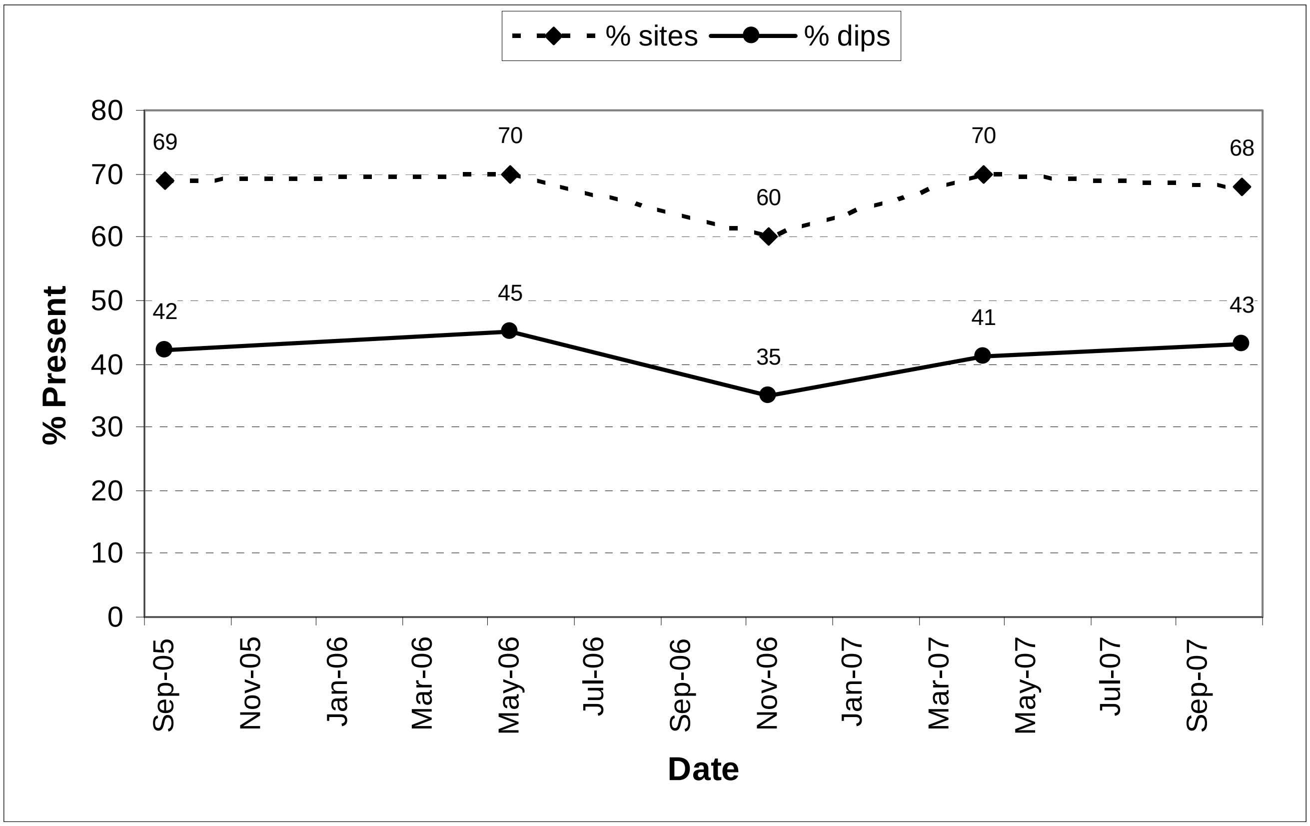


Figure 13. Percentage of sites (N = 50) and percentage of dips (N = 200) in which fountain darters were present during spring and fall 2005-2007.

Visual Observations

Fountain darters were observed in the deepest portions of Landa Lake (depths greater than 2 m) during each sampling event, including all low-flow and high-flow events to date. The quantitative sampling results are limited to a single grid per sampling event; therefore an accurate estimate of the true population size within the sample area is not possible. A much more labor-intensive effort would be required to provide such an estimate. These data simply provide an indication of the relative abundance of the fountain darters that are found in areas similar to that sampled and allow some insight into trends that may be occurring over time. Table 8 shows the number of fountain darters observed in the 7.8 m^2 grid per sampling event.

Table 8. The number of fountain darters observed in Landa Lake per grid/sampling event.

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

Spring 2007 exhibited a continued decreasing trend in the number of darters in the deepest areas of Landa Lake despite a large increase in bryophyte coverage. Although there was ample coverage of bryophytes (75%), only 27 darters were observed. This low number may reflect recent bryophyte growth, but the darters had yet to occupy the habitat. In contrast, 87 darters were observed in fall 2007, the second highest number recorded since the study's inception. With bryophyte coverage also at its highest percentage recorded in the study (along with Fall 2005), it is apparent that fountain darters prefer higher bryophyte densities for cover and foraging. Continued observation will likely reinforce data (from drop netting also) suggesting that fountain darters prefer this habitat to others.

Gill Parasite Evaluation

Below is a brief summary of results from the special *C. formosanus* cercarial drift study conducted by Ms. Anne Bolick in 2007. For a more complete discussion of results and conclusions please see Ms. Bolick's thesis in Appendix D.

Neither total stream discharge (measured at the USGS gauge) nor wading discharge (measured at each transect when collections were taken) were found to be a useful predictor of cercarial abundance. Cercarial abundance differed between sites and increased with distance downstream. Downstream sites received cercarial drift from their immediate area, as well as areas upstream. Abundance differed in relation to sun intensity as well, with higher cercarial counts on sunny days than cloudy days. Season also seemed to influence abundance of cercariae with the period from late fall to early spring having the highest abundance of the parasite. In order to reduce confounding effects of site, sunlight intensity, and season, the dataset was restricted to include only samples from the upstream most site (Houston Street) on sunny days from late fall to late spring. However, even after removing these confounding effects all measured variables were still found to be poor predictors of cercarial abundance.

Results suggest that fountain darter populations in the Comal River will not experience increased infection pressures from *C. formosanus* as a result of reduced discharge. However, this result is only applicable over the range of discharges observed during this study. Should extremely low flows occur due to drought conditions, cercarial abundance may increase to a more severe level. However, this study does provide critical baseline data for comparison to cercarial densities in future low-flow situations.

Comal Springs Salamander Visual Observations

The spring season snorkel surveys revealed the presence of Comal Springs salamanders within each sampled Spring Run. Comal Springs salamanders were not observed in the Spring Island Spring Run in fall 2007, where only few individuals have been found during the past three years (fall 2004 -present). An average number of Comal Springs salamanders were observed in the remaining sampling reaches during the fall season. No Comal Springs salamanders were observed in any areas with excessive sediment. The total number of Comal Springs salamanders observed at each survey site during each sampling event is presented in Table 9.

Two salamanders were found in the Spring Island spring run in 2007, where four had been found in 2006. An increased number of fist sized rocks coupled with large patches of *Amblystegium* in the Spring Island outfall contributed to 23 individuals observed in spring 2007. Only 11 individuals were located in fall. Coverage of byrophytes was similar to spring, but green algae also covered more of the area and may have led to fewer salamanders where the algae were present.

Salamander numbers gradually increased from spring 2006 (12) to the fall 2007 (18) in Spring Run 1. With the higher flows of 2007, there were less vegetation present in this reach, but it appeared little else had changed (number of fist sized rocks). Salamander numbers in Spring Run 3 were similar to other years, and remained consistent between spring 2006 and fall 2007 (13 and 13, respectively). It appears that higher flows had little effect on overall salamander populations in the spring runs with the total number observed (42) only slightly below average.

Table 9. Total number of Comal Springs salamanders observed at each survey site during each sampling period.

SAMPLE PERIOD	SPRING RUN 1	SPRING RUN 3	SPRING ISLAND SPRING RUN	SPRING ISLAND EAST OUTFALL	TOTAL BY SAMPLE
August 2000	9	13	11	1	34
September 2000	5	14	6	5	30
Fall 2000	8	4	4	2	18
Winter 2001	16	9	8	1	34
Spring 2001	20	7	17	6	55
Summer 2001	23	15	4	4	46
High-flow 1 2001	31	12	1	6	50
Fall 2001	11	8	13	7	39
High-flow 2 2001	18	2	6	5	31
Winter 2002	18	9	7	3	53
Spring 2002	10	15	6	5	62
High Flow 2002	18	7	3	16	67
Fall 2002	20	10	8	9	47
Spring 2003	20	21	6	13	60
Summer 2003	25	10	3	13	51
Fall 2003	31	10	3	19	63
Spring 2004	36	14	7	12	69
Summer 2004	27	14	4	14	59
Fall 2004	20	2	2	35	59
Spring 2005	18	10	2	11	41
Fall 2005	22	7	0	16	45
Spring 2006	12	13	2	8	35
Fall 2006	14	11	2	29	56
Spring 2007	15	10	2	23	50
Fall 2007	18	13	0	11	42
Average	**18.6**	**10.4**	**5.1**	**11.0**	**45.0**

Macroinvertebrate Sampling

In 2007, drift net sampling around spring openings and regular monitoring of Comal Springs riffle beetles in several locations were designed to assess habitat requirements and population dynamics of the federally listed invertebrate species.

Drift Net sampling

A total of 14 taxa were captured from 144 hours of sample time at the three drift net sites in Comal Springs during 2007 (Table 10). Table 11 displays the physico-chemical data collected at these sites during sampling. Total discharge in the Comal River was approximately 322 cfs during the May sample and 418 cfs during the November sample.

Species of the genus *Stygobromus* and *Lirceolus* continued to be most abundant at all sites (Table 10). *Stygobromus pecki* (Peck's cave amphipod) was the dominant amphipod (among identifiable individuals) at all sites. Most amphipods caught in this study were only a few millimeters long, which suggests that smaller individuals may be more susceptible to expulsion from the aquifer. Those individuals that were too small to identify to species were recorded as *Stygobromus* sp. and most likely consisted of both *S. russelli* and *S. pecki*.

Another specimen of a rare subterranean amphipod *Parabogidiella* sp. was collected in November 2007. Two specimens of this blind amphipod in the Bogidiellidae family were also collected in November 2006. However, the more recent specimen appeared to have most of its body parts and will likely be useful for a future species description. In May, a single specimen of *Stygobromus bifurcates* was collected which represents a new county record. This species is related to *S. russelli* and is recorded from wells and caves in Kendall, Travis, Hays, Coryell, Lampasas, and San Saba Counties. This is the 4th species of *Stygobromus* collected at Comal Springs (*S. pecki, S. russelli, S. flagellatus, S. bifurcates*). No other locality (> 100 spp. in North America) is recorded to have this many co-occurring species except San Marcos Springs in Hays County (*S. flagellatus, S. russelli, S. bifurcates, S. longipes*). Specimens from this and previous surveys at Comal will be incorporated into a genetics project with Texas State University to better determine systematics, hybridization, dispersal, isolation, and connectivity between populations of *Stygobromus* in Texas.

A total of 4 *Heterelmis comalensis* and 11 *Stygoparnus comalensis* were found in the Spring Run sites in 2007. As in previous years, *H. comalensis* and *S. comalensis* were only found in Spring Runs 1 and 3. One *S. comalensis* was collected in the upwelling site in 2003, but no *H. comalensis* have been discovered there. A possible reason for the lack of these organisms in the upwelling could be due to the source of the spring. The water in Spring Runs 1, 2, and 3 is connected to the shallower recharge zone in the aquifer, while water in the springs in Landa Lake and upstream are connected to the deeper artesian flow system (G. Schindel, pers. comm., 2007). These springs (Landa and upstream) are warmer (Table 11), and because the water comes from a deeper source, there may not be an ample food source for the beetles.

Table 10. Total numbers of troglobitic and endangered species collected in drift nets during May and November, 2007. Federally endangered species are designated with (E). A = adult beetles. L = larvae.

	Run 1	Run 3	Upwelling	Total
Total Drift Net Time (hrs)	48	48	48	144
Crustaceans				
Amphipoda				
Crangonyctidae				
Stygobromus pecki (E)	18	27	57	102
Stygobromus russelli	2	1	1	4
Stygobromus bifurcatus		1		1
Stygobromus spp.	121	120	406	647
All *Stygobromus*	141	148	464	753
Hadziidae				
Mexiweckelia hardeni	23	6	1	30
Sebidae				
Seborgia relicta	5	3	3	11
Bogidiellidae				
Artesia subterranea	1			
Parabogidiella n. sp			1	1
Isopoda				
Asellidae				
Lirceolus (2spp.)	67	57	12	136
Cirolanidae				
Cirolanides texensis		1		1
Arachnids				
Hydrachnoidea				
Hydryphantidae				
Almuerzothyas n. sp	22	3		25
Insects				
Coleoptera				
Dytiscidae				
Comaldessus stygius	2 A	15 A		17
Haideoporus texanus		3 L		3
Dryopidae				
Stygoparnus comalensis	5 L	6 L		11
Elmidae				
Heterelmis comalensis	2 L	2 (1L, 1A)		4

Table 11. Results of water quality measurements conducted in 2007 during drift net sampling efforts at Comal Springs.

	Spring Run 1		Spring Run 3		West Shore Upwelling	
Date	May	Nov	May	Nov	May	Nov
Temperature (°C)	23.1	23.1	23.3	23.2	23.8	23.8
Conductivity (mS)	0.552	0.529	0.6	0.530	0.549	0.529
pH	6.8	7.3	6.9	7.3	6.8	7.3
Dissolved Oxygen (mg/L)	5.4	6.2	5.2	5.5	5.4	5.4
Current Velocity (m/s)	0.8	0.8	0.4	0.6	0.3	0.3

As in previous years, water quality variables remained relatively constant at all sites in 2007, indicating a stable environment for the organisms at the observed discharges. As described above, temperatures in the West Shore Upwelling of Landa Lake are slightly warmer than those in the spring runs due to the source of the water.

Comal Springs Riffle Beetle

Comal Springs riffle beetle sampling conducted as part of this study provides basic information on the population dynamics and distribution of the species among sample sites. The total number of beetles collected in 2007 (758) was somewhat lower than that collected in 2005 and 2006 (Table 12, Figure 14) because several cotton cloth lures were disturbed or missing. This was probably due to animals (e.g., raccoons) digging them out of the spring orifices. Average numbers were less than last year and similar to 2004 when several lures were also missing. Despite the low numbers, two new *Heterelmis* locations were documented in the Spring Island area.

During the spring sampling, two *Stygoparnus* adults were collected on a lure in a small spring upwelling through sand, woody debris, and silt in the Spring Island area. In the fall, a *Stygoparnus* larva was collected on a lure in run 3. This is the first time a dryopid larva has been collected on lures in Comal Springs. The larvae of this family are considered terrestrial living in moist areas near flowing water. The lure was planted in a shallow upwelling near the shoreline. It is possible that the larvae may be semi-aquatic moving above and below the water line and between airspaces trapped under the water line created by air bubbles percolating up through spring upwellings into the lake and spring runs of the Comal System.

As in previous years, beetles tended to be patchily distributed with wide ranges of abundance between sites and seasons. Therefore, temporal patterns in overall abundance of Comal Springs riffle beetles are extremely variable (Figure 14). A large increase in abundance of beetles was apparent in spring 2004 when the current method of sampling beetles using cotton rags placed in spring openings was initiated. In 2003, beetles were actively sampled by examining rocks near spring areas, which resulted in much lower catch rates than the current methodology. Since sampling with cotton rags began, the number of Comal Springs riffle beetles has varied between 293 and 648 per sample period. Although this limited amount of data does not allow for detailed analysis of population trends at this time, it will provide critical baseline data for comparison to that collected during potential critical periods in the future.

Table 12. Total numbers of Comal Springs riffle beetles (*Heterelmis comalensis*) at each survey site during each sampling period.

Sample Period	Spring Run 3	West Shore Landa Lake	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**

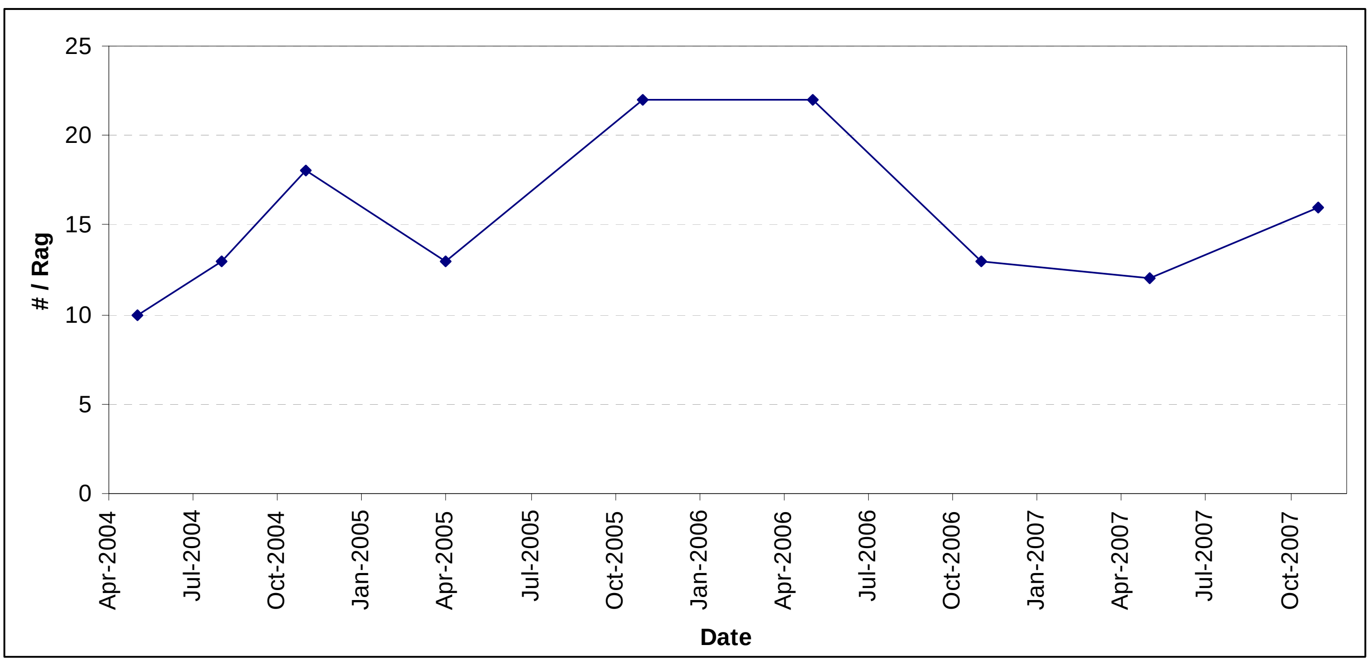


Figure 14. Density (#/rag) of Comal Springs riffle beetles (*Heterelmis comalensis*) combined for each sampling date for 2004 – 2007.

Master Naturalist Observations

Water quality data collected by Master Naturalist volunteers was averaged by site for the 2007 sampling period (January – December) and is presented in Figures 15 and 16. Carbon dioxide concentrations were highest in areas near spring outflows (Houston St. and Gazebo) and decreased downstream. Uptake of carbon dioxide by aquatic plants and the interaction of dissolved gases in the river with the atmosphere resulted in higher pH values in downstream areas (Figure 16). At all sites, dissolved CO_2 measurements were higher in 2007 than in 2006, while the inverse was true for pH values at all sites.

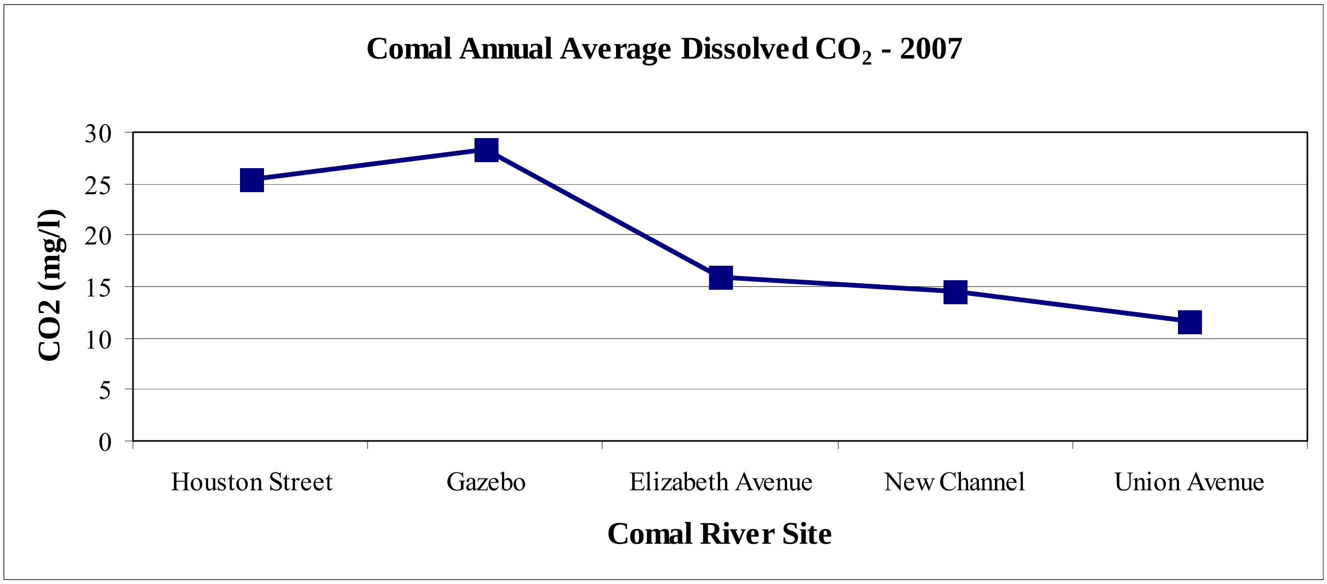


Figure 15. Average carbon dioxide concentrations from weekly samples taken at five sites in the Comal Springs/River ecosystem from January through December 2007.

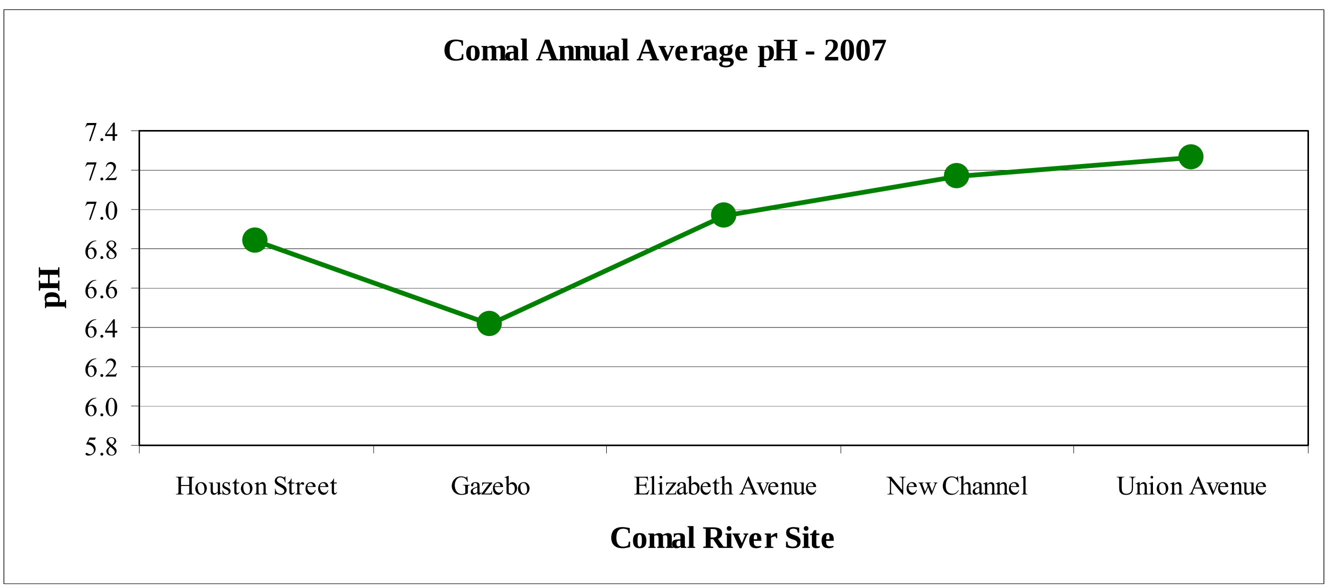


Figure 16. Average pH from weekly samples taken at five sites along the Comal River from January through December 2007.

Recreational use data collected at each of the sites were similar between years, with the most intense use near popular park and tubing areas (61% usage at the New Channel; 17% usage at Union Avenue). The majority of the recreation observed at the New Channel and Union Avenue sites was tubing activity. A moderate amount of recreational users were observed near the Gazebo site at Landa Lake park (16.5% usage), where the majority of people were enjoying the park on foot. Few recreational users were observed at the Houston Street (5% usage) and Elizabeth Avenue (0.5% usage) sites, likely due to limited public access in the area.

Tubers at the New Channel (left) and Union Avenue (right) monitoring sites

Exotics / Predation Study

Because there were no Critical Period events triggered in 2007, no samples were made for the exotics / predation component of this study.

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Cantu, V. 2003. Spatial and temporal variation of *Centrocestus formosanus* in river water and endangered fountain darters (*Etheostoma Fonticola*) in the Comal river, Texas. Masters thesis, Texas State University. 58pp.

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APPENDIX A:
AQUATIC VEGETATION MAPS

Upper Spring Run Reach

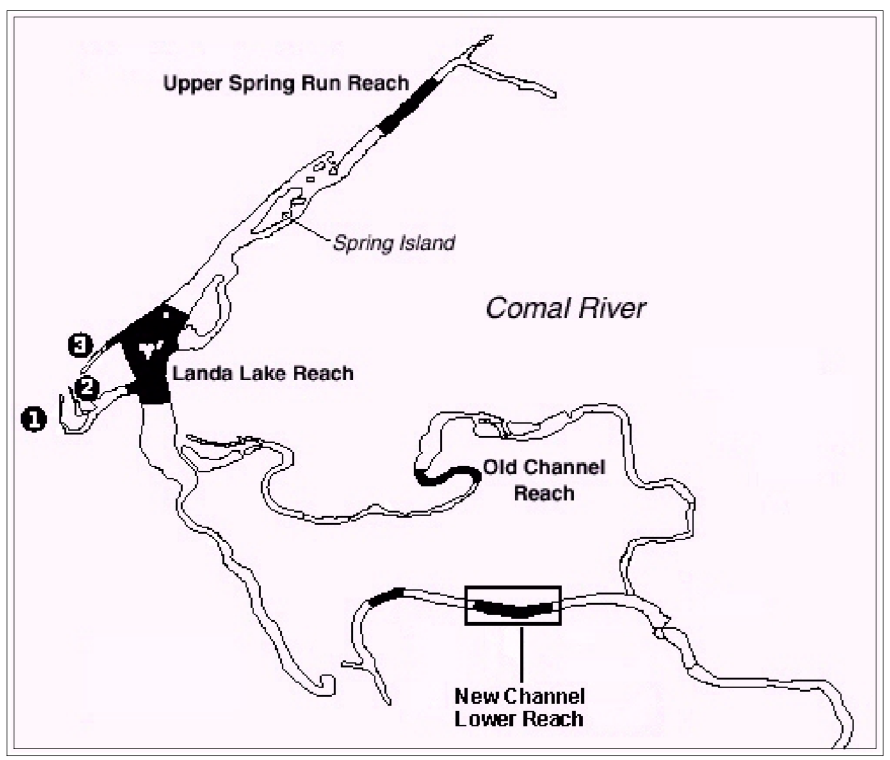
Comal River Aquatic Vegetation

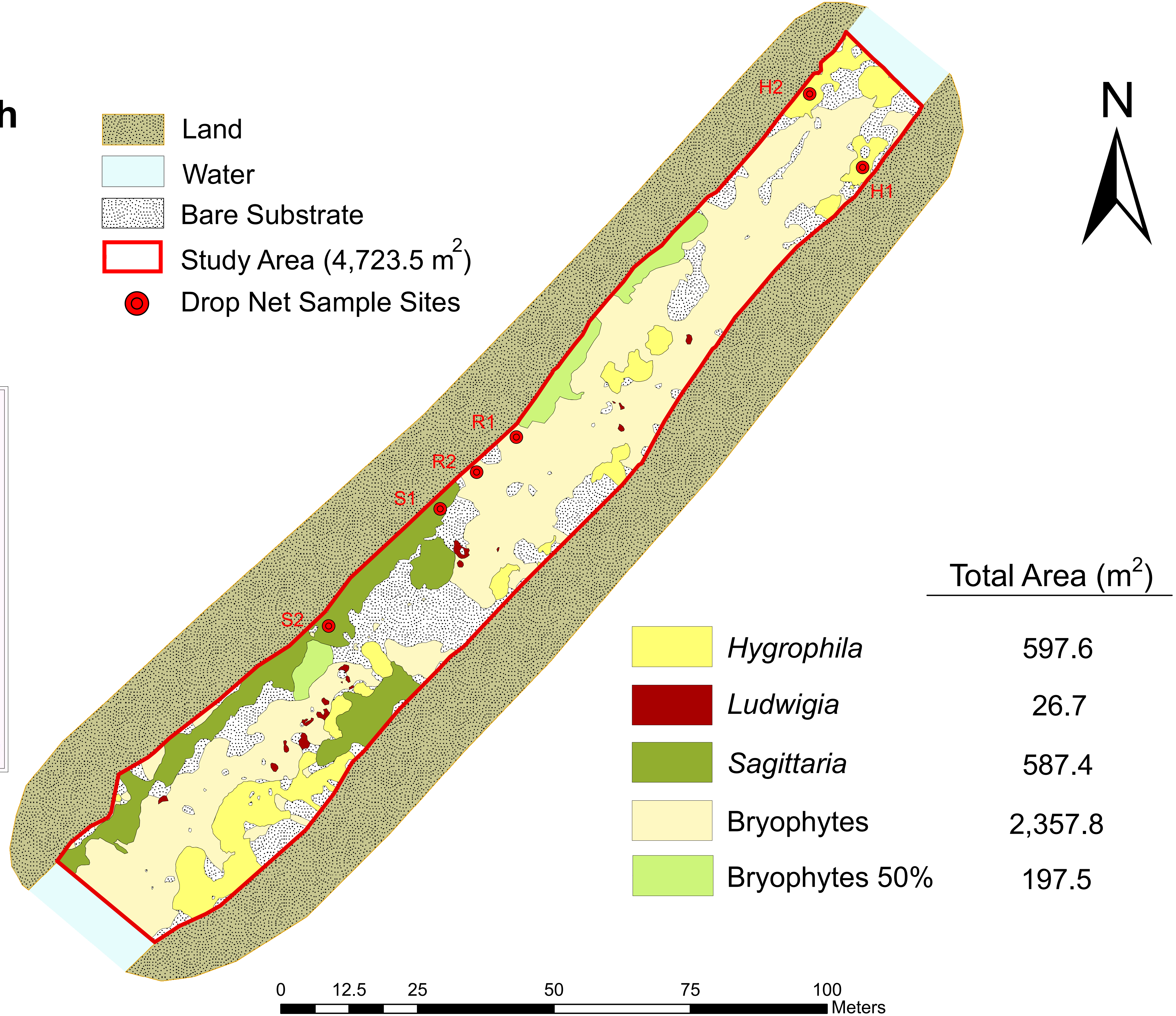
Upper Spring Run Reach

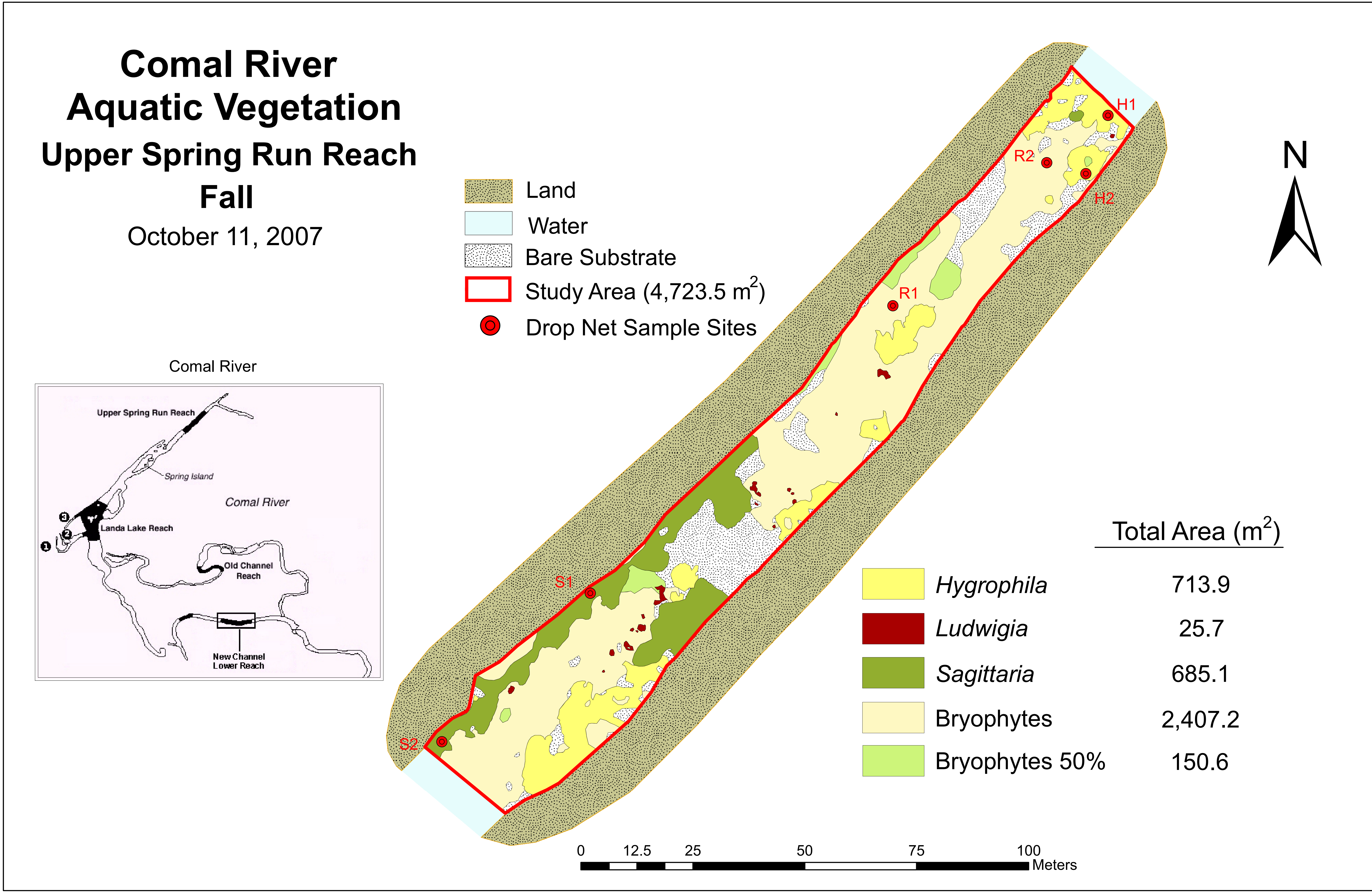
Spring

April 23, 2007

Comal River







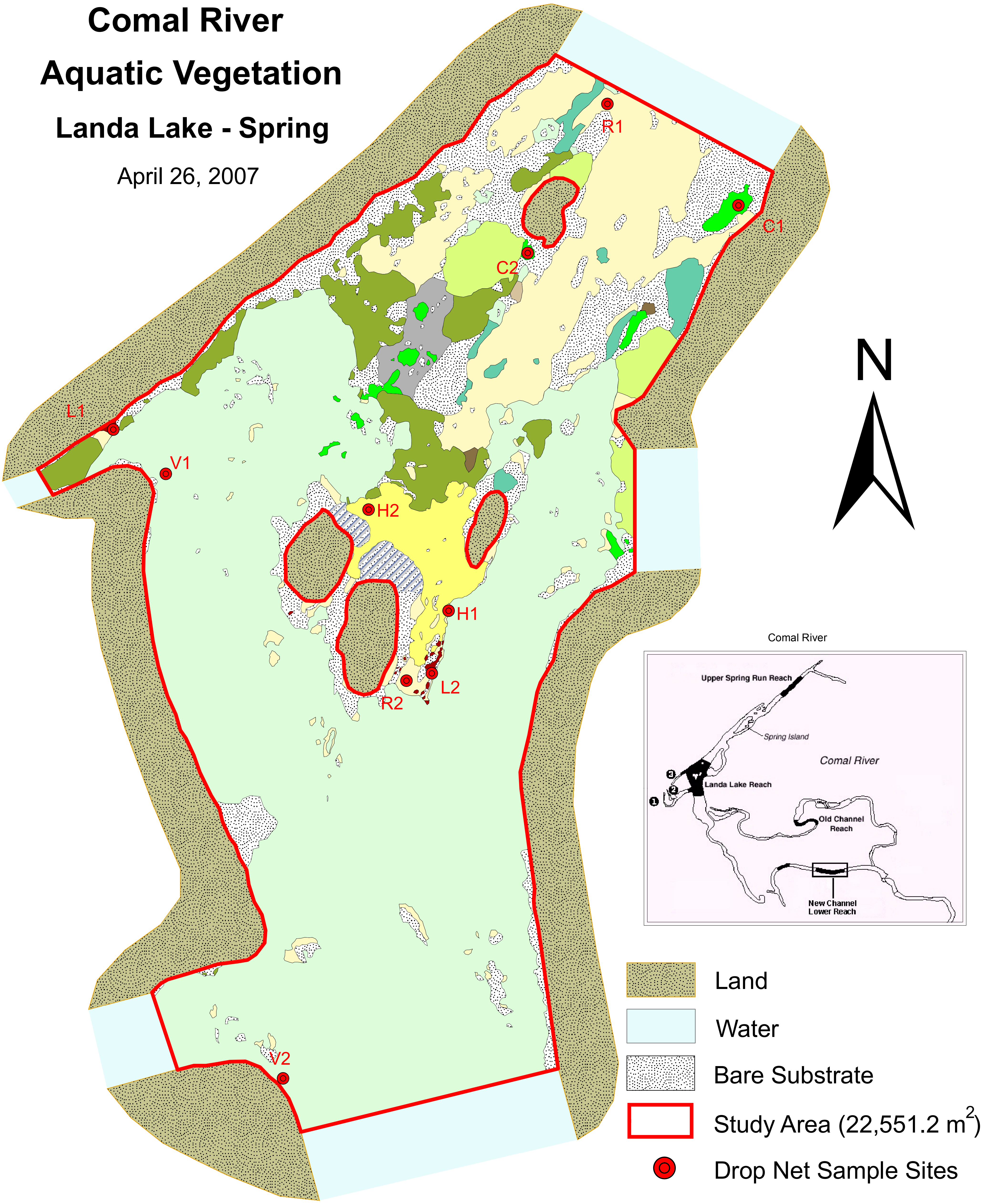
Comal River
Aquatic Vegetation
Upper Spring Run Reach
Fall
October 11, 2007
Land
Water
Bare Substrate
Study Area (4,723.5 m2)
Drop Net Sample Sites
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
H1
R2
H2
R1
S1
S2
N
Total Area (m2)
Hygrophila 713.9
Ludwigia 25.7
Sagittaria 685.1
Bryophytes 2,407.2
Bryophytes 50% 150.6
0 12.5 25 50 75 100 Meters

Landa Lake Reach

Comal River Aquatic Vegetation

Landa Lake - Spring

April 26, 2007



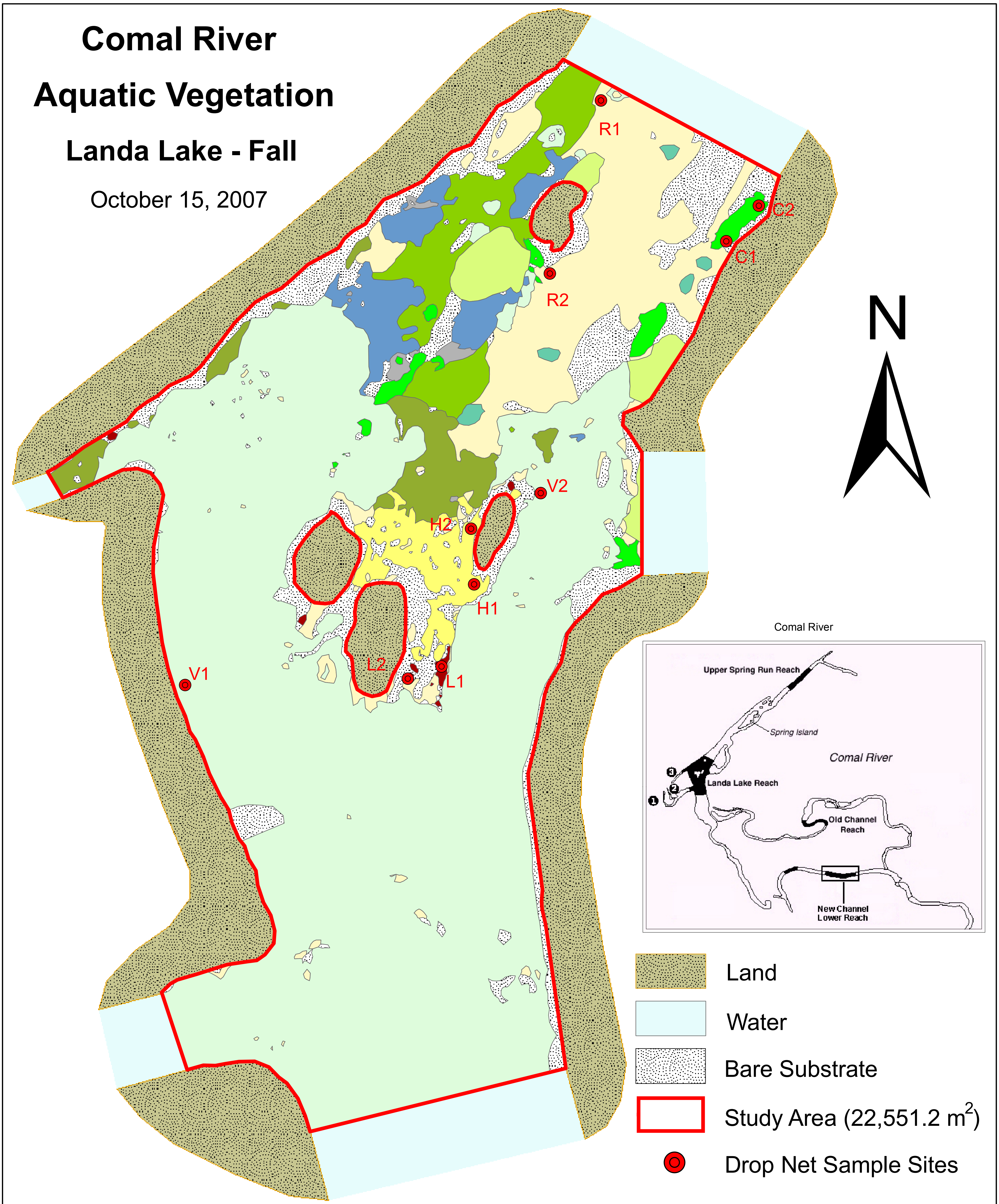
	Total Area (m^2)
Floating Vegetation Mat	184.7
Algae	245.0
Bryophytes	2,778.5
Cabomba	181.0
Hygrophila	620.4
Ludwigia	21.9

	Total Area (m^2)
Nuphar	558.1
Sagittaria	1,217.4
Vallisneria	13,412.6
Bryophytes 50%	282.3
Sagittaria / Bryophytes	20.2
Vallisneria / Bryophytes	10.6
Vallisneria / Sagittaria	10.2

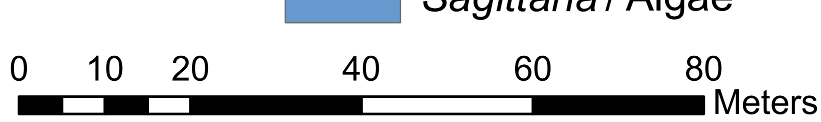
Comal River Aquatic Vegetation

Landa Lake - Fall

October 15, 2007



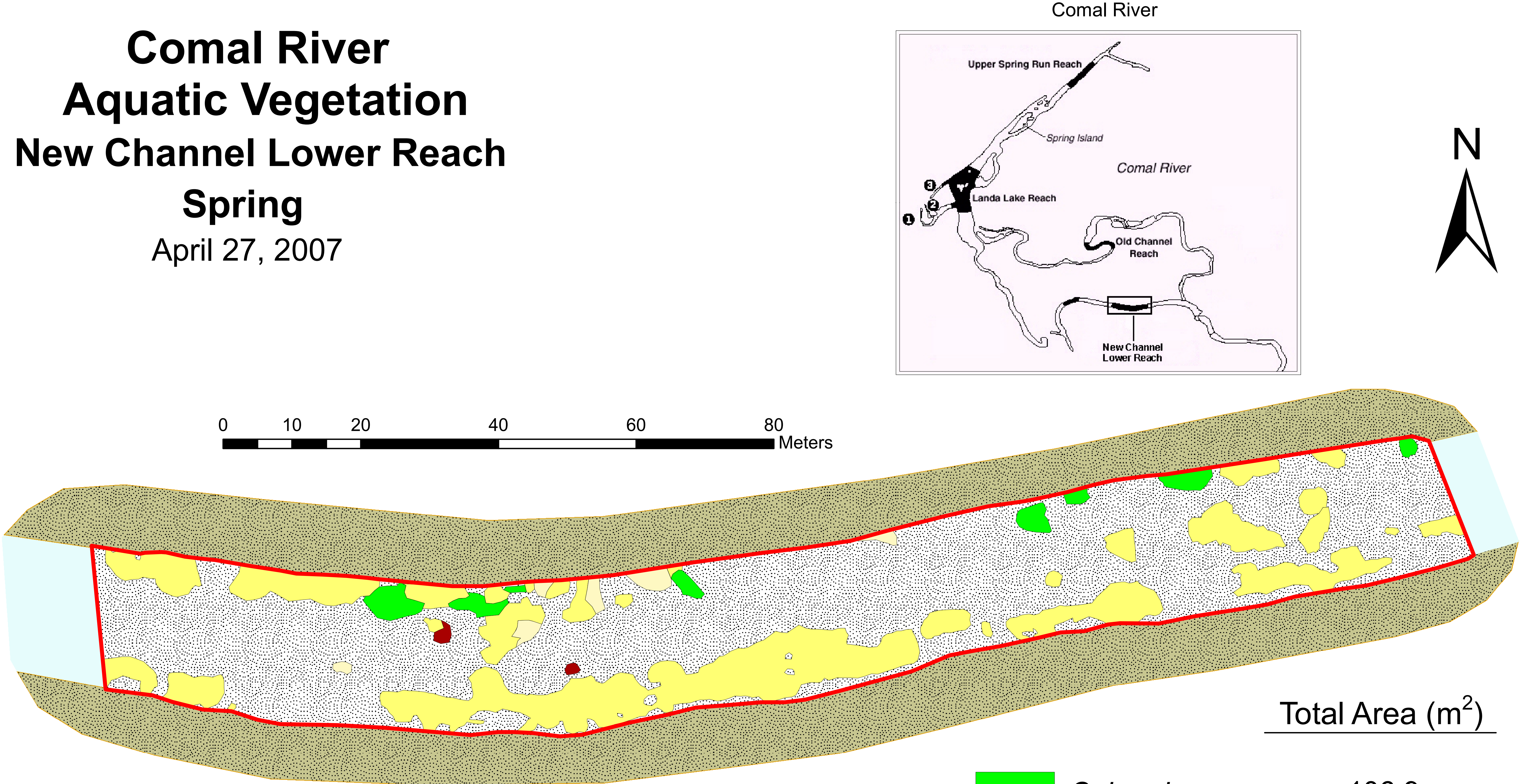
	Total Area (m^2)		Total Area (m^2)
Algae	72.1	*Sagittaria*	651.1
Bryophytes	2,601.0	*Vallisneria*	13,601.9
Cabomba	272.5	Bryophytes 50%	70.3
Hygrophila	549.4	Bryophytes / Algae	988.3
Ludwigia	37.1	*Sagittaria* / Algae	697.1
Nuphar	474.9		



New Channel Reach

Comal River
Aquatic Vegetation
New Channel Lower Reach
Spring

April 27, 2007

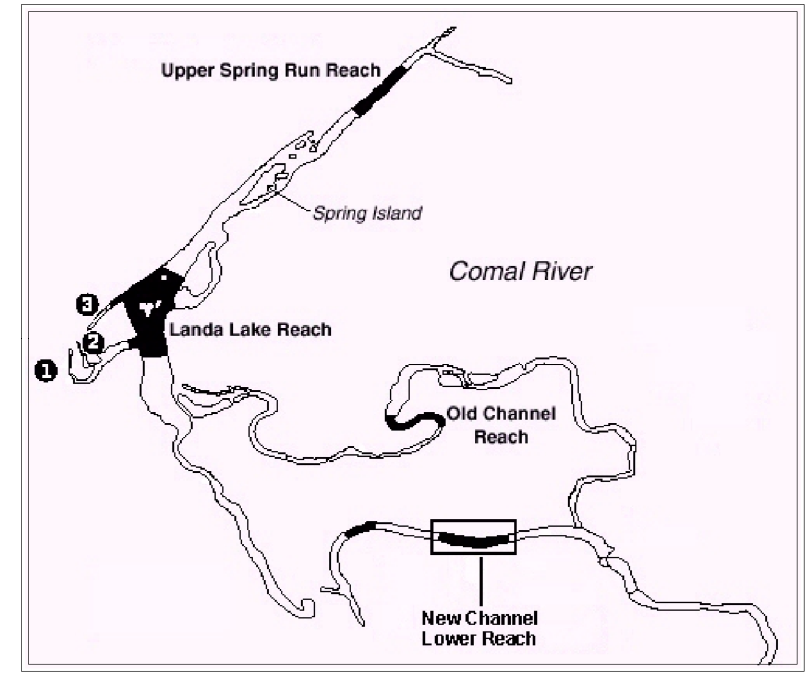


- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m^2)

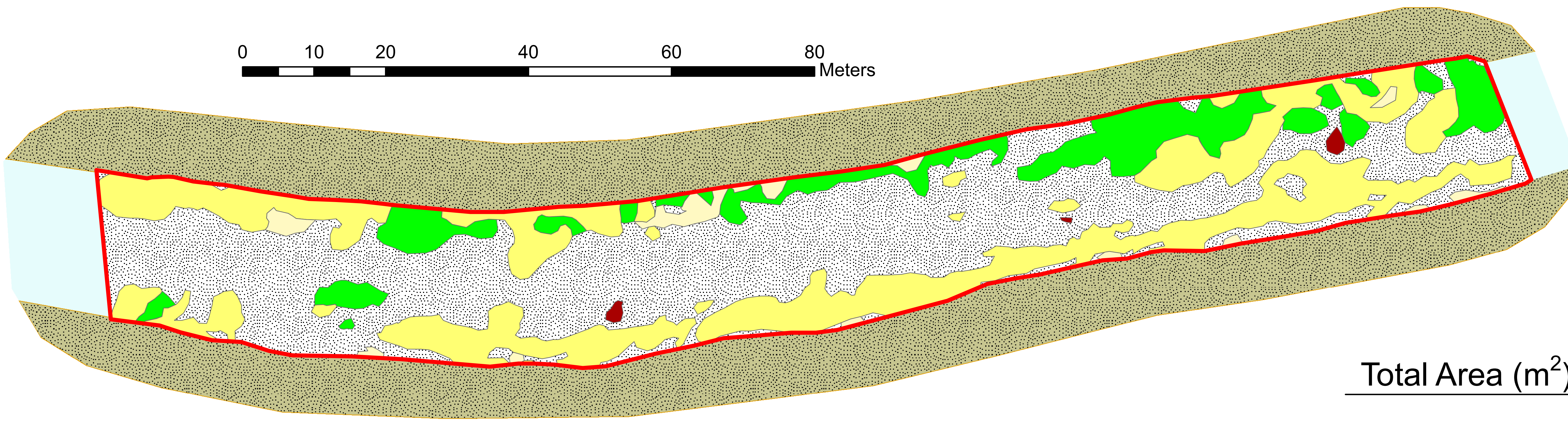
	Total Area (m^2)
Cabomba	106.9
Hygrophila	1,107.6
Ludwigia	8.4
Bryophytes	49.9

Comal River
Aquatic Vegetation
New Channel Lower Reach
Fall
October 18, 2007

Comal River



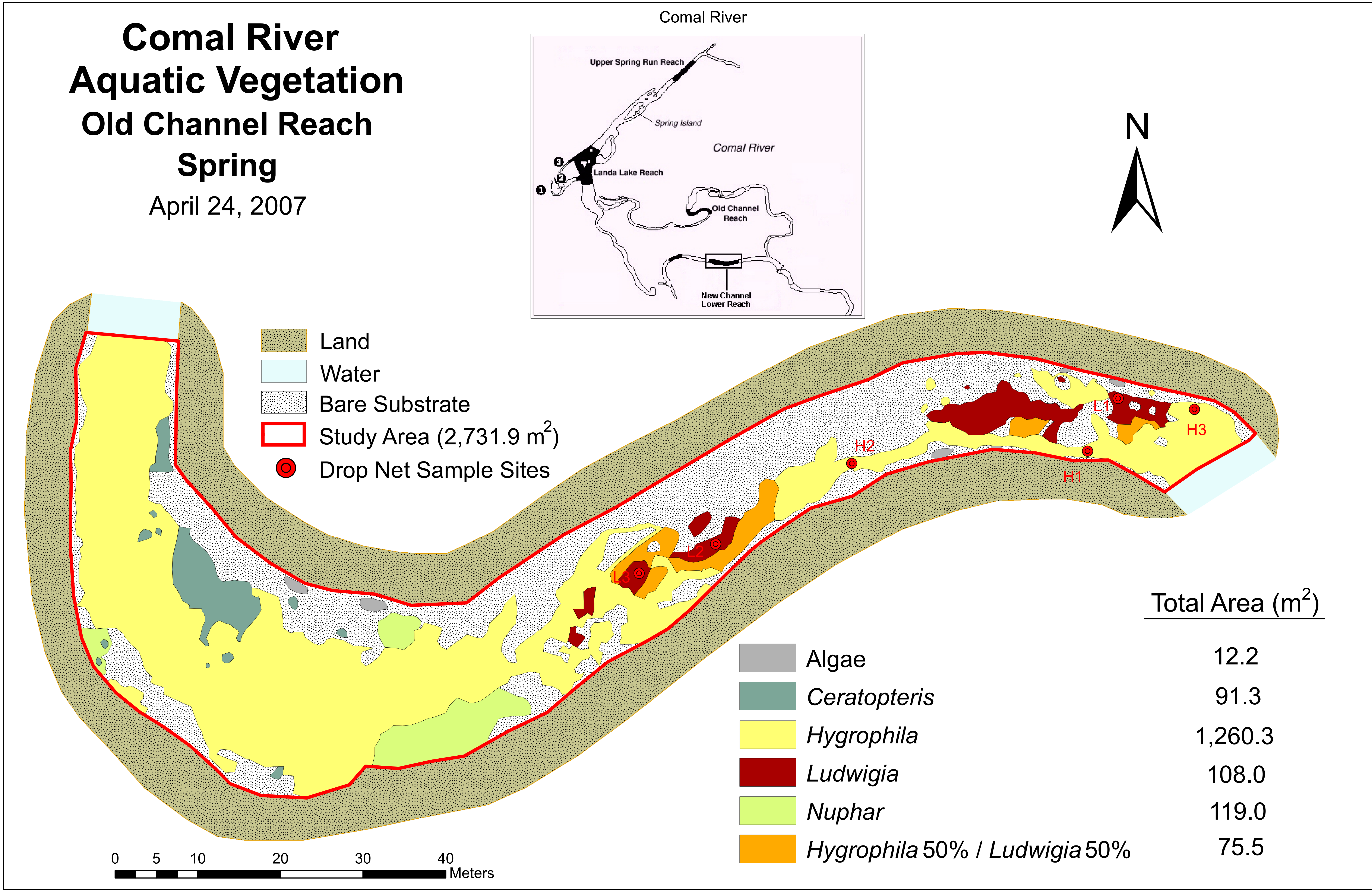
N

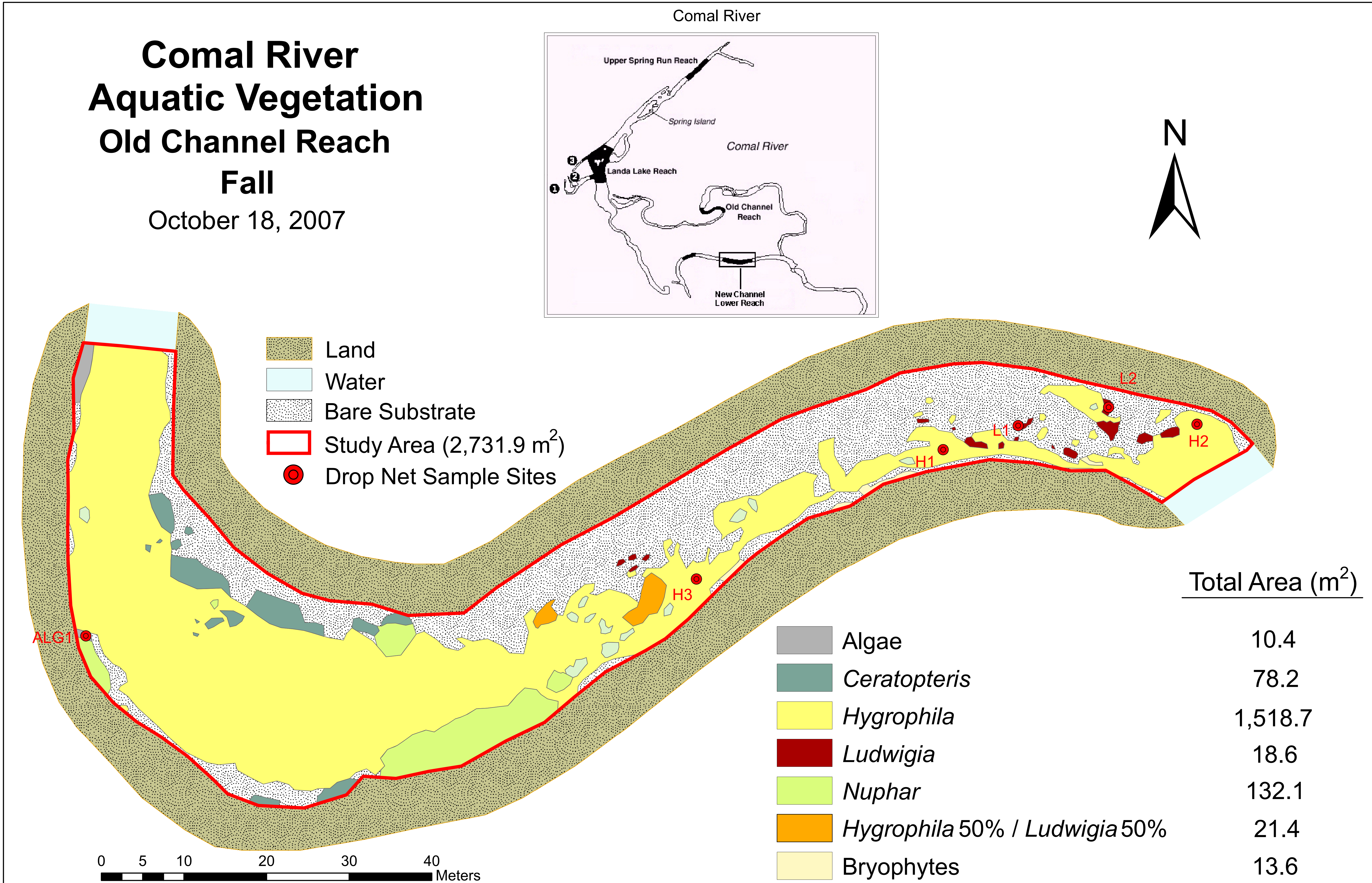


- Land
- Water
- Bare Substrate
- Study Area (4,177.8 m^2)

	Total Area (m^2)
Cabomba	535.5
Hygrophila	1,300.0
Ludwigia	13.3
Bryophytes	92.9

Old Channel Reach


Comal River
Aquatic Vegetation
Old Channel Reach
Spring
April 24, 2007
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
H1
H2
H3
L1
L2
L3
Total Area (m2)
Algae 12.2
Ceratopteris 91.3
Hygrophila 1,260.3
Ludwigia 108.0
Nuphar 119.0
Hygrophila 50% / Ludwigia 50% 75.5
0
5
10
20
30
40
Meters

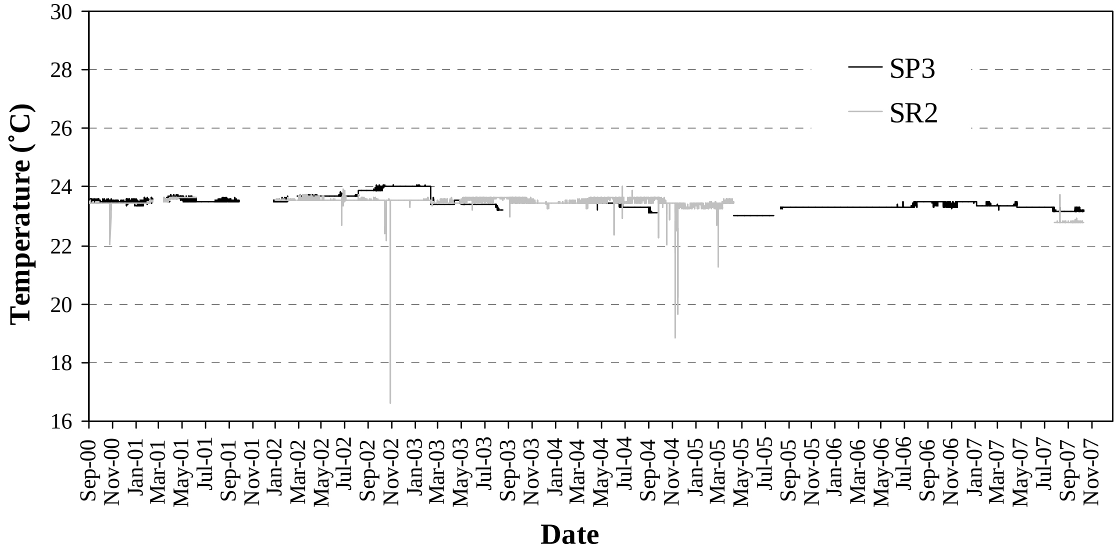


Comal River
Aquatic Vegetation
Old Channel Reach
Fall
October 18, 2007
Comal River
Upper Spring Run Reach
Spring Island
Comal River
Landa Lake Reach
Old Channel Reach
New Channel Lower Reach
N
Land
Water
Bare Substrate
Study Area (2,731.9 m2)
Drop Net Sample Sites
L2
L1
H1
H2
H3
ALG1
Total Area (m2)
Algae 10.4
Ceratopteris 78.2
Hygrophila 1,518.7
Ludwigia 18.6
Nuphar 132.1
Hygrophila 50% / Ludwigia 50% 21.4
Bryophytes 13.6
0 5 10 20 30 40
Meters

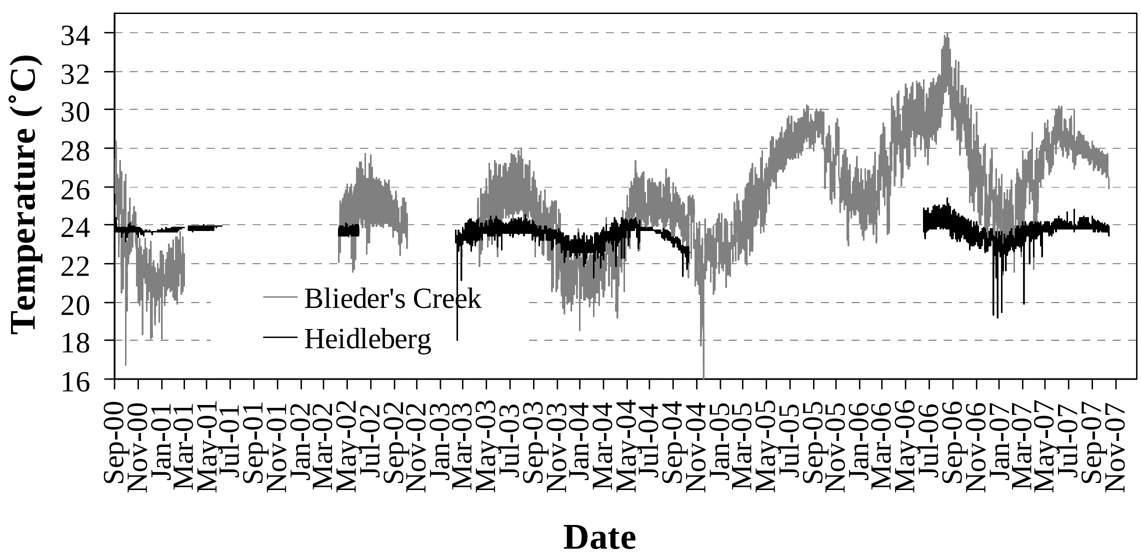
APPENDIX B:
DATA AND GRAPHS

Water Quality Data
and
Thermistor Graphs

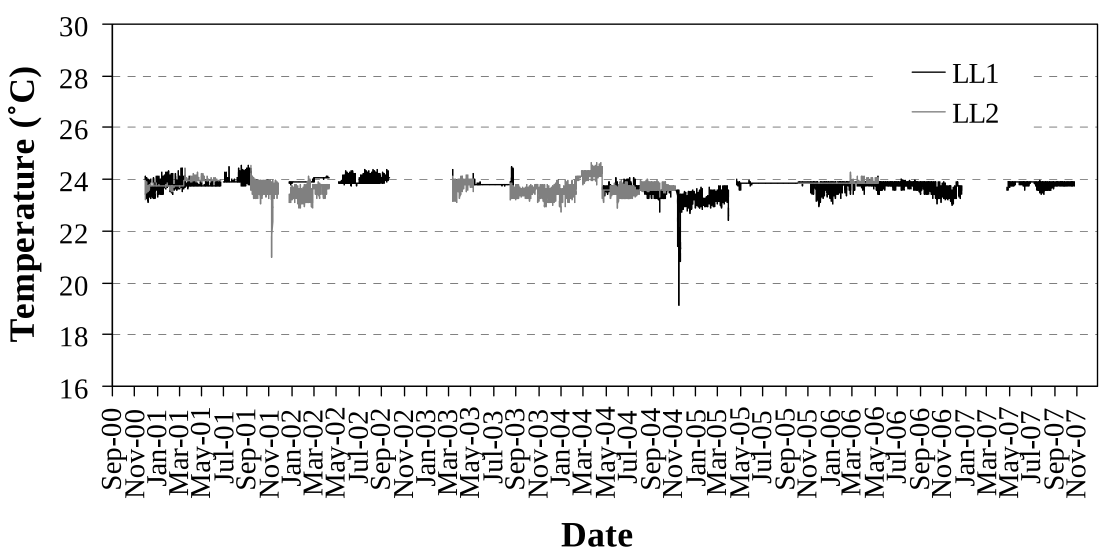
Thermistor Data: Spring Runs 2 & 3



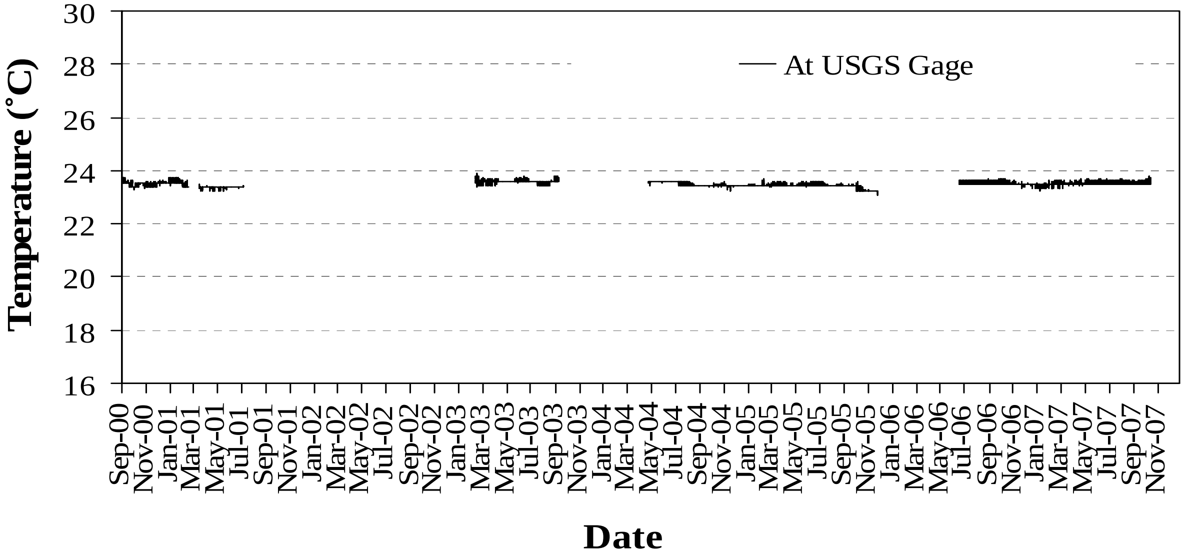
Thermistor Data: Comal Headwaters



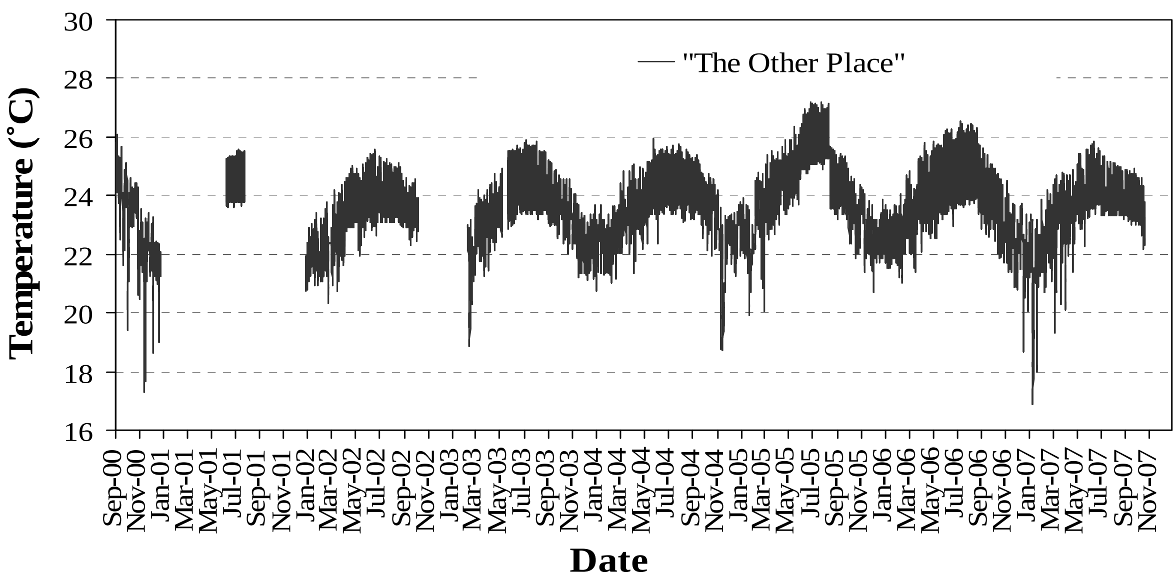
Thermistor Data: Landa Lake Bottom



Thermistor Data: Spring Run 1



Thermistor Data: The Other Place



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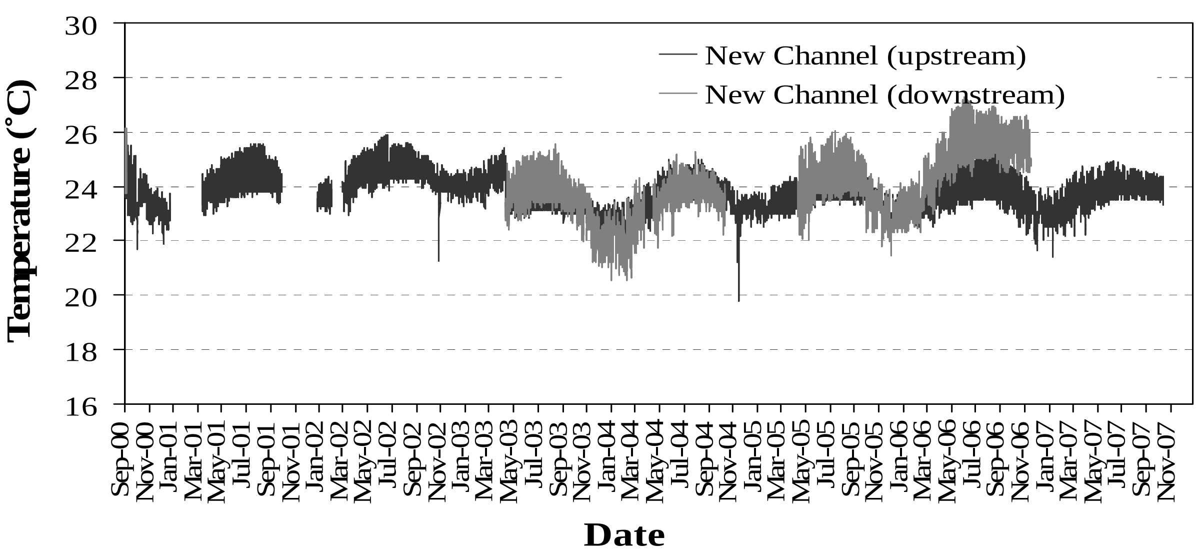

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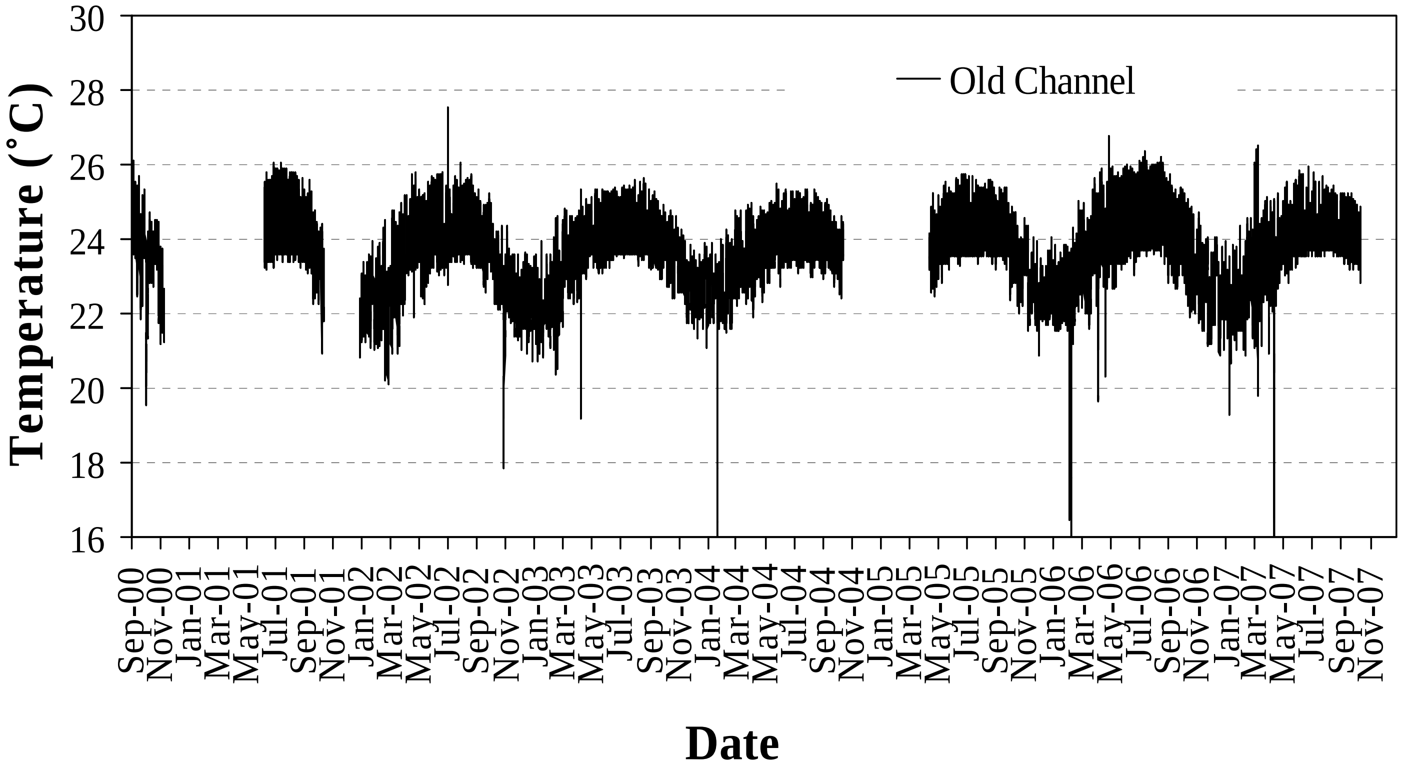
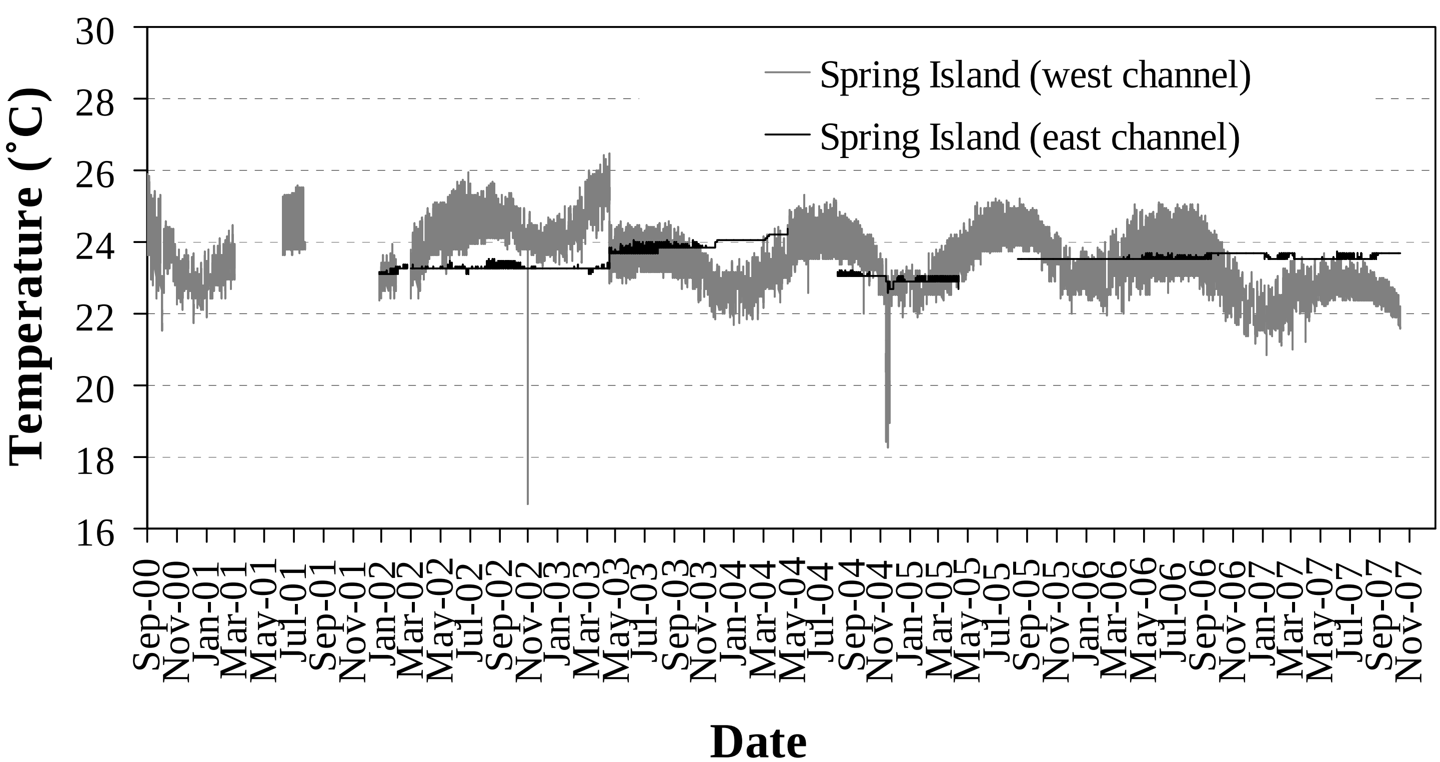
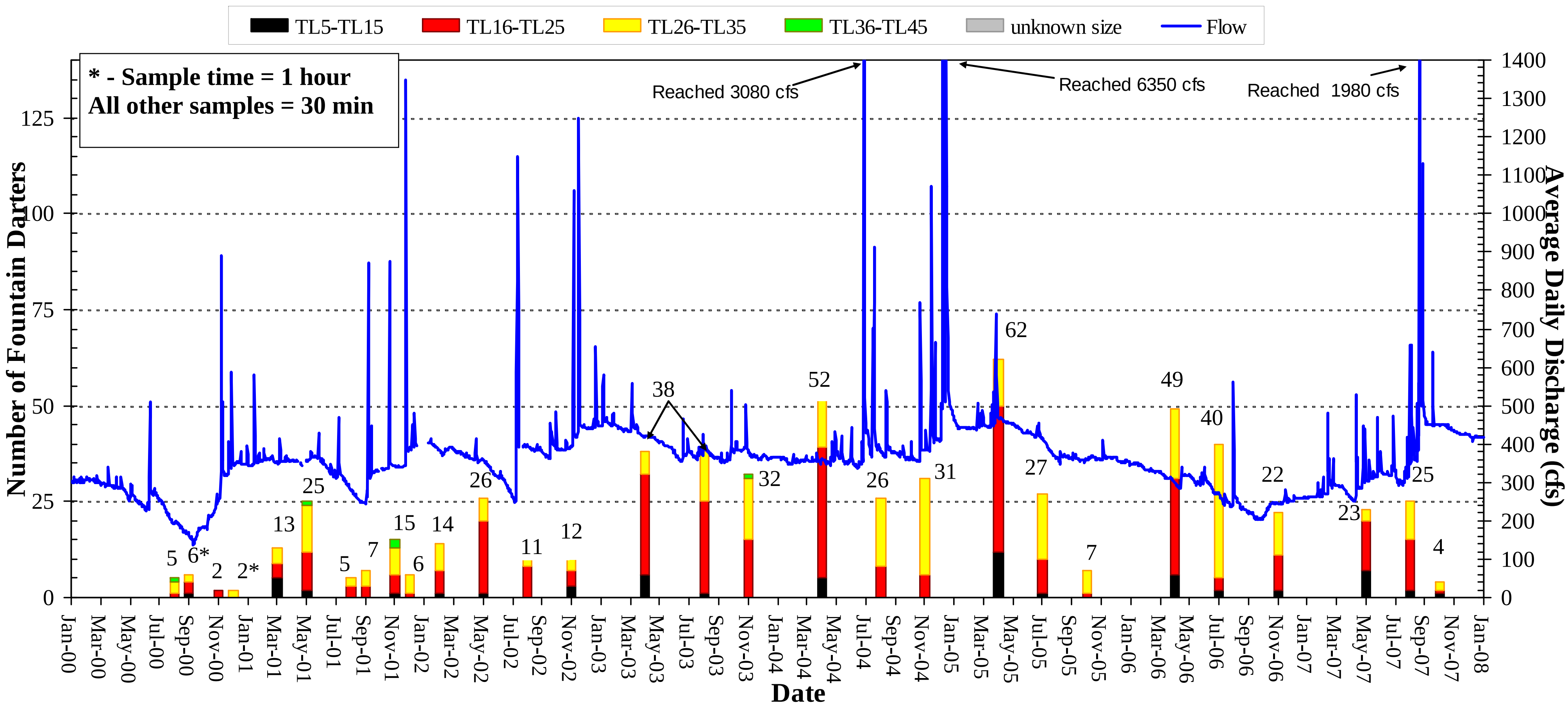


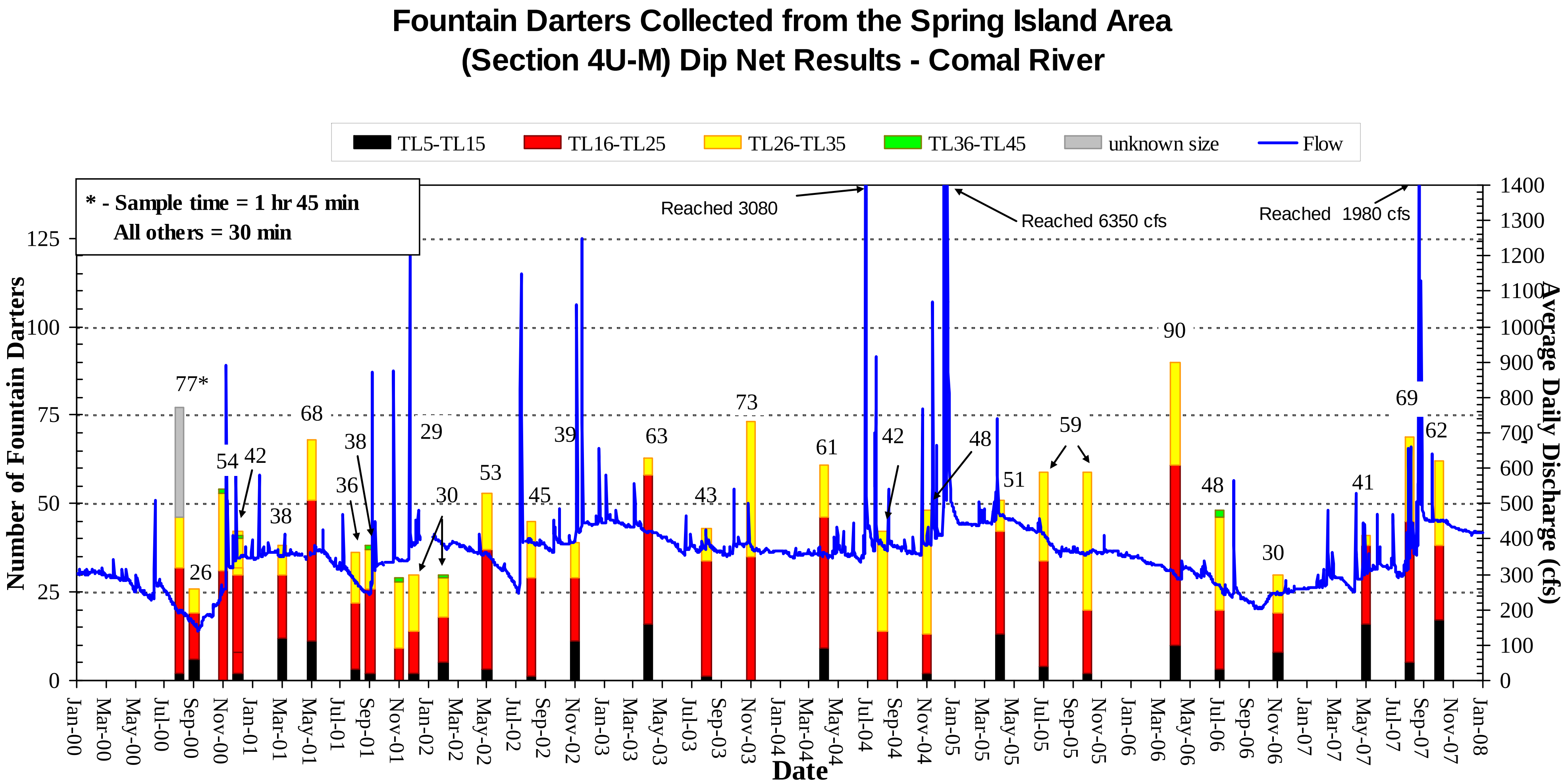
Thermistor Data: Spring Island



Dip Net Graphs

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




Fountain Darters Collected from the Spring Island Area
(Section 4U-M) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hr 45 min
All others = 30 min
Reached 3080
Reached 6350 cfs
Reached 1980 cfs
Number of Fountain Darters
Average Daily Discharge (cfs)
Date
77*
26
54
42
38
68
36
38
29
30
53
45
39
63
43
73
61
42
48
51
59
90
48
30
41
69
62
Jan-00
Mar-00
May-00
Jul-00
Sep-00
Nov-00
Jan-01
Mar-01
May-01
Jul-01
Sep-01
Nov-01
Jan-02
Mar-02
May-02
Jul-02
Sep-02
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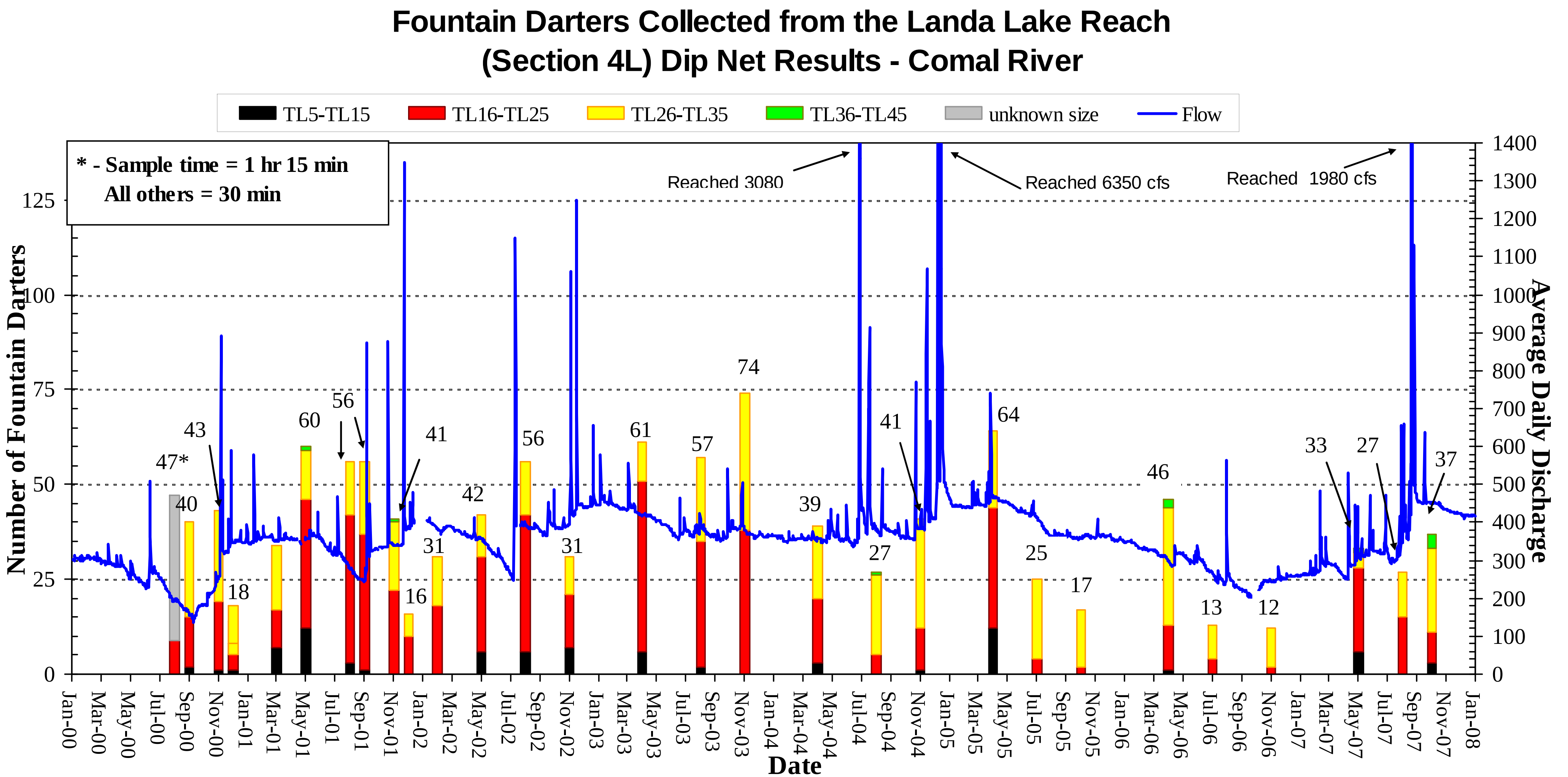
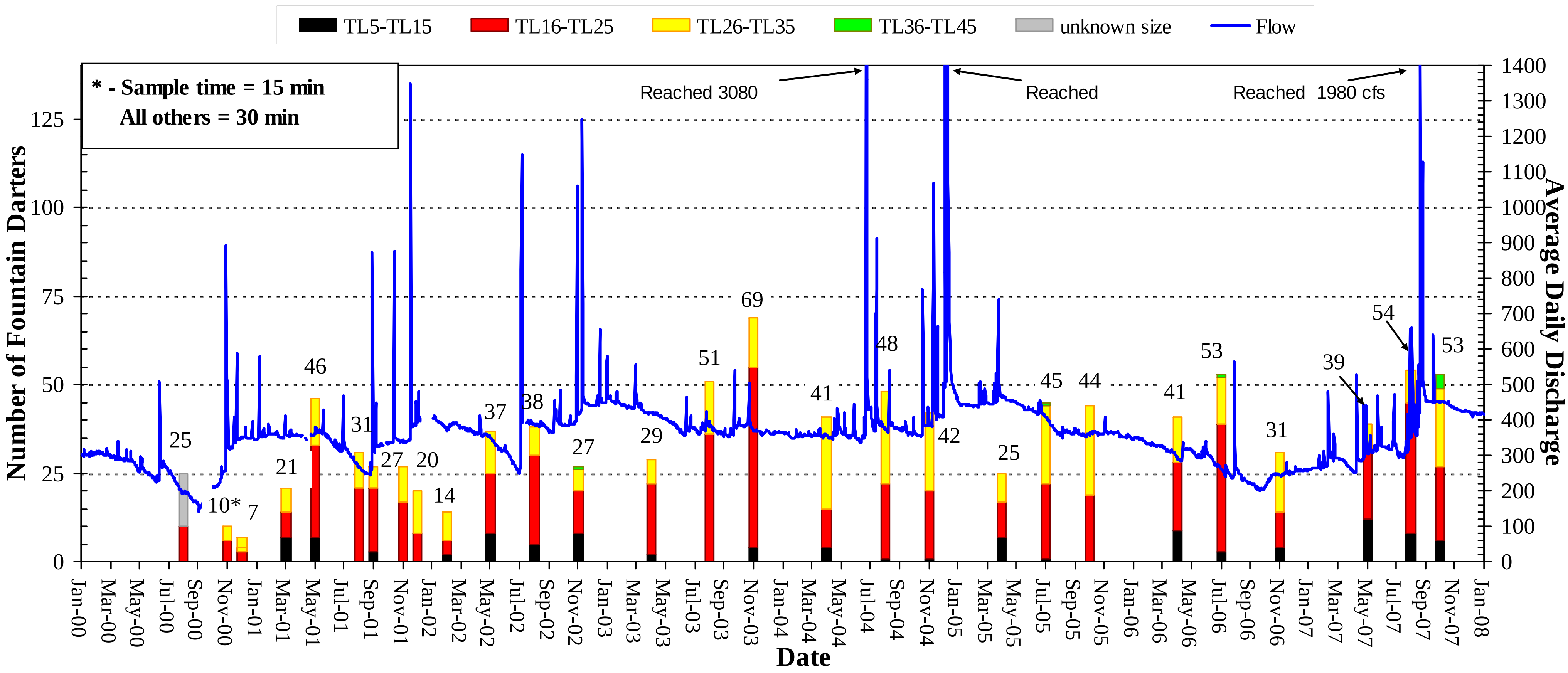
May-07
Jul-07
Sep-07
Nov-07
Jan-08

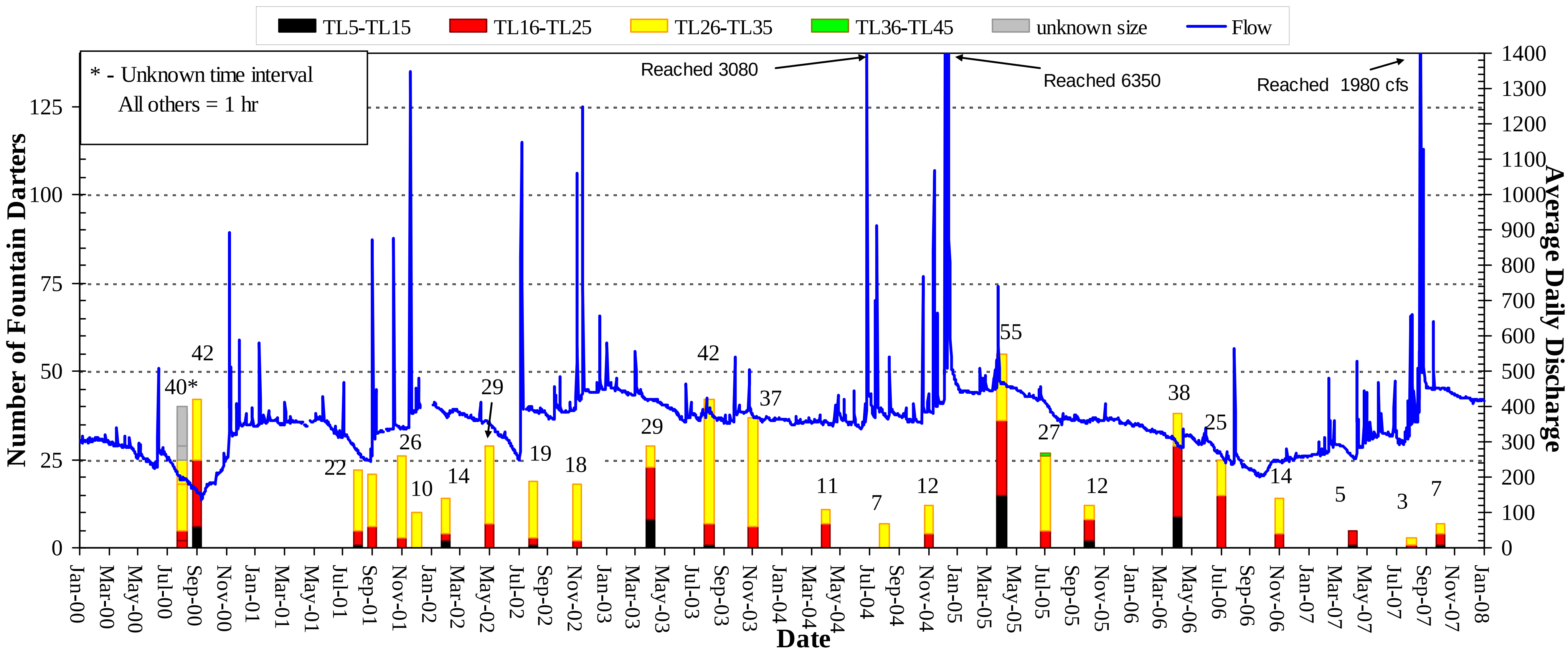


Fountain Darters Collected from the Landa Lake Reach
(Section 4L) Dip Net Results - Comal River
TL5-TL15
TL16-TL25
TL26-TL35
TL36-TL45
unknown size
Flow
* - Sample time = 1 hr 15 min
All others = 30 min
Reached 3080
Reached 6350 cfs
Reached 1980 cfs
Number of Fountain Darters
Average Daily Discharge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

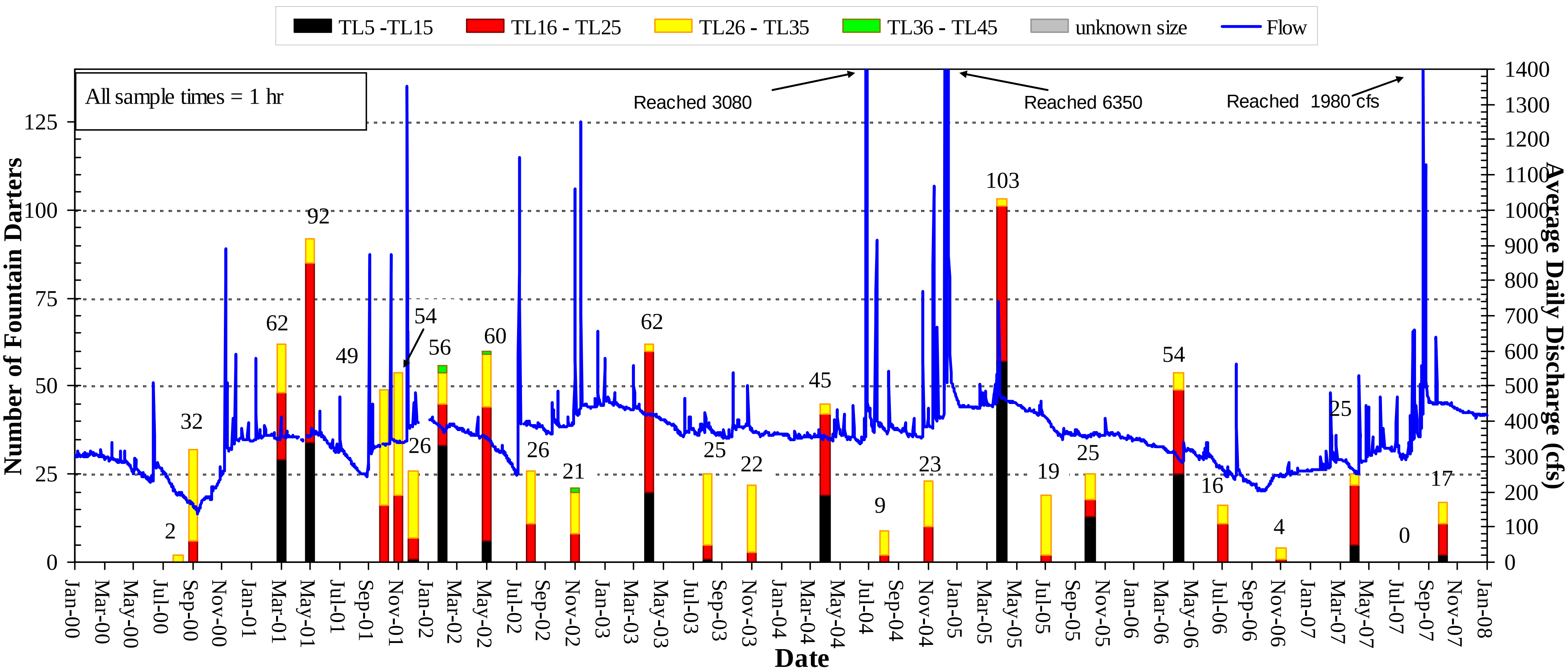
Fountain Darters Collected from the Landa Lake Reach (Section 5) Dip Net Results - Comal River



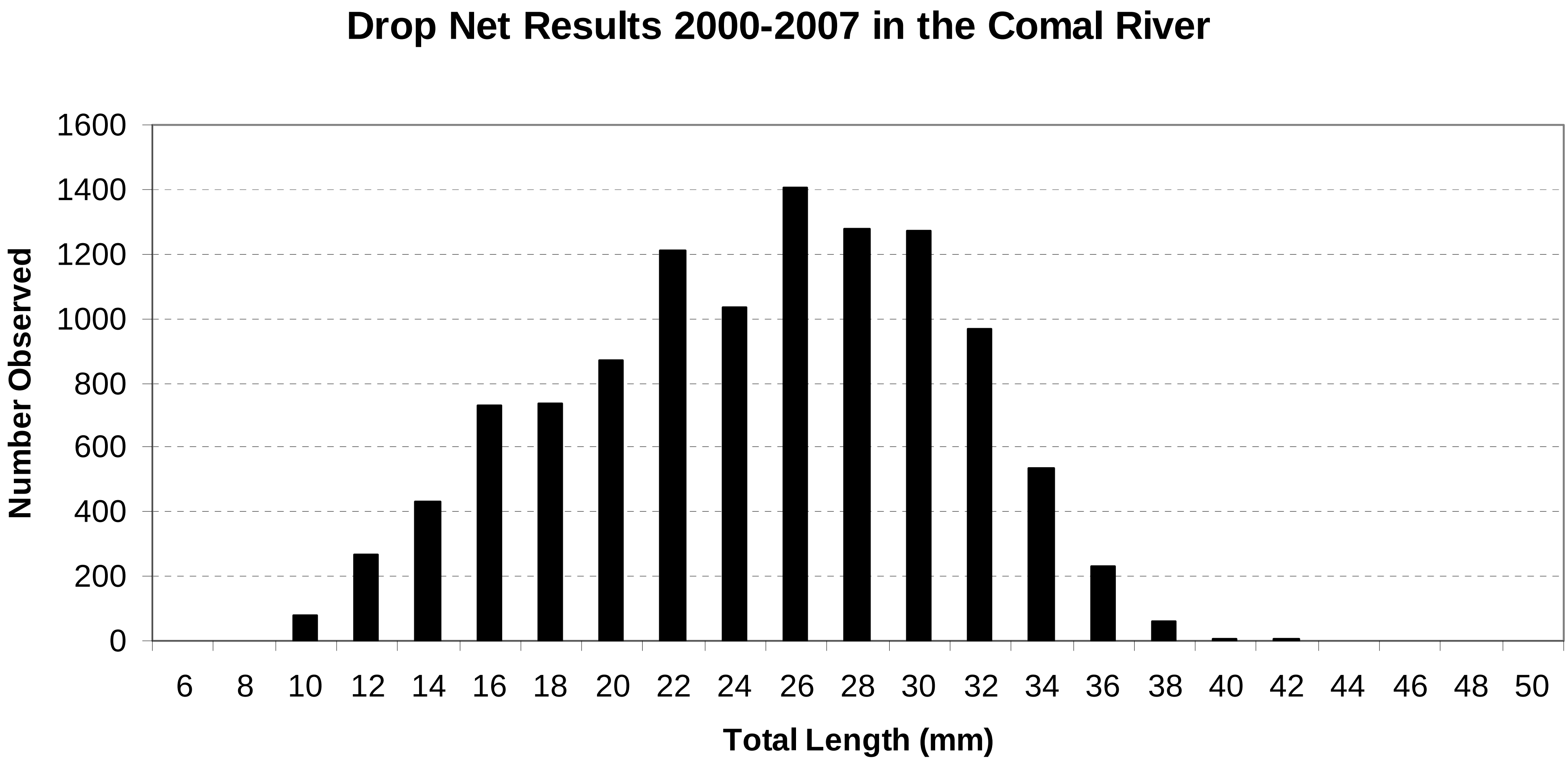
Fountain Darters Collected from the New Channel Reach (Section 10) Dip Net Results - Comal River



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



Drop Net Graph



Drop Net Results 2000-2007 in the Comal River
Number Observed
1600
1400
1200
1000
800
600
400
200
0
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
Total Length (mm)

APPENDIX C:
DROP NET RAW DATA

(not available online)

APPENDIX D:
Bolick (2007) Thesis

THE EFFECTS OF SPRING FLOW ON THE ABUNDANCE OF HETEROPHYID

CERCARIAE IN THE COMAL RIVER, NEW BRAUNFELS, TX

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Anne E. Bolick, B.S.

San Marcos, Texas

December 2007

THE EFFECTS OF SPRING FLOW ON THE ABUNDANCE OF HETEROPHYID

CERCARIAE IN THE COMAL RIVER, NEW BRAUNFELS, TX

Committee Members Approved:

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David G. Huffman, Chair

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Thomas M. Brandt

Approved:

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Dean of the Graduate College

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I am deeply indebted to Valentin Cantu, Kelly McDermott, and Pamela Holmes for their encouragement and time spent in the field. Also it is difficult to overstate my gratitude to Tiffany Kuhlman and Erin O'Docharty. Thank you so much for your support and your friendship. I could not have done it without you.

Lastly, and most importantly, I wish to thank my parents, John and Mary Bolick. Thank you for encouraging me to go to graduate school in the first place, and all your love and support.

This manuscript was submitted on October 24, 2007.

TABLE OF CONTENTS

LIST OF TABLES

Table **Page**

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Setting and History

Comal Springs in New Braunfels, Comal County, Texas is the largest spring system in Texas (Heitmuller and Williams 2006) with a mean annual discharge of 8.04 cms (USFWS 1996). It consists of four major springs, all of which are fed by water from the Edwards Aquifer. These springs are impounded to form Landa Lake, which constitutes the headwaters of the Comal River.

The Comal River is one of two rivers supporting wild populations of the endangered fountain darter, *Etheostoma fonticola* (Schenck and Whiteside 1976). *Etheostoma fonticola* requires clean clear water, adequate stream flows and prefers vegetated floor habitats and constant water temperatures (USFWS 1996). The fountain darter was listed as an endangered species on October 13th, 1970, and its local extinction in the Comal River was most likely caused by the drought of the mid 1950s (USFWS 1996). This severe drought resulted in a cessation of discharge from the Comal Springs that lasted from June until November of 1956 (USFWS 1996). The Comal River was later repopulated with 457 adult fountain darters collected from the nearby San Marcos River (USFWS 1996).

In 1996, while collecting fountain darters from the Comal River for a USFWS refugium program, workers observed that the gills of many fish were heavily infected with trematode metacercariae that had not been seen before in the river. The metacercariae have since been identified as *Centrocestus formosanus* (Mitchell et al. 2000) and the source of the infection has been traced to cercariae emerging from the exotic red-rimmed melania snail, *Melanoides tuberculata* (Salgado-Maldonado et al. 1995; Scholz and Salgado-Maldonado 2000). The trematode is now a concern because of its potential to negatively affect U.S. hatchery fishes and fish in the wild (McDermott 2000; Mitchell et al. 2000, 2002).

Study Organism

The parasite *Centrocestus formosanus* (Trematoda: Heterophyidae) is an invasive digenetic trematode that was originally described in Taiwan and is widely distributed in Asia (Scholz and Salgado-Maldonado 2000). It is now cosmopolitan in many warm waters of the world, including the spring-fed San Antonio, Comal, and San Marcos rivers of central Texas.

The life cycle of digenetic trematodes involves at least two hosts, one is a vertebrate and the other is usually a mollusk (Roberts and Janovy Jr. 2000). The *C. formosanus* life cycle has three stages (Figure 1). Vertebrates that have been shown experimentally to be suitable definitive hosts for *C. formosanus* include birds, dogs, cats, mice, and occasionally amphibians (Yamaguti 1975; Salgado-Maldonado et al. 1995). In central Texas the natural definitive host seems to be the Green Heron, *Butorides virescens* (Kuhlman 2007). In the Green Heron, the adult trematodes localize in the colon where they lay eggs that are later passed through host feces (Yamaguti 1975; Kuhlman 2007).

One complicating factor that may ultimately change the assignment of blame in this issue was discovered by Kuhlman (2007) while attempting to recover *C. formosanus* adults from piscivorous birds taken in the area. Not all heterophyid specimens recovered could be confidently assigned to *C. formosanus*, and indeed, many adult specimens are now suspected of being in the genus *Phagicola* (Kuhlman 2007). This being said, the fountain darters in the Comal River are heavily impacted by metacercariae that are definitely heterophyid. Two species of heterophyid cercariae were seen during the study, *Centrocestus formosanus* (until resolved) and *Haplorchis pumilio*, which both utilize the same snail host (Lo and Lee 1996). Reference to cercariae from this point forward will refer to *C. formosanus* cercariae, unless noted otherwise.

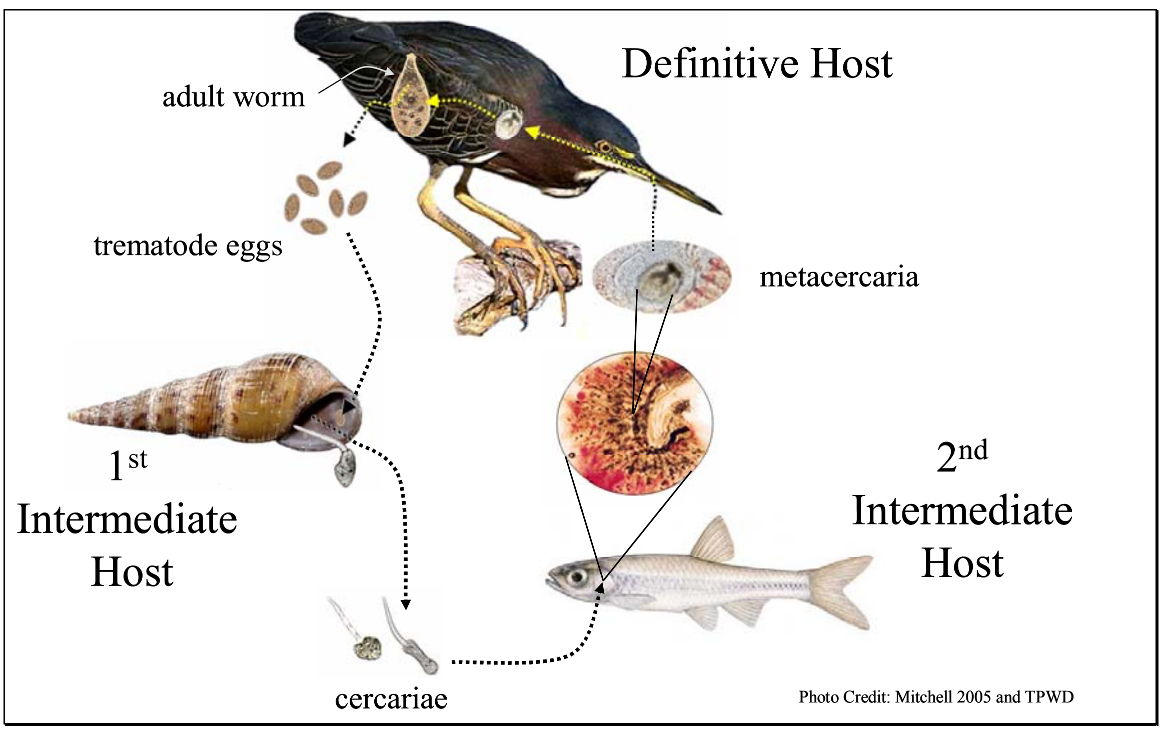


Figure 1. Diagram of *C. formosanus* lifecycle.

The first intermediate host for *C. formosanus* in the Comal River is *Melanoides tuberculata* (Mitchell et al. 2005) an exotic snail found in tropical, subtropical, and temperate regions (Amaya-Huerta and Almeyda-Artigas 1994). The snail has an elongate conical shell with the length being more than twice the width (Duggan 2002; Mitchell and Brandt 2005). The shell color is dark brown with reddish brown basal whorls, giving it the common name the red-rimmed melania (Duggan 2002). *Melanoides tuberculata*, which has the capacity to colonize rapidly and densely in many types of habitats (Amaya-Huerta and Almeyda-Artigas 1994), was first reported from Texas waters in 1964 (Murray 1964).

Centrocestus formosanus can infect the snail via two routes: The snail can ingest the eggs and the miracidium later hatches inside the host, or the eggs can hatch in the water and the free-swimming miracidium can penetrate the snail (Schell 1970; Roberts and Janovy Jr. 2000). Inside the snail, the miracidium metamorphoses into a sporocyst, followed by asexual reproduction within the sporocyst that results in many rediae larvae (Roberts and Janovy Jr. 2000). Both the redia and the sporocyst are able to produce cercariae (Schell 1970), which emerge through the exhalent respiratory current of the snail as free-swimming larvae (Lo and Lee 1996) that are infective to fishes, the second intermediate host (Martin 1958; Yamaguti 1975). This process results in embryonic amplification in which thousands of cercariae are produced (Kiesecker 2002).

While the cercariae are in the water column, they can infect several species of fish. The route of infection is by passively entering the mouth of the fish with the respiratory current and then making contact with the gills (Salgado-Maldonado et al.

1995). The cercariae immediately beginning attaching to the gill filaments, cast off their tails, and begin cyst formation (Salgado-Maldonado et al. 1995).

The metacercarial cysts occur on gill filaments in areas closely associated with bloods vessels (Madhavi 1986). Severe gill lesions and cartilage hyperplasia due to metacercariae have reportedly caused respiratory problems leading to death of infected fish (Balasuriya 1988; Velez-Hernandez et al. 1988; Alcaraz et al. 1999; Mitchell et al. 2000). The life cycle is completed when an infected second intermediate host is consumed by a suitable definitive host.

Relevant Previous Work

Following the first report of this parasite in central Texas, various strategies have been employed to monitor the abundance and impact of the parasite in the Comal River. Mitchell et al. (2000) monitored the mean intensity (number of cysts per fish) and prevalence (percent of fish infected) in the red-rimmed melania and fish in both the San Marcos and Comal rivers from 1997 to 1998. Although these studies revealed the severity of *C. formosanus* infections, no consistent seasonal trends in parasite abundance were reported.

In 2003, Cantu examined the spatial and temporal variation of *C. formosanus* in the Comal River by studying cercarial abundance in river water, and intensity and prevalence of the parasite in caged fountain darters. Cantu (2003) observed a significant positive association between the abundance of cercariae in the water near a cage and the number of cysts actually acquired by the caged darters.

Cantu's estimates of cercarial abundance in river water were based on only three samples taken right along side the cages, because he was concerned only with estimating how much infection pressure the caged darters had experienced. Consequently, Cantu's cercarial abundance estimates cannot be used as estimates of the overall total abundance of cercariae drifting through the entire cross section of the stream at his study sites.

Cantu (2003) also recommended that for studies designed to monitor changes in parasite abundance in the ecosystem over time, sampling the cercarial drift using the filtration technique would be more practical than counting metacercarial cysts on fish. He found that the filtration technique requires fewer resources, provides results with better precision, and does not require the sacrifice of an endangered fish.

Later, Lozano (2005) attempted to determine the minimum sampling effort required to obtain a stable estimate of the number of cercariae drifting through a site. He collected 45 cercarial samples along a single cross-section of the Comal River, but determined that five samples per cross section would be sufficient to accurately represent total cercarial drift for the rest of his study. Lozano (2005) also examined the relationship between his estimates of cercariae drift through the site relative to stream discharge at the site using a modification of Buchanan and Somers (1969) standard procedure for estimating wading discharge.

In the mid 1950's, Comal Springs ceased flowing and this is thought to be the most likely cause of the local extinction of darters in the Comal River (USFWS 1996). The species was successfully reintroduced using 457 darters, but in 1996, a drought brought stream discharge down to 2.35 cms (USGS), a level considered low enough to jeopardize survival of the darters again (Table 2 in USFWS 1996). During 1996 biologists began to find fish with swollen gills. This was caused by reaction to heavy infections with metacercariae of a trematode later identified as *C. formosanus*. In a follow-up study prompted by this development, Mitchell et al. (2000) determined that infection with these metacercariae could severely and permanently damage the respiratory system of individual darters, and estimated that infection intensity exceeding 800 cysts per fish was life threatening to the darters. Some of the darters collected from the Comal River at this time had many more than 800 metacercarial cysts per fish (as many as 1500+, Mitchell et al. 2002) indicating that the darter population was not only jeopardized by low stream discharge, but this threat was substantially compounded by the presence of this parasite.

Statement of the Problem

Infection pressure on fountain darters is a function of cercarial density (Cantu 2003). If the stream discharge rate passing by shedding snails is reduced by say, 50%, there will be a consequent proportionate doubling of cercarial density (count/L) downstream from the snails, essentially doubling the infection pressure on the darters downstream from the snails. Thus, during a drought that reduces discharge rates in the Comal River, darters downstream from shedding snails will experience increasing infection pressures as flow decreases. Thus, the presence of the parasite in the Comal ecosystem is expected to cause the darters to become threatened with local extinction at higher discharge rates (less severe drought) than if the parasite were absent.

Additionally, there is anecdotal evidence that the emergence rate of *C. formosanus* cercariae into the drift community of the Comal River does not remain constant when discharge rates change, but may increase when discharge from the springs decreases (T. Brandt, U.S. Fish and Wildlife, personal communication). If cercarial emergence rate is independent of current velocity, then as discharge rates change cercarial density would double if the discharge rate was reduced by 50%. But, if cercarial emergence rate increases as stream discharge decreases then reducing stream discharge by 50% would more than double the cercarial density and infection pressure on the darters. That would mean that, during a drought, the existing wild population of darters would not just experience an increase in cercarial infection pressure proportionate to volumetric reduction of water, but a compounded increase in infection pressure caused by the hypothetical increase in cercarial emergence rate caused by reduced discharge.

Since discharge rate goes down during an extended drought, and since the respiratory efficiency of the fish is now impacted by the trematode, the wild population could possibly go extinct earlier in a drought and at much higher discharge rates than those thought to have caused the previous extinction. So, if cercarial emergence rate does indeed increase as spring discharge decreases, then the presence of this worm in the Comal River may exacerbate the negative impact of drought on the survival of wild populations of the fountain darter beyond what would be expected from simple proportionate increase in cercarial density.

Project Objectives

The objectives of this project are:

1. To study cercarial drift in the Comal River over a period of at least 1 year to determine if there is evidence that cercarial shedding rate is increased when discharge decreases, and
2. To examine other physiochemical variables to determine if any of them may help explain any trends discovered in objective 1.

CHAPTER II

MATERIALS AND METHODS

Preliminary Study

A preliminary study was conducted from January to April 2006 in which six sites along the Comal River were sampled. A transect was established at each site and 10 equidistant points along the transect were selected for sampling. Three 5-L water samples were taken at each of the 10 points, one approximately 10 cm from the bottom, one at 60% depth, and one approximately 10 cm from the surface. This resulted in 30 water samples taken at each site. The difference between the sample taken at 60% depth and the sample taken at 10 cm from the surface was virtually indistinguishable, and therefore the final design did not include a sample 10 cm from the surface. The design of the preliminary study could not be extended into a long-term study because it would have required resources that exceeded the resource budget. Therefore, budget constraints required that the number of sampling sites be reduced to three.

Sampling Sites

Three sites along the Comal River were selected for this study (Figure 2). The Houston Street site (HS, 29.720777° Lat, -98.128097° Long) was located directly below Spring Five, which is one of the larger springs in this portion of the river. The next downstream site, Liberty Avenue (LA, 29.718766° Lat, -98.130305° Long) was located 300 m downstream from the Houston Street site at the west end of Liberty Avenue. These two sites were chosen because the springs that provide discharge through this stretch of the river were expected to exhibit the earliest reduction in flow as a drought progresses. Approximately 750 m downstream from LA, the flow is impounded by a dam which forms Landa Lake. Water flows from Landa Lake into two channels: the New Channel and Old Channel.

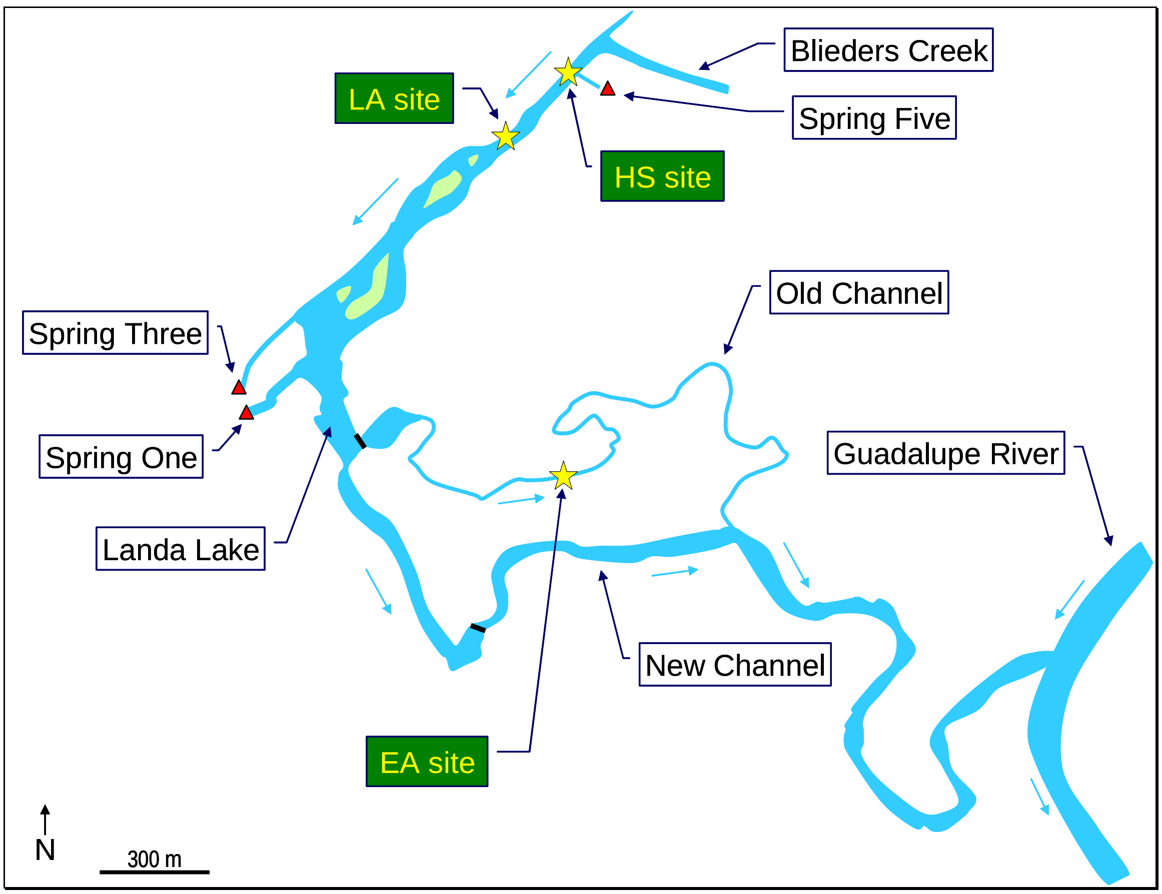


Figure 2. Study sites where *Centrocestus formosanus* cercariae were collected from the Comal River.

The third site, Elizabeth Avenue (EA, 29.710133° Lat, -98.128703° Long) is located in the Old Channel. The EA site is historically one of the most stable areas in the river because a culvert regulates flow from Landa Lake into the Old Channel, in order to reduce scouring from floods events. The Old Channel and New Channel converge 2.5 km downstream from Landa Lake and the river flows south another 2.5 km before the confluence of the Comal River with the Guadalupe River (USFWS 1996).

Site characterization data collected from each of the three sites included wading discharge, current velocity (Marsh-McBirney portable flowmeter, Model 2000, Frederick, Maryland), temperature, and dissolved oxygen (DO meter Model 58, YSI, Yellow Springs, Ohio), while total stream discharge was obtained from USGS. At times of sampling, degree of insolation ("sunny," "partly cloudy," "mostly cloudy," or "overcast") and the presence of piscivorous birds was also recorded.

Sampling Protocol: Final Design

A transect was established across the river at each site, from left bank to right bank (facing downstream), and six equidistant points along the transect were selected for sampling (Figure 3). Two 5-L samples of water were taken at each of the six points, one at approximately 10 cm from the bottom and one at sixty percent depth from the surface (V6). If the depth at a transect point was too shallow for two samples to be taken, then only one sample was taken at sixty percent depth.

Each of the three sites was sampled every two weeks for 12 months (June 2006-2007) between 0930 and 1230 hours (when possible), unless weather or flooding prohibited. The morning time period was chosen because snails infected with *C. formosanus* have been reported to release more cercariae diurnally than nocturnally (Lo and Lee 1996), which is similar to other positively phototactic trematode species (McClelland 1965). The six equidistant points along the transect were sampled in an alternating pattern in order to randomize diel effects over the sampling interval (Figure

3). Sampling was also conducted on sunny days when possible because cercarial abundance differed significantly between sunny and cloudy days (Lozano 2005).

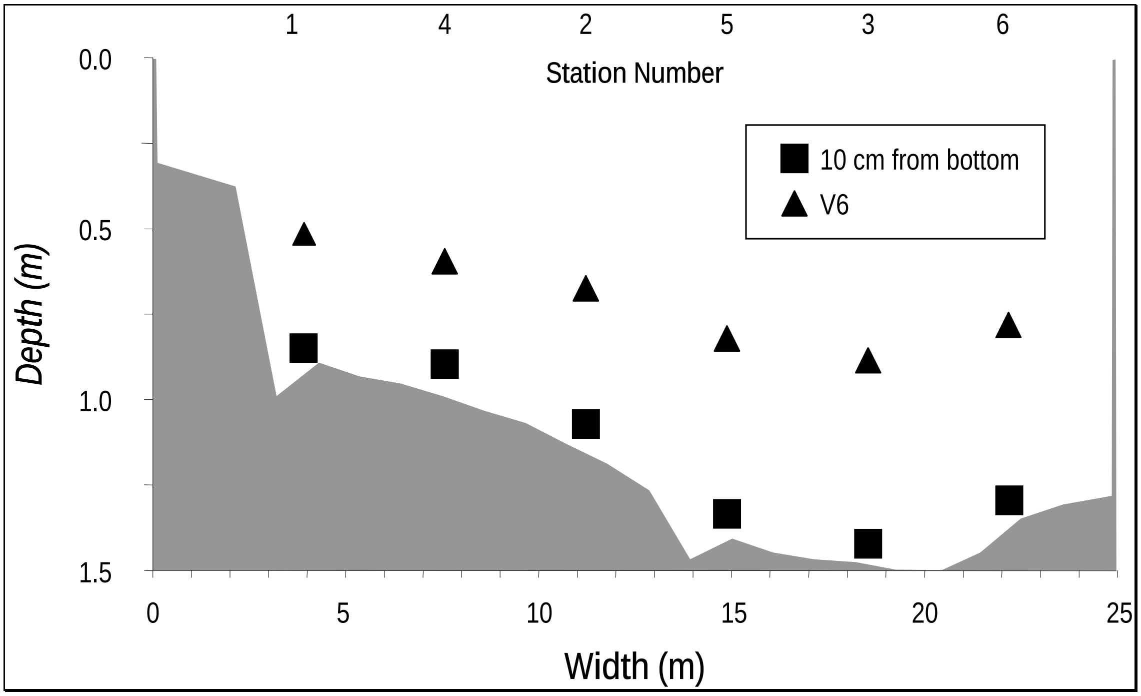


Figure 3. Downstream view of a hypothetical cross section of the Comal River illustrating the locations where samples were collected. The station number represents the sequence in which samples where collected.

A battery operated pump (Attwood aerator pump, Model A500, Lowell, Michigan) was used to transfer 5-L of river water through a 1/4” flexible tube into a 10-L bucket. To collect cercarial samples at various depths, the pump was attached to a flow rod. Current velocity at each cercarial sample point was taken first, while allowing any sediment disturbed during wading to settle. After current velocity was recorded, the pump opening was then pointed into the current to collect the water samples.

The LA and HS sites were sampled on the same date, since they are on the same stretch of the river, with LA sampled first followed by HS. To allow for cercarial sampling to be completed in the recommended diel timeframe, recording of wading discharge was delayed until after all water samples for the day had been collected. The EA site was sampled within one day of the other two sites.

Cercarial Counts

Immediately after collection, the cercariae in each water sample were fixed by adding 5 ml of formalin to the 5 L sample of river water to make 0.1% formalin solution. This was done to prevent cercariae from squeezing themselves through the filter during the filtration process. The sample was then stirred and poured through a filtration apparatus (Figure 4) modified from Theron (1979) and Prentice (1984). Cercariae in the water sample were small enough to pass through two prefilters with 220 μm and 86 μm mesh, respectively, but then collected on a 30 μm final filter. The waste water was collected in a 22.7 L waste container below the final filter and taken back to the laboratory to be treated with formalin neutralizer for proper disposal (Detox Formalin Neutralizer, Scientific Device Laboratory, Des Plaines, Illinois).

After all the water from a sample had passed through the apparatus, the final filter was removed, placed in a petri dish, stained with 1.5 ml of Rose Bengal, and preserved with 3 ml of 10% formalin on site. The petri dish was then sealed with Parafilm (Pechiney Plastic Packaging, Chicago, Illinois) to prevent desiccation until analysis. Sampling at each site typically resulted in 12 final filters.

At the laboratory, a paper counting grid (60 X 60 mm) was placed into a petri dish (95 mm diameter) and a final filter from a water sample was placed on the grid. Water (~ 20 ml) was then added to the dish to help reduce glare. All cercariae from each final filter were counted under a dissecting microscope (100 X) and the total number was recorded.

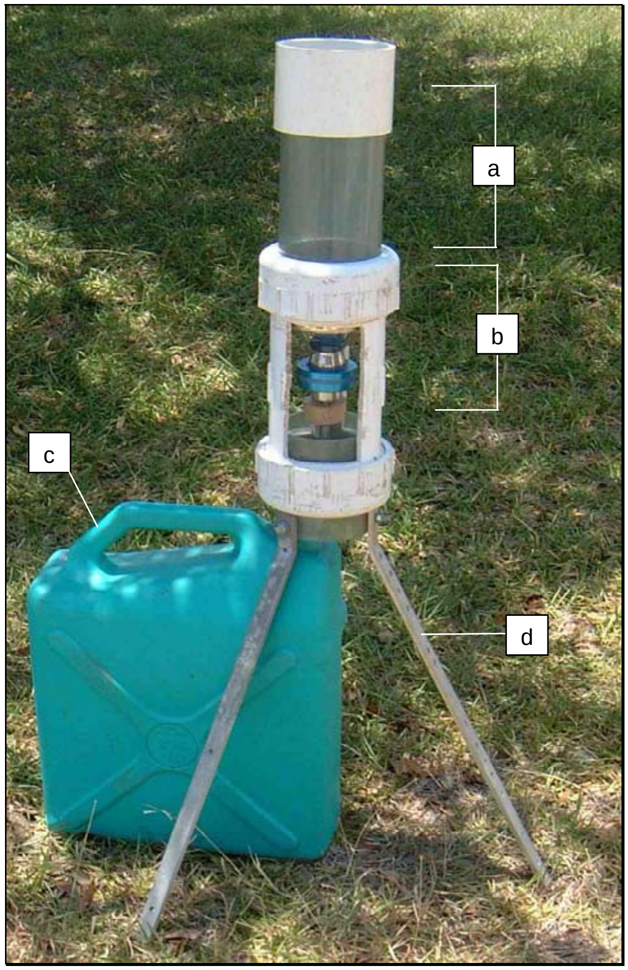


Figure 4. Modified filtration apparatus. a) prefilters (220µm and 86µm). b) sample filter mount with 30 µm monofilament filter. c) 22.7 L formalin collection container. d) filter apparatus support.

To prepare for the next sampling session, the filters were soaked in a 10% solution of sodium hydroxide and then sprayed with hot water to dislodge cercariae (Prentice 1984) so that the filters could be reused.

Effort Required

During the one year study period (apart from the preliminary study), a total of 48 days were spent in the field and 336 hours were expended collecting samples. HS and EA each resulted in 12 samples per visit and 11 samples were collected at LA per visit. This resulted in a total of 840 water samples (4200L) collected during the study.

During the one year period, (apart from the preliminary study), a total of 42 days were spent in the laboratory. Approximately 15 minutes was required to count the number of cercariae per filter, therefore 180 hours total was spent counting cercariae. The number of *C formosanus* cercariae counted was 17,563 and the number of *Haplorchis pumilio* cercariae counted was 132. Therefore, the total number of cercariae counted during the study was 17,695.

Numerical Methods

The number of *C. formosanus* cercariae per liter was calculated by dividing the total number of cercariae counted from a final filter by 5L. The number of cercariae/L was transformed to the meet parametric assumption of normality. The transformation used was the square root of the number of cercariae plus three eighths $\sqrt{N / \mathrm{L}+3/8}$ (Zar 1999). Means are reported in the form: mean ± SE (min-max).

Cercarial abundance (number of cercariae/L) will be regressed independently against total stream discharge, wading discharge, current velocity, temperature, and dissolved oxygen to determine if cercarial abundance varies in response to changes in any of these variables. Regression analyses were tailored to the data post hoc to find a trendline that best fits the model. Regressions used in this study were linear and third order polynomial.

Total stream discharge and wading discharge were recorded in cubic meters per second (cms). Total stream discharge values were obtained from USGS, based on a gage located below the confluence of the Old Channel and New Channel (Lat 29°42'21", Long 98°07'20"), while wading discharge was calculated at each site at the time of sampling. Wading discharge calculations were adapted from the method of Buchanan and Somers

(1969). Wetted stream width was divided into approximately 25 equal segments (n=25). Stream depth was then recorded at the junctions of the segments resulting in n-1 depths (Figure 5). If stream depth at the segment junction was 0.76 m or less, one velocity measurement was taken at 60% depth. If depth exceeded 0.76 m, two velocity measurements were taken, one at 20% depth and the other at 80% depth from the surface. Later, partial discharge at each segment was calculated by multiplying depth at junction × segment width × mean velocity. The partial discharges from a stream cross section were summed to determine the wading discharge for the cross section on that sampling date.

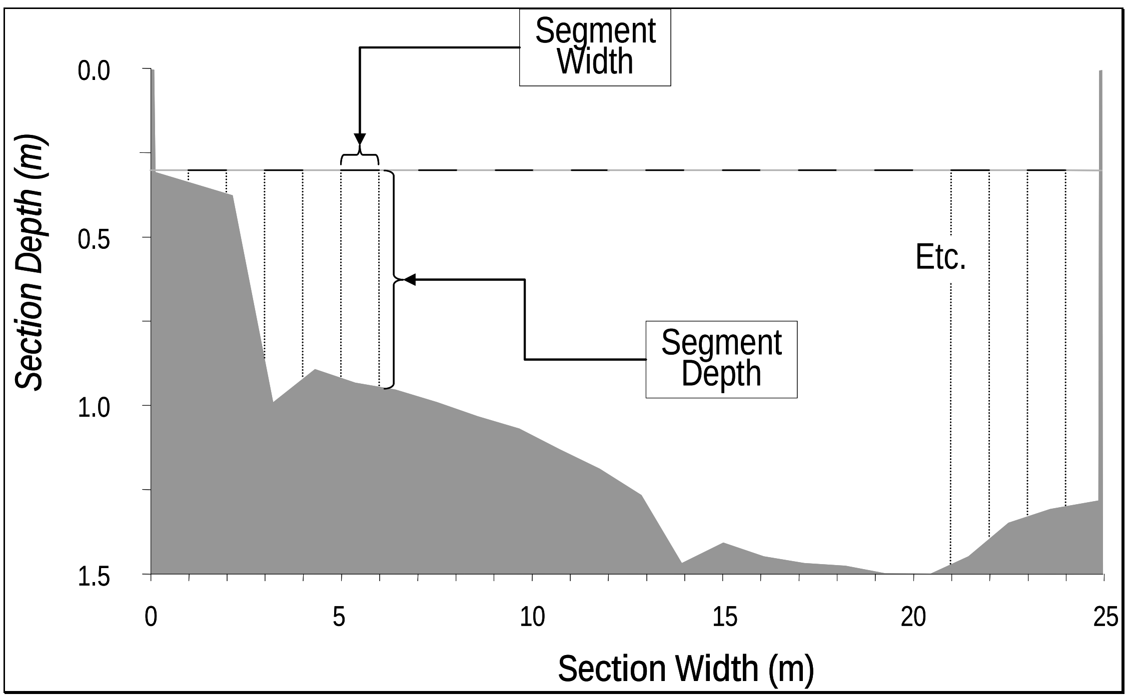


Figure 5. Hypothetical cross section of the Comal River illustrating method of computing wading discharge.

Current velocity was recorded in meters per second (m/s), temperature in degrees Celsius (°C), and dissolved oxygen in milligrams per liter (mg/L). Percent oxygen saturation in water was calculated using the temperature and dissolved oxygen data (WOW 2004a).

Data in this study were recorded in metric units, the equivalents to imperial units are as follows: 1 m^3=35.315 ft^3, 1 m=3.281 ft, 1 L=0.264 gal, and 1 °C=33.8 °F (WOW 2004b).

CHAPTER III

RESULTS

Descriptive Statistics

The mean total stream discharge during the study was 7.61 cms ± 0.04 (5.78-12.49). Total stream discharge during the study was slightly lower than the historical mean (total annual mean from 1933 to 2005 is 8.58 cms).

The mean wading discharge for HS was 0.38 cms ± 0.01 (0.02-0.82). The mean wading discharge for LA was 0.45 cms ± 0.02 (0.12-0.93). The mean wading discharge for EA was 1.42 cms ± 0.01 (1.17-1.62).

The mean current velocity recorded along with the water samples taken at HS was 0.02 m/s ± 0.001 (0.00-0.08). The mean current velocity for samples at LA was 0.02 m/s ± 0.001 (0.00-0.08). The mean current velocity for samples at EA was 0.19 m/s ± 0.01 (0.003-0.44).

The mean temperature during the study was 23.48 °C ± 0.04 (21.0-26.1). The mean amount of dissolved oxygen during the study was 7.30 mg/L ± 0.04 (5.5-10.4).

During the study the mean number of cercariae was 4.18/L ± 0.16 [0.00(in nine samples)-60.6] across all sites. The mean number of cercariae per cross section ranged from 0.48-12.32/L, and by using wading discharge to calculate the number of liters per cross section this indicates that there could be between 1.21 and 1,813 cercariae passing through a cross section at one time.

During the 48 days spent in the field, Green Herons were observed nine times across all sites, with two being the greatest number observed on one sampling date. The LA site had the greatest number of observations during the one year period with a total of seven.

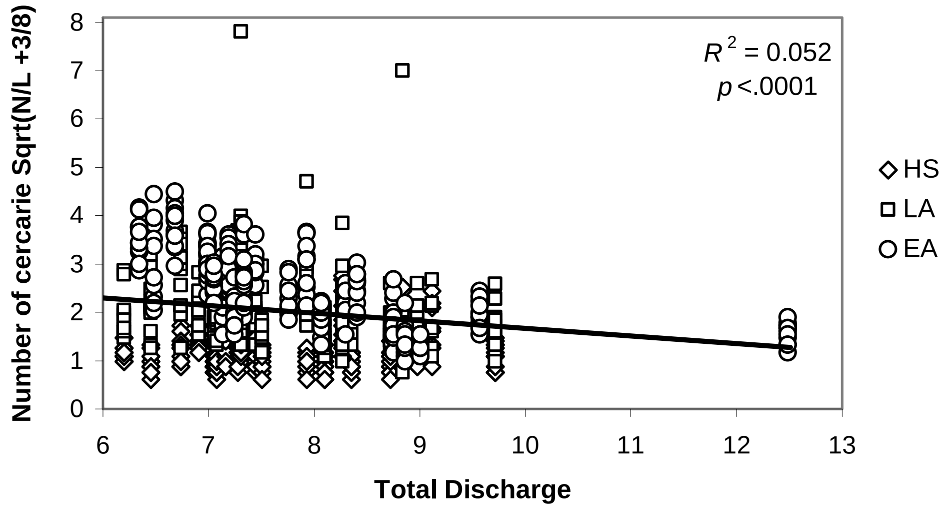
<u>Overall Counts</u>

Effects of discharge

Linear regression was used to search for effects of discharge on cercarial abundance. The number of *C. formosanus* cercariae decreased as total stream discharge increased [$p(F_{1,838} \geq 46.38) < 0.0001$]. However, because only about 5% of the variance in cercarial counts could be explained by total stream discharge (Figure 6, $R^2=0.052$), the variable was dismissed as a useful predictor of cercarial abundance. The number of *C. formosanus* cercariae increased as wading discharge increased [$p(F_{1,780} \geq 206.24) < 0.0001$]. However, because only about 21% of the variance in cercarial counts could be explained by wading discharge (Figure 6, $R^2=0.209$), the variable was dismissed as a useful predictor of cercarial abundance.

Seasonal Effects

Nonlinear regression was used to search for effects of season on cercarial abundance, and a seasonal relationship was observed [$p(F_{3,836} \geq 17.60) < 0.0001$]. The minimum cercarial count predicted by the regression was in March, and the maximum predicted cercarial count was in November. Even though this model had a very small *p* value, season was dismissed as a useful predictor of cercarial abundance because only about 6% of the variance in cercarial counts could be explained by season (Figure 7, R^2=0.059).



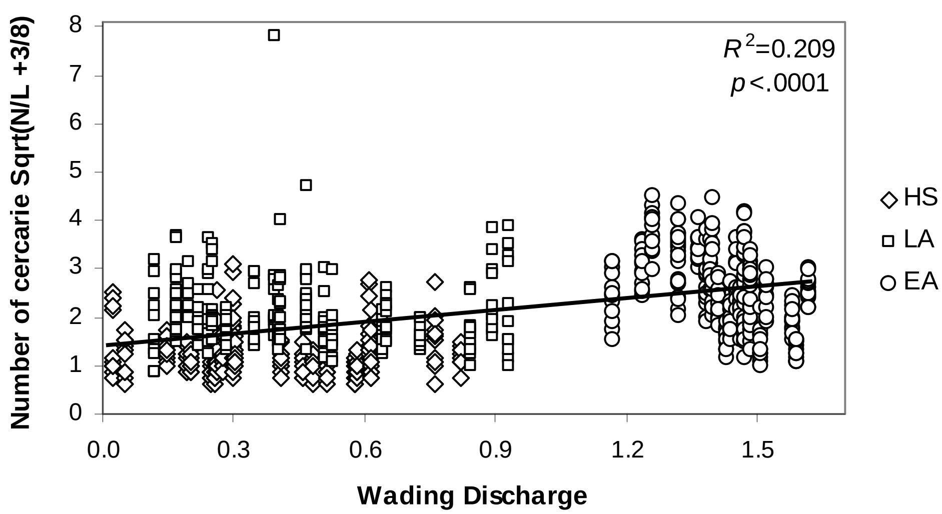


Figure 6. Effects of total and wading discharge (cms) on the abundance of *C. formosanus* cercariae.

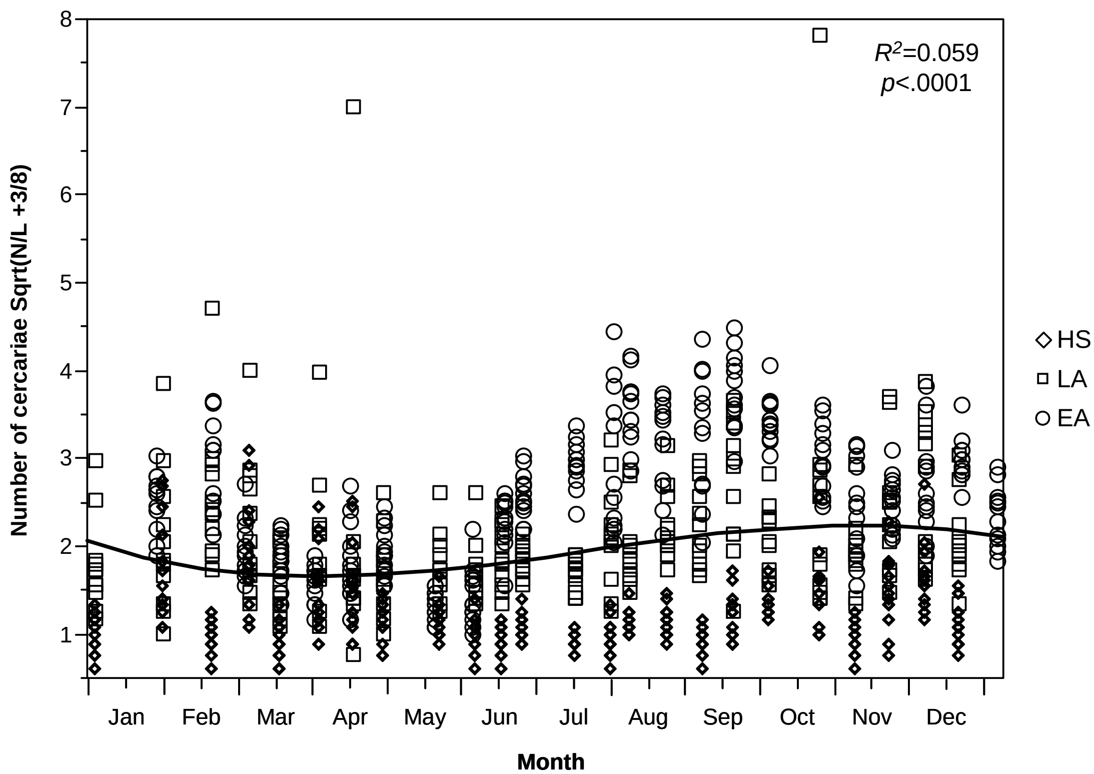


Figure 7. Effects of season on the abundance of *C. formosanus* cercariae

Effects of Site

One-factor ANOVA was used to search for effects of site on cercarial abundance. The mean number of *C. formosanus* cercariae counted at EA [6.75/L ± 0.024 (.60-19.80)] was found to be higher than LA [4.53/L ± 0.33 (0.20-60.60)], followed by HS [1.31/L ± 0.08 (0.00-9.20); $p(F_{2,837} \geq 280.64) < 0.0001$]. Therefore, it appears that the further downstream a site was, the higher the cercarial abundance. Table 1 provides the ANOVA table and means table for the transformed counts.

Effects of Insolation

One-factor ANOVA was used to search for effects of insolation on cercarial abundance. The degree of insolation on a sampling date was subjectively classified as "sunny," "partly cloudy," "mostly cloudy," and "overcast." However, because insolation is a random factor, unequal allocation of these four categories among the three sites complicated the analysis, and some insolation categories were never recorded at some of the sites. Consequently, all sampling dates with insolation recorded as "overcast," "mostly cloudy," or "partly cloudy" were subsequently pooled into a new category referred to as "cloudy" from this point forward. This resulted in two categories for insolation, "sunny," and "cloudy."

After these modifications, the mean number of *C. formosanus* cercariae counted on sunny days [4.49/L ± 0.23 (0.00-60.6)] was found to be higher than on cloudy days [3.72/L ± 0.19 (0.00-18.60); $p(F_{1,838} \geq 4.38) = 0.037$]. Table 2 provides the ANOVA table and means table for the transformed counts.

Table 1. a) One factor ANOVA table for the effects of site on the abundance of *C. formosanus* cercariae. b) Transformed means table for effects of site using one factor ANOVA.

a

Source of Variation	DF	SS	MS	F	*p*
Site	2	260.078	130.039	280.640	<0.0001
Error	837	387.838	0.463		
Total	839	647.916			

b

Site	Number	Mean	Std Error
HS	288	1.224	0.040
LA	264	2.073	0.042
EA	288	2.552	0.040

Table 2. a) One factor ANOVA table for the effects of insolation on the abundance of *C. formosanus* cercariae. b) Transformed means table for the effects of insolation using one factor ANOVA.

a

Source of Variation	DF	SS	MS	F	*p*
Insolation	1	3.367	3.367	4.378	0.037
Error	838	644.550	0.769		
Total	839	647.916			

b

Insolation	Number	Mean	Std Error
Cloudy	337	1.869	0.048
Sunny	503	1.998	0.039

<u>Interaction between Insolation and Site</u>

The analyses of insolation and site effects both resulted in very small *p* values; however, the reported *p* values may be erroneous because of possible interactions between insolation and site due to the unequal allocation of insolation categories. In order to determine if there was a significant interaction between these two variables, a two factor ANOVA with replication was executed. A two factor ANOVA requires a balanced data set (equal sample sizes) and so a random subset of sunny days was taken to equal the total number of cloudy days. Though this reduced the degrees of freedom for the analysis, the resulting cell size in the model was 96 sunny/cloudy pairs. The analysis did not reveal a significant interaction between insolation and site (Table 3 p=0.722).

Table 3. Insolation and site effects on the abundance of *C. formosanus* cercariae using a two factor ANOVA.

Source of Variation	DF	SS	MS	F	p
Weather	1	6.109	6.109	15.879	<0.0001
Site	2	186.203	93.101	242.008	<0.0001
Interaction Wx:Site	2	0.251	0.125	0.326	0.722
Error	570	219.281	0.385		
Total	575	411.843			

Effects of Variables by Site

The abundance of *C. formosanus* cercariae was regressed against several variables within each site. Total stream discharge was found to be a poor predictor of cercarial abundance at all three sites because 40% or less of the variance in cercarial counts could be explained by total stream discharge (Figure 8). The R^2 value for total stream discharge at EA (R^2=.407) was the highest value of any regression in the study, and while it was high compared to all other R^2 values calculated, it was not consistent across all sites. Also, the trend at EA is being driven by a single event of high flow (12.49 cms), which if considered an outlier would bring the R^2 value down to 7.4%. Wading discharge was found to be a poor predictor of cercarial abundance at all three sites because 20% or less of the variance in cercarial counts could be explained by wading discharge (Figure 9).

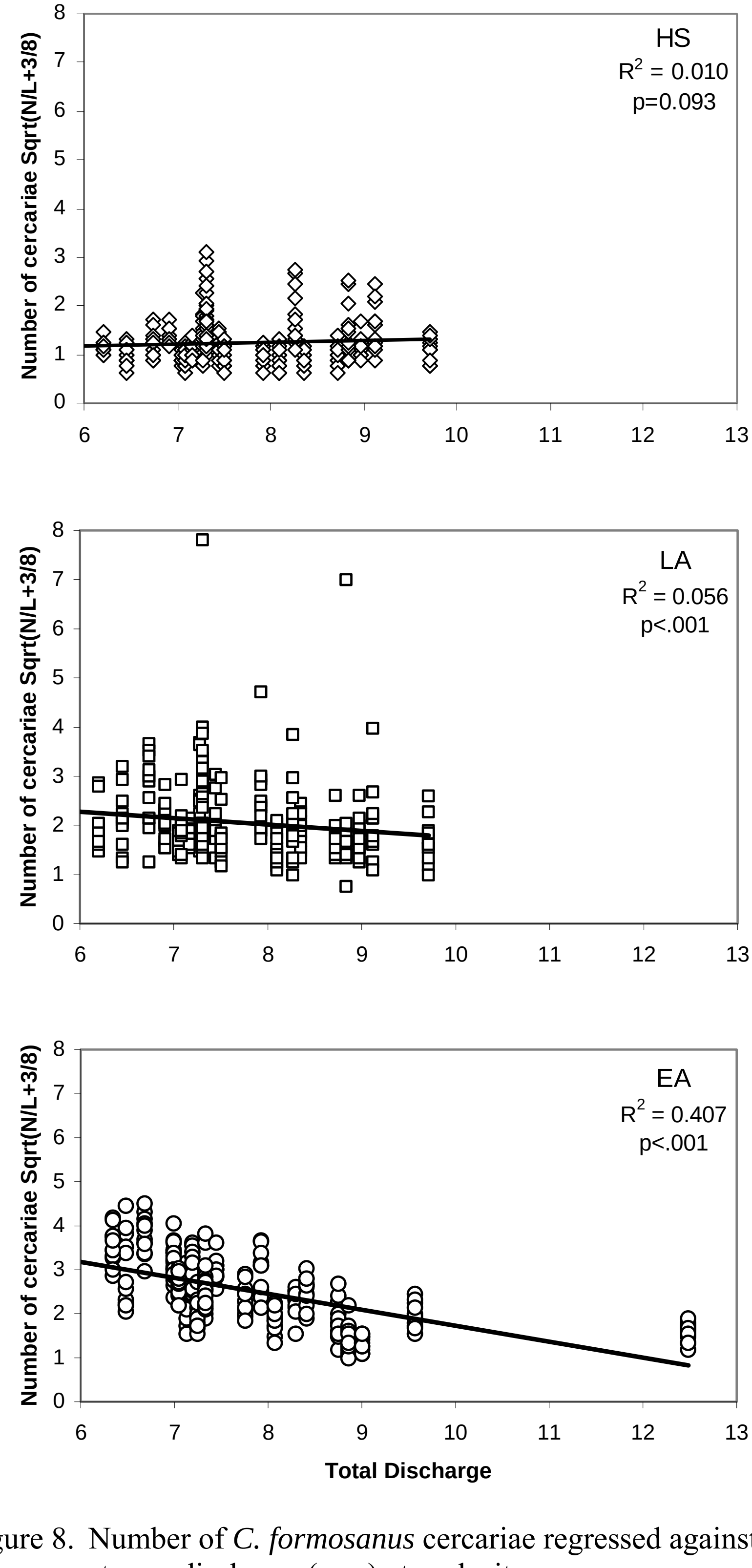


Figure 8. Number of *C. formosanus* cercariae regressed against total stream discharge (cms) at each site.

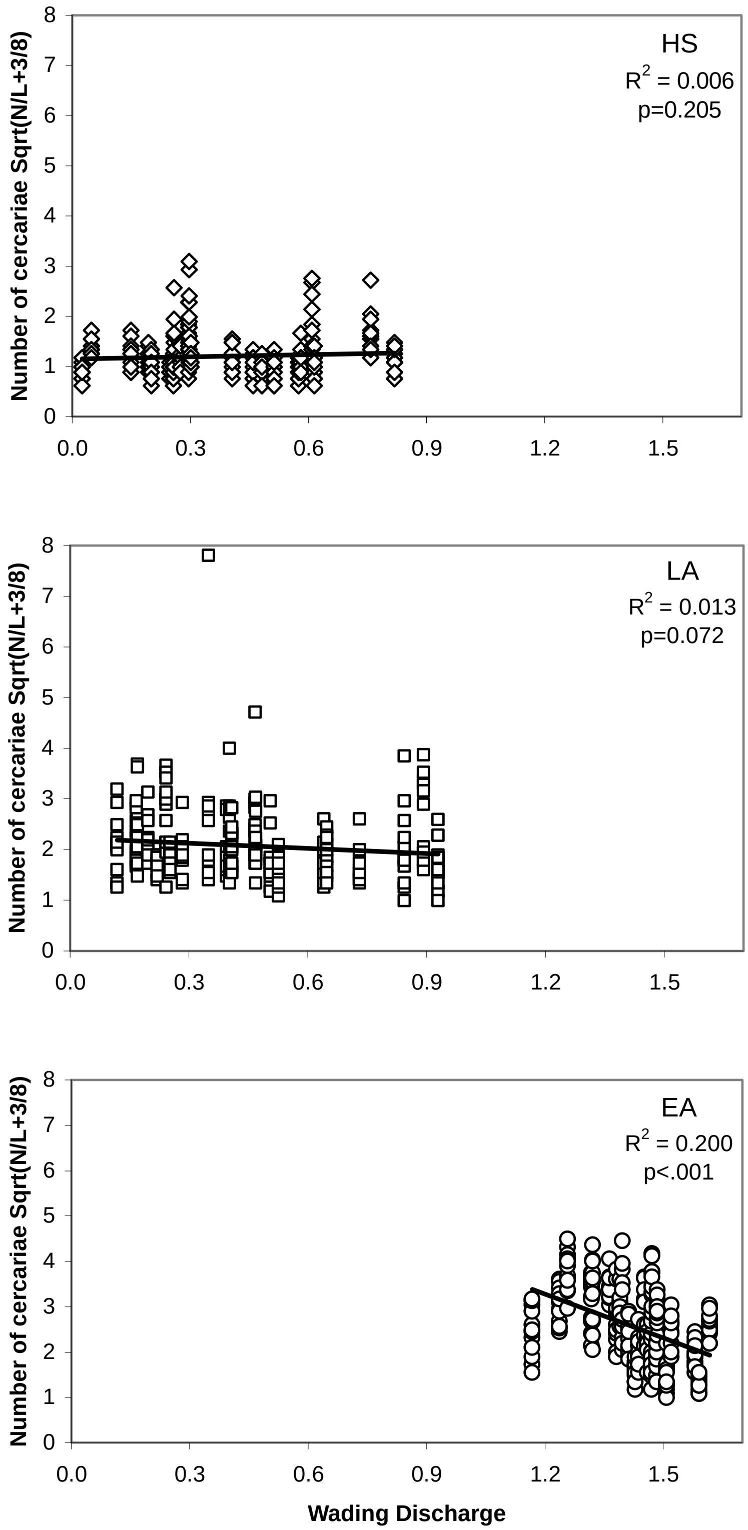


Figure 9. Number of *C. formosanus* cercariae regressed wading discharge (cms) at each site.

Current velocity was found to be a poor predictor of cercarial abundance at all three sites because 0.4% or less of the variance in cercarial counts could be explained by current velocity (Figure 10).

Temperature was found to be a poor predictor of cercarial abundance at all three sites because 12.2% or less of the variance in cercarial counts could be explained by temperature (Figure 11).

Dissolved oxygen was found to be a poor predictor of cercarial abundance at all three sites because 9.5% or less of the variance in cercarial counts could be explained by dissolved oxygen (Figure 12).

Percent oxygen saturation was also found to be a poor predictor of cercarial abundance at all three sites because 7% or less of the variance in cercarial counts could be explained by percent oxygen (Figure 13).

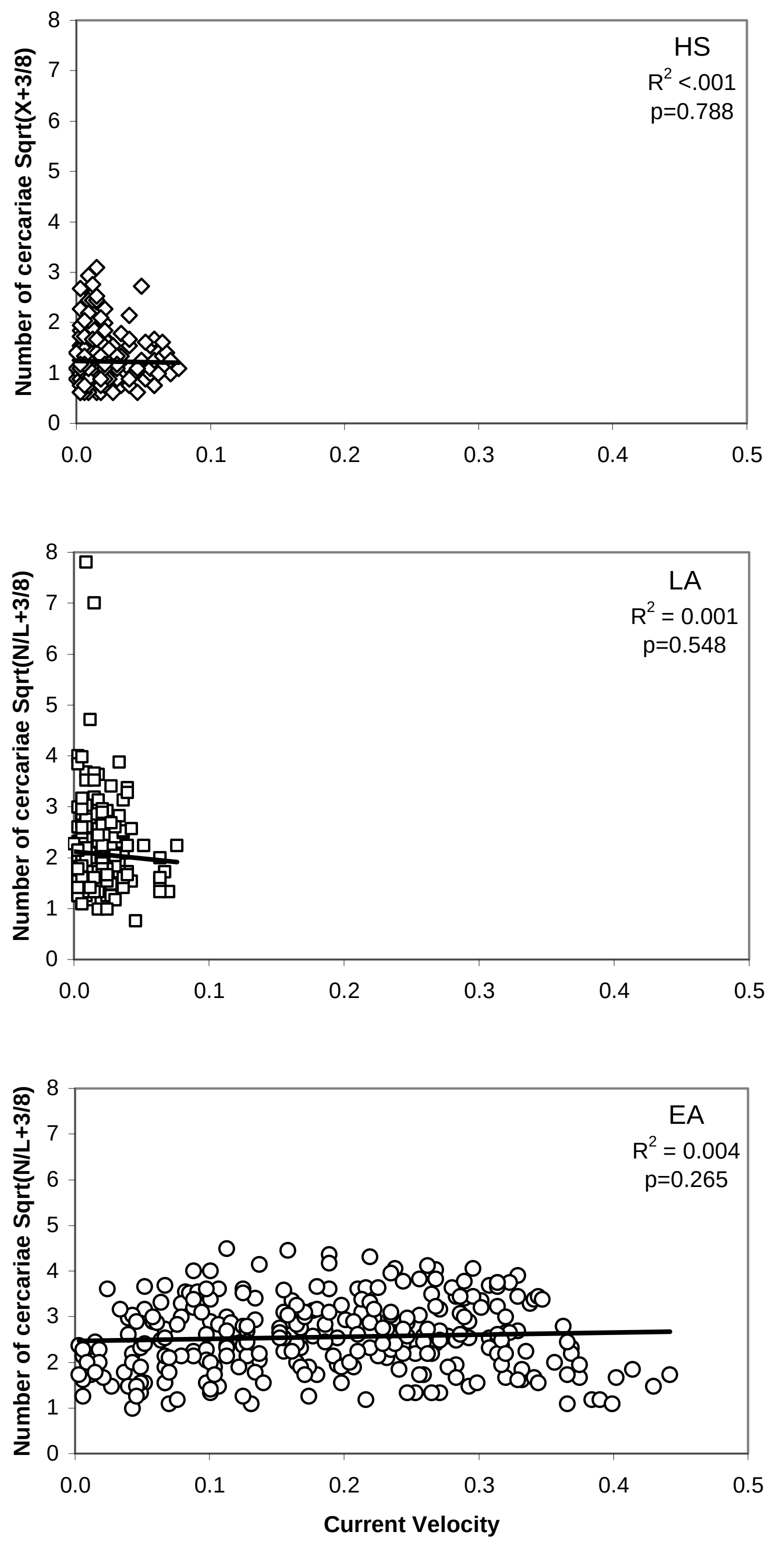


Figure 10. Number of *C. formosanus* cercariae regressed against current velocity (m/s) at each site.

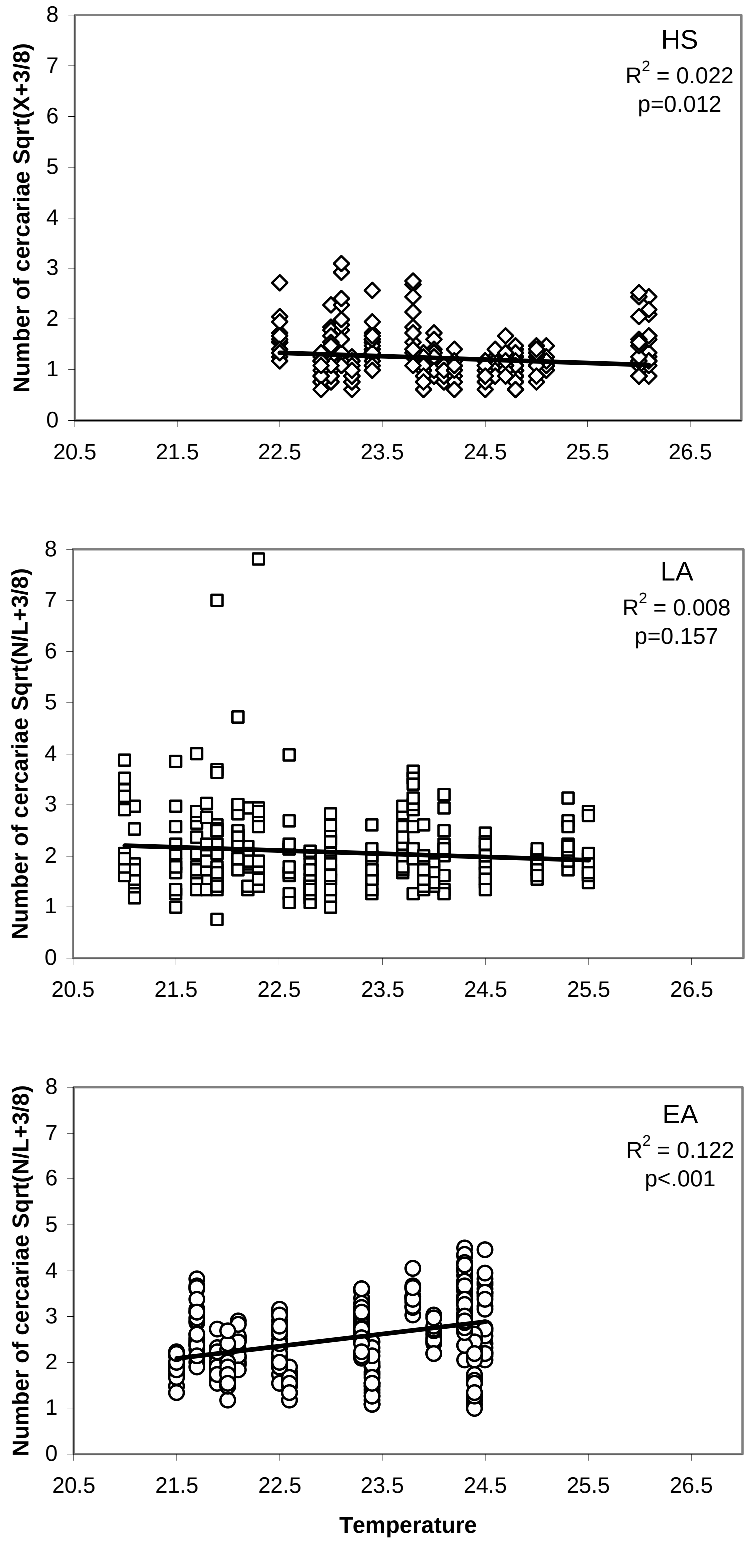


Figure 11. Number of *C. formosanus* cercariae regressed against temperature (°C) at each site.

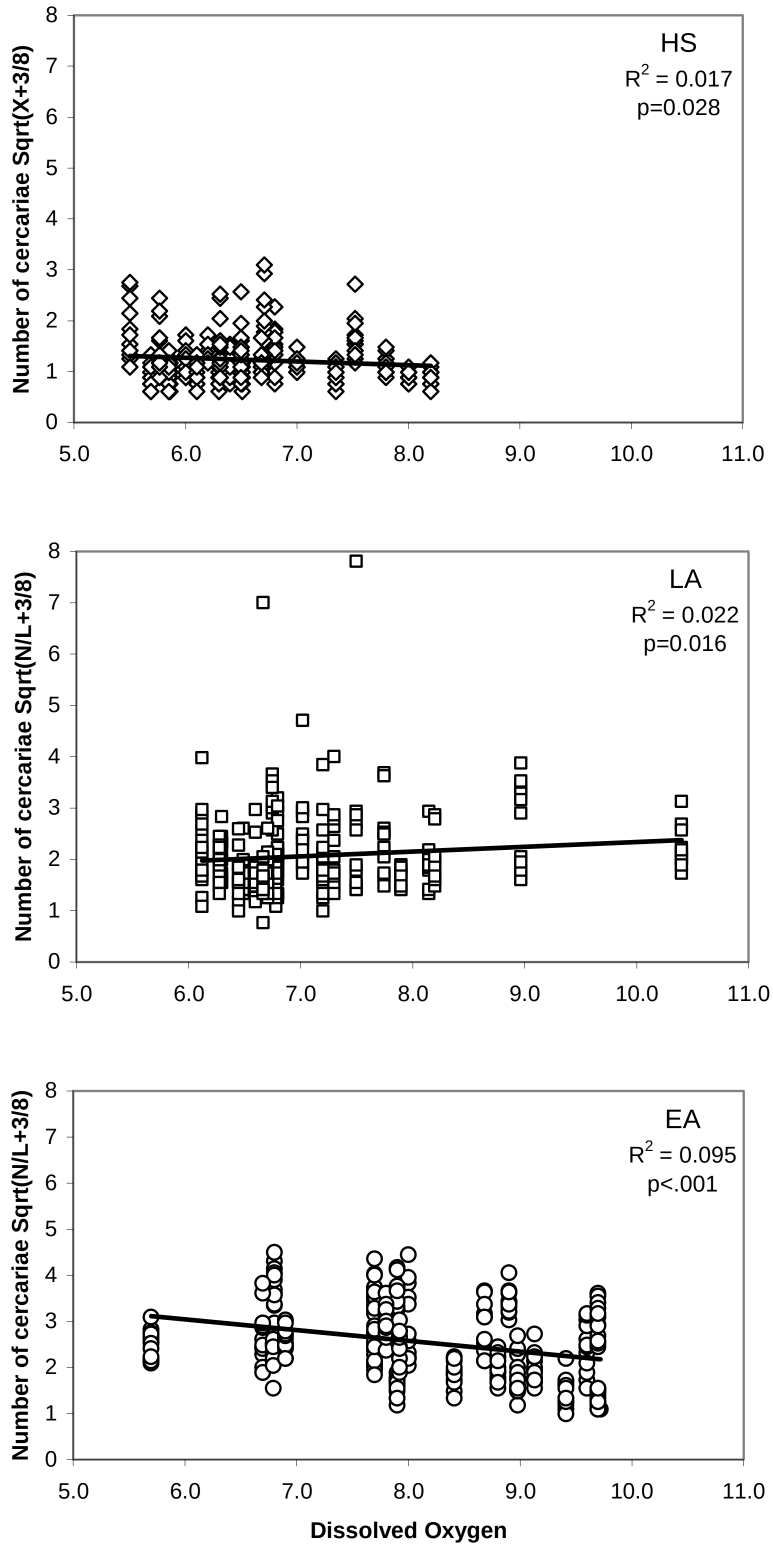


Figure 12. Number of *C. formosanus* cercariae regressed against dissolved oxygen (mg/L) at each site.

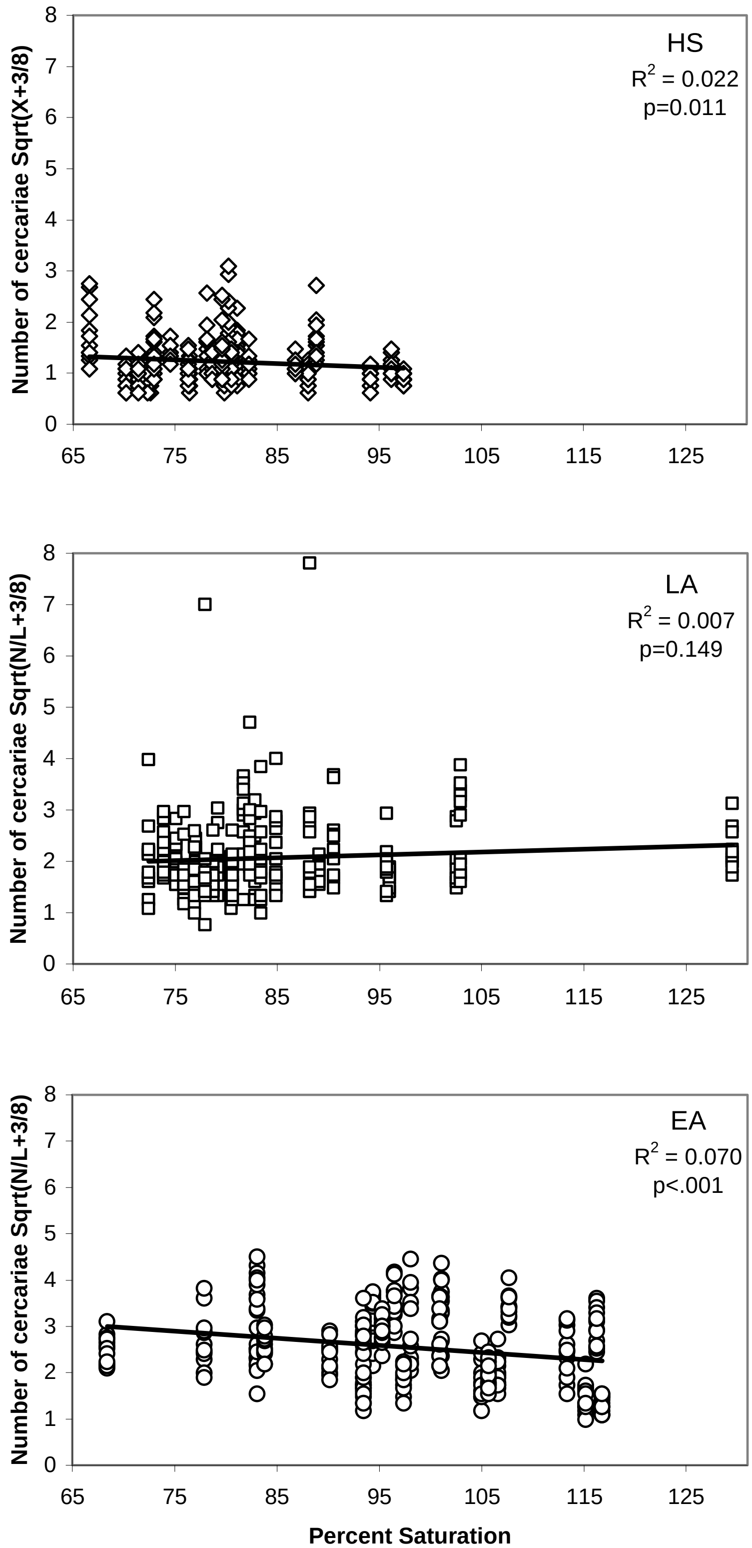


Figure 13. Number of *C. formosanus* cercariae regressed against percent saturation at each site.

Removing the Confounding Effects of Site and Insolation

Cercarial abundance differed by site with increasing numbers of cercariae as distance downstream increased. Cercariae drifting through a site could come from any point upstream, and this makes it difficult to explore relationships between cercarial abundance and discharge-related effects, especially at EA, because of the uncertainty about the location where a given cercaria was originally shed.

Since the LA and EA sites receive water from upstream, the data from these sites are likely to be contaminated by cercarial-laden drift from upstream. Therefore, the cercarial count data from the uppermost site, HS, was considered to be more responsive to local conditions, and so data from this site became the focus of subsequent analyses.

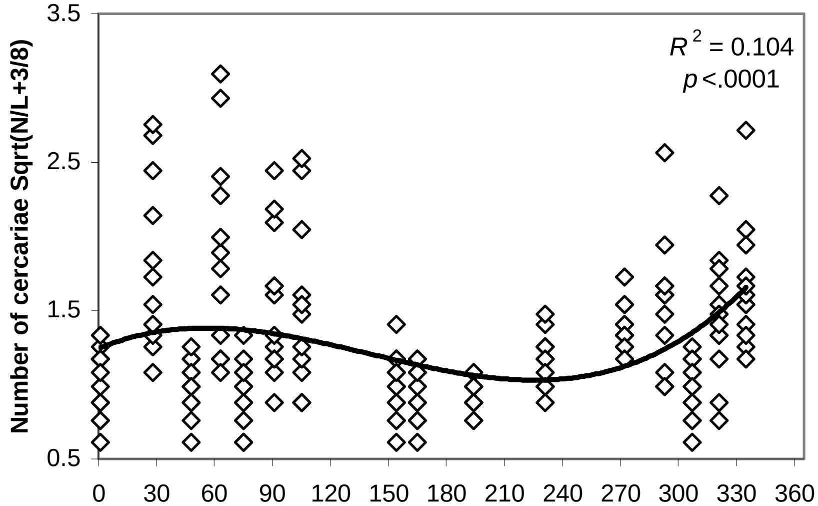
Because insolation had an affect on cercarial count and the focus is now HS, cercarial abundance collected on sunny days only was regressed against stream variables at the HS site. Even after removing cumulative and insolation effects, all variables were found to be poor predictors of cercarial abundance because 16.68% or less of the variance in cercarial counts could be explained by any variable (Figures 14-20).

Removing the Confounding Effects of Season

Even after the dataset was restricted as described above, there were still seasonal patterns in the distribution of cercarial abundance. Thus, any apparent trends in cercarial abundance related to other variables could still be confounded by covariance with season. The seasons generally having the highest cercarial abundance were late fall through late spring (Julian days 250-120), and so only these sample dates were included in the final analyses in an attempt to reduce confounding effects of uncontrolled interaction between season and the other variables.

Though these efforts to reduce the potentially confounding effects of site, insolation, and season reduced the degrees of freedom for the analysis by half, the resulting model still had 144 degrees of freedom. However, even after removing site effects, insolation effects, and seasonal effects, all variables were found to be poor predictors of cercarial abundance because 13.36% or less of the variance in cercarial counts could be explained by all variables (Figures 14-20).

a



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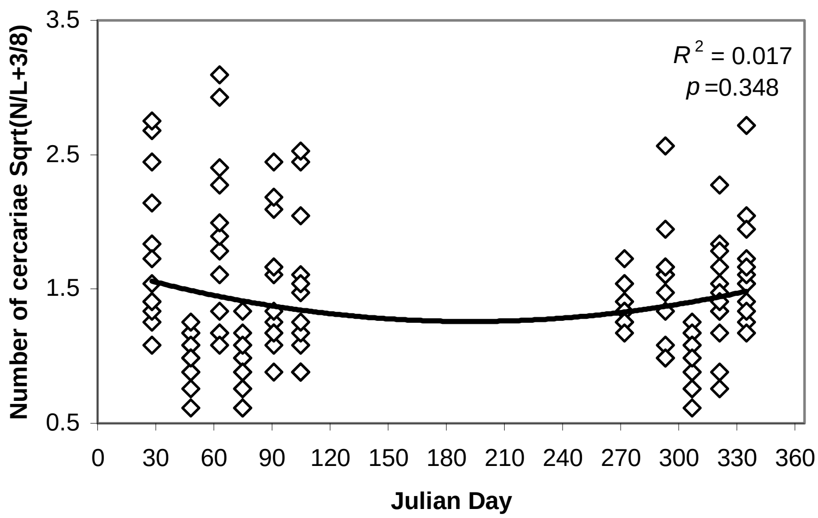
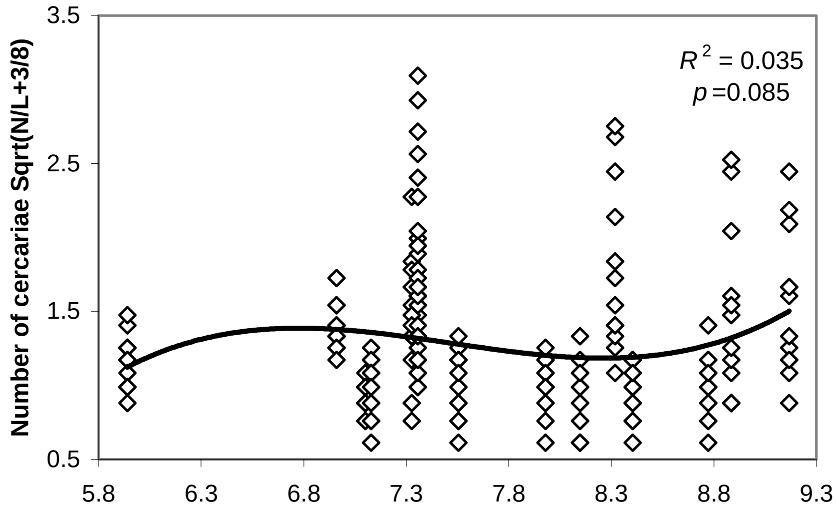


Figure 14. Number of *C. formosanus* cercariae at HS regressed against a) Julian day on sunny days only and b) Julian day on sunny days only and during times of peak emergence.

a



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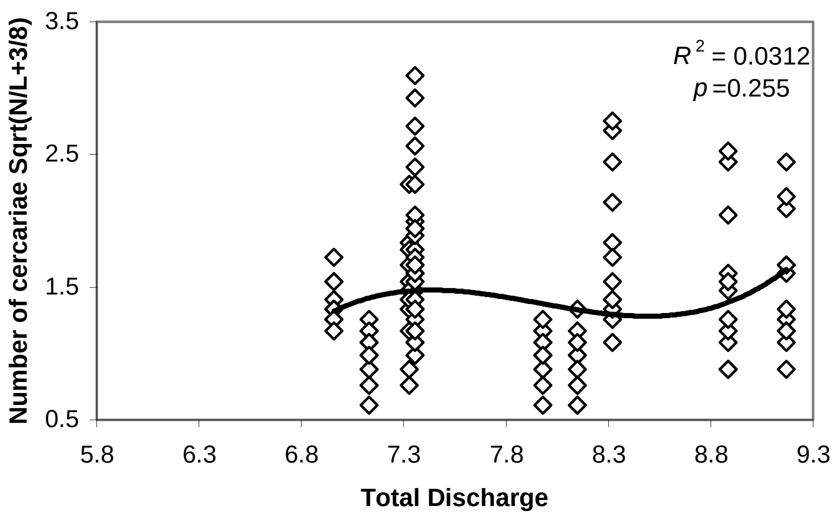
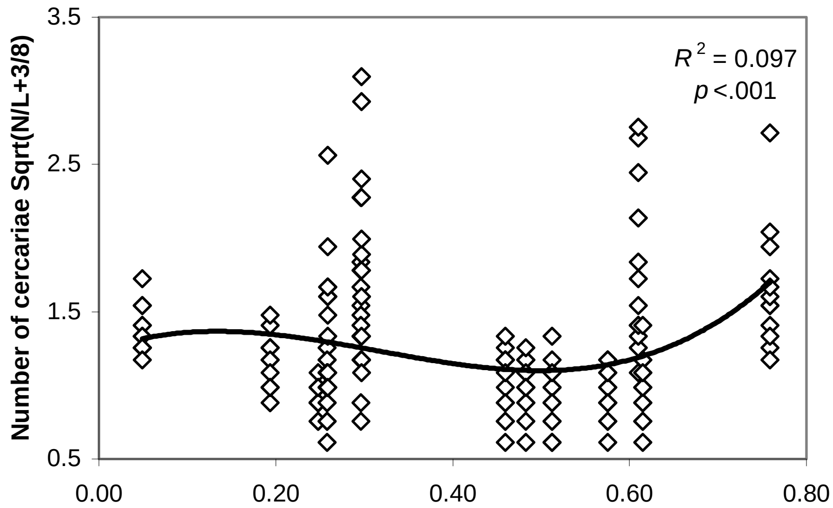


Figure 15. Number of *C. formosanus* cercariae at HS regressed against a) total stream discharge (cms) on sunny days only and b) total stream discharge on sunny days only and during times of peak emergence.

a



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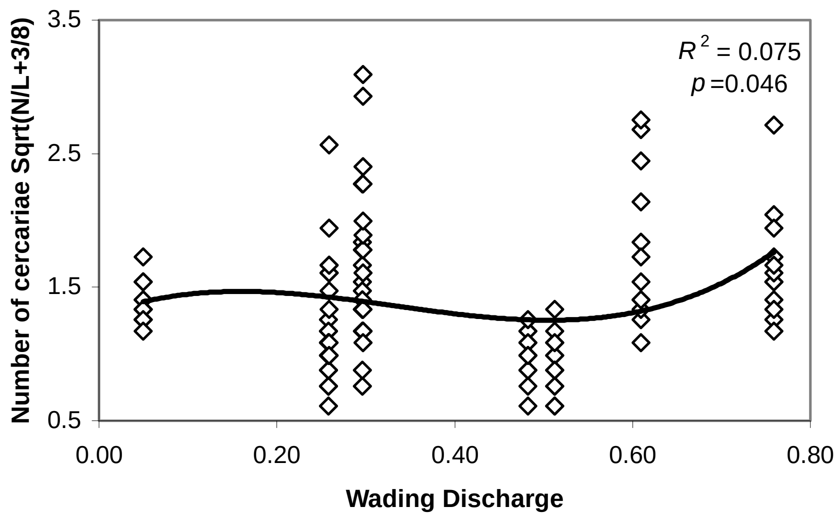
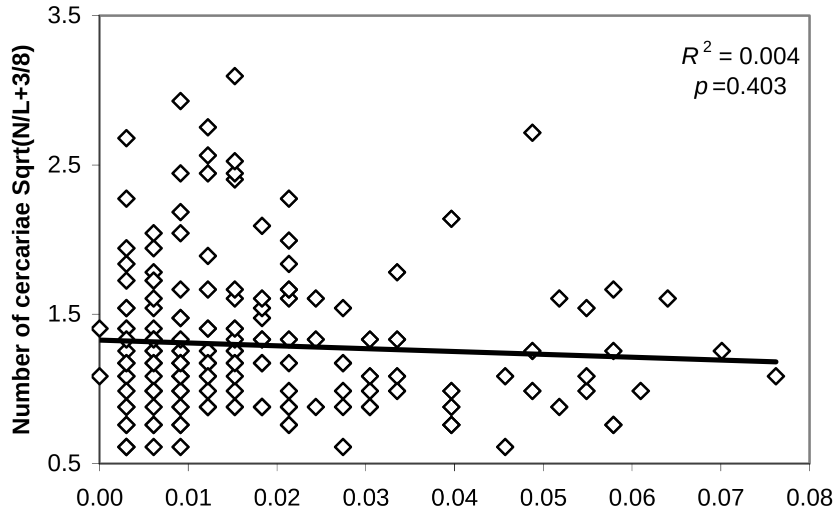


Figure 16. Number of *C. formosanus* cercariae at HS regressed against a) wading discharge (cms) on sunny days only and b) wading discharge on sunny days only and during times of peak emergence.

a



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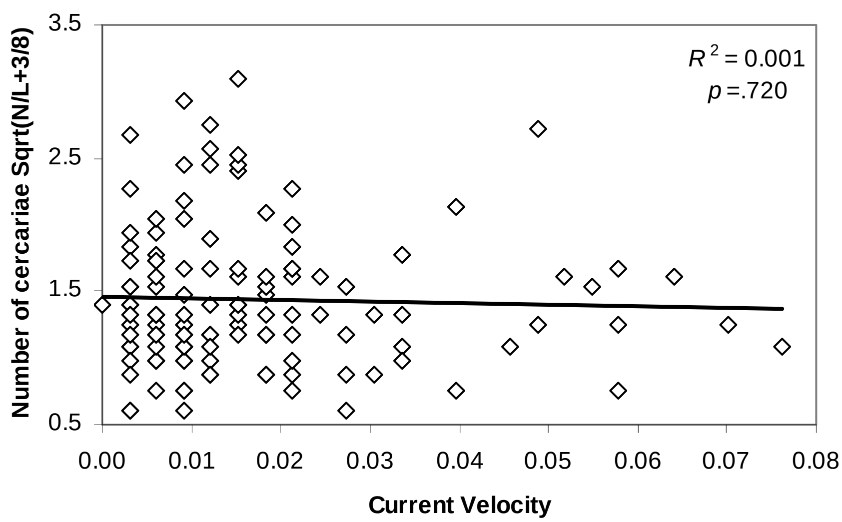
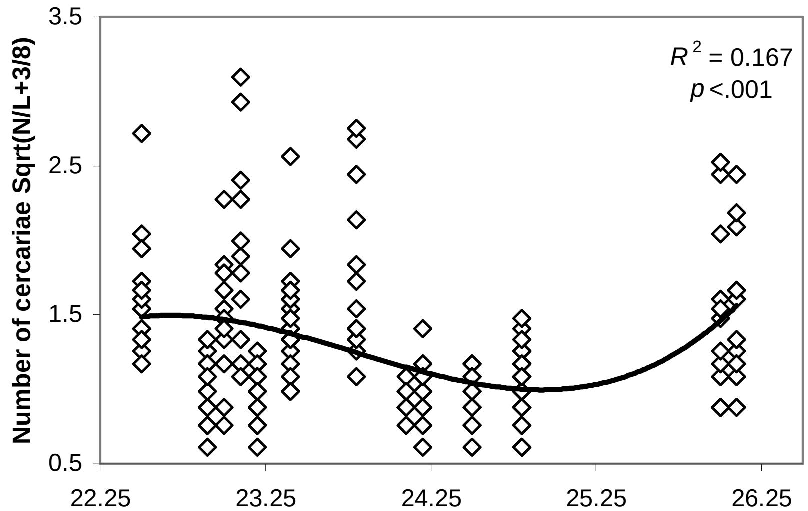


Figure 17. Number of *C. formosanus* cercariae at HS regressed against a) current velocity (m/s) on sunny days only and b) current velocity on sunny days only and during times of peak emergence.

a



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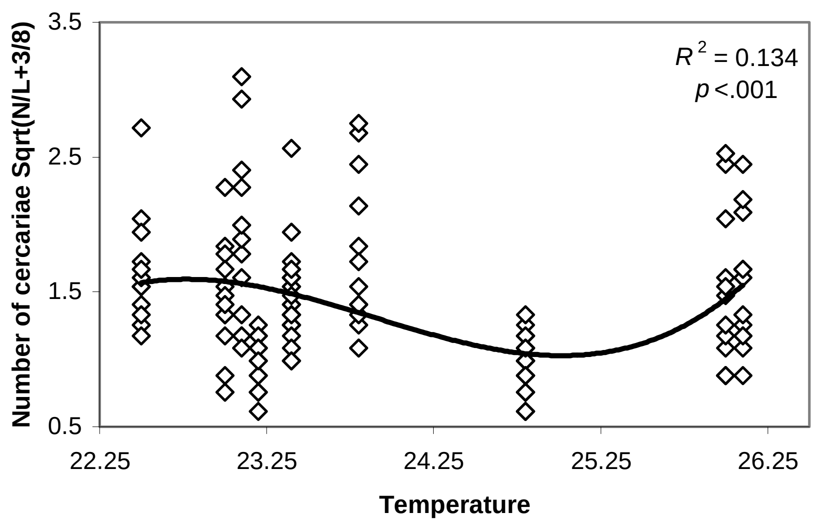
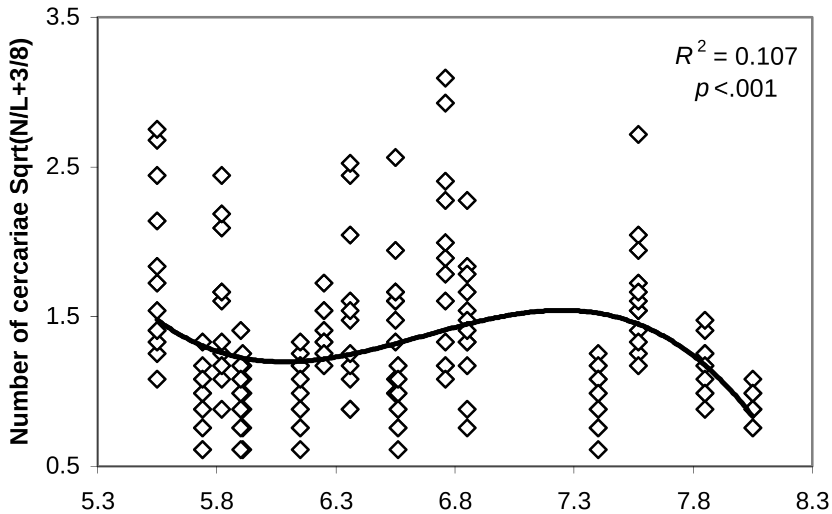


Figure 18. Number of *C. formosanus* cercariae at HS regressed against a) temperature (°C) on sunny days only and b) temperature on sunny days only and during times of peak emergence.

a



b

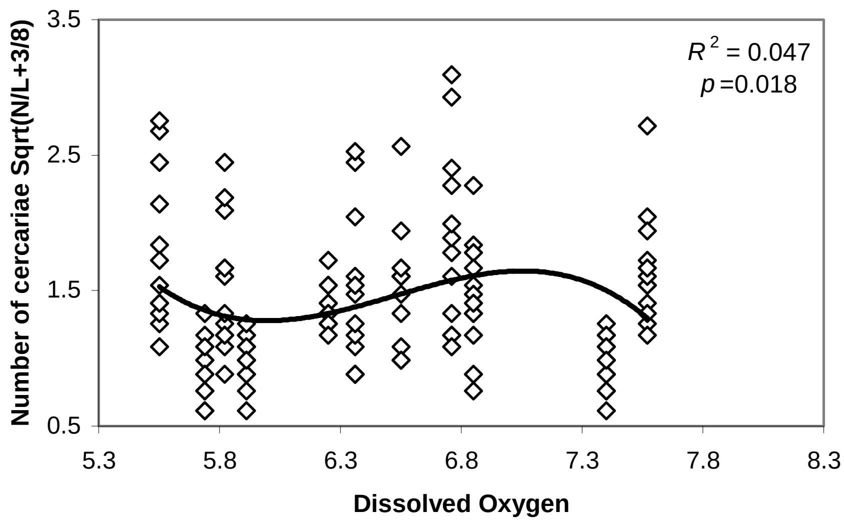
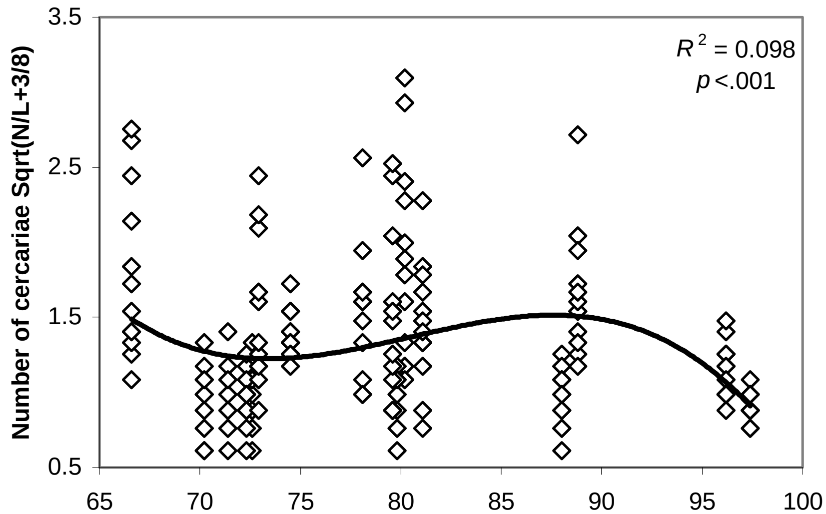


Figure 19. Number of *C. formosanus* cercariae at HS regressed against a) dissolved oxygen (mg/L) on sunny days only and b) dissolved oxygen on sunny days only and during times of peak emergence.

a



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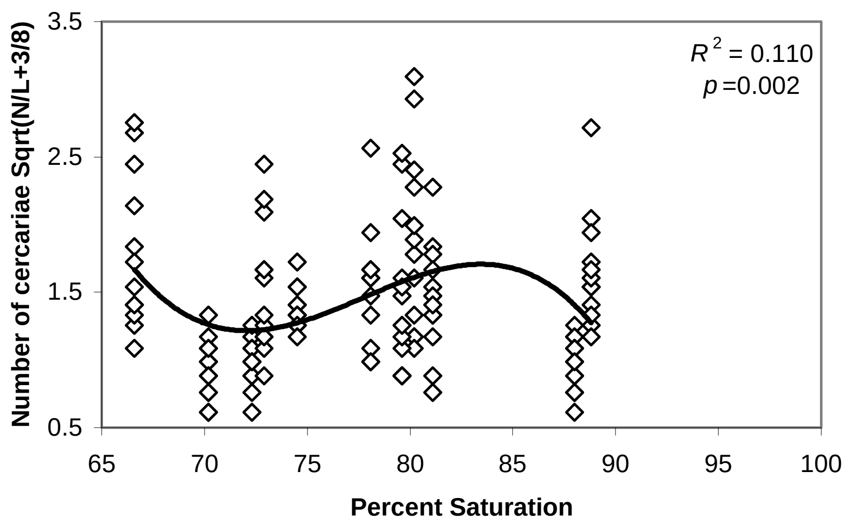

Figure 20. Number of *C. formosanus* cercariae at HS regressed against a) percent oxygen saturation on sunny days only and b) percent oxygen saturation on sunny days only and during times of peak emergence

CHAPTER IV

DISCUSSION

Objectives and Expected Results

The objectives of this study were to determine (1) if cercarial emergence rate in the Comal River increased with decreasing discharge, and (2) if discharge or any other site-characterization variable could be used to predict cercarial abundance. This information was expected to contribute to the management of fountain darters in the Comal River.

Conclusions

Sites in this study with the stillest waters (HS and LA) had lower cercarial abundance than the faster flowing site (EA). Given a snail shedding cercariae at a constant rate, the cloud of cercariae around that snail would be denser if that snail were in the slow flowing sites than if it were in the fastest flowing site. However, since the life expectancy of *C. formosanus* cercariae in the drift (100% survival for 50 hours at 25°C, Lo and Lee 1996) exceeds the transit time from the most upstream site (HS) to the most downstream site (EA), cercariae collected at EA were not only from snails shedding at the EA site, but also from many snails shedding cercariae upstream from EA. This cumulative effect swamped any tendencies for cercarial abundance to be higher in slow-flow conditions at HS and LA. For this reason, the HS site, which is near the very beginning of the river, was thought to have the least contaminated data regarding any effects of local conditions on cercarial abundance. Furthermore, total stream discharge measurements were always reported from the USGS gage downstream from the furthest downstream study site, therefore total stream discharge reports may not be representative of discharge effects on snails at the HS site. Wading discharge, on the other hand, was recorded locally, and was expected to be more representative of the current velocity experienced by snails shedding cercariae at the HS site at the times cercarial samples were collected. On these grounds, total discharge was eliminated as a useful variable for predicting cercarial abundance, and any observed trends between total stream discharge and cercarial abundance were dismissed.

Another potentially confounding factor was uncontrollable variation in insolation. Sunny days had higher cercarial counts than cloudy days, which agreed with the results of a previous study (Lozano 2005). Light is the key stimulus in emergence of *Schistosoma mansoni* and *S. haematobium* cercariae (McClelland 1965) and *C. formosanus* cercariae is similar in that emergence can take place in the dark, but the numbers are greatly reduced (Lo and Lee 1996). In order to eliminate potentially confounding effects of insolation on cercarial abundance, all analyses were performed only on data collected on days recorded as sunny.

Even when data were restricted as described above, there were seasonal patterns in the distribution of cercarial abundance, and so only data collected from late fall through late spring (Julian days 250-120) were included in the final analyses.

With the above restrictions in place, there was still a sufficient range of wading discharge at the HS site (0.05-0.76 cms) to provide evidence of any discharge-related effects on cercarial abundance, but no useful association between wading discharge and cercarial abundance was observed.

One troubling caveat remains in the filtered HS data. The cercarial abundance data for Julian days 1 – 90 alternated dramatically between successive sampling days (Figure 14), and no cause for such alternation could be found in the other variables recorded.

Predictors of Cercarial Abundance

In all of the regressions none of the variables explained a large proportion of the variance in cercarial abundance and therefore all variables were dismissed as useful predictors of cercarial abundance.

One reason for this outcome could be due to the fact that discharge did not decrease into drought conditions during the study. Spring Five flowed during the entire year of sampling. If Spring Five, which is the main water source for the upper sites, had stopped flowing, then perhaps cercarial abundance would have changed more dramatically with wading discharge.

Implications

The results of this study imply that the wild fountain darter population in the Comal River will not experience increased cercarial infection pressure due to reduced discharge within the observed range of this study.

Suggestions for Future Research

The results of this study now provide baseline data to which future cercarial samples can be compared, but this study also raises a number of questions that would benefit from more research.

A study utilizing a living stream could be useful in discovering if there is a relationship between cercarial abundance and discharge while having control over variables. For example, a living stream study could be designed to explore a wider range of discharge values and simulate severe drought conditions that were not observed in the field. Interactions between variables could also be managed and therefore the outcome could be less confounded.

The issue of how many species may be represented in the metacercariae and cercariae remains an interesting challenge, and must be resolved before the results of this study can be used to develop management strategies. Studies such as this can still provide information that may be useful in mitigating the exacerbating effects of theses parasites on the fountain darter. The resolution of the taxonomic questions will not threaten the validity of this study, but may provide a more enlightened perspective on any results uncovered in this study.

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VITA

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