VARIABLE FLOW STUDY

Seven Years of
Monitoring and Applied Research

Prepared for

Edwards Aquifer Authority

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EXECUTIVE SUMMARY

The Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal and San Marcos Springs Aquatic Ecosystems (**Variable Flow Study**) was initiated in Fall 2000. The development of the Variable Flow Study was a collaborative effort starting as a prospective study within the Edwards Aquifer Optimization Program (EAOP) in the late 1990's. The technical advisory group (TAG) of the EAOP created the framework for this study with the focus of addressing concerns about potential impacts of low recharge and corresponding low spring discharge on the threatened and endangered species found within the Comal and San Marcos springs ecosystems. Upon further discussions with the TAG, and scientists from the resource agencies, Edwards Aquifer Authority (Authority), and consultants, the Variable Flow Study quickly evolved into a more holistic study with the goal of assessing variable flow conditions via an ecosystem approach while including monitoring and special studies specific to individual threatened and endangered species.

Throughout the course of the Variable Flow Study to date, a plethora of scientists have provided scope input or comments, assisted in study design, and/or have participated in fieldwork activities and data analysis. Four of the five special studies were designed and conducted in conjunction with U.S. Fish and Wildlife Service (USFWS) National Fish Hatchery and Technology Center (NFHTC) scientists or Texas State University (TSU) professors and graduate students. Additionally, the NFHTC provided in kind services by use of facilities and oversight for several of the special studies and routine monitoring activities. Numerous technical reports have been generated during the course of the Variable Flow Study; many of them in coordination with resource agency scientists and university professors. One journal article and three Master's theses have been published to date with a fourth Master's thesis in preparation. Overall, the interactions and associations with resource agency and academic professionals over the past seven years have provided the Variable Flow Study with a high level of peer review, comment, and critique.

During this seven-year period, valuable information has been gathered on current population dynamics of each listed species found in the Comal and San Marcos Springs ecosystems (except those that are primarily subterranean) and their habitat conditions. These data were collected under a Comprehensive Monitoring effort that included regularly scheduled samples to provide baseline information and during Critical Period events that were triggered by infrequent, extremes in discharge that might affect each species. A detailed description of the Comprehensive and Critical Period components along with special studies is provided within this summary report.

The seven-year study period has been characterized by variable discharge conditions ranging from floods to extended drought. This substantial variation in discharge has provided valuable information on flow related responses of the biological communities. Water quality parameters measured over the course of the study varied only slightly, and appeared to be influenced primarily by groundwater inputs from the Edwards Aquifer. Overall, the water quality observed throughout the seven-year study period was within the range of that required by the threatened and endangered species.

One of the major findings of the Variable Flow Study is the importance of aquatic vegetation to the biological community. Aquatic vegetation within both systems provides physical habitat for threatened and endangered species and other aquatic life, provides a direct food source, supports diverse and abundant macroinvertebrate communities that subsequently provide a food source for organisms higher in the food web, and assists in regulating water quality of the spring ecosystems. This report discusses several highlights observed over the study period including aquatic vegetation to water quality dynamics (in particular to Carbon dioxide [CO_2]), and trends relative to natural cycles and anthropogenic activities.

Texas wild-rice has shown a considerable increase over the seven-year study period nearly doubling in aerial coverage since Fall 2000. Also evident, are the reductions in Texas wild-rice that occur after floods (1998 and 2001) and to a lesser degree after low discharge events (1996 and 2006). The total coverage of Texas wild-rice following both high- and low-discharge events rebounded the subsequent year, respectively. Fountain darter populations within both systems experienced inter- and intra-annual variation but remained stable over the seven-year study period. Several important observations for fountain darters were documented relative to year-round versus seasonal reproduction and native versus non-native vegetation preferences. As with the fountain darter population, the populations of San Marcos Salamanders and Comal Springs Salamanders have remained stable throughout the study period.

Several highlights surrounding the Comal invertebrates have occurred over the course of the study. These start with the expansion of the known range of Comal Spring riffle beetles within the Comal system and also discovery of this species in the San Marcos system. Additionally, through the use of innovative and less intrusive sampling techniques, larger populations of Comal Springs riffle beetles have been documented. Finally, a major finding involved documenting the ability of Comal Springs riffle beetles and Peck's Cave amphipods to survive and reproduce in captivity.

Another highlight of the Variable Flow Study has been the opportunity to educate the public regarding the ecological communities of the Comal and San Marcos Springs ecosystems. Through the organization and commitment of the Authority, the project team has given numerous presentations to biological work groups, non-profit organizations, and universities. As part of the Authority's education outreach program, the project team has also had the pleasure of interacting with area San Antonio middle school teachers on site at Comal Springs and collaborating with the Lindheimer Master Naturalists of Comal County.

Perhaps, the most exciting part of a multi-year, multi-faceted study such as the Variable Flow Study, is that we are starting to get a solid glimpse of some key biological linkages to discharge within these dynamic systems. Two case studies are presented in this report that outline documented biological changes resulting from high- and low-discharge conditions. The former focuses on high-discharge related impacts to the aquatic community in the old channel of the Comal Springs ecosystem. Scouring and exotic vegetation establishment has subsequently led to a major decline in fountain darter density within this once high-quality fountain darter habitat. The latter case study documents the direct and indirect impacts of the low-discharge condition experienced in 2006 at San Marcos Springs. Overall, increased recreational activity during 2006 caused the greatest impacts on the threatened and endangered species in the San Marcos Springs ecosystem.

The established database and comprehensive and critical period monitoring through the Authority's Variable Flow Study continues to provide an excellent measure of ecosystem condition and, after 2006, some very strong insight into biological responses to low discharge conditions. This data will be extremely valuable in initiating discussions with the Authority, TAG, and state and federal agencies during the Recovery Implementation Program (RIP). As such, the final section in this report explores some recommendations for continued assessment, identifies restoration and applied research opportunities, points out key management implications outside of the Authority's jurisdiction, and concludes with proposed strawmen flow regimes for Comal and San Marcos Springs.

Long-term monitoring remains a critical component of assessing the ecological health of the Comal and San Marcos Springs ecosystems. Since direct impacts measured over the past seven years relative to some very low discharge conditions were very minor or non-existent, additional field data at lower discharge conditions are necessary to fully assess conditions that might jeopardize the existence of one or several of these species in the wild. Therefore, opportunities such as environmental restoration and experimentation are discussed relative to evaluating response mechanisms of the threatened and endangered species to low discharge conditions. It is proposed that *in situ* field experiments (upon further feasibility evaluation) could provide valuable information for management decisions, prior to witnessing extreme conditions in the wild. The goal is to establish this information in advance so that properly informed management decisions could possibly prevent or minimize impacts during these rare events.

It is also evident that management activities outside of the jurisdiction of the Authority are direct contributors to impacts to threatened and endangered species in both systems. These factors include such items as exotic species (both plant and animal), aquatic vegetation mats, sedimentation, and recreation. Finally, this document proposes flow regime strawmen for both Comal and San Marcos Springs. The sole purpose of this proposal is to provide a framework to stimulate discussions during the RIP process, and provide a good starting point for potential *in-situ* experimentation.

The Variable Flow Study is the most comprehensive biological evaluation that has ever been conducted on Comal and San Marcos Springs. Variable flow conditions encountered to date have provided an excellent confirmation that the study design is well suited to address the concerns of variable flow and water quality on the biological resources in both ecosystems. Over the course of this study (2000-2007), both ecosystems have experienced ups and downs documenting inter- and intra-annual variability, but overall remained healthy. Although healthy from a system-wide perspective, specific areas within each system (e.g. old channel – Comal, Sewell Park – San Marcos) pose areas of concern due to factors such as exotic vegetation establishment or extensive sedimentation.

1.0 INTRODUCTION

The ongoing Variable Flow study, conducted from Fall 2000 through Summer 2007 (time period concerning this summary report), was initiated in response to concerns about the impact of low recharge and corresponding low spring discharge on the threatened and endangered species found within the Comal and San Marcos springs ecosystems. Management of the water resources within the Edwards Aquifer is dependent on accurate knowledge of the quantity and quality of water required by each species as well as other habitat needs. Changes in springflow associated with fluctuating recharge conditions may have a measurable effect in water quality and habitat availability. Some fluctuation in habitat conditions is beneficial, but severe changes in springflow may reduce habitat suitability and the ability of that habitat to effectively support individual species' populations over long periods of time. Prior to initiating this study, the best available biological data simply did not allow for discernment of habitat shifts from "good conditions" to "poor conditions" for a given species resulting from the total discharge from each spring system. Therefore, the Variable Flow study was initiated to gather more information on population dynamics of each species under a range of discharge conditions in order to enhance predictive ability of population-level responses to a given discharge.

The design of the Variable Flow study focused on evaluating the critical questions of population responses to changes in water quality and quantity occurring in the San Marcos and Comal Springs ecosystems. The monitoring and research plan was developed in coordination with the Edwards Aquifer Authority (Authority) and the U.S. Fish and Wildlife Service (USFWS) in May 2000 with additional input from a Technical Advisory Group (TAG) during the scoping process. The study includes monitoring and applied research efforts directed toward each of the eight threatened and endangered species found in the Comal and San Marcos Springs ecosystems. These include two fish species, fountain darter (*Etheostoma fonticola*) and San Marcos gambusia (*Gambusia georgei*), two salamanders, Texas blind salamander (*Eurycea rathbuni*) and San Marcos salamander (*Eurycea nana*), one plant species, Texas wild-rice (*Zizania texana*) and three invertebrates, Comal Springs dryopid beetle (*Stygoparnus comalensis*), Comal Springs riffle beetle (*Heterelmis comalensis*), and Peck's cave amphipod (*Stygobromus pecki*). Of these, only the San Marcos salamander is listed as threatened by the USFWS; the rest are all listed as endangered species. The San Marcos gambusia is likely extinct since no individuals have been collected since 1982, despite an intensive search effort in 1990 (USFWS 1996). One additional species that was monitored during this study was the undescribed Comal Springs salamander (*Eurycea sp.)*

Each of these species has a restricted distribution limited to springs associated with the Edwards Aquifer, and many are found in either Comal Springs or San Marcos Springs, but not both. Prior to initiation of this study, only the fountain darter was believed to occupy both spring ecosystems, but recent collections of the Comal Springs riffle beetle in Spring Lake at the headwaters of the San Marcos River (R. Gibson, USFWS, pers. comm.) reveal that this species is also found in both ecosystems. Information gathered during the Variable Flow Study (BIO-WEST 2002a) also indicates that the Comal Springs riffle beetle is more widely distributed in the Comal Springs ecosystem than previously believed. Among the other species, San Marcos gambusia, San Marcos salamander and Texas wild-rice occur only in the San Marcos River, while the Texas blind salamander is found in the aquifer below San Marcos and nearby springs. Two of the three invertebrates, Comal Springs dryopid beetle and Peck's cave amphipod, are found only in Comal and nearby springs (i.e., Hueco and Fern bank springs).

During this study, valuable information was gathered on water quality and habitat conditions for many of these species as well as monitoring data on current population dynamics. For those species that are primarily subterranean; Texas blind salamander, Comal Springs dryopid beetle and Peck's cave amphipod, information is less extensive due to sampling limitations. There were multiple components incorporated into this study because sampling efforts were unique to each species/tasks. This summary report provides an overview of:

- the development of the Variable Flow Study and subsequent modifications,
- the study components and sampling regime,
- results/trends/highlights of seven years of monitoring,
- two discharge related case studies,
- recommendations for future activities,
- potential management implications, and
- a description of strawmen flow regimes for discussion and evaluation.

The countless interactions (study design, scope review, document review and comment, study participation, etc.) with resource agencies and academic professionals over this seven year period has provided the Variable Flow Study with a high level of peer review making it an important resource to assist decision makers. The biological information collected via the Variable Flow Study over these past seven years represents a major portion of the best available biological data for the Comal and San Marcos Springs ecosystems.

Information collected over these seven consecutive years has confirmed that the Comal and San Marcos Springs ecosystems are truly dynamic. Habitat changes and population responses have been documented over a range of high, average, and lower discharge conditions. Not each threatened and endangered species has reacted to the same degree, with Texas wild-rice and fountain darters providing the earliest glimpse into changing conditions. Over the course of this study (2000-2007), both ecosystems have experienced fluctuating conditions typical of dynamic systems, with aquatic habitat and populations of threatened and endangered species remaining in good condition.

2.0 VARIABLE FLOW STUDY DEVELOPMENT

The development of the Edwards Aquifer Authority Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal and San Marcos Springs Aquatic Ecosystems (Variable Flow Study) stemmed from the Technical Studies development component of the Edwards Aquifer Optimization Program (EAOP). During the late 1990's, a Technical Advisory Group (TAG) for the EAOP was formed consisting of resource specialists / scientists from the following entities:

- Edwards Aquifer Authority (Authority)
- San Antonio Water System (SAWS)
- San Antonio River Authority (SARA)
- U.S. Fish and Wildlife Service (USFWS)
- Texas Parks and Wildlife Department (TPWD)
- Texas Commission on Environmental Quality (TCEQ – formally TNRCC)
- U.S. Geological Survey (USGS)
- Natural Resource Conservation Service (NRCS)
- Edwards Aquifer Research and Data Center (EARDC) – Texas State University (formerly Southwest Texas State University)

The TAG developed a list of studies for consideration including a biological evaluation of both the Comal and San Marcos Springs ecosystems. The Authority issued a Request for Proposal (RFP) and selected an environmental consulting firm to assist in the preparation of the biological sampling program. A workshop was held in May 2000 to discuss a proposed sampling protocol for both springs systems. The workshop was attended by resource professionals from the Authority, TPWD, USFWS Austin Ecological Services (ES), USFWS National Fish Hatchery and Technology Center (NFHTC), and scientists from the consulting team, EARDC, and Texas State University (TSU) Aquatic Station. Discussions were conducted at the workshop and subsequently, comments were incorporated into a final sampling program entitled, "Edwards Aquifer Authority Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal and San Marcos Springs Aquatic Ecosystems." The program was further reviewed and accepted by the USFWS and TPWD during late summer 2000.

During August 2000, the discharge at Comal Springs fell below 200 cubic feet per second (cfs) triggering the first critical period sampling event under this program. Prior to the initiation of the sampling program, a joint press conference with the Authority and USFWS was conducted in Landa Park at Comal Springs to discuss the low discharge conditions at Comal Springs, water restrictions, endangered species implications, and the Variable Flow study. Former General Manager, Greg Ellis spoke on behalf of the Authority and former Field Supervisor, Dave Frederick spoke on behalf of the USFWS.

Over the past seven years, the need for modifications to the program arose based on the analysis of collected data and identified information gaps. These changes and/or additions of special studies have been developed and approved via coordination between the Authority, USFWS ES and NFHTC, and TPWD. An overview of the sampling program components, modifications, and special studies is presented in Section 3.

Throughout the course of the Variable Flow Study to date, the following scientists have provided scope input or comments, assisted in study design, and/or have participated in fieldwork activities and data analysis:

- Dr. Thomas Arsuffi (Texas Tech University – formally with TSU)
- Dr. Alan Groeger (TSU)
- Dr. Glenn Longley (TSU – EARDC)
- Dr. Timothy Bonner (TSU)
- Dr. David Huffman (TSU)
- Dr. Robert Doyle (Baylor University)
- Dr. Thomas Brandt (USFWS – NFHTC)
- Mr. Joe Fries (USFWS – NFHTC)
- Ms. Paula Powers (USFWS – NFHTC)
- Mr. Randy Gibson (USFWS – NFHTC)
- Dr. Mara Alexander (USFWS – NFHTC)
- Dr. Kathy Phillips (USFWS-NFHTC)
- Dr. David Bowles (formerly TPWD)
- Dr. Randy Moss (formerly TPWD)
- Mr. Doyle Mosier (TPWD – formerly TCEQ)
- Ms. Jackie Poole (TPWD)
- Dr. Katheryn Kennedy (formerly USFWS – ES)
- Mr. Patrick Connor (USFWS – ES)

This list is not all inclusive as many other resource specialists, graduate students, and Authority personnel have participated in this program. Of the five special studies that have been conducted to date, all but the Comal Springs Range Expansion Study were designed and conducted in conjunction with USFWS – NFHTC scientists or TSU professors and graduate students. Additionally, the NFHTC provided in kind services by use of facilities and oversight for each of these four special studies. The following list describes the participation.

- Comal Springs Riffle Beetle Laboratory Study – Mr. Randy Gibson, Dr. Joe Fries
- Aquatic Vegetation – Carbon Dioxide Study – Ms. Paula Powers
- Fountain Darter Laboratory Study – Dr. Thomas Brandt, Dr. Timothy Bonner
- Parasite Evaluation Study – Dr. Thomas Brandt, Dr. David Huffman

Numerous technical reports have been generated during the course of the Variable Flow Study, many of them in coordination with above mentioned resource professionals / scientists. Additionally, one journal publication has resulted from these studies.

McDonald, D.L., T.H. Bonner, E.L. Oborny, and T.M. Brandt. 2007. "Effects of Fluctuating Temperatures and Gill Parasites on Reproduction of the Fountain Darter, *Etheostoma fonticola*". Journal of Freshwater Ecology, Volume 22, Number 2, pages 311-318.

It is the intent of the Authority and project team to pursue journal publications relative to several of the topics/studies that have been conducted via the Variable Flow Study over the past seven years.

Over the course of the study period to date, work directly on the Authority's Variable Flow study has led to the publication of three Master's theses (listed below) through Texas State University. A fourth Master's thesis is currently being written by Anne Bolick under the supervision of Dr. David Huffman (TSU) and Dr. Thomas Brandt (NFHTC) regarding the effects of low flow relative to the trematode parasite and fountain darter interactions in the Comal System. This thesis is scheduled for completion in late 2007 / early 2008.

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.

Norris, C. W. 2002. Effects of variable flows on invertebrate drift in Comal Springs, Texas. Masters thesis, Southwest Texas State University. 112pp.

McDonald, D. L. 2003. Effects of fluctuation temperature and an introduced trematode on reproduction and mortality of *Etheostoma fonticola*. Masters thesis, Texas State University. 35pp.

Through the above described interactions and associations with resource agency and academic professionals over the past seven years, the Variable Flow Study has received a high level of peer review, comment, and critique.

3.0 VARIABLE FLOW STUDY COMPONENTS

The Variable Flow Study consists of three main components, including: 1) Comprehensive Sampling, 2) Critical Period Sampling, and 3) Special Studies. The first two were included in the original sampling protocol, along with a provision in the scope of work that stated: "should additional investigations become necessary based on the information collected, then special studies would be designed and implemented."

3.1 Comprehensive Sampling

The original study design recommended quarterly (winter, spring, summer, fall) sampling of multiple biological components. A full comprehensive sampling event was typically conducted within a two-week period and included the following components:

- Water quality evaluation
  - Standard parameters (DO, pH, specific conductivity, temperature)
  - Conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids)
  - Thermistors for a continuous record of water temperature
  - Fixed station photographs for qualitative evaluation of temporal variation
- Detailed mapping of aquatic vegetation (fountain darter habitat) in study reaches
  - Texas wild-rice annual survey (San Marcos only)
- Texas wild-rice physical observations (San Marcos only)
- Fountain darter sampling
  - Drop nets
  - Dip nets (time constrained surveys)
  - SCUBA / snorkel surveys
  - Parasite evaluation
- San Marcos and Comal salamander observations
- Macroinvertebrate sampling (Comal only)
- Exotic species/ Predation evaluation

Detailed methodologies are presented in the Variable Flow Study annual reports. A description of objectives is presented below followed by several one-page study component overviews. For reference, **Figures 3.1** and **3.2** show the Upper San Marcos and Comal Springss, respectively.

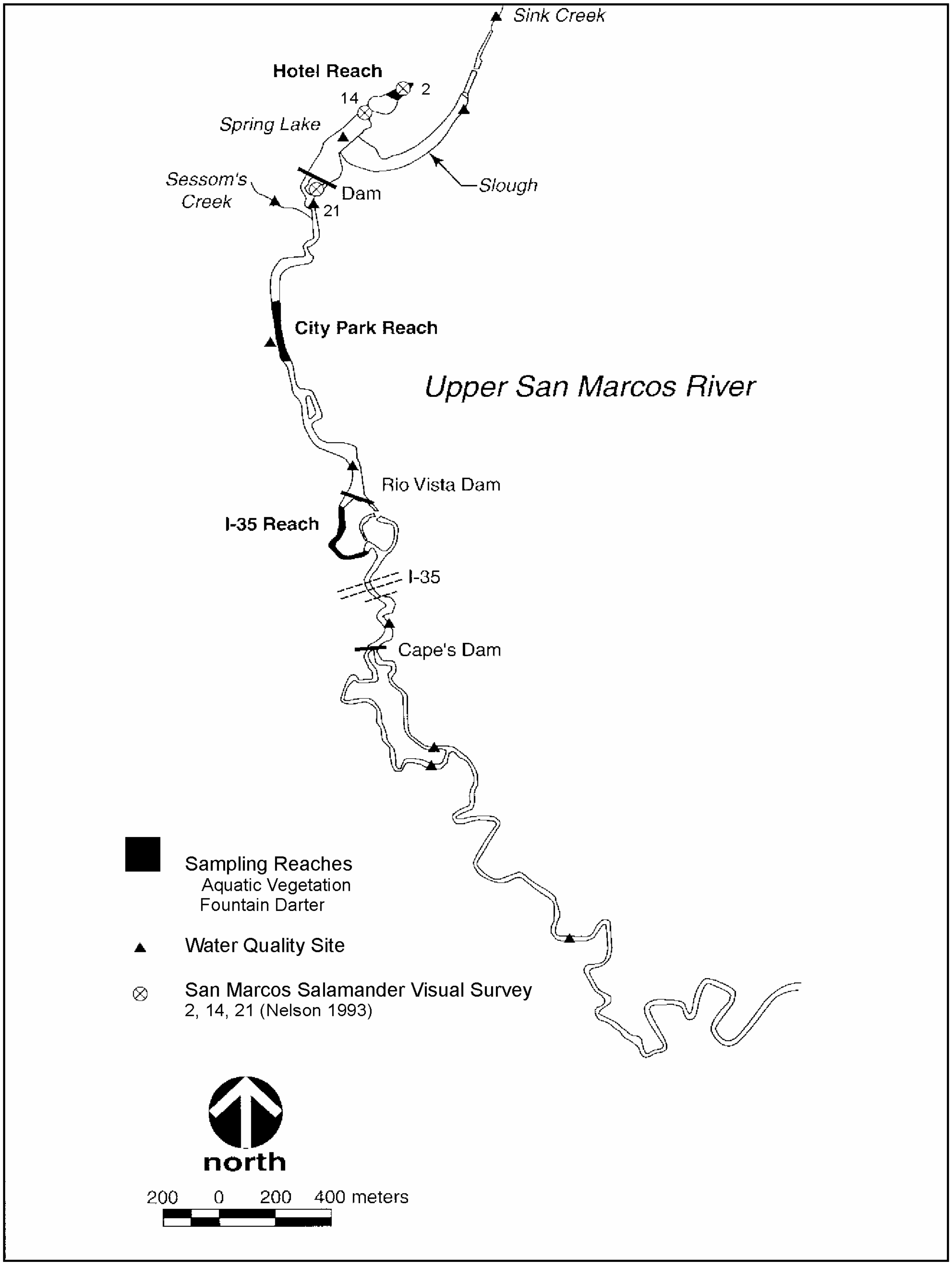


Figure 3.1 – Upper San Marcos Springs / River

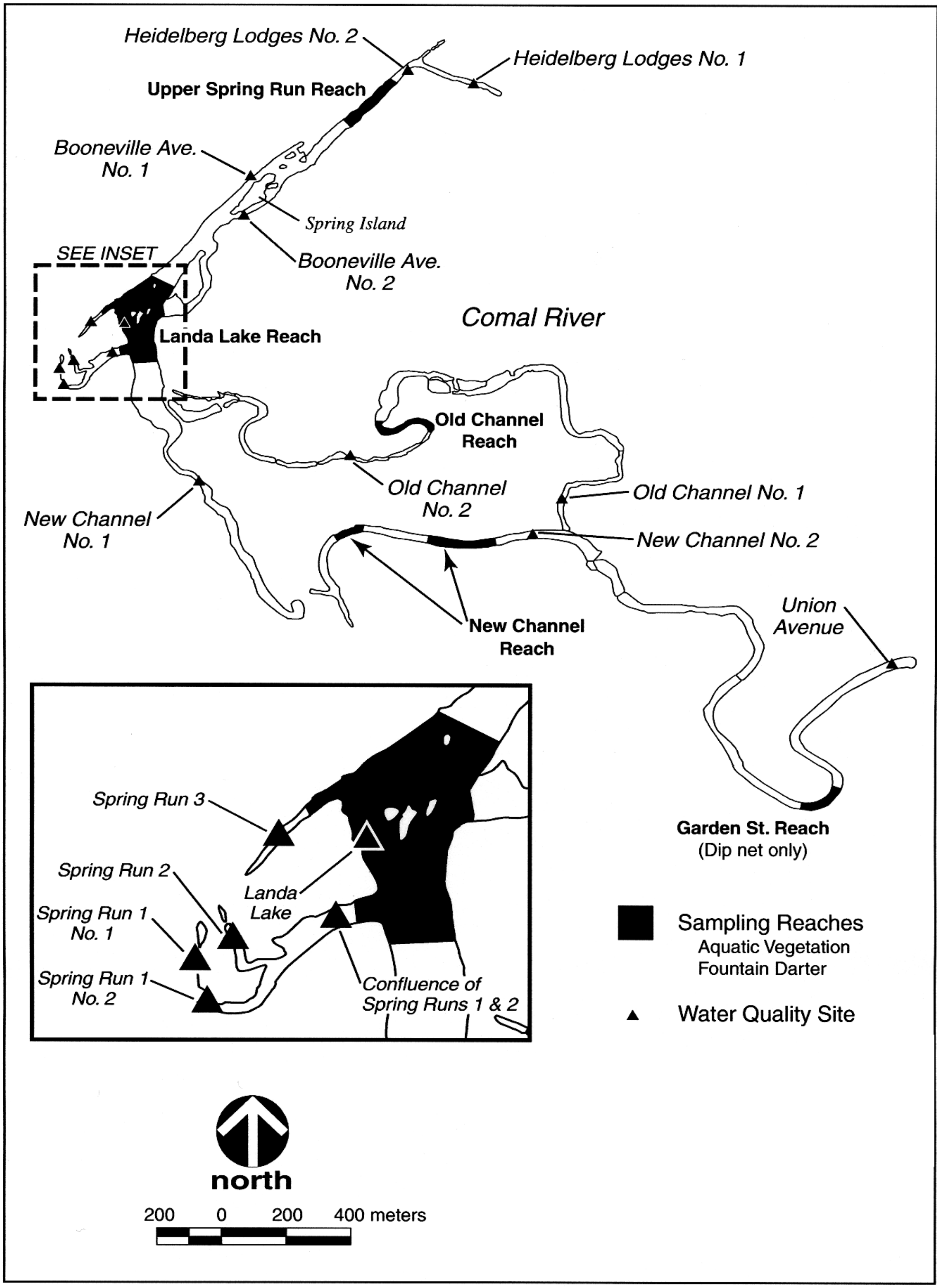


Figure 3.2 – Comal Springs/River

The objectives of the water quality analyses are to delineate and track water chemistry throughout the ecosystem; monitor controlling variables (i.e., flow, temperature) with respect to the biology of each ecosystem; monitor any alterations in water chemistry that may be attributed to anthropogenic activities; and evaluate consistency with historical water quality information. This was conducted with quarterly water quality sampling throughout the entire Comal and San Marcos Rivers for two years including standard parameters (water temperature, dissolved oxygen, pH, and conductivity) and conventional water chemistry parameters (nitrate, total nitrogen, ammonia, soluble reactive phosphorus, total phosphorus, alkalinity, and total suspended solids). A continuous record of water temperature was also maintained throughout the study at multiple locations within the Comal and San Marcos Springs ecosystems using temperature loggers (thermistors). Fixed station photographs documented physical changes in habitat at multiple locations throughout the Comal Springs ecosystem.

The primary habitat sampling component to this study involved aquatic vegetation. The presence or absence of aquatic vegetation and plant species composition have a substantial influence on the distribution and the abundance of several of the threatened/endangered species, particularly the fountain darter. Because it is a primary variable in fountain darter population dynamics, very precise maps (<1m accuracy) of vegetation composition in each sample reach were created during each sample event to document the abundance of all aquatic vegetation and monitor fluctuations associated with season and discharge. Additionally, physical measurements and mapping of Texas wild rice within "vulnerable" areas was conducted quarterly, and the entire river population of Texas wild rice was mapped annually. At the beginning of the study period (Fall 2000), Texas wild-rice stands throughout the river were assessed and documented as being in "vulnerable" areas if they possessed one or more of the following characteristics: (1) occurred in shallow water, (2) revealed extreme root exposure because of substrate scouring, or (3) generally appeared to be in poor condition. Monitoring activities associated with "vulnerable" stands were designed following discussions with Dr. Robert Doyle, currently with Baylor University, and Ms. Paula Power, formerly with the USFWS National Fish Hatchery and Technology Center, San Marcos.

Of the sampling efforts directed at individual species, the fountain darter component was the most extensive. To establish a clear understanding of fountain darter habitat associations and evaluate population responses to changes in flow, sampling was conducted using two methods in multiple sample reaches. Drop netting, enclosing a $2m^2$ area and sampling exhaustively, provided valuable quantitative information on fountain darter densities in each of the dominant aquatic vegetation types and allowed for evaluation of potential seasonal and discharge-related responses of the population. Dip netting provided information on fountain darters using habitat along the river margins, which was not typically sampled with the drop net and also augmented information on smaller size-classes. Qualitative visual observations were also made of fountain darters and salamanders in the deepest portions of Landa Lake and select locations in Spring Lake. One additional component of the fountain darter portion of this study was an investigation of potential impacts of an exotic trematode parasite, *Centrocestus formanosus*, which was first discovered in the Comal River in 1996. The parasite completes part of its life cycle within the digestive gland of an exotic snail, *Melanoides tuberculata*, which is abundant within the Comal Springs ecosystem. When in the water column, the parasite can attach to the gills of the fountain darter. The initial focus of the parasite study component was to examine variation in spatial and temporal concentration of the parasite in the water column throughout the Comal Springs ecosystem. From that work, routine parasite sampling was conducted at select stations to maintain a continuous record. In 2006, a special study was designed to focus on the direct linkages of the parasite to discharge in the Comal system.

The Comal Springs salamander has been monitored throughout this study to evaluate relative distribution among known habitat locations and to examine population responses to changes in springflow. Timed surveys in the spring runs provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge. Qualitative visual observations in the deeper portion of Landa Lake were also used to verify presence/absence in that area during each sample effort. The San Marcos salamander has also been monitored throughout this study to evaluate relative distribution among known habitat locations and to examine population responses to changes in springflow. The sample areas include two locations in Spring Lake and one site just downstream of the Spring Lake dam in the eastern spillway. Timed surveys in each of these areas provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge.

Macroinvertebrate sampling in the spring runs at Comal is an integral component of this study primarily because of the presence of the three endangered macroinvertebrate species (Comal Springs riffle beetle, Comal Springs dryopid beetle, and Peck's cave amphipod). At the outset of this project, drift nets were used in the middle of Spring Runs 1-3 to explore the movement of organisms downstream of the spring openings. The focus was to determine whether a single species might be used to serve as an indicator for measuring community response to changes in springflow. That portion of the study, completed in 2002 (BIO-WEST 2003a) yielded drift rates, densities, and patterns of selected aquatic invertebrates in the spring runs. In 2003, there was a shift of focus to the spring openings in order to evaluate the frequency with which the subterranean species are expelled from the aquifer. The range of the Comal Springs riffle beetle within Comal Springs has been extended from initial reports and the species has been discovered in Spring Lake. Subsequently, a new focus has been an added that involves a less intrusive sampling technique (cotton rag placement and retrieval) to evaluate populations of these species with respect to discharge on a more broad spatial scale.

The final sample component was to estimate the density of various exotic fish species and to evaluate the potential that predators may be consuming one or more of these threatened/endangered species in large numbers. This was conducted by using gill nets and evaluating stomach contents of captured fish for the presence of any threatened/endangered species (primarily fountain darters and salamanders). Because of limited sample sizes early in the study, rod-and-reel sampling was added to supplement gill net sampling for this study component. Rod-and-reel sampling allowed researchers to target larger sunfish and small- to intermediate-sized bass, which are the most likely predators on fountain darters and salamanders. As a result of using both rod-and-reel and gill net sampling, sufficient baseline data had been collected by the final 2002 sample to discontinue this component except during "Critical Period" events.

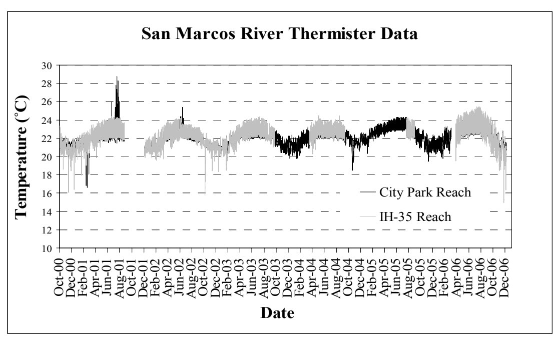
WATER QUALITY
Physical and Chemical Measurements

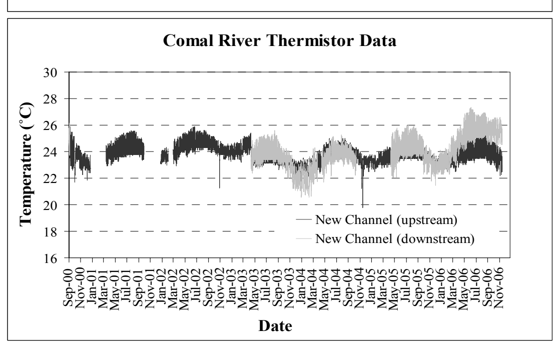
Objective: Maintaining desirable water quality conditions in aquatic habitats is important to the health of both flora and fauna. Continuous water temperature logging and periodic comprehensive water quality testing serve to assess current conditions as well as conditions following extreme climatic events.

Location: Nine locations within Spring Lake; nine locations within the upper San Marcos River; twelve locations in the Comal River system.

Task Description: The initial water quality component of this study was conducted to develop baseline conditions (2000-2002) by comprehensive sampling in both systems. Baseline values were determined for the following parameters: temperature, pH, conductivity, dissolved oxygen, nitrate, soluble reactive phosphorus, alkalinity, total suspended solids, ammonia, total nitrogen, and total phosphorus. Comprehensive water quality sampling was also conducted in both systems during a high-flow period in 2002 and in the San Marcos system during a prolonged low-flow event in 2006. Continuous water temperature data logging (10-minute logging interval) using thermistors has been a component of water quality monitoring for the entire period of sampling.

Data Analysis and Results: The continuously sampled water temperature data have provided a good view of the conditions experienced by fountain darters and other species throughout both spring systems. In many places, the temperature remains nearly constant due to nearby spring inputs, while those locations farther away from the springs are more influenced by atmospheric conditions. Comprehensive water quality sampling has indicated that pH and dissolved oxygen concentrations generally increase with distance from the spring headwaters, while several other parameters remain fairly constant among sites with little variation between sampling periods. A more complete description of water quality in these two systems can be found in each annual report (BIO-WEST 2002-2007).

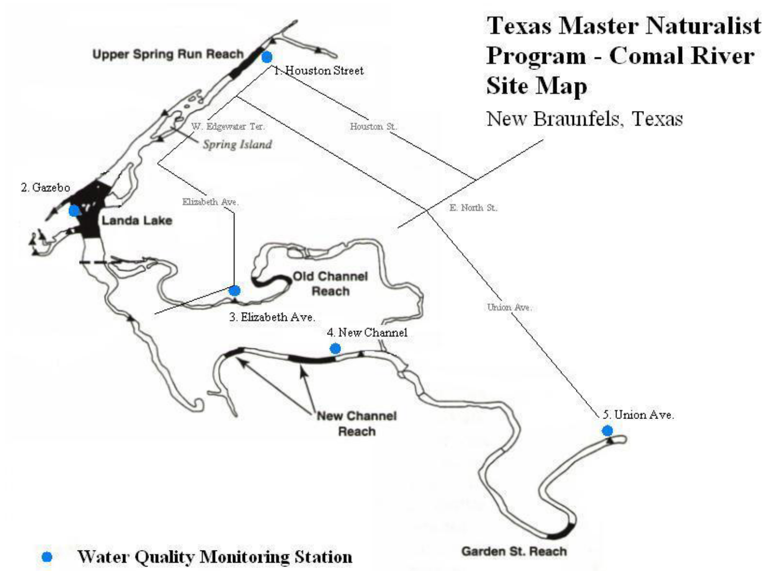




TEXAS MASTER NATURALIST MONITORING
Water Quality and Recreation Surveys

Objective: 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 and outreach through volunteer efforts. Several members of the Lindheimer Master Naturalists of Comal County volunteered to record point water quality and recreation data on a weekly basis in the Comal Springs ecosystem.

Location: Five reaches in the Comal system.



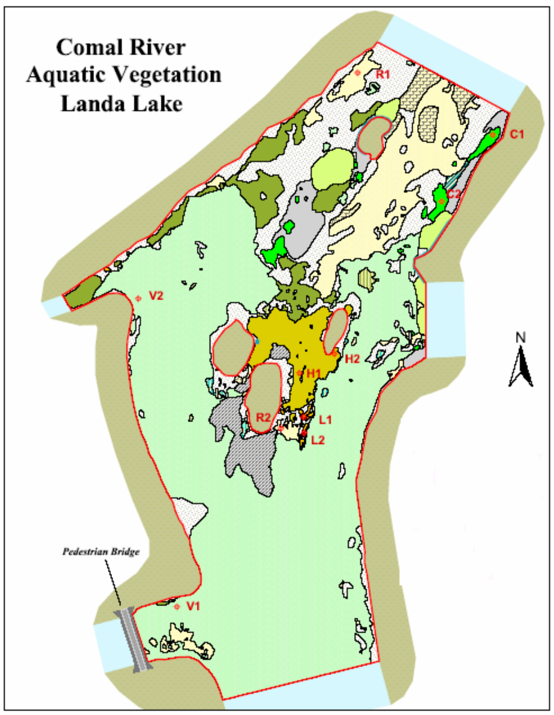
Task Description: Beginning in June 2006, volunteers collected data on water pH and CO_2 levels and conducted recreational use surveys at five locations on a weekly basis. Recreational use data was collected by counting and categorizing a 'snapshot' of the number of tubers, kayakers, swimmers, anglers, etc. in the vicinity of the site. Photos and any notes on the condition of the river were also recorded during each sampling event.

Data Analysis and Results: Water quality data showed that carbon dioxide concentrations were highest in areas near spring outflows (Houston St. and Gazebo sites) and decreased downstream. Recreational user counts indicated the most intense recreation occurred at sites near popular tubing areas (New Channel and Union Ave. sites). These dedicated volunteers have assisted in filling in some key data gaps in the Comal system, and continue their weekly monitoring effort into 2007.

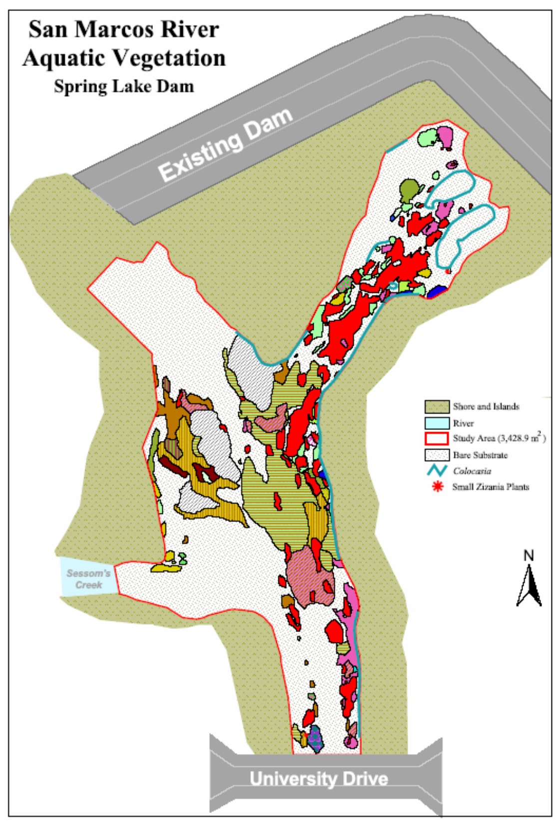
AQUATIC VEGETATION
Mapping

Objective: Aquatic vegetation plays a key role in governing the health of spring-fed aquatic ecosystems. Mapping of this resource continues to assist in the evaluation of changes to the system over time relative to physical habitat and organism populations.

Location: Four reaches in the Comal system; three reaches in the San Marcos system.



Task Description: Aquatic vegetation mapping was conducted using a global positioning system (GPS) unit with real-time differential correction capable of submeter accuracy. The receiver was linked to a Windows CE device with specialized software that displays field data as it is gathered and improves efficiency and accuracy. The GPS unit was placed in a kayak with the GPS antenna mounted on the bow (see photo above).

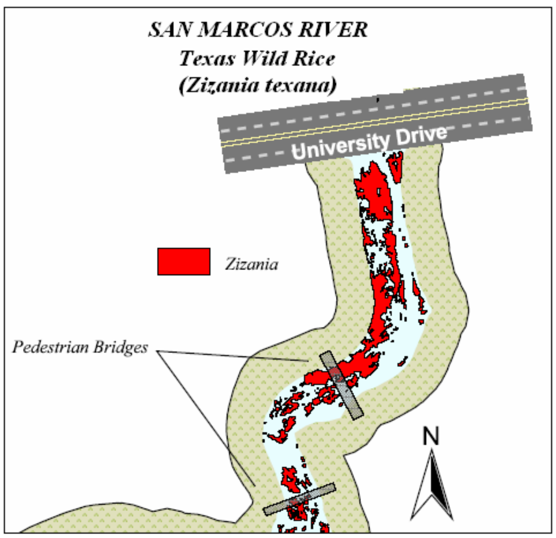


Data Analysis and Results: From Fall 2000 through Spring 2007, the project team mapped the same reaches in the Comal and San Marcos systems. Two example figures are presented on this page with figures of the aquatic vegetation observed during each sample effort found in each of the annual reports (BIO-WEST 2002-2007). It is difficult to make sweeping generalizations about seasonal and other trip-to-trip characteristics, since most changes occurred in fine detail; however, overall trends are discussed in each annual report and are summarized in Section 4.0 (Results/ Trends).

TEXAS WILD RICE
Annual Mapping and Physical Measurements

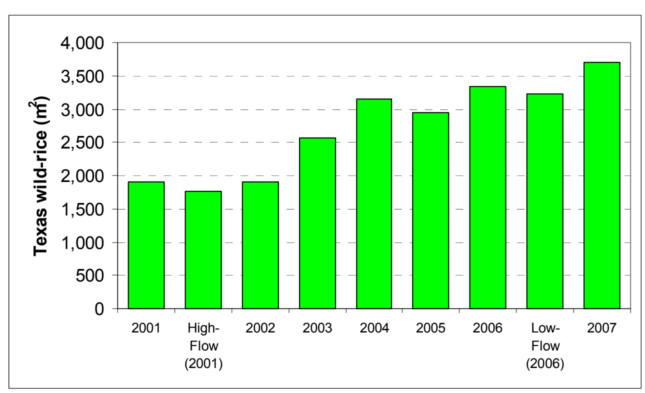
Objective: Texas wild-rice (*Zizania texana*) is a perennial aquatic plant found only in a small area along the upper San Marcos River. It has been listed as a state and federal endangered species since 1978. Monitoring the health and distribution of Texas wild-rice is important to understand its critical habitat requirements.

Location: Upper San Marcos River



Task Description: All Texas wild-rice stands in the San Marcos River are mapped annually using the GPS / computer / kayak method, described previously for aquatic vegetation mapping. Additionally, the aerial coverage of Texas wild-rice stands in vulnerable locations is mapped on a more frequent basis. Qualitative observations associated with stands in these vulnerable areas include noting the percent of the stand that is emergent (and how much of that was in seed), the percent of stands covered with vegetation mats or algae buildup, any evidence of foliage predation, and a categorical estimation of root exposure. Spot velocity, depth and substrate measurements are conducted within these stands, as well as discharge cross-sections upstream of these areas.

Data Analysis and Results: Since the inception of the variable flow study, the entire population of Texas wild-rice has been mapped eight times. Physical habitat measurements and discharge cross-sections have been conducted eighteen times. Maps of the Texas wild-rice distribution observed during each sample effort can be found in each of the annual reports. The overall trend for Texas wild-rice from the inception of the Variable Flow study has been a steady increase in aerial coverage for the overall population. Temporary impacts associated with floods have been documented but are usually followed by periods of rapid recovery. Additionally, Section 4.0 describes the impacts associated with recreational activities during 2006.



FOUNTAIN DARTER
Drop Netting

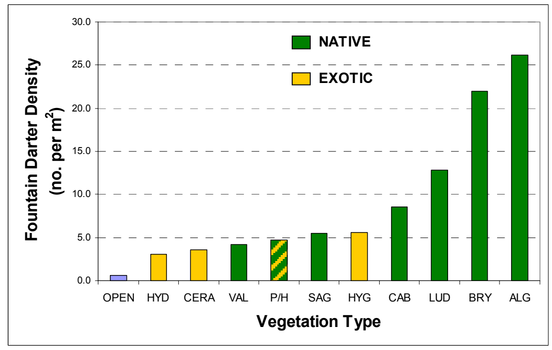
Objective: The fountain darter (*Etheostoma fonticola*) is a small, federally endangered fish that lives only in the relatively thermal-constant spring-influenced headwaters of the San Marcos and Comal Rivers. Drop netting allows for quantitative estimates of fountain darter densities in the various vegetation types. This information, combined with estimates of vegetation coverage acquired through vegetation mapping of each reach can be used to examine trends in the fountain darter population.

Location: Three reaches in the Comal system, and two reaches in the San Marcos system.

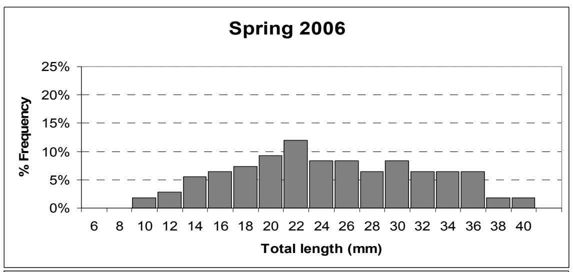
Task Description: Drop netting is conducted by throwing a specially designed rectangular net (encompassing 2 m^2) into randomly selected areas within each major vegetation type. This net quickly sinks to the bottom and entraps organisms in the sample area. A hand-held dip net is used to collect all organisms within the drop net which are then enumerated and/or measured. Habitat measurements such as depth, velocity, substrate, and vegetation coverage are recorded at each sample location. This technique provides information on density of fountain darters within each vegetation type.

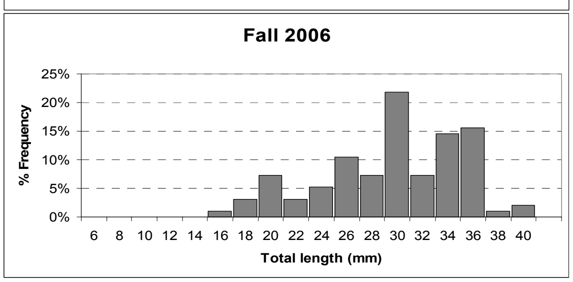
FOUNTAIN DARTER
Drop Netting (continued)

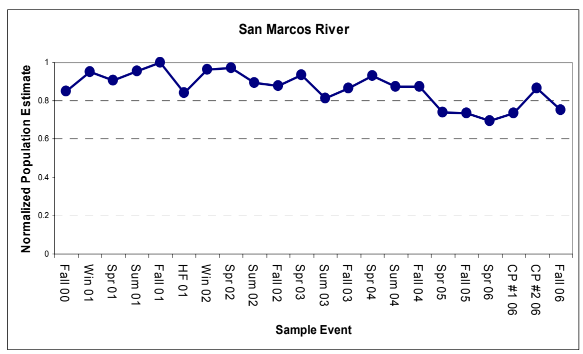
Data Analysis and Results: From Fall 2000 through Spring 2007, the project team has conducted 945 drop net samples in the Comal and San Marcos River ecosystems, catching nearly 12,000 fountain darters. Fountain darters are most numerous in native vegetation types, especially those which grow thick on or near the substrate (i.e., bryophytes and algae).

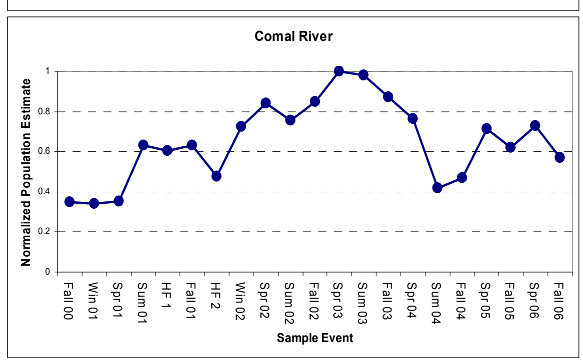


Size structure of fountain darters indicates a healthy population with some reproduction occurring year around in headwater areas. However, a spring peak in reproduction is common in certain areas, and seems to be habitat dependent. A more detailed analysis of this trend is provided in Section 4.









Due to the variation observed thus far, it is difficult to make generalizations about population trends. However, under flows observed during the study period both populations seem to be relatively stable. Larger shifts in estimated population size in the Comal system are a result of greater variation in coverage of high-quality vegetation types (bryophytes and algae) within Comal study reaches. Neither of these vegetation types is abundant in the San Marcos system. A more detailed analysis of fountain darter data can be found in each of the Annual Reports, as well as in Section 4 of this report.

FOUNTAIN DARTER
Routine Dip Netting

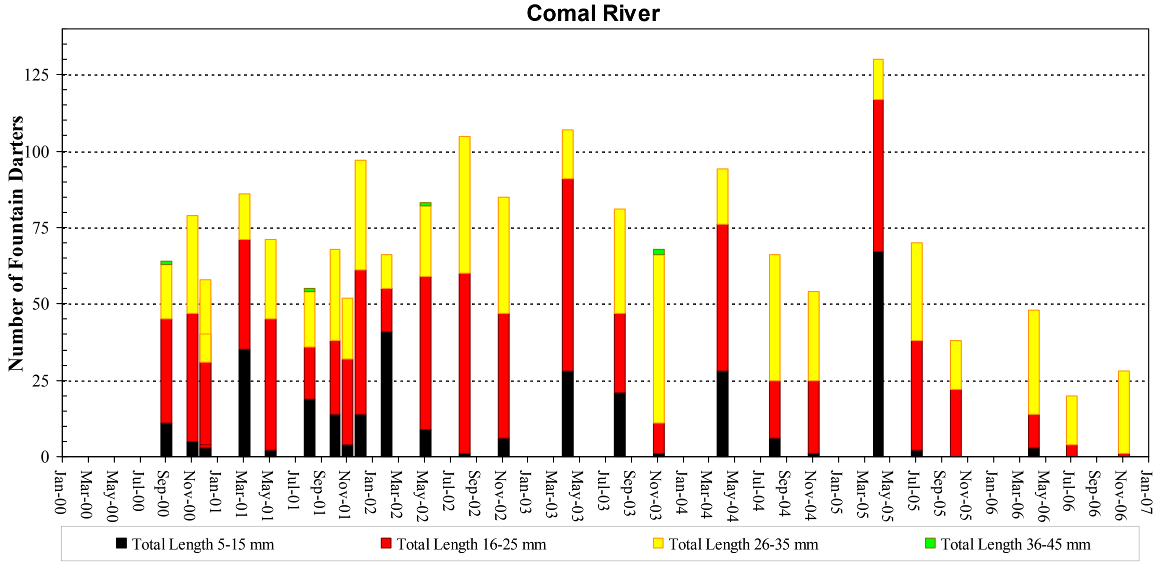
Objective: Dip netting was used to supplement data gathered by drop netting, which provides information on fountain darter size distributions, abundance, and parasite levels.

Task Description: Dip netting involves sampling areas thought to contain fountain darters with a hand-held dip net (40 cm x 40 cm, 1.6 mm mesh). An effort was made to capture as many darters as possible, while working upstream through the sample reach. A predetermined and consistent amount of time was spent at each reach, so that results could be compared between sampling events.

Location: Four reaches in the Comal system; three reaches in the San Marcos system.

Data Analysis and Results: From Fall 2000 through Spring 2007, dipnetting was conducted in each reach 22-26 times. Dip net results for each reach can be viewed in Annual Reports. Length information from dip netting was combined with that collected in drop netting to analyze size distributions of fountain darters. In addition, since effort (time sampled) is constant, abundance can be compared between sample events.

Dip Net Results from the Old Channel Reach
Comal River



FOUNTAIN DARTER
Presence/Absence Dip Netting

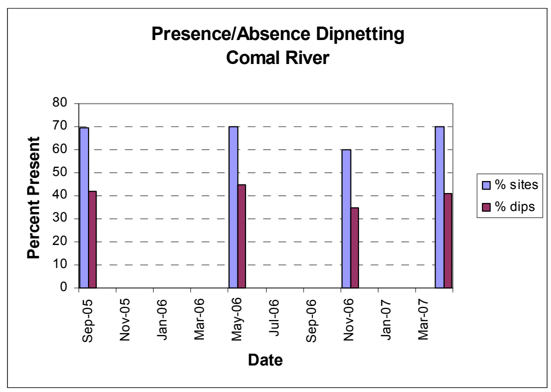
Objective: Presence/Absence dip netting was established as a more cost effective and less destructive means of monitoring the fountain darter population. This method does not provide detailed information on size structure, density, and habitat use. However, given adequate baseline data it is anticipated that this methodology will be able to assist in the detection of significant trends in the population with less effort and fewer impacts to critical habitat.

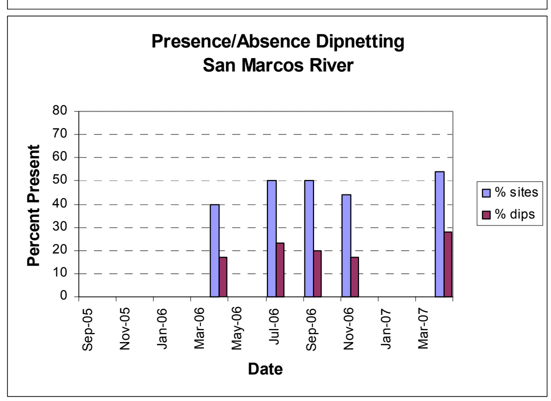
Location: Four reaches in the Comal system; three reaches in the San Marcos system.

Data Analysis and Results:

Presence/Absence dipnetting began in 2005 with a trial run on the Comal River. In three consecutive days, the proportion of sites with darters present varied from 66-72%. Due to the consistent results obtained, this method was expanded to both systems in 2006, and was conducted along with other methods during each sampling event. Although this method does not provide the detailed data provided by other methods, it does provide a quick and easy way of detecting notable changes in the population. Results collected thus far will provide a good baseline for comparison of results in future critical periods.

Task Description: A stratified random sampling design is used to randomly select 50 sites within the major vegetation types in each system. At each site, four dips are taken with a 40 cm x 40 cm (1.6 mm mesh) dip net, and presence or absence of darters is quickly noted in each dip. Trends in the population can be monitored by comparing percentage of sites and/or percentage of dips in which fountain darters were present.





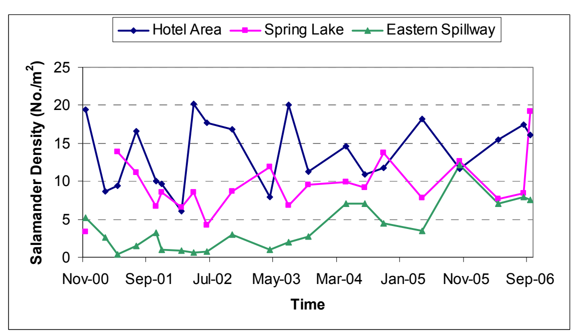
SALAMANDER
Snorkel / SCUBA Surveys

Objective: The Comal Springs salamander and San Marcos Salamander have been monitored throughout this study to evaluate relative distribution among known habitat locations and to examine population responses to changes in springflow.

Task Description: At Comal Springs, timed surveys in the spring runs provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge. Qualitative visual observations in the deeper portion of Landa Lake are also used to verify presence/absence in that area during each sample effort. At San Marcos, timed surveys in each of the three areas provided a means of comparison among sites and among sample efforts to evaluate potential changes associated with season and/or discharge.

Location: Comal system - Spring runs 1, 3, and deeper portions of Landa Lake; San Marcos system - Hotel reach and Riverbed site in Spring Lake, eastern spillway below Spring Lake Dam.

Data Analysis and Results:
As with the fountain darter populations, the populations of San Marcos Salamanders and Comal Springs Salamanders has remained stable throughout the study period. Indirect impacts from recreational activity have been noted as have short-term direct impacts of sedimentation following pulse flow events. However, neither impact has caused a notable change in the population of salamanders studied in the respective representative reaches in either system. Additionally, sampling bias (easier to snorkel during lower discharge conditions) has been noted and is taken into consideration in data interpretation.



3.2 Critical Period Sampling

The Critical Period Monitoring component of the Variable Flow Study applies to both the Comal and San Marcos systems and is based upon established flow trigger levels (including high and low flows) for each. The type and extent of sampling conducted is dependent on the respective trigger level for each spring ecosystem. The sampling is designed to directly relate to parameters sampled and conditions observed during the comprehensive sampling. Thus, a full sampling event during Critical Period monitoring essentially mirrors efforts during the comprehensive sampling. As noted below, some additional components are added to evaluate the status of the systems between Critical Period full sampling events. An overview of the trigger levels and associated sampling activities are presented below for the San Marcos and Comal systems, respectively.

3.2.1 High Flow Monitoring

High flow critical period monitoring is to be conducted only after the following triggering criteria are met, as determined by Authority personnel:

- ☑ The daily average flow exceeds 385 cubic feet per second (cfs) in the San Marcos aquatic ecosystem or 500 cfs in the Comal aquatic ecosystem (total flow through the ecosystem as measured at the USGS gauging station located immediately downstream of the ecosystem); and
- ☑ After conducting a joint visual inspection of the aquatic ecosystem with contractor personnel and consulting with contractor, Authority staff determines that high-flow critical period monitoring is warranted.
- ☑ Additionally, before high flow critical period monitoring is conducted for any high flow event, the monitoring parameters must be pre-approved by Authority personnel.

3.2.2 Low Flow Monitoring

The low flow trigger levels and associated sampling activities are summarized below with more detail available in the Variable Flow annual reports. Low flow Critical Period Monitoring for the San Marcos River is triggered at 120 cfs. The sampling at 120 cfs consists of Texas wild-rice physical habitat measurements to be conducted at each 5 cfs decline or a maximum of once per week. The first full sampling event is triggered at 100 cfs, which is the current "Take" and "Jeopardy" level specified by the USFWS for San Marcos Springs. Subsequent full sampling events are triggered at 85, 60, 25, and 10 cfs. In addition, two recovery full sampling events are to be conducted as the system rebounds from a low-flow period. Between full sampling events, habitat evaluations per every 5 cfs decline would be conducted again not to exceed weekly monitoring.

For the Comal system, the initial low flow trigger for Critical Period Monitoring is 200 cfs, which is the current "Take" level specified by the USFWS. This triggers the first full sampling event with four subsequent full events being triggered at 150 (defined USFWS "jeopardy" level), 100, 50, and 10 cfs, respectively. As in the San Marcos system, two recovery events are scheduled as the flows rebound from drought conditions on the Comal system. The recovery events are dependent on flow stabilization. Typically, these systems rebound from drought conditions with the help of a tropical depression or some weather pattern that produces a large amount of rainfall over the watershed. The flows typically increase rapidly and require a period of stabilization before the collection of biological data is meaningful. The Comal system also has habitat evaluations scheduled between full sampling events, however, at 10 cfs increments again not to exceed weekly observation. An additional component for the Comal system is the detailed riffle beetle habitat evaluation and spring orifice condition documentation that is triggered at 120 cfs and continued at 10 cfs increments as discharge declines.

3.3 Variable Flow Scope Modifications – 2003

From 2000-2002, all sample components were incorporated into each Comprehensive and Critical Period sampling event. In 2003 and 2004, slight modifications were made to the protocol for a sampling event on both ecosystems in which some components were removed from Comprehensive samples. Modifications to the monitoring program were discussed among BIO-WEST, Authority, and USFWS personnel during a meeting in August 2002 and implemented beginning with the Fall 2002 Comprehensive sampling event. The most notable change was to remove the water quality component from Comprehensive sampling events due to the comprehensive assessment that was completed from 2000-2002 which demonstrated that relatively constant water quality conditions occur under a fairly broad range of discharge conditions. However, regular monitoring of water temperature via thermistors remained a part of each sample effort in order to maintain a continuous record of water temperature at all sites. Another component that was removed from Comprehensive sampling events was the predation study because there was little evidence to suggest predators were consuming threatened or endangered species under normal discharge conditions. Specifically for the Comal system, macroinvertebrate sampling was modified to include placing drift nets over spring openings and Comal Springs riffle beetle surveys were added.

One additional modification to the sampling protocol was the removal of the Winter Comprehensive sample. Data collected during the first two years of the study provided sufficient information to adequately describe baseline conditions during this season. Low-flow conditions are most likely to occur during the late summer and early fall, so it was considered to be more critical to have continuous monitoring of conditions immediately preceding and following this time frame each year. Therefore, starting in 2003, three seasonal (Spring, Summer, Fall) sampling efforts were conducted per year under the guidance of the modified sampling protocol.

3.4 Variable Flow Scope Modifications – 2005

Starting in 2005, only two (reduced from three) full comprehensive sampling efforts (Spring and Fall) were conducted with a sampling protocol that was slightly modified relative to 2000 – 2004. In Fall 2004, the proposed modifications were discussed and agreed upon by BIO-WEST, Authority, and USFWS personnel. Modifications to summer sampling included only conducting the dip net sampling for fountain darters, while the spring and fall sampling periods remained the same. The annual mapping of Texas wild-rice during the summer months also continued. These two components maintained the continuity of the sampling plan through the summer. These modifications not only assisted in maximizing the efficiency of the project, but also reduced the disturbance to each system from multiple sampling activities.

3.5 Special Studies

In addition to each of the study components described above, several individual research efforts that developed during the course of this study were conducted by BIO-WEST in conjunction with the USFWS NFHTC and TSU. Each special study is noted with a brief description below:

BIO-WEST, Inc. 2002b. Comal Springs Riffle Beetle Habitat and Population Evaluation. Final Report. Edwards Aquifer Authority. 24 p.

BIO-WEST, Inc. 2002c. Comal Springs riffle beetle laboratory evaluation study: evaluation under variable flow conditions. Final Report. Edwards Aquifer Authority. 27 p.

BIO-WEST, Inc. 2002d. Fountain darter laboratory study: reproductive response to parasites and temperature fluctuations. Final Report. Edwards Aquifer Authority. 12 p. (subsequent publication listed below)

BIO-WEST, Inc. 2004b. Aquatic vegetation laboratory study: Phase 1: Observations of water quality changes and plant growth under various flows. Phase 2: Effects of carbon dioxide level on aquatic plants found in the Comal and San Marcos Springs/River Ecosystems. Final Report. Edwards Aquifer Authority. 25 p.

In 2001, a comprehensive survey of Landa Lake (BIO-WEST 2002a) revealed that the Comal Springs riffle beetle was found in areas outside of the Spring Runs, specifically in one area along the western shoreline of Landa Lake and in the lake around Spring Island. Additional laboratory efforts with the Comal Springs riffle beetle (BIO-WEST 2002b) provided data that suggest Comal Springs riffle beetles oriented toward lateral flow and upwelling flow. A laboratory study of fountain darter reproductive response to parasites and fluctuations in temperature (BIO-WEST 2002c, McDonald et. al 2007) revealed that parasites did not affect reproductive capability, but reaffirmed earlier work by Brandt et al. (1993) and Bonner et al. (1998) that reproduction in the laboratory is reduced at elevated water temperatures regardless of daily temperature fluctuations. In 2003, BIO-WEST conducted a laboratory study on the influence of fluctuations in discharge on water quality and determined that carbon dioxide is an important factor affecting growth and structural support of several plants in the Comal and San Marcos Springs/River ecosystems, including Texas wild-rice (BIO-WEST 2004c). One page overviews of each of these special studies are presented on the following pages.

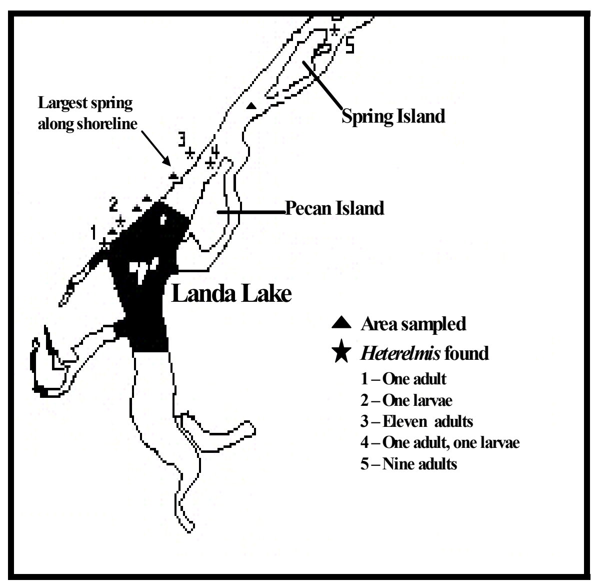
SPECIAL STUDY
Comal Springs Riffle Beetle Habitat Survey

Objective: The Comal Springs riffle beetle (*Heterelmis comalensis*) is an endangered aquatic beetle which was initially thought to have an extremely narrow range including only Spring Runs 1, 2, and 3 of the Comal Springs ecosystem. This study was conducted to further assess the range of Comal Springs riffle beetles, and to quantify density of riffle beetles within these areas.

Task Description: Three separate surveys (3-4 days each) were conducted to search for Comal Springs riffle beetles in spring outflow areas in and around Landa Lake. Where riffle beetles were discovered, a 0.25 m^2 metal frame was used to make quantitative estimates of riffle beetle densities. The frame was placed over the substrate in areas where riffle beetles were discovered and the number of riffle beetles within the area of the frame was enumerated.

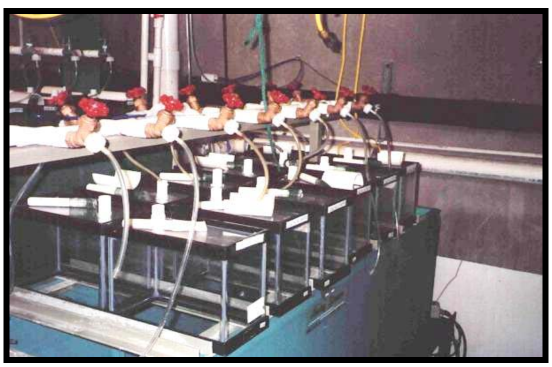
Location: Spring outflows near Landa Lake and Spring Island in the Comal Springs Ecosystem.

Data Analysis and Results: Results indicate Comal Springs riffle beetles occupy several spring outflows beyond Spring Runs 1, 2, and 3, including: seeps along the western shore of Landa Lake, upwellings in the deeper portions of the mid-lake area, and outflows near Spring Island. Although Comal Springs riffle beetles are limited to areas free of silt near spring outflows, these results greatly expand the known range of the beetle. Therefore, the physico-chemical tolerances of the species also may be somewhat broader than initially thought.



SPECIAL STUDY
Fountain Darter Lab Study

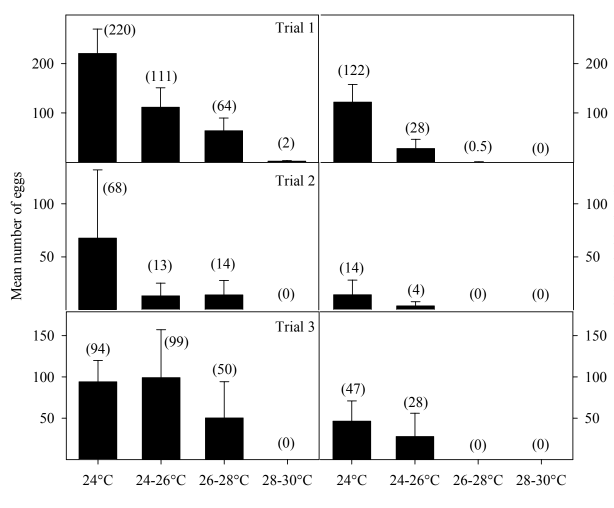
Objective: Temperatures of 22-24°C are thought to be optimal for fountain darter reproduction, whereas temperatures 27°C and higher are known to negatively impact reproduction (Bonner et al. 1998). However, in the lower reaches of the Comal and San Marcos Rivers temperatures often fluctuate between optimal and suboptimal within a 24-hour period. This study was designed to test the impacts of such fluctuating water temperatures on fountain darter egg and larval production. In addition, this study examined the effects of the exotic trematode parasite *Centrocestus formosanus* on fountain darter reproduction.



Location: This study was conducted at the San Marcos National Fish Hatchery and Technology Center by Dusty McDonald and Dr. Timothy H. Bonner of Southwest Texas State University with support from BIO-WEST.

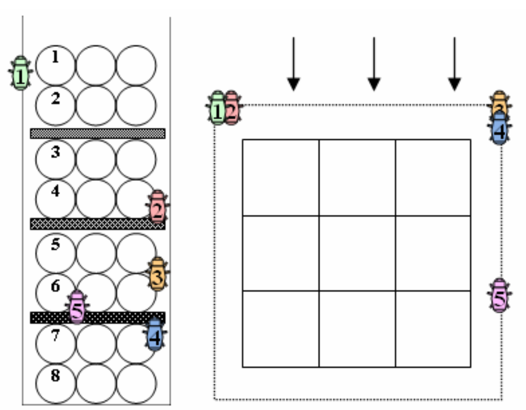
Task Description: Fountain darters (half infected with *C. formosanus* cercariae and half uninfected) were exposed to one of four temperature treatments on a 24-hour cycle: constant 24°C, fluctuating 24 to 26°C, fluctuating 26 to 28°C, and fluctuating 28 to 30°C. Total number of eggs, number of healthy eggs, and larval production were then compared between infected and uninfected fish and between temperature treatments.

Data Analysis and Results: Infection by *C. formosanus* at levels and durations tested in this study did not affect fountain darter reproductive success, and therefore, infected and uninfected fish were combined for temperature analysis. Total egg, healthy egg, and larval production were highest at a constant 24°C, but significantly decreased in variable higher temperature treatments. These laboratory results demonstrate that fountain darter reproductive success is affected by temperatures that fluctuate between optimal and suboptimal in a 24-hour period. However, data from field collections show successful reproduction and recruitment in wild populations when temperatures fluctuate up to 26°C.



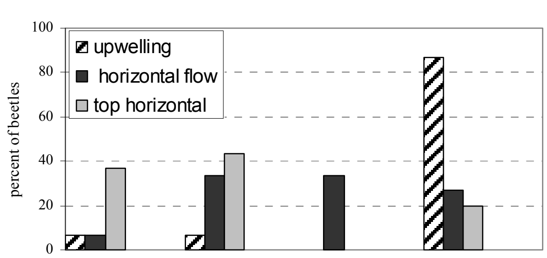
SPECIAL STUDY
Comal Springs Riffle Beetle Lab Study

Objective: A paucity of data exists regarding physiological and ecological requirements of the Comal Springs riffle beetle (*Heterelmis comalensis*). To expand on data collected in the Comal Springs riffle beetle habitat survey, a laboratory study was conducted. Of particular interest is that this species must have persisted through low spring flow events such as the period in 1956 when Comal Springs ceased to flow for approximately five months. Potentially, riffle beetles migrate deeper into the substrate during low flow periods where some subterranean flow may still exist. This lab study was designed to test the relationship between substrate depth, vertical and lateral flow, and riffle beetle location.

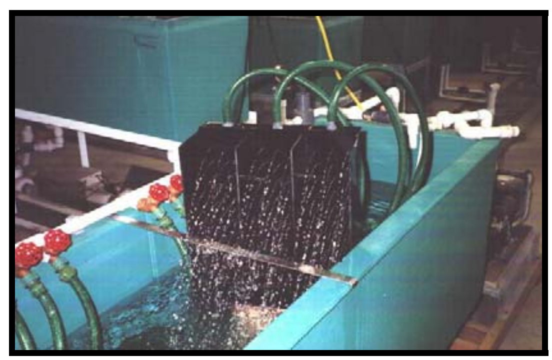


Location: This study was conducted at laboratory facilities of the San Marcos National Fish Hatchery and Technology Center.

Vertical Distribution



Task Description: In the lab, several detailed experimental blocks were constructed with black acrylic plexiglass and Bio Barrel polypropylene media. The construction of this apparatus was designed to mimic the natural gravel and cobble substrate of the riffle beetle and allowed for movement through the media via interstitial spaces. Barrier layers were put in place to slow beetle movement and more accurately reflect the decreasing size of interstitial spaces deeper in the substrate, after test runs revealed that beetles were moving quickly to the bottom of the block. Blocks were designed to allow flow manipulation both horizontally and vertically through the apparatus. Riffle beetle locations were noted after experimenting with combinations of horizontal and vertical flows.



Data Analysis and Results: In this laboratory study, Comal Springs riffle beetles displayed a tendency for downward movement through the substrate and a preference to be in, and move toward moving water (current). When horizontal flow was applied to the block, the beetles were most commonly found towards the front. When a vertical upwelling flow was applied to the bottom, most beetles were collected near or moved toward the bottom. Therefore, it is feasible that these beetles would respond to decreased spring flows by moving downward into the substrate in search of a flow stimulus. It is also likely that these beetles inhabit areas deeper in the gravel and sediment than have previously been sampled.

SPECIAL STUDY
Aquatic Vegetation Lab Study

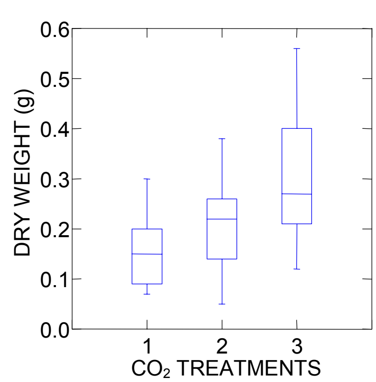
Objective: Submerged aquatic vegetation provides extremely important habitat for the endangered fountain darter. Therefore, understanding factors that influence aquatic vegetation growth and reproduction is critical to maintaining fountain darter populations. Objectives of this study were to evaluate the effects of varying spring flows and resulting water quality parameters on the growth of several aquatic plant species which occur in the Comal and San Marcos Rivers, including endangered Texas wild-rice *Zizania texana*.

Task Description: This study was conducted in two phases. In Phase 1, *Vallisneria sp.* and *Ludwigia repens* plants in outdoor raceways were exposed to varying flows of Edwards Aquifer water. Under each flow level water quality parameters were closely monitored and growth of both species was measured at the end of the study and compared between treatments. In Phase 2, flow levels and temperature were held constant, and carbon dioxide (CO_2) concentrations were manipulated between treatments to examine effects on growth of Texas wild-rice, *Ludwigia repens*, *Vallisneria sp.*, *Hydrocotyle umbellate*, *Riccia sp.*, and *Amblystegium sp.*

Location: This study was conducted at the aquatic nursery/greenhouse facilities of the San Marcos National Fish Hatchery and Technology Center.

Data Analysis and Results: Results from Phase 1 of this study showed that growth of both aquatic plant species tested were greatest under higher flow conditions, mainly as a result of higher CO_2 concentrations. Therefore, in Phase 2 the impact of CO_2 concentrations were tested. When flow and temperature were held relatively constant most plants exhibited increased growth in the higher CO_2 treatment. In summary, dissolved CO_2 concentrations appear to be important in shaping the aquatic plant communities of the San Marcos and Comal Rivers, and therefore, play an important role in maintaining populations of Texas wild-rice and in maintaining quality habitat for fountain darters.

Zizania Below Ground Biomass

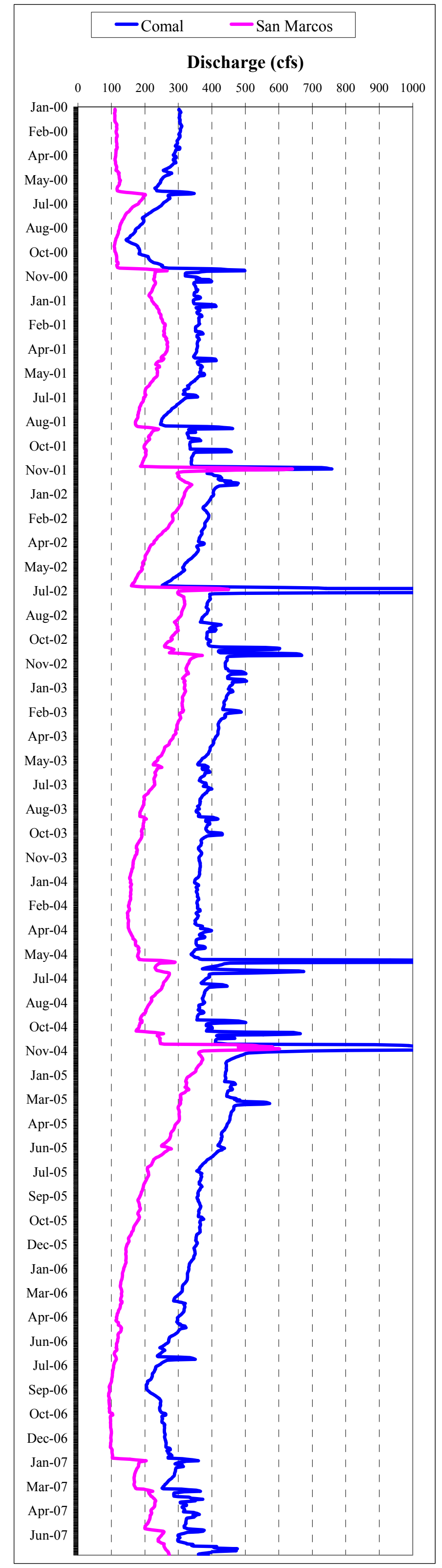


4.0 RESULTS AND TRENDS

Detailed results of the seven-year study period are presented in the Annual Summary reports that have been generated via the Variable Flow project (references included in Section 7). **Figure 4.1** presents a project timeline that outlines the study development, comprehensive and critical period sampling events, and lists select highlights observed over the seven year study period. Discharge conditions recorded at both San Marcos and Comal Springs are embedded in the timeline to provide a better feel for springflow conditions over the study period to date.

The following discussion highlights select results and trends to provide an overview of information collected and knowledge acquired to date. For ease of presentation, the trends overview is broken out by study component with the underlying understanding that the ecological complexity of these systems intertwines each of these components.

VARIABLE FLOW STUDY - PROJECT TIMELINE



YEAR	Study Development / Refinement	SAMPLING PROGRAM Comprehensive	Critical Period	OBSERVATIONS
2000				
	May Workshop - Scope Development			
				Comal Springs - Lowest discharge recorded (< 150 cfs) during 7 year study period. Joint Edwards Aquifer Authority and USFWS press conference at Comal Springs.
	Sampling Protocol Finalized		Comal - Low flow (@ 200cfs)	
			Comal - Low flow (@ 150cfs)	Comal Springs - Spring run 5 ceased flowing over barrier below 150 cfs.
		Fall		
2001	Comal Springs Riffle Beetle Range Expansion Study	Winter		Range of Comal Springs Riffle Beetle greatly expanded.
		Spring		
		Summer		
		Fall	Comal - High flow (Fall 1)	
			Comal / San Marcos - High flow (Fall 2)	Texas wild-rice river population decreased by 7% after high-flow event.
2002		Winter		
	Fountain Darter Laboratory Study			
	Comal Springs Riffle Beetle Laboratory Study	Spring		
			Comal / San Marcos - High Flow (Summer)	High flow scours vegetation, including Texas wild-rice. As in 2001, vegetation community responds with rapid regrowth.
				Fountain darter cage studies show that parasite infestation is worst near island in Landa Lake which supports bird colonies.
	Norris Thesis (TSU) - Invertebrates / Comal	Fall		
2003	Scope revisions - Water quality, predation study, drift net (spring runs) components removed from Comprehensive sampling, remain in Critical Period sampling			As a result recent of scouring events and culvert modifications which alter flows in the Old Channel, *Hygrophila* and *Ludwigia* become established and eventually replace filamentous algae as the dominant vegetation type.
		Spring		
		Summer		
	Aquatic Vegetation / CO_2 Study			Laboratory studies show that CO_2 is important in structuring plant communities.
		Fall		McDonald thesis documents that the parasite is not affecting fountain darter reproduction. Also, refines temperature requirements for fountain darter reproduction.
	McDonald Thesis (TSU) - Fountain Darter			
	Cantu Thesis (TSU) - Parasite / Fountain Darter / Comal			Cantu develops filtration method for assesssing abundance of parasite cercariae in the river.
2004				
		Spring		
			Comal / San Marcos - High Flow (Summer)	Intense scouring of vegetation in the New Channel of the Comal River.
		Fall		
2005	Scope Revision - Summer trip reduced to TWR annual mapping and fountain darter timed dip net			
		Spring		Start of extended drought period ultimately leading to the 2006 low discharge conditions.
		Summer		
				Lack of high pulse flows leads to accumalation of dense vegetation mats over areas containing Texas wild-rice by late summer/fall 2005.
		Fall		Fountain darter population decline in the Old Channel, Comal noted due to exotic vegetation expansion.
				Study initiated to examine abundance of parasite cercariae relative to discharge at six cross-sections in the Comal River.
2006				
		Spring		
				Large stands of Texas wild-rice (234 m^2)are physically removed by recreationalists in the Spring Lake Dam Reach.
			San Marcos - low flow (@100cfs)	
			San Marcos - low flow (@ 90cfs)	San Marcos River - 90 consecutive days with springflow < 100 cfs.
		Fall		San Marcos Springs - Lowest flow recorded (90 cfs) during 7 year study period.
2007				
		Spring		Texas wild-rice in Spring Lake Dam Reach shows recovery from previous impacts.
				Well above average discharge on both systems due to large amounts of spring/summer rainfall.
		Summer		
	Bolick Thesis (TSU) - Parasite / Fountain Darter / Comal			

Figure 4.1 – Project timeline of Variable Flow Study activities.

4.1 Discharge

As evident in **Figure 4.1**, the seven year study period has been characterized by variable flow conditions ranging from floods to extended drought. Substantial variation in discharge has been observed over the course of this study, providing valuable information on flow related responses of the biological communities within the San Marcos and Comal Springs ecosystems. **Figure 4.2** focuses on the total daily discharge less than 600 cfs at both systems from January 2000 through June 2007 to provide a more detailed visual examination. The Variable Flow Study was initiated with a critical period sampling event in late summer 2000 as flow from Comal Springs dropped below 200 cfs. A few weeks later, flow dipped below 150 cfs for a short period, triggering another full low-flow critical period sampling event. These would turn out to be the lowest flows observed in the Comal System over the course of the seven-year study.

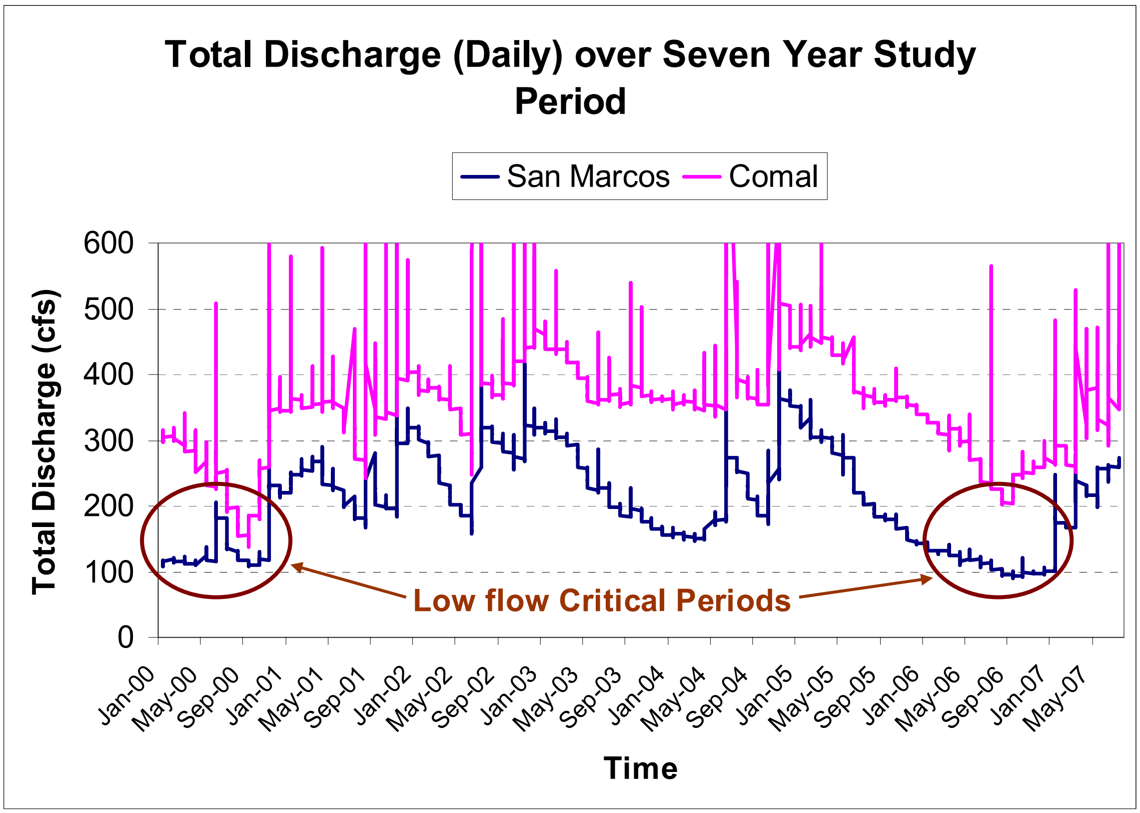


Figure 4.2 – Average daily discharge for both systems over the study period.

From fall 2000 through fall 2004, the central Texas area received greater than average precipitation, and spring flows were above average for most of this period. Although flows were consistently high through this period, seasonal patterns in discharge were still evident, with increasing discharge in winter and spring and declining flows throughout the summer months. Large rainfall events triggered high-flow critical period sampling on both systems in fall 2001, summer 2002, and summer 2004 (**Figure 4.1**). However, spring 2005 was the beginning of an extended drought in the region which resulted in steadily decreasing spring flows in both systems throughout 2005 and much of 2006. This culminated in low-flow critical period sampling events on the San Marcos River in July 2006 when spring flows dropped below 100 cfs, and again in September when discharge dipped below 90 cfs. During this period total discharge from San Marcos Springs were below 100 cfs for approximately 90 consecutive days. Although lower than average, flows from Comal Springs during the same time period did not drop below the trigger level of 200 cfs. Thus far in 2007, extremely wet conditions have recharged the aquifer, and resulted in increasing spring flows throughout the year. At the time of the preparation of this document (August 2007), the J-17 index well was recording nearly 700 feet above mean sea level, which is approximately 40 feet above the historical average.

In summary, the variation in discharge documented over the course of this study has allowed for documentation of biological conditions under average conditions, high flow conditions (2001, 2002, 2004), and a couple of low-flow conditions (Comal 2000, San Marcos 2006) on both systems.

4.2 Water Quality

Water quality parameters measured over the course of the study varied only slightly, and appeared to be influenced primarily by groundwater inputs from the Edwards Aquifer. Temperature is consistent throughout both systems, especially near spring influences (varying <1°C per year). However, farther downstream increased interaction with ambient air causes slightly larger fluctuations. At the downstream most sites monitored in each system (Other Place site [Comal], Animal Shelter site [San Marcos]) fluctuations vary up to 7°C throughout the year, except for occasional larger, short-term declines due to winter stormwater runoff. The continuous recorded thermistor data have and continue to provide a wealth of information. In summary, water temperatures observed throughout the seven year study period were within the range of those required by fountain darters for survival and reproduction.

Other water quality parameters monitored during the study include conductivity, pH, dissolved oxygen (DO), carbon dioxide (CO_2), alkalinity, total suspended solids (TSS), nitrate nitrogen, ammonium, total nitrogen, soluble reactive phosphorus (SRP), and total phosphorus. Of these, DO is perhaps the most biologically important parameter, and concentrations noted throughout the study have never been a cause for concern. Carbon dioxide concentrations, which are high in spring areas, tend to decrease in downstream reaches due to uptake by plants and interaction of the water column with the atmosphere. As a result of a decrease in CO_2 concentrations, pH increases in downstream areas. The only conventional parameter measured that exceeded TCEQ water quality standards was nitrate, as it occasionally exceeded the screening level (1.0 mg/L). However, this does not appear to be a result of anthropogenic inputs, but instead stems from the fact that Edwards Aquifer waters are naturally high in nitrate. Overall, water quality over the seven year study period within both systems was consistently of high quality and posed no serious threat to aquatic life use.

4.3 Aquatic Vegetation

Over the course of the Variable Flow Study, aquatic vegetation has clearly been shown to be a key component of the aquatic community. Aquatic vegetation within both systems provides physical habitat for threatened and endangered species and other aquatic life, provides a direct food source, supports diverse and abundant macroinvertebrate communities that subsequently provide a food source for organisms higher in the food web, and assists in regulating water quality of the spring ecosystems. A few of the highlights observed over the study period include the aquatic vegetation to water quality dynamics (in particular to Carbon dioxide [CO_2]), and trends relative to natural cycles and anthropogenic activities.

4.3.1 Growth Dynamics

During the Aquatic Vegetation laboratory study (BIO-WEST 2004c), all six plant species (four angiosperms and two bryophytes) in all treatments increased in biomass over the six-week study period. Thus, even when CO_2 was low, growth still occurred. However, most plant species tested had a significantly lower biomass (total, above ground, or below ground) in the low CO_2 treatments compared to the other treatments. From this study it was clear that the ranges of CO_2 in the high concentration treatment (when water temperature is held constant) provided for greater plant growth and health. A reduction in growth or a change in growth strategy occurs when CO_2 concentrations are within the ranges of the low concentration treatment (under constant water temperatures). It is also clear from the laboratory studies that water quality parameters change in response to changes in discharge.

So, how does this translate to a natural setting? First, the same water quality to water quantity trend holds in the natural environment as increases or decreases in water quantity will affect the water quality by collectively changing water temperature, CO_2, pH, conductivity, and DO concentrations. From a system wide evaluation, CO_2 concentrations measured in the Comal and San Marcos Springs ecosystems over the seven year study period tend to be similar, at most stations, to the concentrations represented by the low and moderate CO_2 treatments in the aquatic vegetation laboratory study (BIO-WEST 2003a, 2003b). Under typical conditions, there is a downstream gradient with CO_2 as concentrations decrease with distance downstream from the spring openings. As such, the diversity and biomass of aquatic vegetation is highest near the spring openings (i.e. spring runs and Landa Lake in the Comal Springs ecosystem and Spring Lake in San Marcos Springs ecosystem).

In the spring of 2002 and following the large recharge event in the summer of 2002, CO_2 concentrations near the spring openings at both Comal and San Marcos were more similar to the high CO_2 treatment used in the aquatic vegetation laboratory study. During these time periods there was a rapid growth of aquatic vegetation in both systems including a substantial increase in the amount of bryophytes in Landa Lake. Although other water quality parameters (temperature, nutrients, etc.), physical properties (substrate, channel depth, light, etc.), and physical processes (scouring of old vegetation and sediment accumulation) are important to the dynamics of the aquatic vegetation community, CO_2 appears to be key factor that may have strongly influenced these observed changes. The relationship between CO_2 and aquatic vegetation has previously been poorly understood or defined for the Comal and San Marcos Springs ecosystems.

In addition, the CO_2 concentrations were in the upper range of the low CO_2 treatment or lower range of the moderate CO_2 treatment in key habitat areas during the summer and fall of 2000 when total discharge at Comal Springs was below 150 cfs and total discharge at San Marcos Springs was below 110 cfs, and again during the extended low discharge condition at San Marcos in summer 2006 (below 100cfs). Nonetheless, the interesting observation is that at these CO_2 levels, all aquatic vegetation in the laboratory study continued to grow over the experimental period, although growth was lowest in the low CO_2 treatment. From the results of this experiment, one can speculate that aquatic vegetation growth will be reduced under low discharge conditions in the Comal and San Marcos Springs ecosystems. The aquatic vegetation mapping that was conducted during these low discharge periods did not reflect notable changes in aquatic vegetation which is consistent with the laboratory results as the aquatic vegetation community should have been slightly expanding during these periods and not decreasing. As previously stated, many other factors also influence aquatic vegetation condition in a natural setting, and thus further investigation is warranted via measurements during extreme conditions. In the interim, low-flow experiments conducted in a natural setting could provide a wealth of data concerning this and many other topics vitally important to the survival of endangered species in the Comal and San Marcos Springs ecosystem.

4.3.2 Texas wild-rice Restoration Observation

Another key observation from the aquatic vegetation laboratory study was the way the angiosperms (for this example, Texas wild-rice) focused their energy on above ground biomass production under low CO_2 conditions. Simply stated, although growth was still occurring during these conditions, the focus of the majority of the growth was on the plant itself so that it could grow quickly to the water surface and obtain the CO_2 from the atmosphere. In doing this, Texas wild-rice sacrificed the development of strong roots (below ground biomass). So, in the San Marcos River, a plant further downstream may appear to be as healthy as a plant nearer to the springs, but under conditions of lower CO_2, that downstream plant may have a very limited root structure. As such, the slightest of flashy discharge conditions could remove that plant. This knowledge is important to restoration efforts because the most logical place to reintroduce Texas wild-rice appears to be areas that not only have suitable physical habitat conditions but also proper CO_2 concentrations to allow for full plant and root development.

4.3.3 Aquatic Vegetation Trends

Over the course of the study period, changes in the aquatic vegetation community have typically been controlled by three main events: floods, natural growth processes, and recreation.

Floods

High discharge events in both the Comal and San Marcos system have taken the lightly rooted vegetation (e.g. bryophytes) and in many cases more securely rooted vegetation (e.g. Texas wild-rice) and removed them from sections of the river in part or in their entirety. The upper spring run reach of the Comal system is a good example of where a flashy discharge event has completely scoured out the entire bryophyte community. The high discharge events in the San Marcos system typically reduce the overall coverage of Texas wild-rice in the system following those events. Although temporary impacts are clearly evident from these extreme events, the following periods have characteristically witnessed extensive regeneration and re-growth. This is simply nature's way of flushing the system.

Natural processes
In addition to floods, we have observed trends in aquatic vegetation growth which at this time, we can only attribute to natural processes. One such trend is simply related to seasonal influences. A more perplexing trend is most evident in the bryophyte growth in the Comal system. During the middle of this study period we observed a couple years of higher than average discharge conditions without major pulses. During the start and middle of this time period, the bryophytes in Landa Lake and the upper spring run reach were booming. Our CO_2 theory, coupled with lack of flooding conditions, and seasonal understanding lead to the assumption that the bryophyte community would continue to experience seasonal changes but prosper overall. However, once the bryophyte community got to a certain level, it started to demonstrate a system-wide decline in coverage over approximately a six-month period. This decline was independent of the high CO_2 concentrations in the system and not caused by a major flow pulse event. Since the decline was not observed for the other vegetation types in these reaches, and happened over an extended period, herbicides are not likely the cause. It is hypothesized that the bryophyte community simply reached, or more likely, exceeded its carrying capacity and could not expand anymore, thus stimulating a natural thinning process. During this time of high bryophyte growth, there were two to three foot high columns of bryophytes in the deeper portions of Landa Lake. Subsequent to the thinning, the bryophyte community quickly rebounded and is currently quite abundant in the Comal system.

Recreation
A third observation relative to aquatic vegetation growth in these two systems is recreational impacts. Each summer, regardless of discharge, reduction of native and exotic vegetation in the middle of Landa Lake can be expected due to paddle boat activity. For the San Marcos system, the mapping clearly shows decreases in aquatic vegetation due to recreational activity during summer in the City Park reach and Spring Lake dam reach. The Texas wild-rice vulnerable area measurements also clearly document recreational impacts within the Sewell Park reach. As discussed further in Section 6, the level of these recreational impacts is related to the discharge condition of the system. The lower the discharge, the more area within the river channel that is easily accessible, thus resulting in more foot traffic and subsequent disturbance to the vegetation.

4.4 Texas wild-rice

The Texas Parks and Wildlife Department (TPWD) has been conducting annual mapping of Texas wild-rice from the early 1990s. **Figure 4.3** shows the trends in the San Marcos system since 1994 which encompasses the Variable Flow study period. Several trends are evident in **Figure 4.3** including a considerable overall increase (more than doubled) from the early 1990s to present day. It can also be seen that reductions did occur after the 1998 flood (TPWD data) and Fall 2001 flood (BIO-WEST data). In both cases, the total amount of Texas wild-rice increased the subsequent year. It can also be seen that the extremely low discharges experienced in 1996 (TPWD data) and 2006 did relate to slight reductions during the subsequent mapping effort. Similar to the high flow temporary impacts, the total coverage of Texas wild-rice following the low-discharge events also rebounded. A more detailed discussion of impacts experienced during the 2006 low discharge event will be conducted in Section 5.

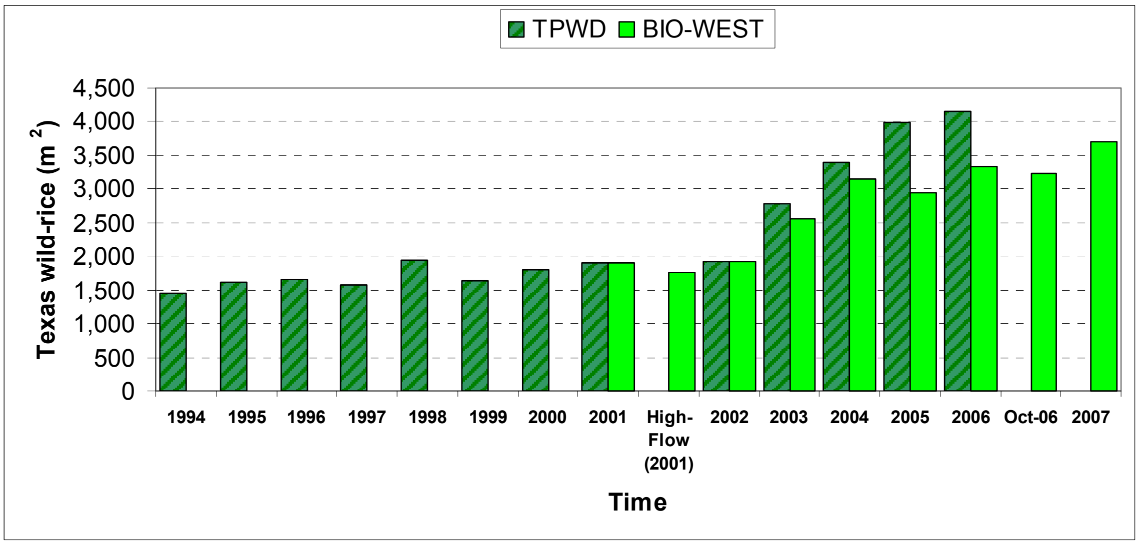


Figure 4.3 – Texas wild-rice coverage in the San Marcos River over time.

It is also recognized in **Figure 4.3**, that the total coverage calculated by the BIO-WEST project team differs somewhat from the total area measured by TPWD for some years, most notably 2005. Since mapping typically occurs during the same time of year, this variation is due to differences in mapping techniques and procedures, primarily in decisions to map Texas wild-rice plants as individual plants or as combined stands. BIO-WEST maps plants individually when open area is present between plants, whereas the TPWD methodology often lumps plants into stands and measures the entire perimeter. The latter approach translates to slightly greater overall estimates of coverage. The important aspect at this point is consistency within sample methodology with both methods providing a valuable double check for the other.

4.5 Fountain Darters

4.5.1 Native verses Non-native vegetation

One of the more prominent trends observed over the course of the study is the strong relationship between fountain darter density and aquatic vegetation type. When fountain darter density data is combined from both systems, it is evident that native vegetation types which provide substantial cover at the substrate level (Bryophytes, filamentous algae, *Ludwigia*) support the largest number of darters, whereas fewer darters are usually found in exotic vegetation such as *Hydrilla*, *Hygrophila*, and *Ceratopteris* which grow thickest near the surface (**Figure 4.4**). In addition to providing more cover for fountain darters, native vegetation types also tend to harbor more amphipods, one of the favorite food items of fountain darters.

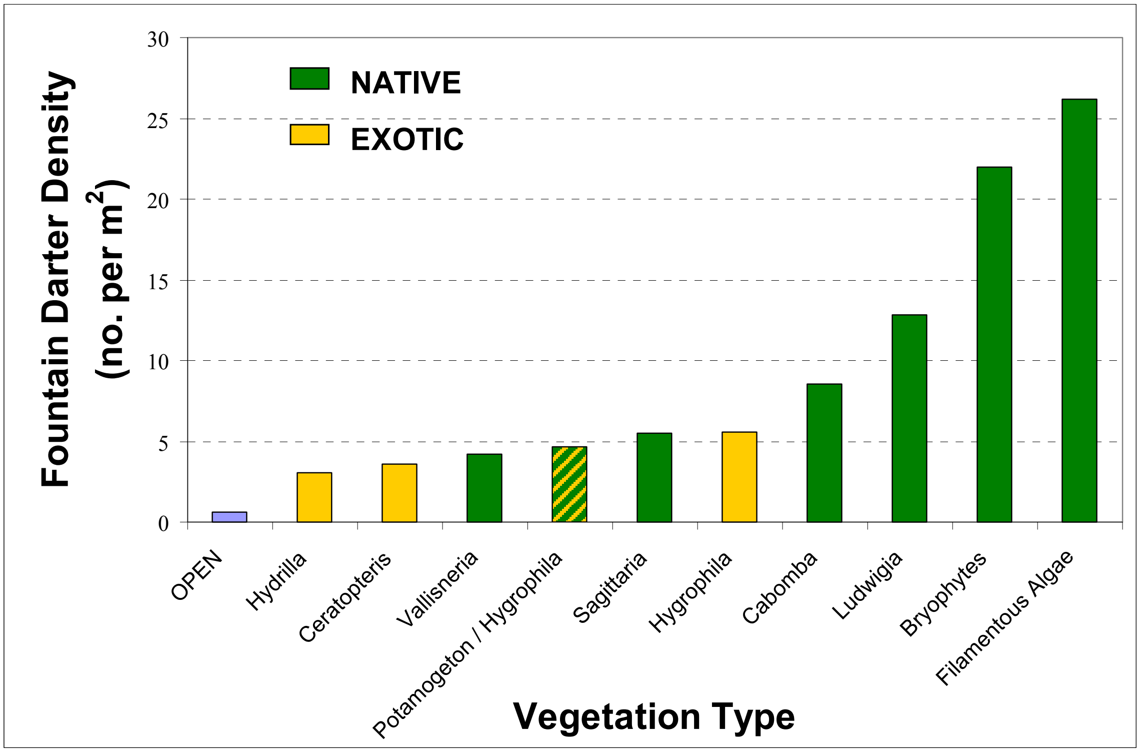
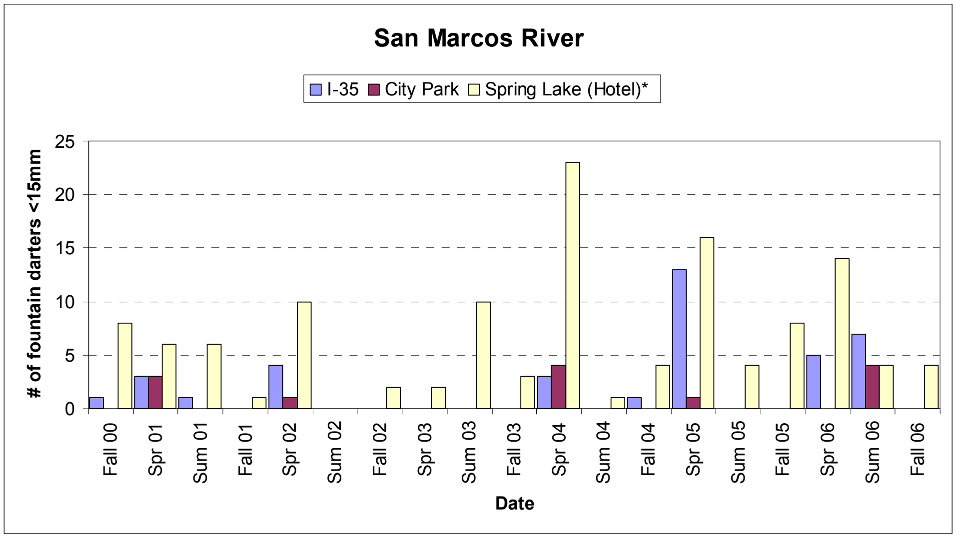


Figure 4.4 – Fountain darter density per aquatic vegetation type.

4.5.2 Habitat Quality

One interesting trend observed in the fountain darter population over the course of the study is the relationship between reproduction and habitat quality. Dipnet results suggest that reproductive success may be related to vegetation type, current velocity, and general habitat quality. In high-quality habitats near spring outflows (e.g., Landa Lake, Spring Lake) length-frequency analysis reveal that reproduction occurs year-round. However, in downstream areas with different vegetation types and higher current velocities, analyses suggest a strong spring reproductive peak with little reproduction during the remainder of the year. This trend is evident in the number of small darters (< 15 mm) collected from the various reaches in each system (**Figure 4.5**). Given that 15 mm fountain darters are approximately 58 days old (Brandt et al. 1993), presence of this size class provides a good indicator of recent reproduction.



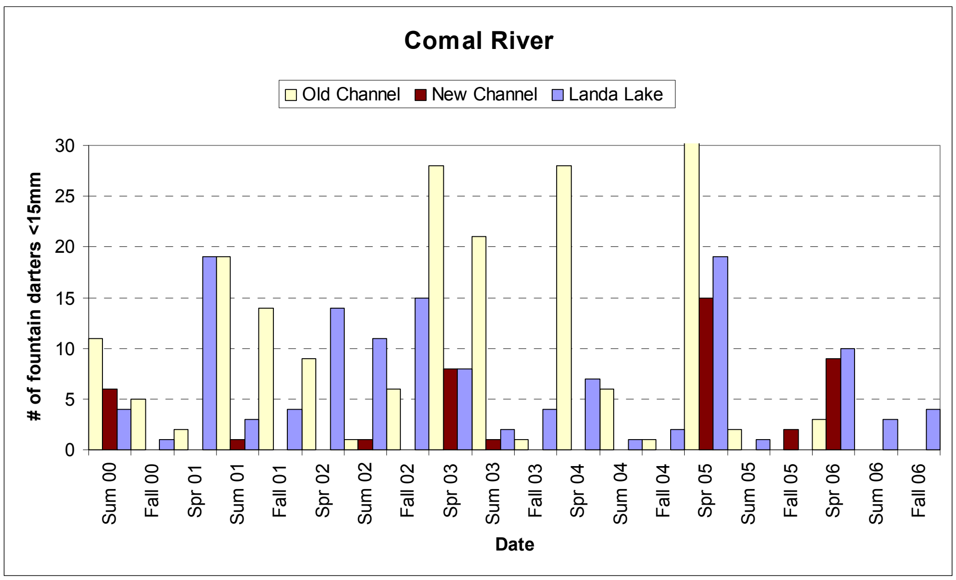


Figure 4.5 – Fountain darter reproduction trends in the San Marcos (top) and Comal (bottom) systems.

In the Comal system, it is important to note that manipulations to a culvert in the Old Channel have caused significant increases in discharge and thus led to large-scale changes in vegetation communities in this reach (discussed in more detail in Section 5). This has resulted in fewer darters being collected in the Old Channel reach since fall 2005. Prior to this modification, both Landa Lake and the Old Channel exhibited presence of small darters (<15 mm) in almost every collection. Data from drop netting and vegetation mapping shows that these areas were dominated by high-quality vegetation types such as bryophytes and filamentous algae. However, the New Channel reach, which is dominated by exotic vegetation, shows the presence of small darters mainly during spring seasons.

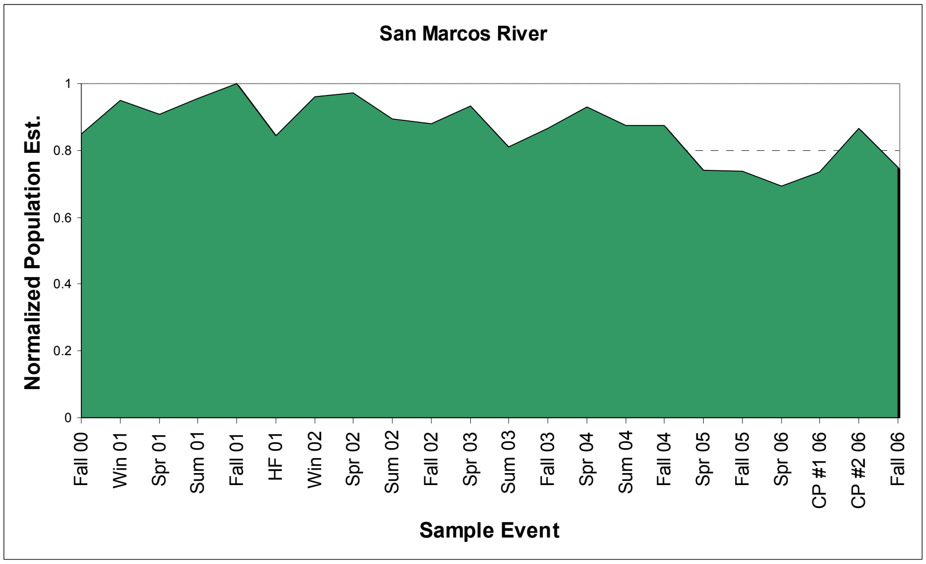
In the San Marcos River, a similar trend is evident. In Spring Lake (Hotel reach), where filamentous algae dominates the sample area and velocities are minimal small darters are present in almost every sample suggesting year-round reproduction. However, in City Park and I-35 reaches, which are both dominated by exotic vegetation and characterized by higher average current velocities, substantial numbers of small darters are observed mainly during spring collections.

Previous studies on fountain darter reproduction, based on combined samples from Spring Lake and the upper 4.8 km of the San Marcos River, have shown that they spawn year-round with peaks in early spring and again in August (Schenck and Whiteside 1977). Data from several reaches sampled in this study support this conclusion. However, our data (seven years of seasonal data from multiple reaches in both systems) also suggest that reproductive success is influenced by habitat quality and varies substantially between reaches within each system.

4.5.3 Population Trends

The high degree of variation in drop net samples observed thus far is not unexpected, but does make it difficult to characterize specific population trends. However, based on the extensive database of drop net sampling for fountain darters in representative reaches within both the Comal and San Marcos systems, several generalized trends in overall annual populations over the study period are discussed. The basic conclusion from this data is that over the range of discharges observed during the study period, the fountain darter populations clearly fluctuate, but have remained stable in both systems. Larger fluctuations in estimated population size in the Comal system are a result of greater variation in coverage of high-quality vegetation types (bryophytes and algae) within Comal study reaches. Neither of these vegetation types is abundant in the San Marcos system.

A cursory glance at **Figure 4.6** might have the reader ask, "What does *remained stable* really mean?", as it looks like the population at San Marcos is decreasing and the Comal population is all over the graph. One might speculate based on Figure 4.6 alone, that the trend for the San Marcos River fountain darter population is decreasing. Forgetting that the data used for that figure only includes the river portion of the San Marcos system and not Spring Lake, and understanding the physical changes (increased sedimentation, disturbance associated with the new Rio Vista dam, increased recreational use) that have occurred in the San Marcos River over the study period, one might start to piece together reasons for such a decline. This again emphasizes the importance of the multi-faceted monitoring program and scientific design of the Variable Flow Study. The observations in Spring Lake via dip netting and SCUBA surveys do not support the speculation of decreasing system-wide fountain darter populations in the San Marcos system. Finally, the size class distribution data collected and analyzed also support that the population of fountain darters in the San Marcos system over the study period has remained stable. We feel that the size class distribution data will be our first indicator of changing populations as is discussed in greater detail in the Section 5.



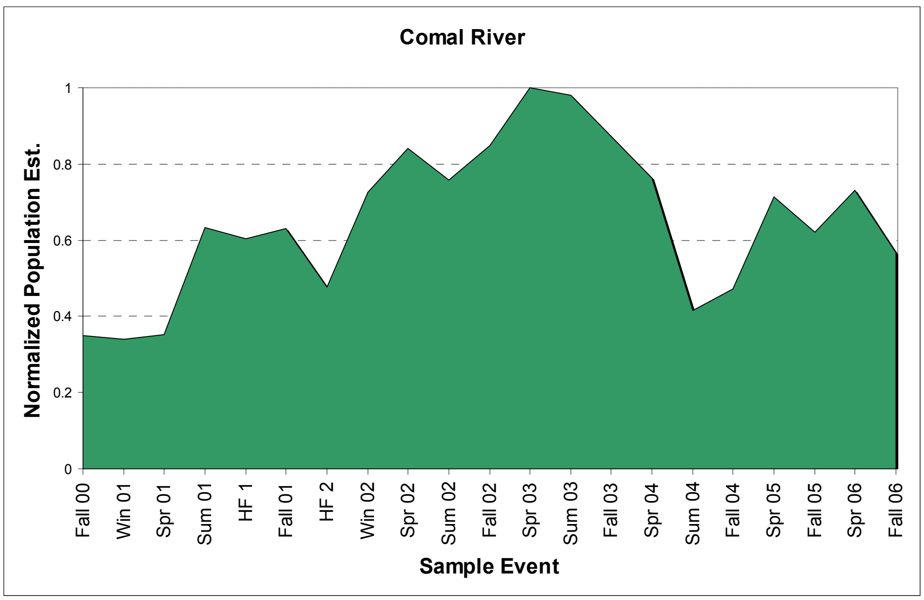


Figure 4.6 – Normalized fountain darter population estimates within the San Marcos (top) and Comal (bottom) systems.

For the Comal system, the lower fountain darter population values prior to Summer 2001 are an artifact of the original scope of work not including mapping or drop netting in bryophytes. The bryophyte sampling was initiated in Summer 2001 when the importance of that vegetation type became evident. The apparent decline from Fall 2003 to Summer 2004 was likely a combination of two main events. As previously described, this was a period of continued above-average discharge, but also the period when the Comal system was experiencing a natural thinning of bryophytes. Secondly, a major high-flow pulse occurred during Summer 2004, which scoured out a great deal of the remaining bryophytes along with modest quantities of filamentous algae. A rebounding of the fountain darter population was evident with the re-establishment of the bryophytes later that year. However, this subsequent increase is likely offset somewhat by the declining fountain darter populations in the Old Channel reach starting in 2005 due to exotic vegetation expansion. As with the San Marcos fountain darter data, the size class distribution data collected on the Comal system imply the population (although clearly fluctuates based on availability and quality of habitat) has remained stable over the seven year study period.

4.6 Salamanders

As with the fountain darter population, the populations of San Marcos Salamanders and Comal Springs Salamanders have remained stable throughout the study period. Indirect impacts from recreational activity have been noted as have short-term direct impacts of sedimentation following pulse flow events. However, neither impact has caused a notable change in the population of salamanders studied in the respective representative reaches in either system. Additionally, sampling bias (easier to snorkel during lower discharge conditions) has been noted and is taken into consideration during data interpretation.

4.7 Comal Invertebrates

Several highlights surrounding the Comal invertebrates (Comal Spring Riffle Beetle, Comal Springs Dryoptid Beetle, and Peck's Cave amphipod) have occurred over the course of the study. These start with the expansion of the known range of Comal Spring riffle beetles within the Comal system (BIO-WEST 2002a). At the start of the study period, it was assumed that the Comal Springs riffle beetle was only present within the fast moving waters of the spring runs. However, individuals have since been consistently found in upwelling areas of Landa Lake, as well as upwelling areas upstream of Spring Island. Another advance in the knowledge of Comal Springs riffle beetles was discovered through the development of a different sampling technique than previously used in this system and for the Variable Flow Study. This technique consists of using cotton rags placed for set periods of time followed by retrieval and counting. This has replaced the old, more intrusive technique of using quadrats within the spring run and turning rocks to investigate. Probably more important than the reduction in intrusiveness, is the effectiveness of this new technique. Many more Comal Springs riffle beetles have been collected using this technique, documenting that larger populations of this species are present in the Comal system than was originally thought at study inception. Using this new technique, USFWS biologists also documented the presence of the Comal Springs riffle beetle in Spring Lake in San Marcos. This was an important discovery as it vastly extends the range of this species.

Large numbers of Pecks Cave amphipods continue to be collected during the Variable Flow sampling project, so many in fact, that we had to change our USFWS scientific collection permit because it was impossible not to collect greater numbers than originally anticipated. Comal Springs dryoptid beetles are also consistently found, but in much fewer numbers that the other two invertebrates of interest. To date, we have not been able to link any of these three invertebrates to changes in the discharge regime, suggesting that the ranges of discharge observed during this study have not exceeded conditions acceptable for these species.

Finally, a major discovery during the study period was the ability to hold Comal Springs riffle beetles and Peck's cave amphipods for extended periods of time in refugia. Also, by making slight manipulations to refugia conditions, reproduction of both species was documented at the USFWS NFHTC in San Marcos. This was a major discovery, countering the assumption of several experts who told us early on this simply would not be possible for these species in captivity because they would not likely survive, let alone reproduce, outside of the natural environment. To date, Comal Springs riffle beetle adults have survived for extended periods of time and eggs and larvae have successfully pupated into adults in captivity. Peck's cave amphipods have survived in captivity for nearly three years at a time, with reproduction occurring throughout that period. The young that were subsequently raised in captivity also reproduced producing F2 populations that also survived. Although, there has not been a major effort in the development of refugia for these species, limited evaluation and experimentation has been extremely positive.

4.8 Parasite Study

Although the fieldwork for the intensive parasite evaluation has just recently been completed and the data analysis is currently being conducted, a few preliminary trends are evident. Observations show that the Elizabeth Avenue site (Old Channel Reach) consistently has the highest trematode density, followed by Liberty Avenue and Houston Street sites (both in the Upper Spring Run Reach). While no seasonal trend is evident, discharge and flushing flows do appear to have an effect on parasite concentrations. For example, the density of cercariae was the lowest following pulse flows with higher densities of cercariae being observed during the lowest discharges.

4.9 Education and Community Involvement

Another highlight of the Variable Flow Study has been the opportunity to educate the public regarding the ecological communities of the Comal and San Marcos Springs ecosystems. Through the organization and commitment of the Authority, the project team has given numerous presentations to biological work groups, non-profit organizations, and universities. Audiences have ranged from highly technical scientific experts, to environmental stewards, to students pursuing environmental science degrees at St. Mary's University in San Antonio, to the general public.

As part of the Authority's education outreach program, the project team has also had the pleasure of interacting with area San Antonio middle school teachers on site at Comal Springs. The following photographs (**Figure 4.7**) highlight some of the activities over the years from those interactions.

Figure 4.7 – Photographs of Authority field trips involving San Antonio area middle school teachers.

Another highlight of the study period was the cooperative agreement formed between the Authority and the Comal chapter of the Texas Master Naturalist Program. The Texas Master Naturalist Program is a partnership among the Texas Cooperative Extension, TPWD, and numerous local partners designed to provide natural resource education, outreach, and other services through volunteer efforts. The program currently supports over 2,750 volunteers across the state of Texas (http://masternaturalist.tamu.edu).

In 2006 and 2007, volunteers from the Master Naturalist program (following a training session by BIO-WEST) assisted the project team by collecting water quality and recreation data on the Comal Springs ecosystem. Volunteers collected water quality data at five sites on a weekly basis. In addition to water quality measurements, recreational use data was collected at each site by counting the number of tubers, kayakers, swimmers, anglers, etc. Photos were taken at each sampling event and any other notes on recreational use or condition of the river were recorded. This type of community involvement is a win-win situation, providing valuable scientific data for the project as well as increasing the educational understanding of this ecosystem.

5.0 CASE STUDIES

Seven years of study have demonstrated the vast ecological complexity of the Comal and San Marcos Springs ecosystems. Perhaps, the most exciting part of a multi-year, multi-faceted study such as the Variable Flow Study, is that we are starting to get a solid glimpse of some key biological linkages to discharge within these dynamic systems. To better explain these biological linkages, the following two case studies have been included. The first outlines biological changes resulting from high discharge conditions with the second example documenting biological responses of low discharge conditions.

5.1 Old Channel, Comal River

Discharge related impacts to the aquatic community in the old channel of the Comal Springs ecosystem have clearly taken place over the past several years. As shown in **Figure 5.1**, the discharge in the old channel at the start of the monitoring period (fall 2000) was approximately 40 cfs. This level of discharge was fairly consistent because of the culvert system that was in place during that time. In 2003, a USFWS sponsored project was implemented that added a new culvert setup for the old channel. The new culvert system allowed for increased discharge capacity. Subsequent to the completion of that project, significant rainfall occurred, increasing recharge and causing the Comal Springs system to flow well above average conditions. These conditions prompted the manipulation of the new culvert structure to allow greater flow through the old channel, which released pressure on the embankment adjacent to the swimming pool. As shown in **Figure 5.1**, the discharge during the spring 2003 nearly tripled and since that time has been a function of culvert operations.

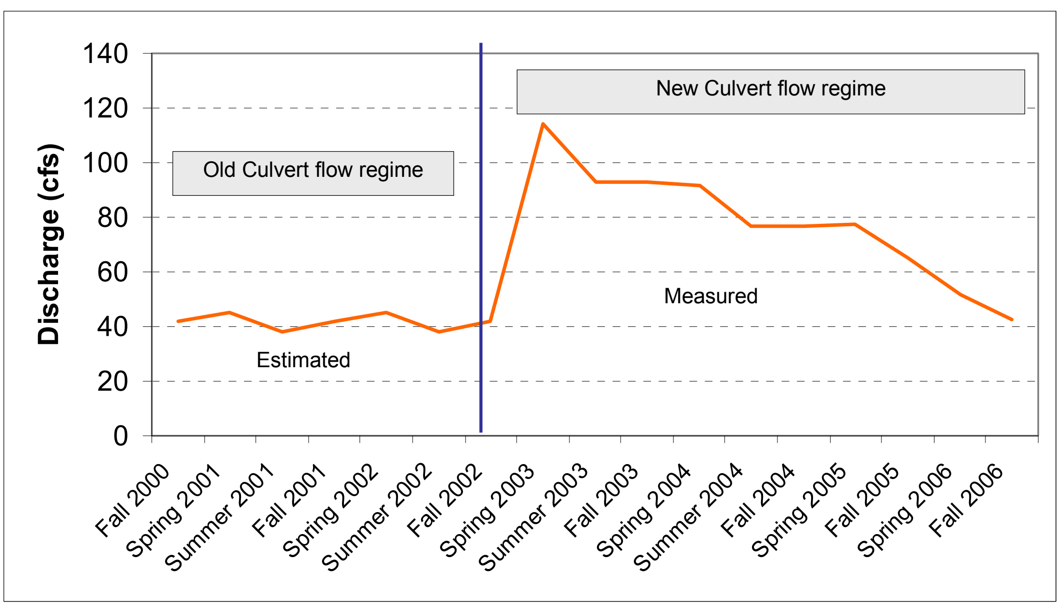


Figure 5.1 – Discharge changes in the Old Channel, Comal River.

Prior to the increased discharge conditions, the old channel was characterized by large expanses of filamentous algae. During the three-fold increase in discharge, most of this native vegetation type was completely scoured out of the routine monitoring site. A period of very limited vegetation growth within the channel was then followed by the establishment of native *Ludwigia* and non-native *Hygrophila*. *Hygrophila* proceeded to take over areas of *Ludwigia* and at present (2007) dominates the aquatic vegetation community of the Old Channel study reach. **Figure 5.2** shows the described aquatic vegetation response.

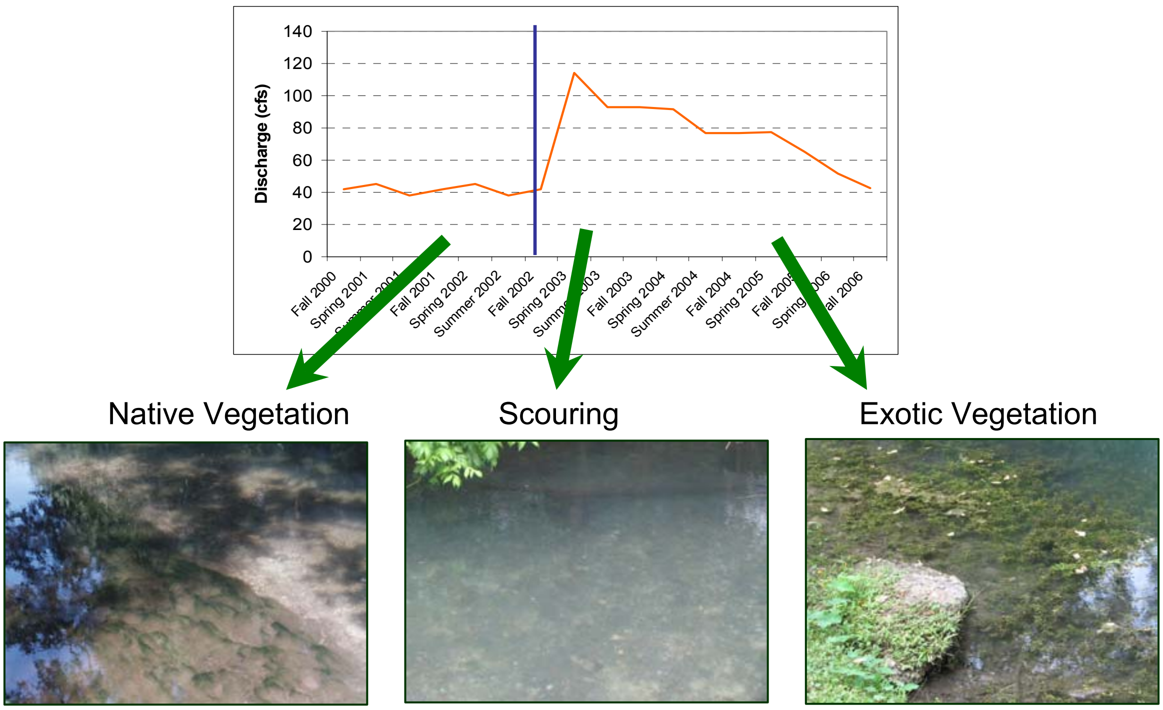


Figure 5.2 – Aquatic vegetation response to changing discharge in the Old Channel, Comal River.

Over the course of the study, fountain darter abundance per vegetation type has been documented. **Figure 5.3** shows the number of darters per meter squared found in the various native (green) and non-native (gold) vegetation types. It is evident that native vegetation is highly preferred by the fountain darter. For example, there is a five-fold increase in fountain darter density in filamentous algae compared to *Hygrophila*.

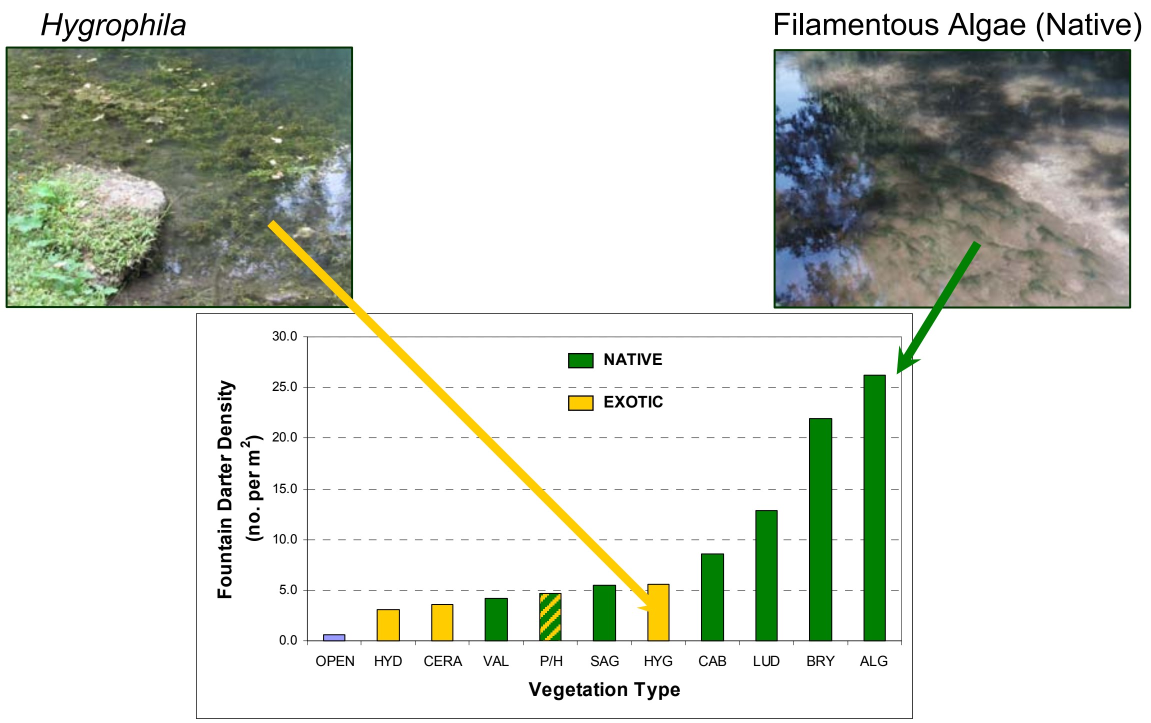


Figure 5.3 – Fountain darter density to aquatic vegetation type.

Figure 5.4 shows the resulting fountain darter population dynamics experienced in the Old Channel study reach over the same time period. With the filamentous algae, the old channel supported a high abundance of fountain darters as well as a normal size class distribution. Year-round reproduction was also evident under these conditions. From 2003-2005, more variable conditions were evident in the Old Channel reach. However, when the exotic vegetation started to dominate in the Old Channel reach, the fountain darter population declined considerably and the size class distribution shifted to larger adults. Typical with other areas of lower quality habitat in these systems, the reproductive pattern of the fountain darter shifted back to spring time only.

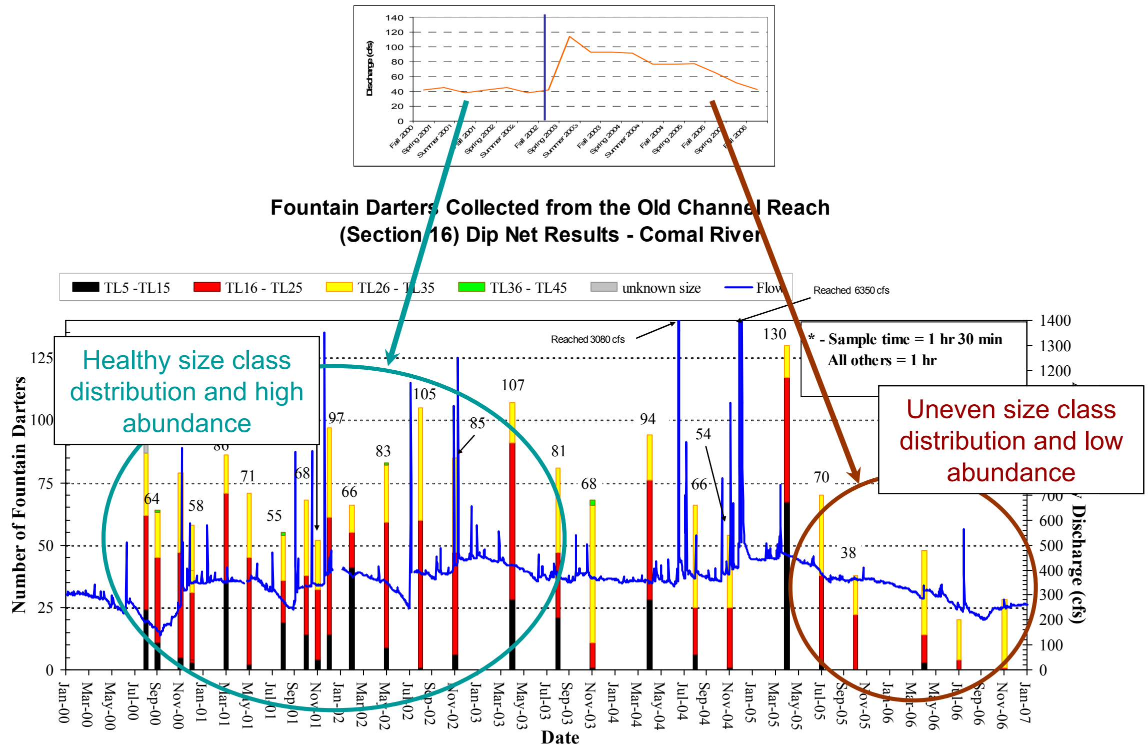


Figure 5.4 – Fountain darter size class and abundance data from dip netting samples in the Old Channel, Comal River over the seven year study period.

It is exciting how the Variable Flow monitoring program has allowed the determination of these key ecological linkages:

Discharge ---- Aquatic Vegetation ---- Fountain Darter population dynamics

Through the physical habitat, the fountain darter dynamics can be linked directly to discharge. It is also clear from this example that aquatic vegetation is a key indicator. With knowledge of this habitat to fountain darter dynamics linkage, potential impacts to the fountain darter population via aquatic vegetation were detectable (although unknown at that time) as early as 2003. However, fountain darter data did not show a change until late 2005.

5.2 San Marcos, 2006 low discharge

The extended period of limited recharge leading up to and extending throughout 2006 caused discharge in the San Marcos River to decline to levels not experienced since 1996. Total discharge in the San Marcos River declined below 140 cfs in early January and remained below this level for the remainder of the year (**Figure 5.5**). The lowest recorded average daily discharge in 2006 was 90 cfs in early September. **Table 5.1** shows various discharge levels and total and consecutive days below those respective levels experienced in 2006.

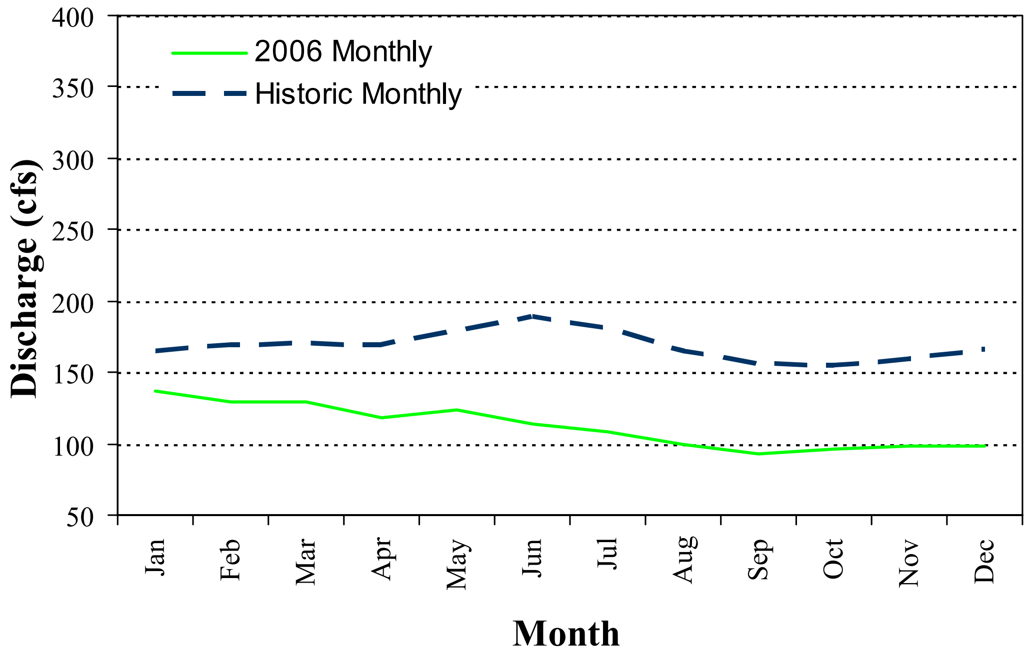


Figure 5.5 – Mean monthly discharge in the San Marcos River during the 1956-2006 period of record.

Table 5.1 – Total discharge levels and associated durations during 2006.

Discharge (cfs)	Days	
	Total	Consecutive
140	356	355
130	309	232
120	241	181
110	180	172
100	115	90
95	47	29

When reviewing the annual discharge in the San Marcos River since the installation of the USGS gage in fall 1956, the 2006 average annual discharge of 112 cfs was the eighth lowest on record. This period of limited recharge and lower than average discharge provided an excellent opportunity to observe biological conditions and direct and indirect impacts associated with these discharge levels. A detailed overview of these observations and impacts on the surface dwelling threatened and endangered species (fountain darter, San Marcos salamander, and Texas wild-rice) are presented in the 2006 Annual Report (BIO-WEST 2007b) and summarized below.

5.2.1 Fountain Darters

The overall fountain darter population did not exhibit any major changes during the 2006 discharge regime. In fact, population estimates of darters actually increased during the critical period sampling events. It has been hypothesized that this would occur during lower discharge conditions as a function of clumping associated with reductions in aquatic vegetation (e.g. available habitat). However, as described in BIO-WEST (2007b), large reductions in available habitat were not measured in 2006. Therefore, this increase in population may simply represent biological variation inherent in this type of sampling. A quick glance at the 2006 spring and fall size class distribution data for fountain darters in the San Marcos River (**Figure 5.6**) may lead one to believe that the shift to larger individuals in the Fall 2006 versus the normal distribution in the Spring 2006 represents a major change. However, upon examination of the previous six years' data of fall collections within the San Marcos River, it is clear that this is simply a seasonal phenomenon (**Figure 5.7**).

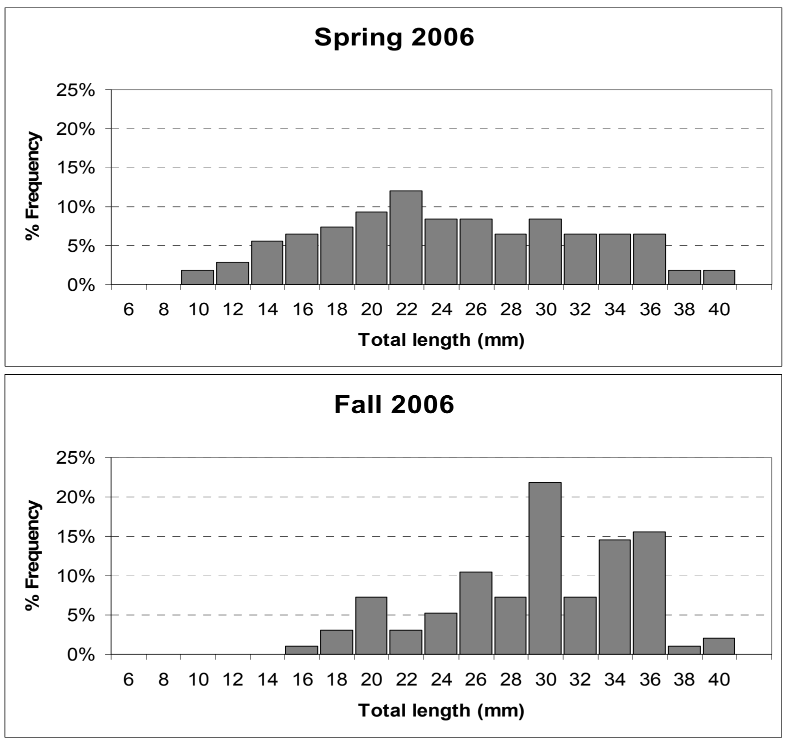


Figure 5.6 – Fountain darter size class distribution from drop net sampling during spring and fall 2006 comprehensive sampling.

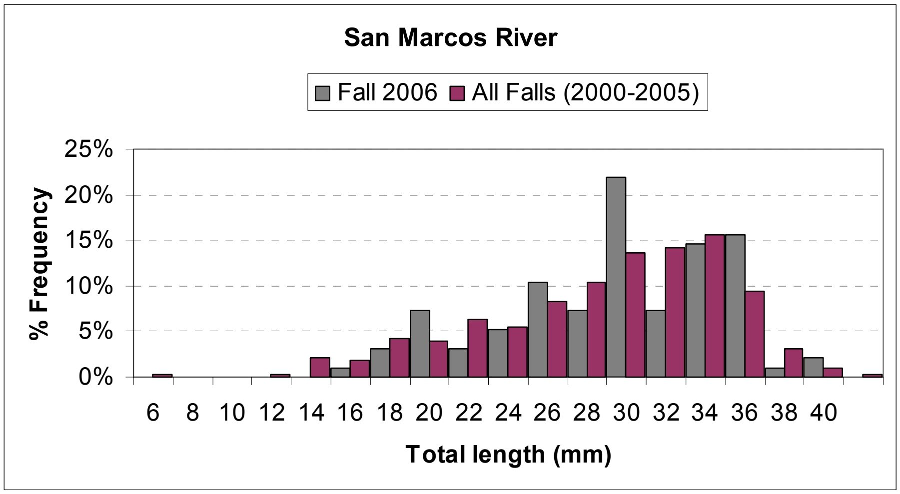


Figure 5.7 – Fountain darter size class distribution from drop net sampling during all Fall comprehensive sample events.

One interesting note was the observance of reproduction of fountain darters during the early and late summer months in the drop trap sampling from the City Park and I-35 reaches (**Figure 5.8**). Reproduction during this time period in these reaches has not been previously reported with this technique during the study.

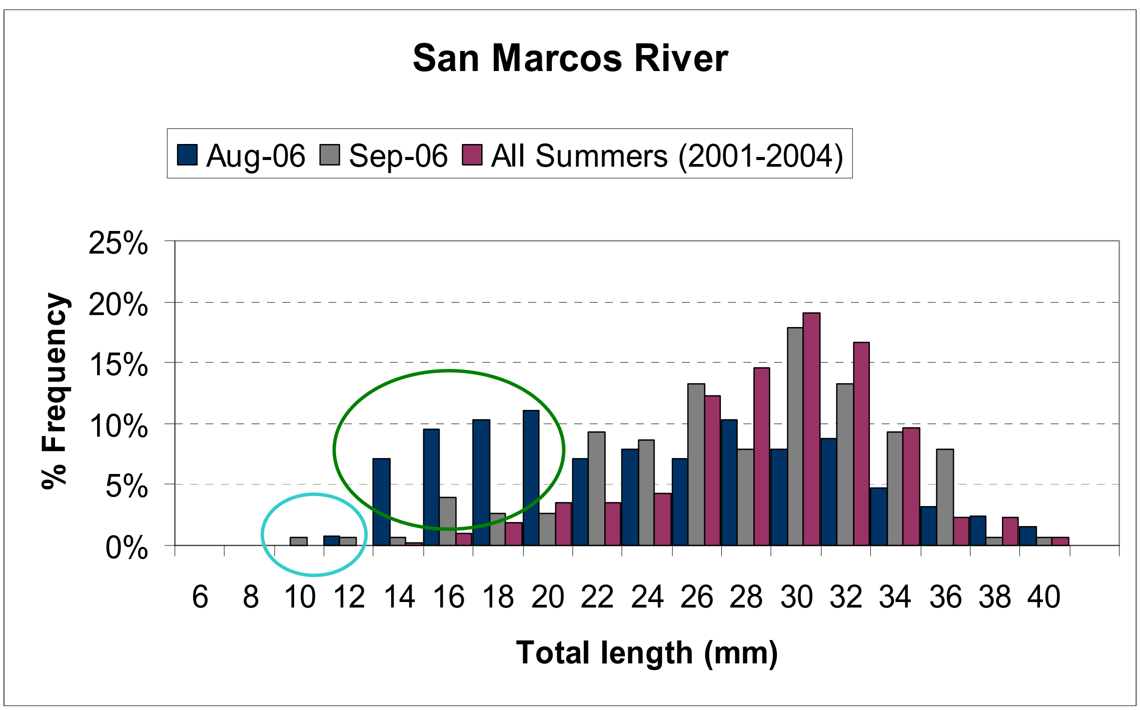


Figure 5.8 – Fountain Darter size class distribution data from drop net sampling during summer.

This summer reproduction event noted by the drop net sampling, coupled with the increased reproduction documented by the dip net samples during this time period, suggests several possible explanations:

1) This event might represent a biological response that was triggered by the onset of lower discharges and slight reductions in available habitat. Could this have been a stress related biological response? The fact that very limited reductions in aquatic habitat were experienced (most of them due to recreational impacts) and water quality conditions were not out of the ordinary, it seems unlikely that this was a stress related response.
2) It may simply reflect inherent biological variation in this complex system. However, one would have expected to pick this up in one of the previous four years of summer sampling with two separate techniques (drop net and dip net).
3) It might have been that lower discharges through these reaches (City Park and I-35) actually created better habitat conditions for fountain darter reproduction. As discussed earlier, year-round reproduction does occur in both the Comal and San Marcos systems, but predominantly in areas of high quality habitat. This high quality habitat is often characterized by certain types of aquatic vegetation and lower velocities (e.g. Landa Lake, Spring Lake, Old channel – prior to the discharge increase). In the San Marcos system, the I-35 and City Park reach maintain lesser quality habitat compared to Spring Lake, and subsequently only typically facilitate spring time fountain darter reproduction. The reduction in discharge to conditions more similar to Spring Lake might have cued reproduction outside of the normal time period typical of these reaches. However, based on a one time occurrence or snapshot, it is impossible to conclude this is the only explanation.

This phenomenon will be closely monitored during future low discharge conditions in an attempt to better understand affects of increasing habitat quality or a potential predictor of stress related biological responses. Based on the fall size class distributions similarity to the previous years as described above, it does not appear that this summertime reproductive phenomenon in these reaches played a noticeable role in the overall population structure.

5.2.2 San Marcos Salamanders

Overall, there was no observed direct impact of the measured discharges on the San Marcos salamander population in 2006. The densities reported in 2006 were very similar to previous years and available habitat within Spring Lake was also very comparable with no signs of increased siltation or excessive vegetation growth around spring outlets. The shallower water depths below Spring Lake Dam as a result of the low discharge conditions in late summer did cause some indirect effects on San Marcos salamander habitat via increased recreational activity. Recreation in the immediate areas below the dam increased during this period with a lot of rocks being physically moved by people to create structures, dams, underwater rock art, and artificial channels (**Figure 5.9**). Although not captured in the snorkel surveys, the physical perturbation associated with this recreation as well as the habitat modification likely had some impacts on the resident salamander population below Spring Lake dam.

Figure 5.9 – Physical disturbance below Spring Lake Dam, September 2006.

5.2.3 Texas wild-rice

The most notable impacts to a species in the San Marcos River during the low discharge conditions in 2006 were the direct and indirect effects of the measured discharge on Texas wild-rice. The declining discharge coupled with the dramatic increase in sedimentation over the past 10 years led to areas of river bottom becoming completely exposed during the late summer months. The Sewell Park reach was the best example of this condition. A small island was actually created in the middle of what had previously been a large expanse of Texas wild-rice. On this island, other plants established themselves resulting in fragmentation of the Texas wild-rice. In addition, larger portions of Texas wild-rice in this reach were emergent and thus, more prone to herbivory by waterfowl and *Nutria* than in previous years. Also, because these plants were in such shallow areas, thick vegetation mats from cuttings in Spring Lake often got caught on these plants and covered them for extended periods of time. This led to achlorotic leaves, and suppression of reproductive culms because the Texas wild-rice could not push through these heavy mats.

Figure 5.10 – Texas wild-rice in Sewell Park, September 2006.

Figure 5.10 provides a nice snapshot of conditions in Sewell Park in September 2006. The photographs show the poor health of the stands (upper right) and emergent vegetation along the river right (bottom right), and healthy stands in the deeper portion on river left (left). The greatest impacts observed during 2006 were the indirect effects of the lower discharge conditions on Texas wild-rice. The most prominent indirect effect was that from increased recreation in these shallow areas. These areas were shallow because of a combination of low discharge and the extensive sedimentation that has occurred over the past decade. For example, in the Sewell Park reach, "paths" developed in the shallow areas where Texas wild-rice was located because it was easier for people to wade in these areas during lower discharge conditions. This led to extensive fragmentation both within the Sewell Park and Spring Lake Dam Reaches. People were observed walking in these areas and parking their kayaks/tubes/inflatable floats on top of plants, leading to plants being pulled out or trampled.

The greatest single impact was observed in the Spring Lake Dam reach adjacent to the Clear Springs Apartments where a 73% decrease in total Texas wild-rice area was observed in late summer 2006 (**Figure 5.11**). Upon visual observation during a field investigation, it was evident that large patches of Texas wild-rice had been physically pulled out with only solitary leaves and root-wads to indicate where these plants had been.

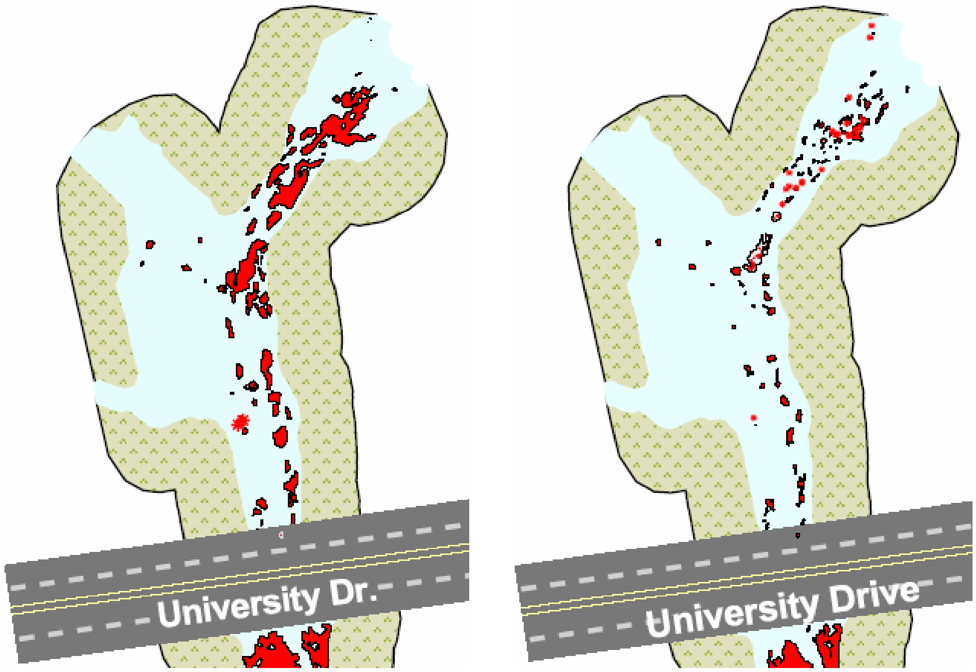


Figure 5.11 – Texas wild-rice aerial coverage (August 2006 – left / September 2006 – right).

Further evidence of manipulation of the river by people was present immediately downstream of Spring Lake Dam. Artificially created walls of rocks emerging from the water column served to further channelize this area blocking flow to several Texas wild-rice plants. **Figure 5.12** shows the sizable decrease from Spring 2006 to August 2006. However, if this had been caused directly by low discharge, a continued decline would have been expected from September 2006 to Fall 2006, as the discharge in the San Marcos River remained at low levels. There is no question that direct human activity was responsible for the reduction in Texas wild-rice in the Spring Lake Dam reach during this time period.

To put the Spring Lake dam physical disturbance into perspective, the overall coverage of Texas wild-rice in the San Marcos River actually increased by 50.7 m^2 from Summer 2005 to the second Critical Period sampling in late Summer 2006. From spring 2006 to the second Critical Period sampling, the overall Texas wild-rice coverage declined by 335.3 m^2 or approximately 11%. If you subtract the 234 m^2 that was clearly physical disturbance, this leaves an overall 2006 decline (101 m^2/approx. 3%) that might be directly attributable to lower discharges. If you look just at the Texas wild-rice deemed to be in the most vulnerable locations there was a reduction from 980.7 m^2 in spring 2006 to 772.9 m^2 in fall 2006 or approximately a 21% decline. As this is greater than the overall coverage change, as expected, it also points out that Texas wild-rice not located in vulnerable areas actually increased throughout the lower discharge conditions in 2006. A follow-up consideration is that if the excessive sedimentation in the San Marcos River over the last 10 years had not occurred creating these vulnerable areas, Texas wild-rice likely would have continued to expand even during the eighth lowest average annual discharge on record.

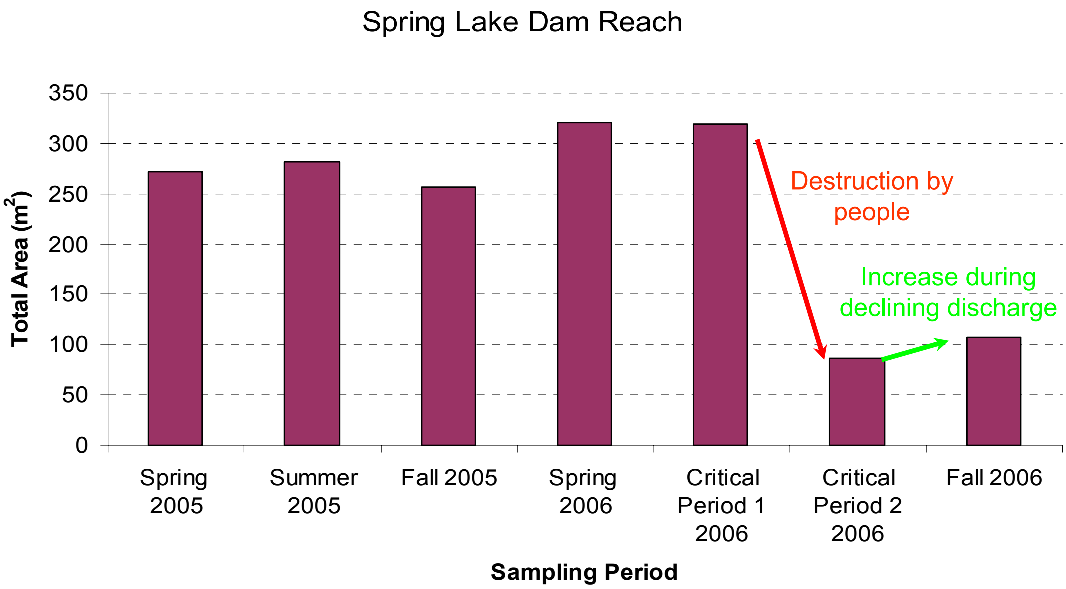


Figure 5.12 – Changes in Texas wild-rice within the Spring Lake Dam reach.

5.2.4 Summary

In summary, increased recreational activity during 2006 caused the greatest impacts on the threatened and endangered species in the San Marcos Springs ecosystem. These effects included destruction of Texas wild-rice and habitat modification for the San Marcos salamander (humans physically moving rocks below Spring Lake Dam) and fountain darter (increased foot traffic through commonly deeper areas). Direct effects of the lower discharge conditions were not evident for the San Marcos salamander at the discharge levels measured in 2006. Direct effects on the fountain darter may have been experienced with compensatory reproduction occurring as a result of lower discharge conditions, however, this is not an absolute, nor did the response have any measurable impact on the fountain darter population in the San Marcos River in 2006. Finally, the greatest direct impact associated with the lower discharges experienced in 2006 was to Texas wild-rice with an overall reduction in coverage (~3% entire river and ~21% in vulnerable areas.)

The data collected in 2006 will be extremely valuable in initiating discussions with the Authority, TAG, and state and federal agencies relative to the condition of the San Marcos Springs ecosystem during lower discharge conditions. It is important to remember that these data must be evaluated in context, which is a one-time event with an extended duration, preceded by an extended period of good biological conditions. *Caution should be taken when speculating how these results might transfer to longer durations of low discharge, lower than observed discharge, or similar discharge preceded by poor ecological condition.*

6.0 RECOMMENDATIONS AND MANAGEMENT IMPLICATIONS

The established database and comprehensive and critical period monitoring through the Authority's Variable Flow Study continues to provide an excellent measure of ecosystem condition and, after 2006, some very strong insight into biological responses to low discharge conditions. This section explores some recommendations for continued assessment, identifies restoration and applied research opportunities, and points out some key management implications outside of the Authority's jurisdiction. The section concludes with proposed strawmen flow regimes for Comal and San Marcos Springss to stimulate discussions during the Recovery Implementation Program and facilitate applied research activities should they be pursued.

6.1 Long-term Monitoring

It is recommended that the Authority's Variable Flow Study be continued with both the comprehensive and critical period components intact, and with the continued opportunity for special studies as deemed appropriate. Extending both components will hopefully yield the desired information on the effects of low discharge on the listed species in the Comal and San Marcos Springs ecosystems. This valuable information will improve the ability to predict the response of each population to such conditions if and when they occur again in the future. Regardless, the continuation of routine monitoring is also extremely valuable due to the dynamic nature of population-level responses to changing habitat conditions. A population that has experienced "good conditions" for an extended period and has expanded in abundance to capitalize fully on extensive availability of high quality habitat will not have the same response to a rapid decline in discharge as a population that had experienced moderate to low quality conditions preceding such an event. The only way to maintain knowledge of current population conditions is through regular monitoring. This monitoring will be vital for documentation of conditions immediately prior to a low-recharge event, since low-flow conditions can occur rapidly due to the geophysical characteristics of the Edwards Aquifer.

6.2 Environmental Restoration and Research

Since direct impacts measured in 2000 (when the Comal system was at a total discharge of less than 150 cfs) and 2006 (when the San Marcos system was under 100 cfs for approximately three consecutive months) were very minor or non-existent, additional field data at lower discharge conditions are necessary to fully assess conditions that might jeopardize the existence of one or several of these species in the wild. Hopefully, these extreme conditions will be rare events in these systems. Logistically, this provides a dilemma for understanding the severity of potential impacts when these events finally unfold. Therefore, to fully prepare for potential impacts during such conditions, interim efforts to evaluate response mechanisms of the threatened and endangered species to low discharge conditions either via laboratory investigation (as conducted in previous years under this program) or via *in situ* field experiments as previously proposed with the "intensive management areas" concept would provide valuable information for management decisions.

One immediate opportunity would be to explore environmental restoration and research feasibility within either the Comal or San Marcos systems. Areas that are well suited for this type of activity are the Old Channel within the Comal system and the eastern spillway below Spring Lake dam in the San Marcos system. For example, the Old Channel has been overrun with exotic vegetation and is not the high quality habitat that it was merely five years ago. There has been a proposal for some time to examine the effectiveness of an Intensively Managed Area (IMA) at Comal Springs by actually testing the concept in the field. The goal of the IMA would be to provide *in-situ* refugia for species when conditions are poor, while also providing the opportunity for research when conditions are favorable. Conducting *in-situ* experiments to evaluate low discharge responses of the endangered species and their habitats would assist in solving a critical piece to the puzzle that remains unanswered even after seven years of intensive monitoring. Discharge conditions within the Comal and San Marcos systems over the past seven years have simply not fallen to levels or experienced durations that have caused significant impacts to any threatened or endangered species. Thus, even though we have called some of the Variable Flow sampling events "Critical Period" or "low-discharge" based on our preconceived thoughts of what low discharge really is, impacts have not been substantial enough to make conclusive linkages as to appropriate jeopardy levels.

At this time, a companion strategy to the proposed *in-situ* refugia / low flow experimentation would be to restore the higher quality (native) vegetative habitat for fountain darters within this Old Channel reach. One proposed area would be between the culvert that directs flow from Landa Lake into the Old Channel and the low-water golf course road (**Figure 6.1**). The length of this segment of river is approximately 275 meters. Flow enters the proposed study reach through two culverts, which are both controlled by the City of New Braunfels along with the USFWS. In addition to the culvert that directs flow from Landa Lake into the head of the Old Channel, there is another culvert that directs water from the lake into the city swimming pool area and then into the Old Channel about 175 meters downstream from the first culvert. The majority of the proposed IMA would be within the downstream-most 100 meters of this reach.

The basic conceptual design of the project is to build/install a diversion structure and pipeline to re-circulate a portion of the flow in the Old Channel from a point near the golf course road back up to a point near where the flow-through swimming pool water enters the channel (**Figure 6.1**). This could potentially provide higher discharge through the immediate project area during periods of limited recharge. It is anticipated that water chemistry conditions would not change substantively using this re-circulation design, but that component would also need to be evaluated prior to implementation. In addition to re-circulation, the pipeline would allow the transfer of water in both directions so that during normal to high flow periods a portion of the flow could be directed around the project area. The ability to lower discharge through the project area, without affecting the total flow available to downstream users such as Schlitterbahn, would provide an ideal situation for experimentation of the impacts of different streamflow conditions.

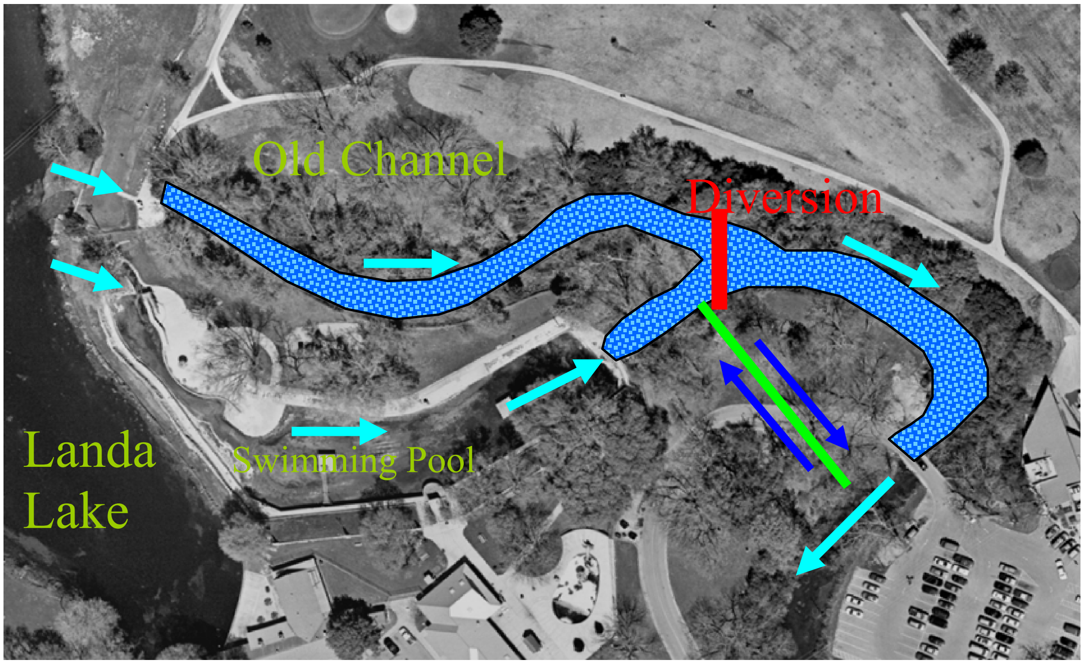


Figure 6.1 – Conceptual design of an Intensive Management Area on the Old Channel, Comal River

The three main activities of: 1) evaluating the Intensive Management Areas approach for effectiveness of preserving habitat and protecting species; 2) conducting flow manipulation experiments to better define critical conditions and effects of different flow patterns; and 3) evaluating cost and effectiveness of re-circulation for preserving habitat and protecting species, could serve as vital components in the upcoming Recovery Implementation Program (RIP).

6.3 Management Implications

A recurring issue that tends to get more attention during low discharge conditions is river management activities outside of the jurisdiction of the Authority. The Variable Flow Study has documented that several of these factors are direct contributors to impacts to threatened and endangered species in both systems, but more evident on the San Marcos River. These factors include such items as exotic species (both plant and animal), aquatic vegetation mats, sedimentation, and recreation. A brief description of existing sedimentation and recreation issues are presented below to highlight reasons for concern.

6.3.1 Sedimentation

Over the course of the study period, the upper San Marcos River has filled in considerably from just below Spring Lake Dam (near Bob Dog island at the mouth of

Sessom's creek) to the reconstructed Rio Vista Dam. This statement is not based on a BIO-WEST sediment transport study or model (as this type of effort has not been part of the Variable Flow Study), but rather on seven years of visual observations and actually being in the river on a regular basis. The build up of sediment in this stretch directly dictates the depth of water and substrate composition. Water depth and substrate are important components to maintaining the proper aquatic vegetation for fountain darter habitat as well as suitable conditions for Texas wild-rice. A master's thesis examining sedimentation in the upper San Marcos River relative to Texas wild-rice distribution was recently completed by Ms. Katherine Griffen, Texas State University, under the direction of Dr. Joanna Curan. Griffen (2006) describes that the sediment input to this upper reach derives from Sink Creek, Sessoms Creek, and through overland flow. She continues with a discussion on the effectiveness of the five flood detention dams that have been built on tributary creeks draining into the San Marcos River and explains how reducing the frequency of higher flows has reduced the ability of the river to flush out the accumulated bed sediment (Griffen 2006).

As sedimentation of the San Marcos River will continue to impact endangered species habitat or the species directly in the case of Texas wild-rice, it is recommended that specific discussions with the entities vested in the protection of the San Marcos River be initiated. An additional recommendation includes a comprehensive evaluation of existing literature (primarily cross-section data) specific to the San Marcos River. Potential sources include Griffen (2006), TPWD cross-sectional work in the 1990's specific to Texas wild-rice, TPWD cross-sections associated with their mid-1990s instream flow study (Saunders et al. 2001), Espey, Huston and Associates (1975) flow investigation report, along with more recent Variable Flow study cross-sectional information associated with Texas wild-rice physical measurements. Following these initial discussions and a comprehensive review of this existing literature, the need for further discussions with these entities regarding potential sediment transport studies, funding, and management will likely transpire.

6.3.2 Recreation

Similar to the trends witnessed with sedimentation, qualitative observations of increasing recreational activity have been noted for both the San Marcos and Comal systems. On the Comal system, the bulk of the recreational activity is fairly isolated to the new channel and below the confluence of the old and new channels. Being at the downstream extent of the Comal River, these areas support the lowest quality habitat for fountain darters and thus, increasing recreation is not nearly the concern as for the San Marcos River. The main focus of the recreation on the San Marcos River starts immediately below Spring Lake Dam and extends to Rio Vista Dam. This upper stretch of the San Marcos River includes high quality habitat for the San Marcos salamander, fountain darter, and supports the highest areal coverages of Texas wild-rice.

During the summertime discharge conditions in 2006, recreation was the largest contributor to threatened and endangered species impacts. Obviously, recreational impacts are facilitated by shallower water depths due to sedimentation and lower discharge. Yet, simply removing people from below Spring Lake dam and Sewell Park during 2006 would have potentially prevented the majority of impacts documented. This report is not meant to be a critique of current recreational management practices, rather it simply states the facts surrounding 2006 impacts to the threatened and endangered species in the San Marcos system. As with the sedimentation issue, it is recommended that specific discussions regarding river recreation with the entities vested in the protection of the San Marcos River be initiated, perhaps as part of the Recovery Implementation Program.

6.4 Flow Regime Strawmen to Stimulate Discussion and Evaluation

Ever since the establishment of the current USFWS take and jeopardy numbers for the Comal and San Marcos Springs ecosystems, there has been controversy and debate over this topic. This section is not meant to be an exhaustive description of "take" and "jeopardy" with supporting documentation nor even a comparison to existing criteria. The sole purpose of this section is to provide a framework to stimulate discussions during the Recovery Implementation Program (RIP) process, and provide a good starting point for potential *in-situ* experimentation.

It is unknown at this time whether the following flow regimes would be protective of the threatened and endangered species in the Comal and San Marcos systems. They simply represent the author's best professional judgment based on the information gathered and reviewed during the seven year Variable Flow Study, including but not limited to historical hydrology and biological information collected as part of the Variable Flow Study and other sources. With all of those caveats in place, it must also be said that a flow regime vs. a single number approach needs to focus on achieving conditions at multiple levels of the flow regime. This includes not only those conditions during extreme drought, but those in the average discharge range, as well as high flow pulses. However, since drought contingency plans center around periods of limited recharge, the following discussion is in fact purposely narrowed to the low end of the spectrum.

The following flow regime strawmen represent total discharge conditions for each respective system. However, as it would not be possible to test the protectiveness of these proposed flow regimes on the entire system until they actually occurred in the wild, it is recommended that *in-situ* experimentation be explored as a means for scaled evaluation. This was discussed briefly in Section 6.2 with the focus on the old channel of Comal Springs. Although the information regarding aquatic habitat changes and fountain darter responses in the Comal system would likely be applicable to the San Marcos system, there are notable differences between the systems that would need to be accounted for. Thus, having an environmental research area within the San Marcos system would also provide valuable information on that system's response to low discharge conditions.

One such area for consideration in the San Marcos system is the eastern spillway immediately below Spring Lake dam. This area supports a diverse community of aquatic vegetation and populations of fountain darters, San Marcos salamanders, and Texas wild-rice. Additionally, methods for re-routing water through the western spillway during times of experimentation appear feasible without major modifications to the system. For example, during the recent reconstruction of the chute in the western spillway near Joe's Crab Shack, the majority of the water was diverted to the eastern spillway.

Up to this point (Summer 2007), only preliminary investigations and discussions have taken place regarding these environmental research areas on either Comal or San Marcos Springs. Upon initial evaluation, the old channel in the Comal system does present the advantage of a more secluded area with limited recreation. However, as previously mentioned, having the ability to test biological responses specifically on the San Marcos system is also important. Clearly, a feasibility study evaluating the proposed and other potential locations, methodologies, stakeholder involvement/participation, permitting requirements, etc. would need to be conducted in advance of implementation.

6.4.1 Comal Springs

At any given discharge, one can attribute some level of "take" to threatened and endangered species. For this discussion, our goal was to outline a starting point for loss of habitat directly related to low discharge conditions observed over the Variable Flow study period. During the Variable Flow Study, springflow ceased horizontal flow over the concrete wall at Spring Run 5 at approximately 150 cfs total discharge in the Comal System. At this total discharge level, the flow through the upper spring run reach became limited. This coupled with the warm weather conditions led to the development of extensive mats of green algae. Under those conditions, fountain darter habitat quality related to discharge was reduced, thus indicating a good starting point for discussion as when impact to endangered species habitat starts to occur. There have been no Comal invertebrates documented in this uppermost stretch of river, so this analysis simply reflects the beginning of impacts at the extent of the fountain darters range. At these discharge levels, conditions observed (albeit for only a short period of time) in Landa Lake and subsequent downstream habitat in the Comal system indicated no signs of aquatic habitat reductions. As mentioned above, no critical period low-discharge sampling efforts on the Comal system were conducted since the Fall 2000 event. During all subsequent discharge conditions at Comal Springs, no observed impact to endangered species were documented that could be strictly related to low discharge.

It must be noted that this section will be describing concepts for protecting the survival of the species in the wild (i.e. avoiding jeopardy). This is NOT equivalent to maintaining high quality conditions and all ecological functions throughout the entire system for the entire year if not necessary for survival. Although fountain darter reproduction is a year-round activity in high quality habitat, the seasonal (spring) activity exhibited in lesser quality habitat still appears to support populations in these reaches. Therefore, one goal for low discharge criteria is to protect spawning conditions for the fountain darter during the springtime months. So for the Comal strawman, March through May is considered springtime, and discharge is proposed to be maintained at a higher level during this time.

Given the information to date, it does not appear ecologically necessary (in the context of survival) to maintain continuous fountain darter reproduction. Therefore, the other nine months of the year will be prescribed a lower discharge condition. This discharge condition is one whose goal is to be protective of a large amount of aquatic habitat within the higher quality habitat areas of the system. It is not meant to be protective of the entire system, as nature does not protect the extent of the range of any species during extreme conditions. The final proposed category is a floating three month period (outside of March – May) at which an even lower discharge condition would be allowed. During this short time period, the most important aquatic habitat (i.e. highest quality) would be maintained at a level to ensure continued existence of the species in the wild. This may mean that the temperature is too warm for successful reproduction during these three months, but clearly the temperature within the highest quality habitat could not be lethal to the organism. **Figure 6.2** provides the strawman flow regime for the Comal system.

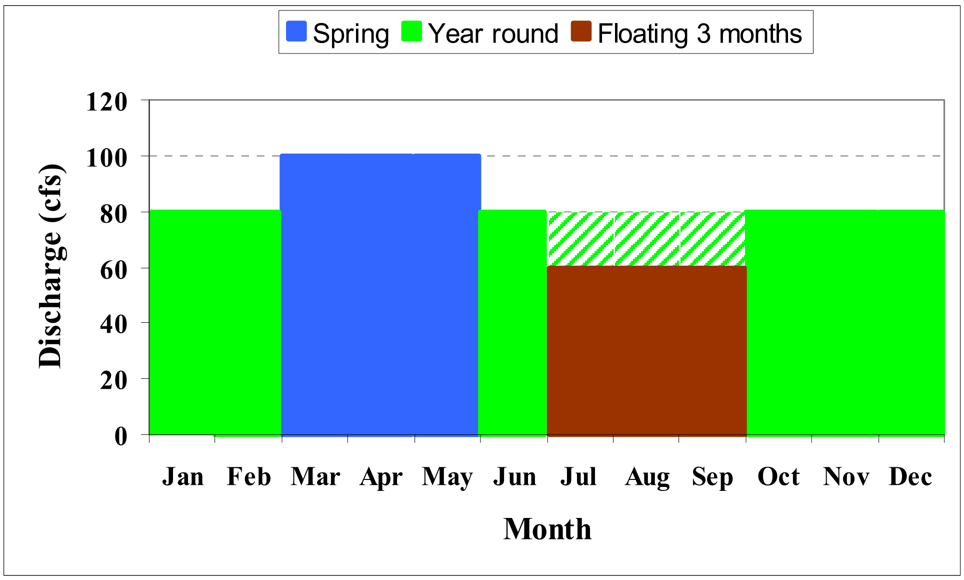


Figure 6.2 – Proposed Strawman Flow Regime for Comal Springs.

The instantaneous requirement would be 80 cfs throughout the year. Of this amount, 40 cfs would be continuously directed through the old channel. This amount is increased during the springtime (March to May) to 100 cfs to protect fountain darter reproduction. Finally, a 60 cfs instantaneous discharge for no more than three consecutive months (outside of March – May) would be allowed during a given year. There would be the requirement that of that 60 cfs, 40 cfs would be directed through the Old Channel. Following the natural flow paradigm, the flow regime pattern in **Figure 6.2** reflects greater recharge in the spring (typically a wetter period) and drought conditions during the late summer as often characteristic of central Texas climatic conditions.

Considering that the Comal system (not including the 1950s drought in which it ceased flowing) went below 60 cfs for over a 100 consecutive days (dropping all the way down to 26 cfs) in 1984 with species survival, this strawman flow regime may be overly conservative. However, until more information on the biological responses to these levels of discharge is acquired, these levels appear to be a good starting point for discussions and potential future experimentation.

6.4.2 San Marcos Springs

During the Variable Flow study period, the San Marcos system dropped to "low" discharge conditions in the Fall 2000, and as discussed in Section 5, for an extended period during 2006. During those times, it was evident that starting as early as 120 cfs, Texas wild-rice in "vulnerable" areas has the potential to be impacted. As noted, a great deal of this impact is associated with sedimentation reducing the water depth near the stand, and recreation causing increased pressure on stand survival. Nevertheless, following our outline, this would be the point at which discharge related impacts are notable.

Focusing on the low discharge spectrum, and following the same general principles as described for the Comal system, the following flow regime strawman for the San Marcos River is presented in **Figure 6.3**.

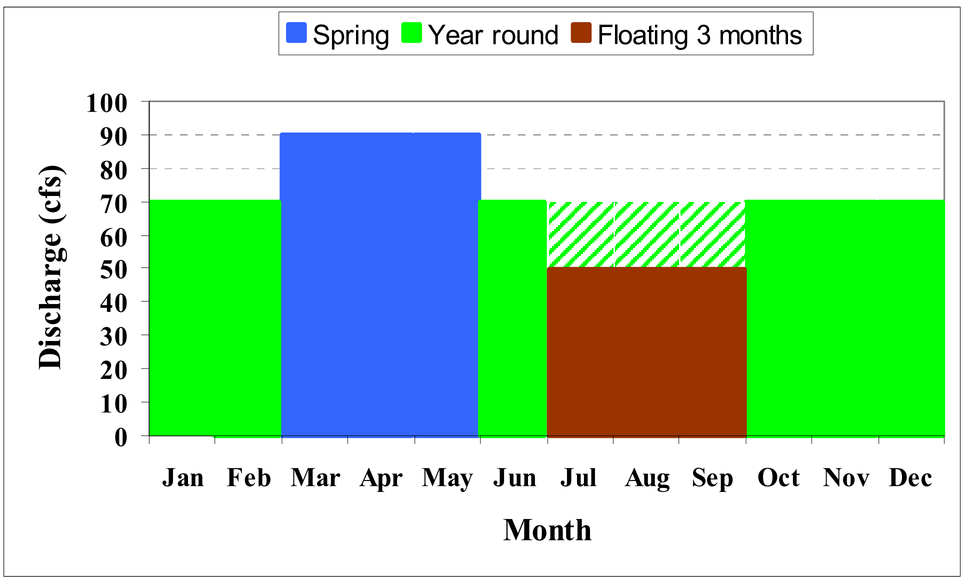


Figure 6.3 – Proposed Strawman Flow Regime for San Marcos Springs.

The instantaneous requirement would be 70 cfs throughout the year. This amount would be increased during the springtime (March to May) to 90 cfs to protect fountain darter reproduction. Finally, a 50 cfs instantaneous discharge for no more than three consecutive months (outside of March – May) would be allowed during a given year. As for the Comal system, the San Marcos river strawman in **Figure 6.3** reflects a typical flow pattern characteristic of central Texas climatic conditions.

Considering that the discharge in the San Marcos system went below 50 cfs during the drought of record, and the species survived, the proposed values again might be conservative. The San Marcos system has the added advantage of maintaining a major portion of the fountain darter and salamander population within Spring Lake, which appeared completely un-impacted during the three month stint below 100 cfs during the summer/fall of 2006. However, until more information is available regarding biological interactions to low discharge conditions in both the river and Spring Lake environment, we feel these values provide a good starting point for discussions and potential future experimentation.

7.0 REFERENCES

BIO-WEST, Inc. 2002a. Comal Springs Riffle Beetle Habitat and Population Evaluation. Final Report. Edwards Aquifer Authority. 24 p.

BIO-WEST, Inc. 2002b. Comal Springs riffle beetle laboratory evaluation study: evaluation under variable flow conditions. Final Report. Edwards Aquifer Authority. 27 p.

BIO-WEST, Inc. 2002c. Fountain darter laboratory study: reproductive response to parasites and temperature fluctuations. Final Report. Edwards Aquifer Authority. 12 p.

BIO-WEST, Inc. 2003a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2002 Annual Report. Edwards Aquifer Authority. 45 p. plus Appendices.

BIO-WEST, Inc. 2003b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2002 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.

BIO-WEST, Inc. 2004a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2003 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.

BIO-WEST, Inc. 2004b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2003 Annual Report. Edwards Aquifer Authority. 30 p. plus Appendices.

BIO-WEST, Inc. 2004c. Aquatic vegetation laboratory study: Phase 1: Observations of water quality changes and plant growth under various flows. Phase 2: Effects of carbon dioxide level on aquatic plants found in the Comal and San Marcos Springs/River Ecosystems. Final Report. Edwards Aquifer Authority. 25 p.

BIO-WEST, Inc. 2005a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal Springs/River Aquatic Ecosystem. 2004 Annual Report. Edwards Aquifer Authority. 70 p. plus Appendices.

BIO-WEST, Inc. 2005b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2004 Annual Report. Edwards Aquifer Authority. 57 p. plus Appendices.

BIO-WEST, Inc. 2006a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2005 Annual Report. Edwards Aquifer Authority. 43 p. plus Appendices.

BIO-WEST, Inc. 2006b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2005 Annual Report. Edwards Aquifer Authority. 33 p. plus Appendices.

BIO-WEST, Inc. 2007a. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the Comal River Aquatic Ecosystem. 2006 Annual Report. Edwards Aquifer Authority. 42 p. plus Appendices.

BIO-WEST, Inc. 2007b. Comprehensive and Critical Period Monitoring Program to Evaluate the Effects of Variable Flow on Biological Resources in the San Marcos River Aquatic Ecosystem. 2006 Annual Report. Edwards Aquifer Authority. 54 p. plus Appendices.

Bonner, T.H., T.M. Brandt, J.N. Fries, and B.G. Whiteside. 1998. Effects of temperature on egg production and early life stages of the fountain darter. Transactions of the American Fisheries Society. 127:971-978.

Brandt, T. M., K. G. Graves, C. S. Berkhouse, T. P. Simon, and B. G. Whiteside. 1993. Laboratory spawning and rearing of the endangered fountain darter. Progressive Fish-Culturist 55: 149-156.

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-San Marcos. 58 p.

Espey, Huston, and Associates, Inc. 1975. Investigation of Flow Requirements from Comal and San Marcos Springs to Maintain Associated Aquatic Ecosystems. Final Report. Texas Water Development Board. 141 p.

Griffin, K. L. 2006. An analysis of changes in Texas wild-rice distribution following the 1998 flood of the San Marcos River, Texas. Masters Thesis, Texas State University-San Marcos. 68 p.

McDonald, D. L. 2003. Effects of fluctuation temperature and an introduced trematode on reproduction and mortality of *Etheostoma fonticola*. Masters thesis, Texas State University-San Marcos. 35 p.

McDonald, D.L., T.H. Bonner, E.L. Oborny, and T.M. Brandt. 2007. "Effects of Fluctuating Temperatures and Gill Parasites on Reproduction of the Fountain Darter, *Etheostoma fonticola*". Journal of Freshwater Ecology, Volume 22, Number 2, pages 311-318.

Norris, C. W. 2002. Effects of variable flows on invertebrate drift in Comal Springs, Texas. Masters thesis, Southwest Texas State University. 112 p.

Saunders, K. S., K. B. Mayes, T. A. Jurgensen, J. F. Trungale, L. J. Kleinsasser, K. Aziz, J. R. Fields, and R. E. Moss. 2001. An Evalution of Spring Flows to Support the Upper San Marcos River Spring Ecosystem, Hays County, Texas. River Studies Report No. 16, Resource Protection Division, Texas Parks and Wildlife Department, Austin, Texas.

Schenck, J. R., and B. G. Whiteside. 1977. Reproduction, fecundity, sexual dimorphism and sex ratio of *Etheostoma fonticola* (Osteichthyes: Percidae). American Midland Naturalist 98:365-375.

United States Fish and Wildlife Service (USFWS). 1996. San Marcos/Comal/Edwards Aquifer rare, threatened, and endangered species contingency plan.