Analysis of the Comal Springs and San Marcos Springs Long-Term Monitoring Dataset

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Executive Summary

Groundwater-dependent ecosystems including the Comal Springs and San Marcos Springs of Central Texas are unique spring ecosystems, the persistence of which faces challenges from dependence of growing human populations on the underlying groundwater resources. Human diversion and depletion of groundwater supplies, degradation of water quality, and disturbance to habitats are potential future threats to these systems. Nearly two decades ago, the Edwards Aquifer Authority (EAA) established a springs-specific, long-term monitoring program to assess water quantity, water quality, and habitats for multiple covered species in each spring system. Data resulting from this monitoring program covering the period 2000-2015 (and on-going), compiled into a central database by the EAA, provided the data used for analysis in this report. This report provides an overview of data in the EAA long-term monitoring program, summarizes spatiotemporal patterns, and provides guidance for how existing monitoring data might be leveraged to address long-term conservation goals for listed species. It is important to note that the analysis presented herein is specific to the EAA springs-specific, long-term monitoring database. A plethora of hydrological, water quality, geological, and biological data has been (and is currently being) collected by EAA in the region over the years, which if not incorporated into this springs-specific database, was not reviewed or included.

As part of the overall Edwards Aquifer Habitat Conservation Plan (HCP) statistical analysis initiative, two additional contracts were issued to additional researchers to address other specific components of the overarching database. Upon contract award, a thorough literature review was conducted, methodologies were established in coordination with EAA's chief science officer, and initial analysis plans were presented to the HCP Science Committee in March 2017.

Water quantity in the Comal Springs and San Marcos Springs ecosystems fluctuated during the 1.5 decades of EAA (now HCP) long-term monitoring considered in this report. Flows during 2000-2007 in both systems were characterized by frequent flow reversals (characteristic of dynamic flow regimes), higher annual 90-day minimum flows, and few extreme low flow events; however, during 2008-20013, flow reversal were fewer, 90-day minimum flows were lower, and extreme low flow events were more common. As flows transitioned towards drier conditions during the latter half of monitoring, the expression of groundwater from numerous spring sources in the Comal Springs system was dynamic, and a single spring system dominated (>75% of flow) discharges during the lowest flows. Recent data (2014-2015) included in the assessment suggested hydrology was transitioning again towards wetter conditions. In fact, excessive rainfall

and recharge over the region in late 2015 through 2016 has resulted in both springs systems witnessing greater than average discharge conditions throughout the entirety of 2016-2017.Water quality in the Comal Springs and San Marcos Springs ecosystems varies through space and time. Water temperature regimes near spring sources are stable (22-23 ^{o}C) within and among years, and temperature fluxes increase with increased distance from spring sources. Physiochemical parameters measured using single-point grab samples during base flows are within the acceptable limits set by the Texas Commission on Environmental Quality (where limits apply), and longitudinal trends in temperature, dissolved oxygen, pH, and other measured variables are apparent. Recent (2014-2015) storm water sampling suggests *E. coli* levels routinely exceed established standards during high flow pulses. Additionally, a number of contaminants have been detected during recent (2013-2015) HCP focused water quality monitoring. Continuous data logged at 15-minute intervals during 2014-2015 at sites in each spring system illustrate the strong effects flow pulses have on physiochemical parameters such as turbidity, conductivity, pH, temperature, and dissolved oxygen.

The primary determinant of physical habitat structure for the Fountain Darter (*Etheostoma fonticola*) and many other species in the Comal Springs and San Marcos Springs systems is submerged aquatic vegetation (SAV). The EAA has tracked changes in the spatial coverage of 26 forms (i.e., families or species) of SAV at four sites in the Comal Springs system and three sites in the San Marcos system since 2000. Additionally, recent (2013-2015) plantings of native vegetation and removal of non-native vegetation have occurred in each system. Monitoring data suggest that as of 2015, native SAV species coverage was greater than non-native coverage at five of seven sites, and approximately equal at the remaining two. Long-term trends in coverage suggest reductions in the coverage of non-native species at all sites except for one. One HCP covered plant species, Texas wild-rice (*Zizania texana*), is included within SAV monitoring but analysis of this species was addressed by a separate contract and thus is beyond the scope of this report.

Abundance or occurrence of six HCP covered species routinely monitored by the EAA, including Fountain Darter, San Marcos salamander (*Eurycea nana*), Comal Springs salamander (*Eurycea* sp.), Comal Springs riffle beetle (*Heterelmis comalenses*), Comal Springs dryopid beetle (*Stygoparnus comalensis*) and Peck's Cave amphipod (*Stygobromous pecki*) were considered in this analysis. Fountain Darter are monitored using drop nets, dip nets, and visual observations. Among these monitoring methods, analysis with random forest models suggest SAV is an important determinant of local abundances for Fountain Darter, but no consistent relationships between SAV forms and darter abundance or occurrence were evident using this assessment approach, likely because of associations with cover in general. Partial dependence plots that allow for assessing relationships with time while accounting for all other variables suggest Fountain Darter abundances in drop nets were stable in both systems during 2000-2015, though occurrences in random dip net monitoring declined slightly in the San Marcos Springs system during 2006-2015. Fountain Darter length-frequency data collected using timed dip net sampling revealed spatiotemporal patterns in both spring systems, and visual observations in the Comal Springs system revealed a strong correlation between SAV coverage and Fountain Darter abundance. San Marcos Salamander abundances monitored using visual observations in the San

Marcos River system attenuated with longitudinal distance from spring sources and the species has shown long-term increases across sites in the San Marcos Springs system. Partial dependence plots revealed recent (2014-2015) declines in Comal Springs salamander abundance at some sites in the Comal Springs system (though more recent data outside the scope of this report illustrates numbers increasing beyond those recorded historically). Comal Springs riffle beetle abundances monitored using cotton lures were not effectively modeled with existing environmental covariates, though abundances remained stable during 2004-2012 when covariates were routinely collected. Abundance of Pecks Cave amphipods and Comal Springs dryopid beetles in drift nets placed at surface outflows appear to be an artifact of spring flow magnitude.

Components from major portions of the EAA long-term monitoring database can be combined to test hypotheses related to meeting long-term biological goals (LTBG) established as a part of the HCP. Specifically, repeated count, open population modeling approaches applied to the existing data would allow for estimating densities and acknowledging uncertainty around these estimates when compared to LTBG targets. This uncertainty can then be included in retrospective models for the entire dataset and as new data are collected and placed into the dataset. Using these hierarchical models will allow for including local-scale determinants of detection as well as system-scale regulators of organism abundances, providing more accurate estimation of population trends to guide management actions implemented throughout the Comal Springs and San Marcos Springs systems.

Table of Contents

Background 5

Recreational Use 6

Water Quantity 8

Stream Flow Measurements 8

Stream Flow Partitioning 12

Water Quality 14

Temperature 14

Grab Samples 18

Data Sondes 23

Surface and Storm Water Sampling 25

Submerged Aquatic Vegetation 42

Covered Species 58

Fountain Darter 58

Macroinvertebrate Sampling 87

Comal Springs and San Marcos Salamanders 93

Comal Springs Riffle Beetle 99

Pecks Cave Amphipod and Invertebrate Drift Sampling 104

Predation gillnet 108

Future Research and Hypothesis Development 111

Fountain Darter 111

San Marcos Salamander 113

Comal Springs Riffle Beetle 114

Peck's Cave Amphipod 115

Other Species and Associated HCP Activities 118

Literature Cited 119

Background

Groundwater-dependent ecosystems are threatened globally by human disturbance to habitats, diversion and depletion of groundwater supplies, and degradation of water quality (Eamus et al. 2016). Ecosystems that rely on expression of groundwater sources at the surface, typically referred to as "exposure spring" ecosystems ("springs" hereafter; Springer and Stevens 2009), are particularly sensitive to these alterations given their narrow distribution (i.e., exist only near groundwater outflows) and inhabitance by diverse and unique fauna (Danielopol et al. 2000). Opportunities exist to address the challenges facing aquatic biodiversity in spring ecosystems, particularly in the realm of applied research relating groundwater-dependent processes and response functions to management and conservation actions (Boulton 2005). Applied research paradigms that might be applied to enhance conservation of springs and their biota include concepts such as biodiversity research, development of bio-indicators, and establishment of critical habitat and refugia (Griebler et al. 2014). In fact, ecology-based evaluation of spring ecosystems is among the most pragmatic approaches to developing sustainable management programs for groundwater ecosystems (Marmonier et al. 2013).

The Comal Springs and San Marcos Springs ecosystems in the Edwards Plateau region of Texas represent microcosms for springs world-wide. These systems are embedded within urban centers and consequently exhibit altered habitats, both are fed by the Edwards Aquifer system for which human demand for water has led to increased pumping, and water quality within the spring outflows is degraded by human activities on the surface (Bowles and Arsuffi 1993). The fauna within these springs include a number of rare and endangered species, including Texas wild-rice (*Ziziana texana*; Poole and Bowles 1999), Peck's Cave Amphipod (*Stygobromus pecki*; Gibson et al. 2008), Comal Springs Riffle Beetle (*Heterelmis comalensis*; Bowles et al. 2003), Fountain Darter (*Etheostoma fonticola*; Schenck and Whiteside 1976), and San Marcos Salamander (*Eurycea nana*; Woods et al. 2010). Beyond their importance to biodiversity, these spring systems are also of value to humans for drinking water (Sharp and Banner 1997) and recreational activities. For example, the number of recreational users throughout the Comal Springs system (Figure 1) over the past 10+ years illustrates consistent use with highest counts expectantly occurring during warmer periods (Figure 2). Protection of the quantity and quality of water within these spring systems, the unique habitats and recreational opportunities they present, and the organisms (including humans) they support will become increasingly challenging in the future under increased human population growth, human demand for water, and climate change (Loaciga et al. 2000; Green et al. 2011). Consequently, science-based decision tools for management of these systems are critical for ensuring their protection into the future, and data routinely collected by the Edwards Aquifer Authority (EAA) during 2000-2015 (and on-going) present opportunities through the Edwards Aquifer Habitat Conservation Plan (HCP) to assess mitigation / restoration / and applied research activities to benefit the conservation and management of the Comal Springs and San Marcos Springs ecosystems.

The goal of this report is to review, summarize, and synthesize data from the EAA springs-specific, long-term monitoring database to assess the efficacy of these data in meeting conservation goals. Specifically, the report reviews trends in aspects of water quantity and quality, submerged aquatic vegetation (SAV) that dominates the habitats within each spring system, and a number of HCP covered species that inhabit each system. For each of these series of data, a brief review of long-term monitoring locations and methodologies is provided, emergent patterns and temporal trends are summarized, and the implications for long-term conservation goals are discussed. Because this approach is intended to be exploratory of existing data, rather than confirmatory of specific hypotheses, statistical analyses for covered species are based on tree-based machine learning algorithms (i.e., random forest models; Breiman 2001) that are intended to highlight pattern-process relationships that might be used to develop future research questions. Consequently, the outputs from these analyses are synthesized at the end of the report to identify future research opportunities and hypothesis development, specifically those that might leverage existing data and target compliance with established goals of the EAA and/or HCP.

Recreational Use

The EAA database contains data collected by volunteers with the Texas Master Naturalist program detailing recreational use of the Comal Springs system. These data were collected from five sites beginning in 2006, including Houston Street near the upstream spring outflows, the Gazebo near Landa Lake, the Old Channel near Elizabeth Avenue, a portion of the New Channel used for tubing, and the tubing take-out point near the U.S. Geological Survey (USGS) gage at Union Avenue (Figure 1). Observations represent fixed point counts of recreational users of the following classes: tubing, swimming, angling, and other miscellaneous uses collected by single observers weekly on Friday afternoons (Figure 2). This method provides an excellent metric for relative magnitude of recreational usage over time, but should not be regarded as a precise estimate of use numbers.

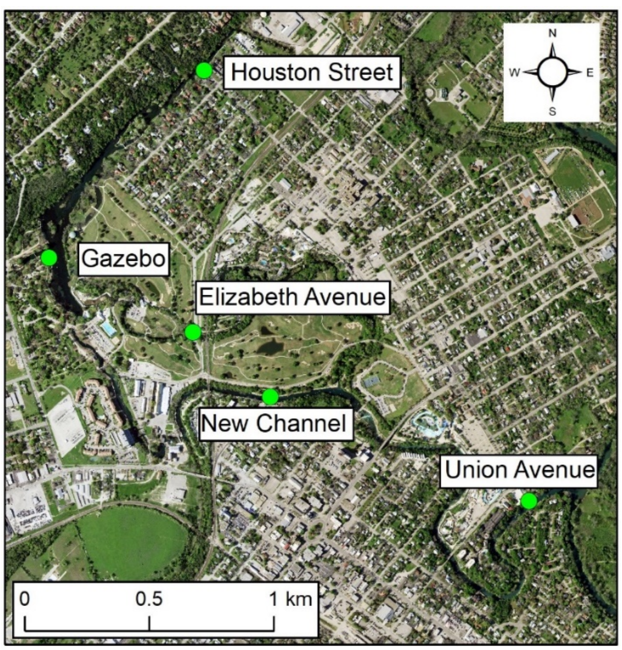


Figure 1. Locations for Texas Master Naturalist observations for number of users at five locations on the Comal Springs ecosystem.

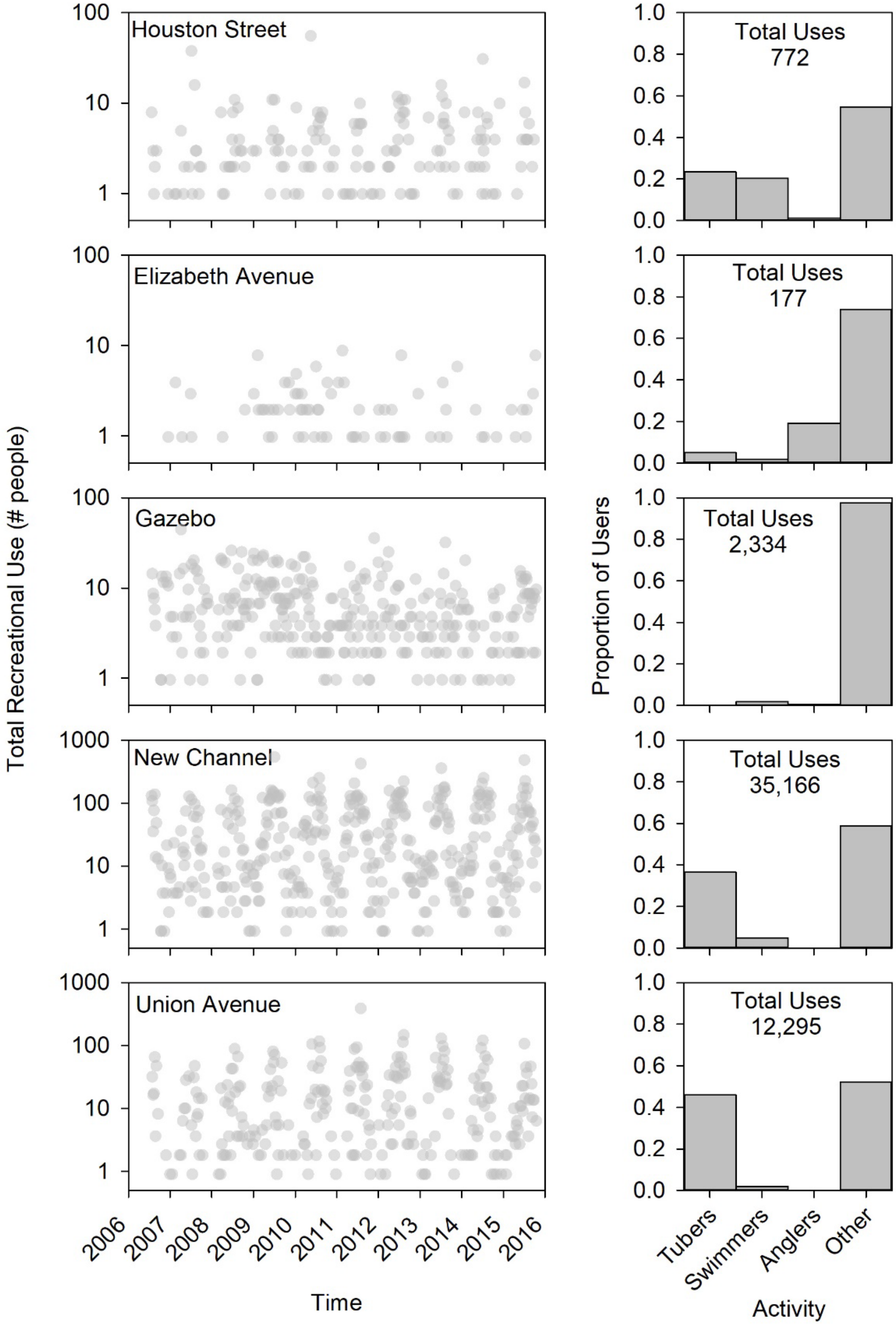


Figure 2. Time series plots for number of recreational users at five sites on the Comal Springs ecosystem (left; note log scale) and proportion of total observations for classes of recreational activities (right) based on Texas Master Naturalist observations. See Figure 1 for locations of sites.

Water Quantity

Water quantity in the Comal Springs and San Marcos Springs systems is monitored through a series of U.S. Geological Survey (USGS) stream flow gages that provide long-term records of historical flow. The spatial distribution of water sources, particularly within the Comal Springs ecosystem, has also been assessed using deployment of an Acoustic Doppler Current Profiler.

Stream Flow Measurements

Stream flow measurements are recorded by the USGS at five locations in the Comal River system and two locations in the San Marcos River system. These data span various historical periods (Table 1) and two stations in each river cover the entire period of biological data collection (2000-2015). The locations of these gages are shown below for the Comal Springs (Figure 3A) and San Marcos Springs (Figure 3B) systems. Daily stream flow data from Comal Springs at New Braunfels, Texas (USGS ID 08168710) and San Marcos Springs at San Marcos, Texas (USGS ID 08170000) were analyzed using Indicators of Hydrologic Alteration (IHA; Richter et al. 1996) to assess temporal variability in flow regime components (Table 2). Although additional flow components relate to the natural flow regime concept (Poff et al. 1997) can be calculated with the IHA software, this report focuses on parameters that have been related to the ecology of organisms and represent habitat-limiting or habitat-forming flows (e.g., Perkin et al. 2017), including low pulse frequency, high pulse frequency, number of reversals, extreme low flow frequency and extreme high flow frequency. Analyses indicate flows were variable during 2000-2015 and transitioned from generally higher minimums, few extreme low flow events, and larger numbers of reversals during early monitoring to lower minimums, more frequent extreme low flow events, and smaller numbers of reversals during more recent monitoring (Figure 4). From an ecology-based study perspective, these gradients in flow regimes within each spring system mean existing data within the EAA database might be used to establish flow-ecology relationships to inform management (e.g., Davies et al. 2014). In particular, "natural experiments" in which flows varied without manipulation present an opportunity to track ecosystem process and organism abundance responses along a gradient of flows that occurred during 2000-2015 (Konrad et al. 2011; Olden et al. 2014).

Table 1. U.S. Geological Survey streamflow gages located in the Comal Springs and San Marcos Springs systems, gage IDs, location descriptions, and the period of record for each gage.

River System	Gage ID	Location description	Period of record
Comal	08168710	Comal Springs at New Braunfels, TX	1927-Present
Comal	08168797	Dry Comal Cd at Loop 337 near New Braunfels, TX	2006-Present
Comal	08168913	Comal River (old channel) near Landa Lake, New Braunfels, TX	2012-Present
Comal	08168932	Comal River (new channel) near Landa Lake, New Braunfels, TX	2011-Present
Comal	08169000	Comal River at New Braunfels, TX	1927-
San Marcos	08170000	San Marcos Springs at San Marcos, TX	1956-Present
San Marcos	08170500	San Marcos River at San Marcos, TX	1994-Present

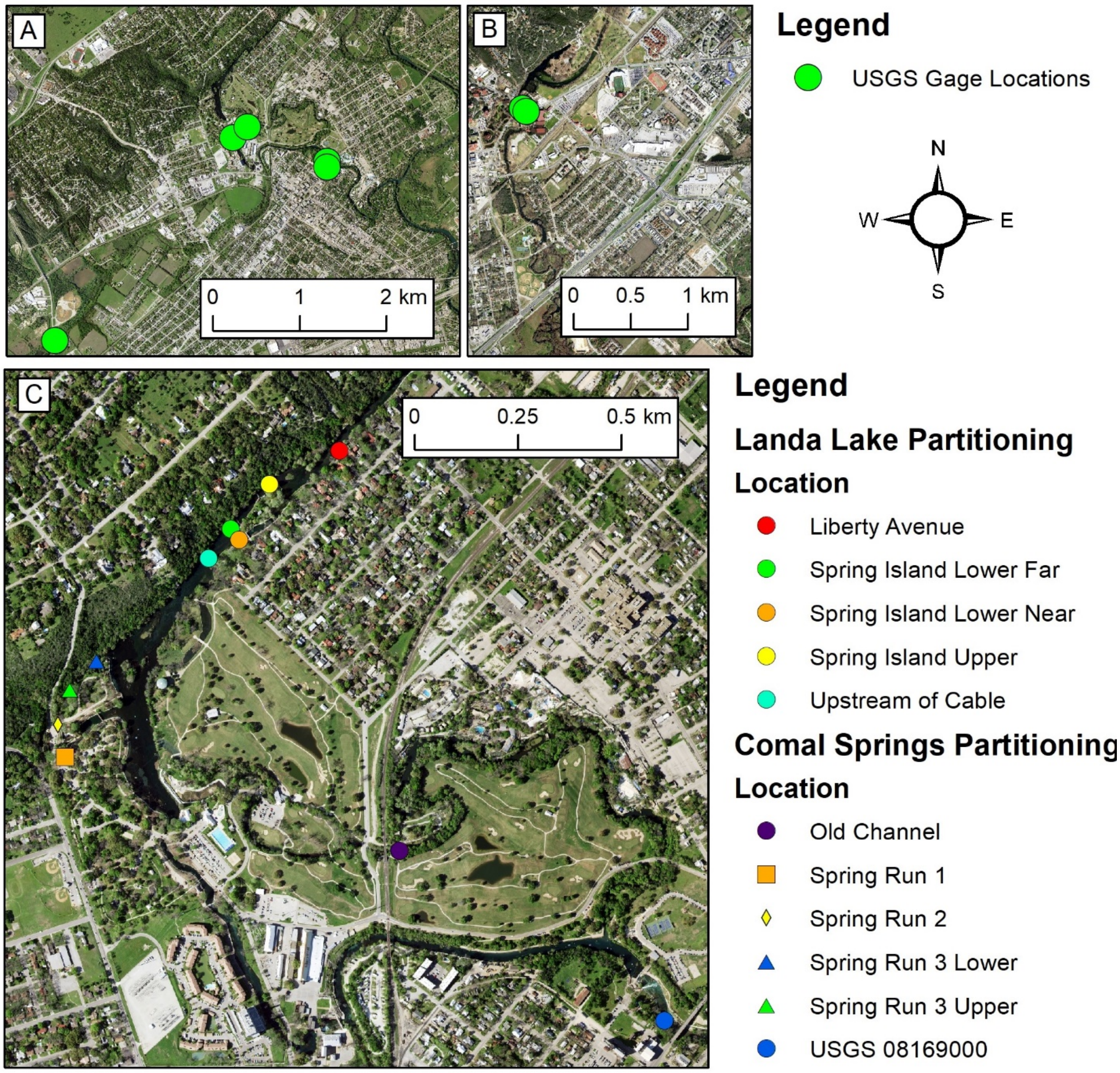


Figure 3. Maps illustrating locations of flow measurements in the Comal Springs and San Marcos Springs systems. (A) The Comal Springs system has five U.S. Geological Survey (USGS) stream flow gages that monitor discharge and (B) the San Marcos Springs system has two (see Table 1). (C) Flow partitioning within Landa Lake and its associated springs have been measured and compared to total discharge measured at USGS gage 08169000.

Table 2. Results from Indicators of Hydrologic Alteration analysis for flow components based on daily stream flows from Comal Springs (USGS ID 08168710) and San Marcos Springs (USGS ID 08170000). See Richter et al. (1996) for detailed descriptions of flow components.

System and year	90-day min.	90-day max.	Base flow	Low pulse freq.	High pulse freq.	Number of reversals	Extreme low flow frequency	Extreme high flow frequency
Comal								
2000	180.1	299.9	0.5502	3	1	114	2	0
2001	289.8	370.5	0.7236	0	4	128	0	0
2002	319.4	431.2	0.6708	0	2	130	0	0
2003	368.6	436.6	0.9061	0	0	127	0	0
2004	354.7	436.3	0.8892	0	1	116	0	0
2005	356	452.2	0.8628	0	0	131	0	0
2006	227.5	317	0.7542	3	0	125	0	0
2007	279.3	447.9	0.6983	0	1	96	0	0
2008	285	390	0.8074	0	1	104	0	0
2009	170.4	293.1	0.655	2	0	70	5	0
2010	330.9	358.1	0.8909	0	5	84	0	0
2011	170.6	309.3	0.7109	1	0	71	6	0
2012	179.3	262.5	0.7187	2	0	68	3	0
2013	129.7	214.3	0.6372	0	0	65	4	0
2014	81.32	163.9	0.5153	0	0	57	3	0
2015	175	314.8	0.5392	1	2	75	0	0
San Marcos								
2000	113.4	184.7	0.7761	2	1	107	0	0
2001	189.9	257.6	0.7343	0	4	106	0	2
2002	192.7	306	0.5862	0	1	108	0	0
2003	173.7	308.5	0.6723	0	0	132	0	0
2004	153.1	282.5	0.6984	0	2	126	0	0
2005	157.8	316.8	0.6124	0	5	129	0	5
2006	94.99	131.7	0.8206	3	0	115	3	0
2007	172.2	388.4	0.3887	0	2	85	0	0
2008	103.6	186.9	0.704	4	0	66	1	0
2009	87.87	171.7	0.7497	0	0	50	2	0
2010	183.8	252.7	0.7624	0	4	63	0	3
2011	91.9	155	0.7547	3	0	54	4	0
2012	135.4	209.6	0.6129	1	2	38	3	1
2013	106	172.2	0.7904	4	1	40	4	0
2014	106.6	150.3	0.8145	3	0	48	0	0
2015	148.4	314.6	0.4854	0	2	56	0	0

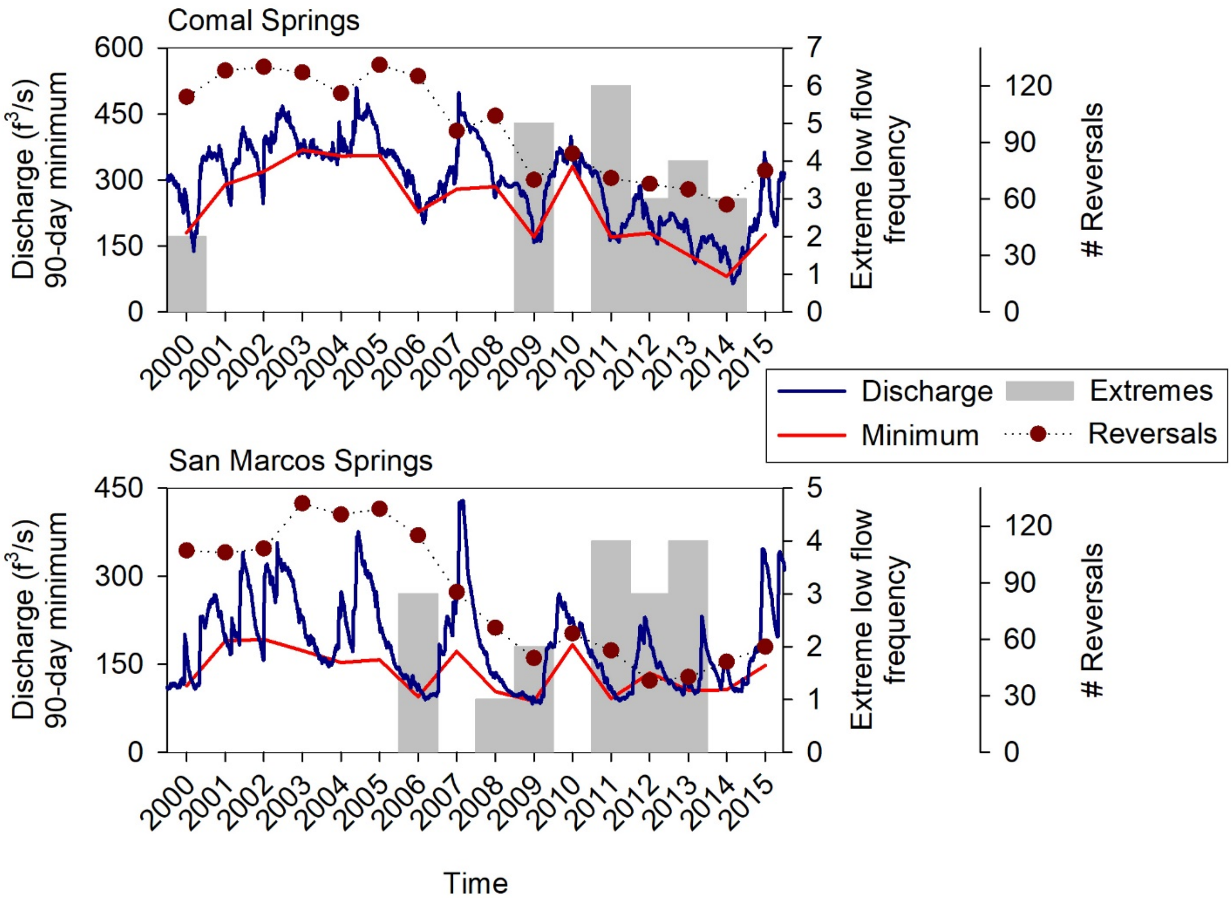


Figure 4. Hydrographs for Comal Springs (upper; USGS gage 08168710) and San Marcos Springs (lower; USGS gage 08170000) illustrating daily discharge (blue line), annual 90-day minimum flows (red line), annual frequency of extreme low flow events (gray bars), and annual number of flow reversals (red points).

Stream Flow Partitioning

In addition to USGS stream flow data, discharge partitioning was conducted at five locations within Landa Lake during 2014-2015 and five locations within spring outflows along the Comal River system during 2003-2015 (Figure 3C). Data were collected using an Acoustic Doppler Current Profiler (ADCP) to assess the relative contributions of springs to overall discharge measured at the USGS gage on the Comal River at New Braunfels, Texas (USGS ID 08169000). These data illustrate relatively consistent spatial distribution of discharge within Landa Lake across discharge levels ranging 67-256 cubic feet per second (CFS) and measured on 10 occasions (Figure 5). Contributions by springs on the west side of Landa Lake and the old river channel were measured during 2003-2015 across a wider range of total discharges (66-446 CFS) and were more dynamic through space and time (Figure 6). The Old Channel was the largest contributor to discharge through time, and the relative contribution by the Old Channel flows to overall discharge decreased as overall discharge magnitude increased as would be expected due to controlled management of the old channel outflow from Landa Lake. Furthermore, the relationship between total discharge and percent contribution was approximately linear at Upper Spring Run and Spring Run 1, but became increasingly non-linear at downstream locations. These data can be paired with monitoring data for covered species to assess fine-scale changes in organism abundance through time (see Future Research and Hypothesis Development section).

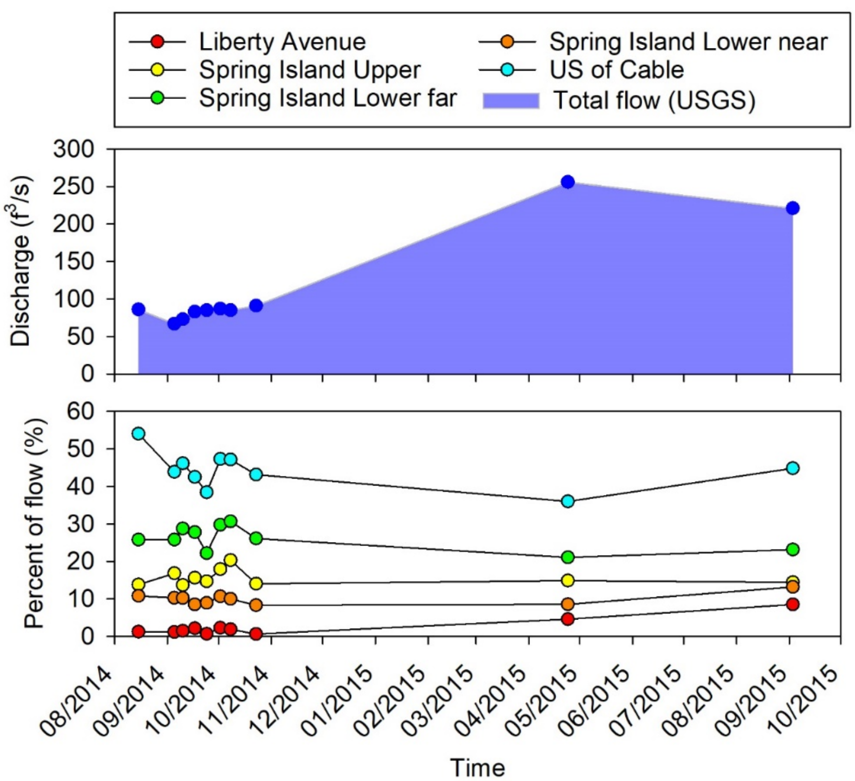


Figure 5. Landa Lake flow partitioning results showing change in stream discharge through time (upper) for the USGS gage at New Braunfels, Texas (USGS ID 8169000) and the percent of flow originating from five locations in Landa Lake (lower). See Figure 3C for locations of sites.

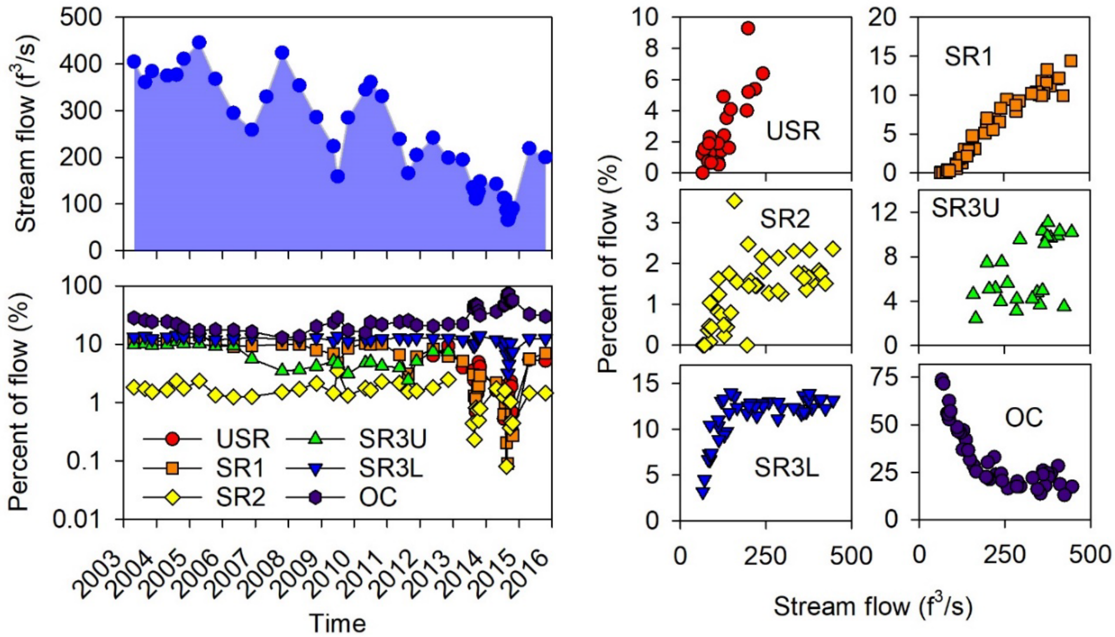


Figure 6. Comal Springs flow partitioning results showing change in stream discharge through time (upper left) for the USGS gage at New Braunfels, Texas (USGS ID 08169000) and the percent of flow originating from six spring sources (lower left; note the log-scaled y-axis), including Upper Spring Run (USR), Spring Run 1 (SR1), Spring Run 2 (SR2), Spring Run 3 Upper Site (SR3U), Spring Run 3 Lower Site (SR3L), and the Old Channel (OC). The six panels to the right illustrate the relationship between total stream discharge and contribution by individual sources. The relationship shown for this relationship in the Old Channel is unique as it is and has historically been a managed outflow, though some changes have been made to the structure and management over time. See Figure 3C for locations of sites.

Water Quality

Water quality has been monitored in the Comal Springs and San Marcos Springs systems using spatially distributed water temperature thermistors, single time point grab samples, continuous monitoring data sondes, and single point surface and storm water samples analyzed for physiochemical and contaminant parameters.

Temperature

Water temperature has been monitored since 2000 at 12 locations in the Comal Springs system (Figure 7A) and 11 locations in the San Marcos Springs system (Figure 7B) using thermistors. Temperature (°C) is logged every 10 minutes, then this data is compiled as 4 hour averages and data cover 3,567-5,593 days depending on location of deployment (Table 3). The spatial distribution of thermistors illustrated longitudinal variability in the Comal Springs system, including thermally dynamic Blieders Creek upstream through thermally stable spring outflows, to the thermally dynamic downstream area (Figure 8). In the San Marcos Springs system, increasingly dynamic thermal regimes with greater distance from the spring source in Spring Lake was evident (Figure 9). These data also illustrate spatial consistencies across years with exceptionally cool water temperatures in Comal Springs (2005) and San Marcos Springs (2007). These data can be matched with spatially distributed sampling of covered organisms to assess fine-scale relationships between water temperature and ecological responses (see Future Research and Hypothesis Development section), though it is important to note the range of temperature values observed even at variable sites is relatively small and is not likely of sufficient magnitude to elicit a biological response.

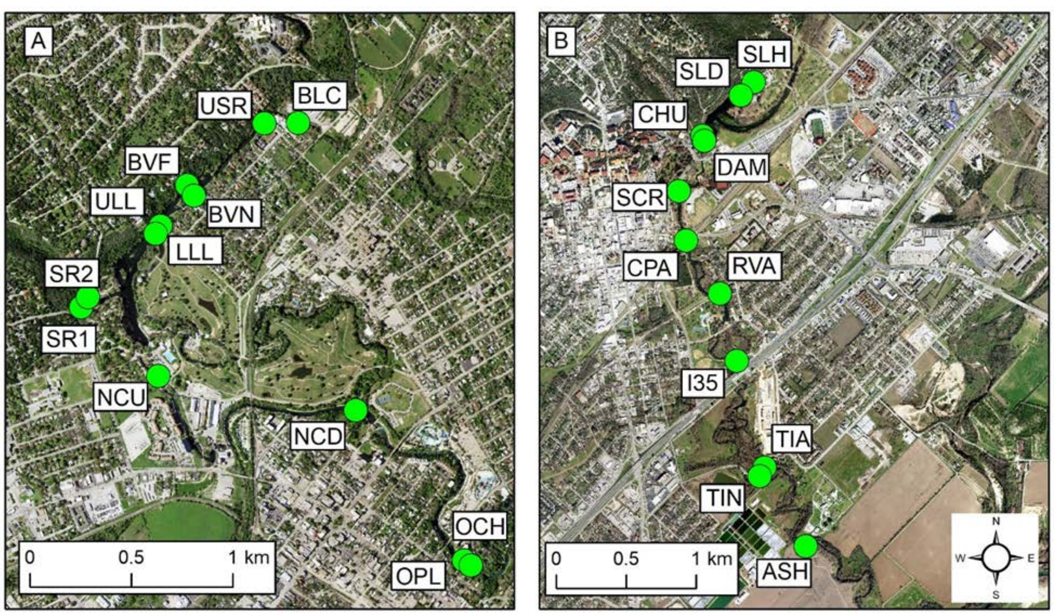


Figure 7. Location of water temperature thermistors in (A) Comal and (B) San Marcos river systems. See Table 5 for site descriptions.

Table 3. Sampling locations for thermistors in Comal Springs and San Marcos Springs systems with site abbreviations, northing and easting coordinates (NAD83 UTM Zone 14N), and number of daily observation days per location.

System and location		Northing	Easting	Observations
Comal				
	Blieders Creek (BLC)	3288153.3	584482.3	5,532
	Heidelberg/Upper Spring Run (USR)	3288148.9	584315.7	5,532
	BV Far (BVF)	3287847.2	583935.6	5,532
	BV Near (BVN)	3287793.6	583970.4	5,532
	Upper Landa Lake (ULL)	3287647	583805.5	5,593
	Lower Landa Lake (LLL)	3287607.1	583781	5,593
	Spring Run 1 (SR1)	3287246.4	583412.9	5,532
	Spring Run 2 (SR2)	3287295	583448.6	5,532
	New Channel Upstream (NCU)	3286908.5	583793	5,532
	New Channel Downstream (NCD)	3286739.1	584766	5,532
	Old Channel (OCH)	3285976.9	585332.3	5,532
	Other Place (OPL)	3286001.3	585298.8	5,532
San Marcos				
	Spring Lake Hotel (SLH)	3307517.1	603294.1	3,567
	Spring Lake Deep (SLD)	3307421.4	603200.6	3,889
	Chute (CHU)	3307133.2	602925.6	5,461
	Dam (DAM)	3307095.8	602941.5	5,316
	City Park (CPA)	3306738.5	602757.8	5,461
	Sessoms Creek (SCR)	3306384.1	602810.8	5,461
	Rio Vista (RVA)	3306005.5	603050.1	5,316
	I-35 (I35)	3305524.5	603172.1	5,316
	Thompsons Island Artificial (TIA)	3304762.3	603370.2	5,461
	Thompsons Island Natural (TIN)	3304701.9	603335.9	5,461
	Animal Shelter (ASH)	3304199.5	603664.1	5,461

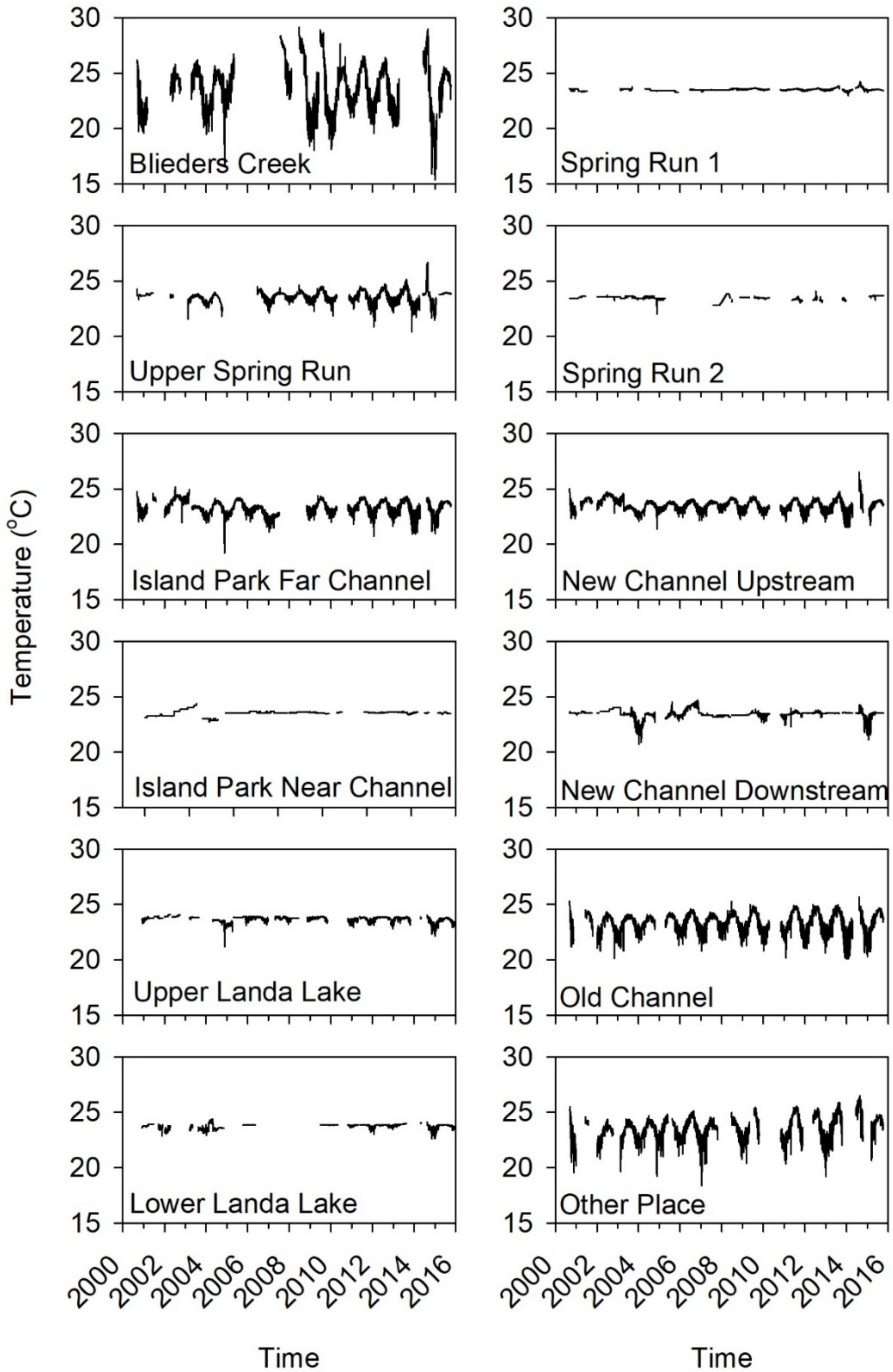


Figure 8. Daily thermal regimes measured at 12 locations in the Comal Springs system.

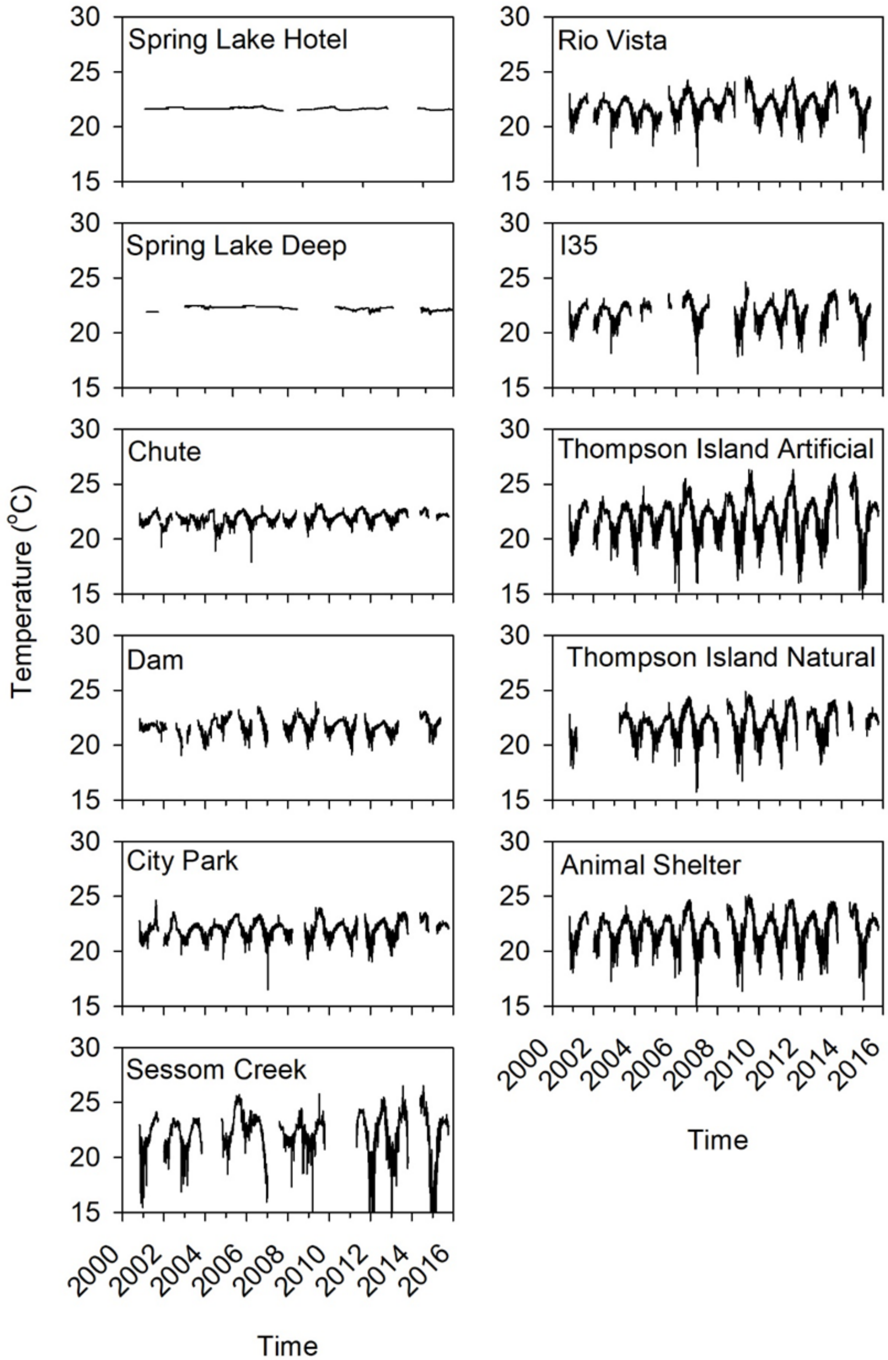


Figure 9. Daily thermal regimes measured at 11 locations in the San Marcos Springs system.

Grab Samples

Water quality grab samples specific to the biological monitoring program were collected at 15 locations in Comal Springs (Figure 10A) and 18 locations in San Marcos Springs (Figure 5B). Sites were sampled up to 16 times in Comal Springs and 14 times in San Marcos Springs. Comal Springs collections were made on 16 dates, including during 2000 (August 28, September 11, November 13), 2001 (March 21, May 24, August 27, November 8), 2002 (March 7, May 30, August 13), 2009 (July 2), 2011 (9-21), 2013 (8-12), 2014 (8-12, 10-31), and 2015 (11-18). San Marcos collections were made on 23 dates, including during 2000 (October 31), 2001 (March 5, March 6, March 15, April 2, May 7, May 14, August 13, August 15), 2002 (February 13, February 14, May 8, May 22, August 5, August 7), 2006 (July 25, September 14), 2009 (January 9, April 10), and 2015 (June 18, November 17). Eleven water quality variables were analyzed from grab samples (Table 5). Although the temporal extent of sampling is limited, the spatial distribution of sites illustrated longitudinal variability (upstream to downstream) in Comal Springs (Figure 11) and San Marcos Springs (Figure 12). These patterns suggest water quality during routine sampling is largely consistent across sites in Comal Springs. In San Marcos Springs, sites located in the slough arm of Spring Lake (i.e., SMS, SMH, SMG) exhibited much more variability relative to other sites, and there was some evidence of longitudinal change in parameters such as dissolved oxygen, nitrate, pH, and turbidity. This site is located in a golf course and is not directly influenced by direct spring inputs, and thus variation here is not mediated by spring influences.

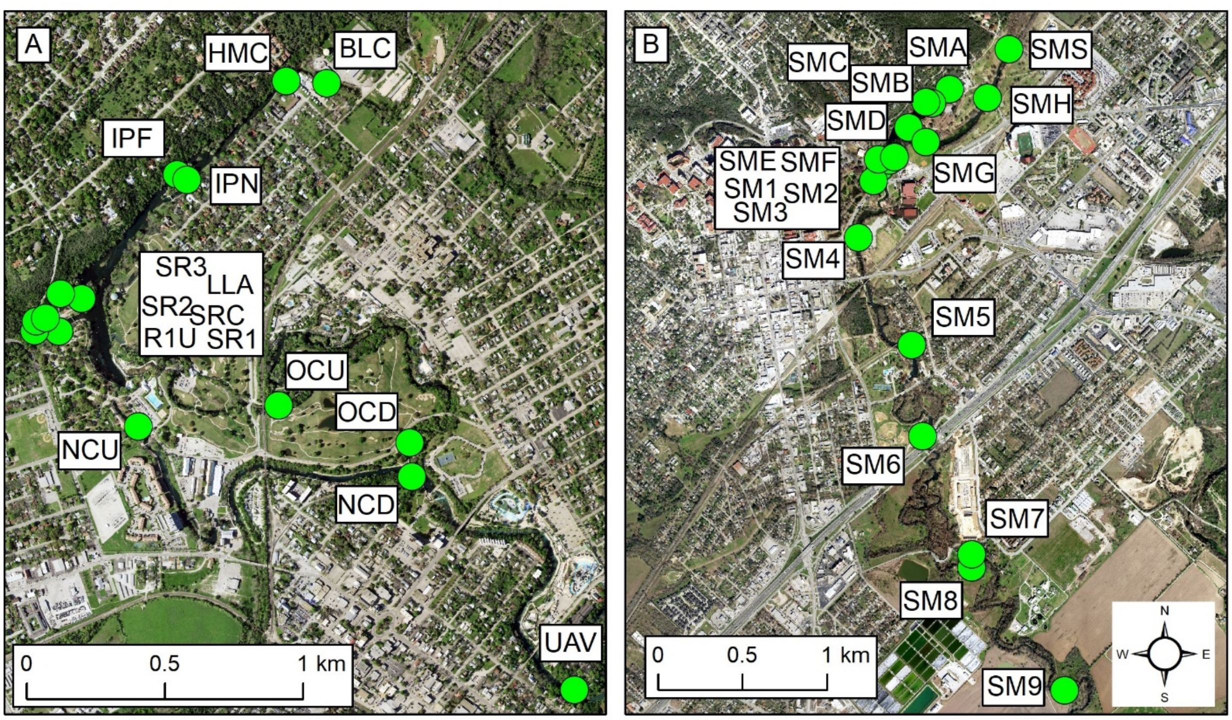


Figure 10. Spatial locations of water quality grab samples in (A) Comal Springs (B) San Marcos Springs. See Table 4 for site descriptions and abbreviations.

Table 4. Sampling locations for water quality grab samples in the Comal and San Marcos river systems with site abbreviations, northing and easting coordinates (NAD83 UTM Zone 14N), and number of observations per site.

System and site	Northing	Easting	Observations
Comal			
Blieders Creek (BLC)	3288154	584473	16
Heidelberg Main Channel (HMC)	3288161	584326	15
Island Park Far Channel (IPF)	3287824	583932	16
Island Park Near Channel (IPN)	3287803	583966	16
Landa Lake (LLA)	3287374	583586	2
Spring Run 1 ("Downstream") (SR1)	3287257	583415	16
Spring Run 1 Upstream (R1U)	3287291	583422	2
Confluence of Spring Runs 1 and 2 (SRC)	3287255	583503	2
Spring Run 2 (SR2)	3287303	583455	16
Spring Run 3 (SR3)	3287392	583510	16
Old Channel Upstream (OCU)	3286988	584299	16
Old Channel Downstream (OCD)	3286853	584774	16
New Channel Upstream (NCU)	3286911	583790	16
New Channel Downstream (NCD)	3286730	584782	16
Union Avenue (and "Other Place") (UAV)	3285957	585369	16
San Marcos			
Sink Creek (SMS)	3303953	603983	12
Downstream of Road (SMH)	3304683	603434.1	14
Boardwalk (SMG)	3304760	603434.1	14
Hotel (SMA)	3307048	602889.8	14
Submarine (SMB)	3307092	602947.9	14
Downstream of Boat dock (SMC)	3306969	602852.9	14
Above Chute (SMD)	3306633	602763.1	14
Upstream of Dam (SME)	3305998	603077.2	14
Landing Dock (SMF)	3305458	603143.5	14
Below Chute (SM1)	3307514	603302.6	14
Below Dam (SM2)	3307430	603196.9	14
Sessom Creek (SM3)	3307437	603163	14
City Park (SM4)	3307098	602878.8	14
Rio Vista Park (SM5)	3307110	602974.6	14
I35 Crossing (SM6)	3307285	603059.6	14
Thompson Island Artificial (SM7)	3307201	603162.9	14
Thompson Island Natural (SM8)	3307461	603527.1	14
Animal Shelter (SM9)	3307750	603653.7	14

Table 5. Water quality parameters measured from grab samples in Comal and San Marcos river systems illustrating median, range (minimum-maximum) and total number of observations per parameter for each river system.

Parameter	Comal			San Marcos		
(units)	Median	Range	Observations	Median	Range	Observations
Alkalinity (mg/L)	220	180-280	195	240	160-380	248
Ammonia (mg/L)	0.049	0.009-0.37	190	0.046	0.001-0.227	231
Conductivity (us/cm)	536	481-672	197	566	446-773	248
Dissolved Oxygen (mg/L)	6.48	3.19-15.10	197	7.75	0.83-18.72	248
Nitrate (mg/L)	1.85	1.00-5.97	196	1.25	0.05-2.62	244
pH	7.36	6.65-8.02	195	7.4	6.80-8.25	248
Soluble Reactive Phosphorus (mg/L)	0.08	0.06-0.13	4	0.08	-	1
Temperature (^{o}C)	23.58	20.39-28.10	197	22.3	13.94-28.73	248
Total Nitrogen (mg/L)	2.18	0.62-3.370	196	1.55	0.032-4.25	248
Total Phosphorus (mg/L)	0.03	0.01-19.73	43	0.03	0.01-0.74	80
Total Suspended Solids (mg/L)	0.021	<0.01-7.10	172	0.013	<0.01-18.00	225

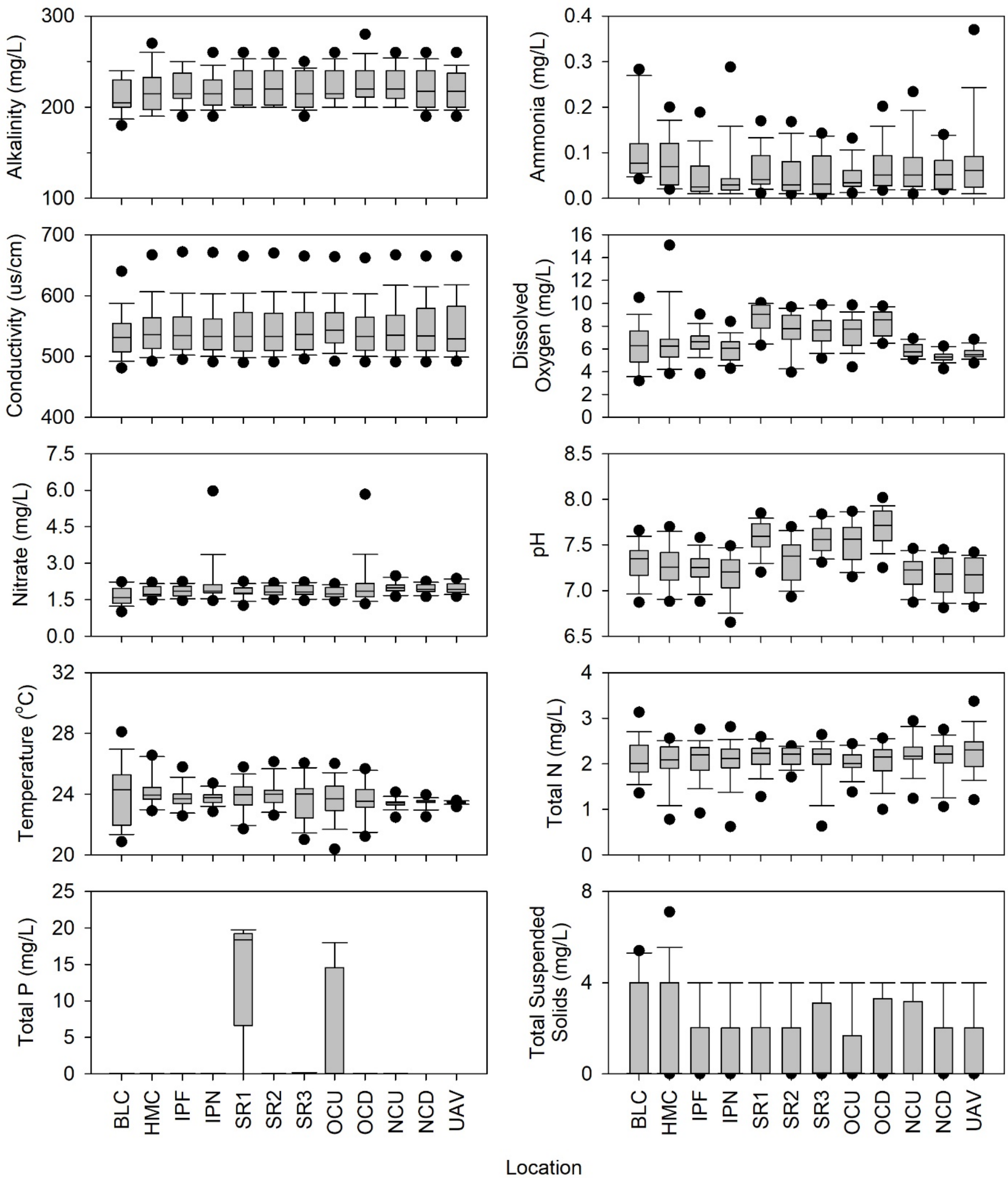


Figure 11. Box plots illustrating spatial distribution in water quality grab sample parameters in the Comal River system. See Table 3 for site abbreviation descriptions.

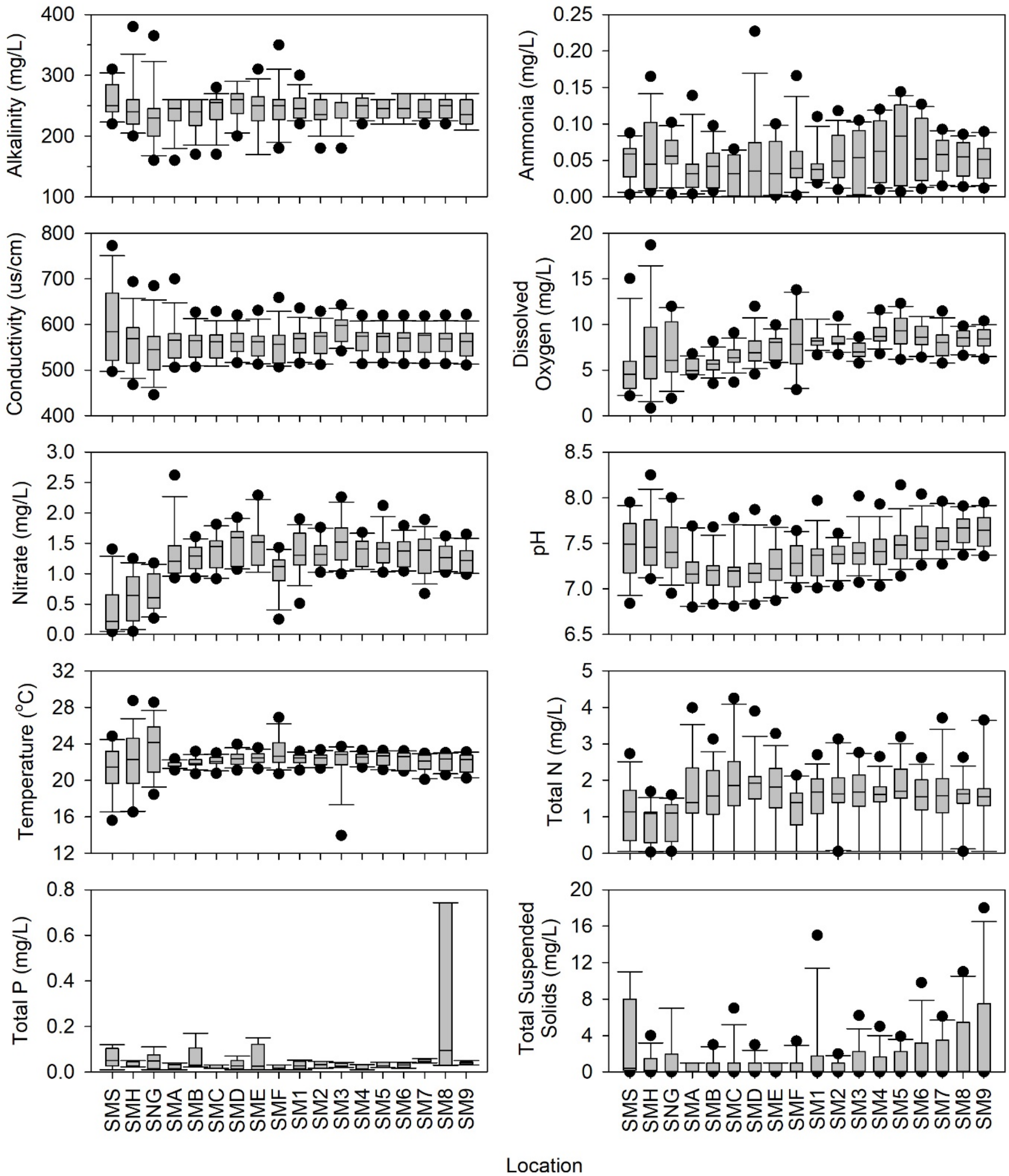


Figure 12. Box plots illustrating spatial distribution in water quality grab sample parameters in the San Marcos River system. See Table 3 for site abbreviation descriptions.

Data Sondes

Water quality standard parameters were monitored using real time data sondes installed specifically for the HCP at 15-minute intervals at sites in the Comal River (New Channel) and the San Marcos River (Rio Vista Park) beginning in 2013. These real time data provide fine-scale resolution of changes in turbidity, conductivity, water temperature, dissolved oxygen, and pH (Figure 13). Inspection of the magnitude of changes and their duration further illustrate the relative stability of water quality parameters in these systems, especially as relevant to potential biological effects. For instance, DO, one of the more critical parameters with respect to persistence of biological organisms, has not been observed to fall to values approaching those which might be expected to affect covered species.

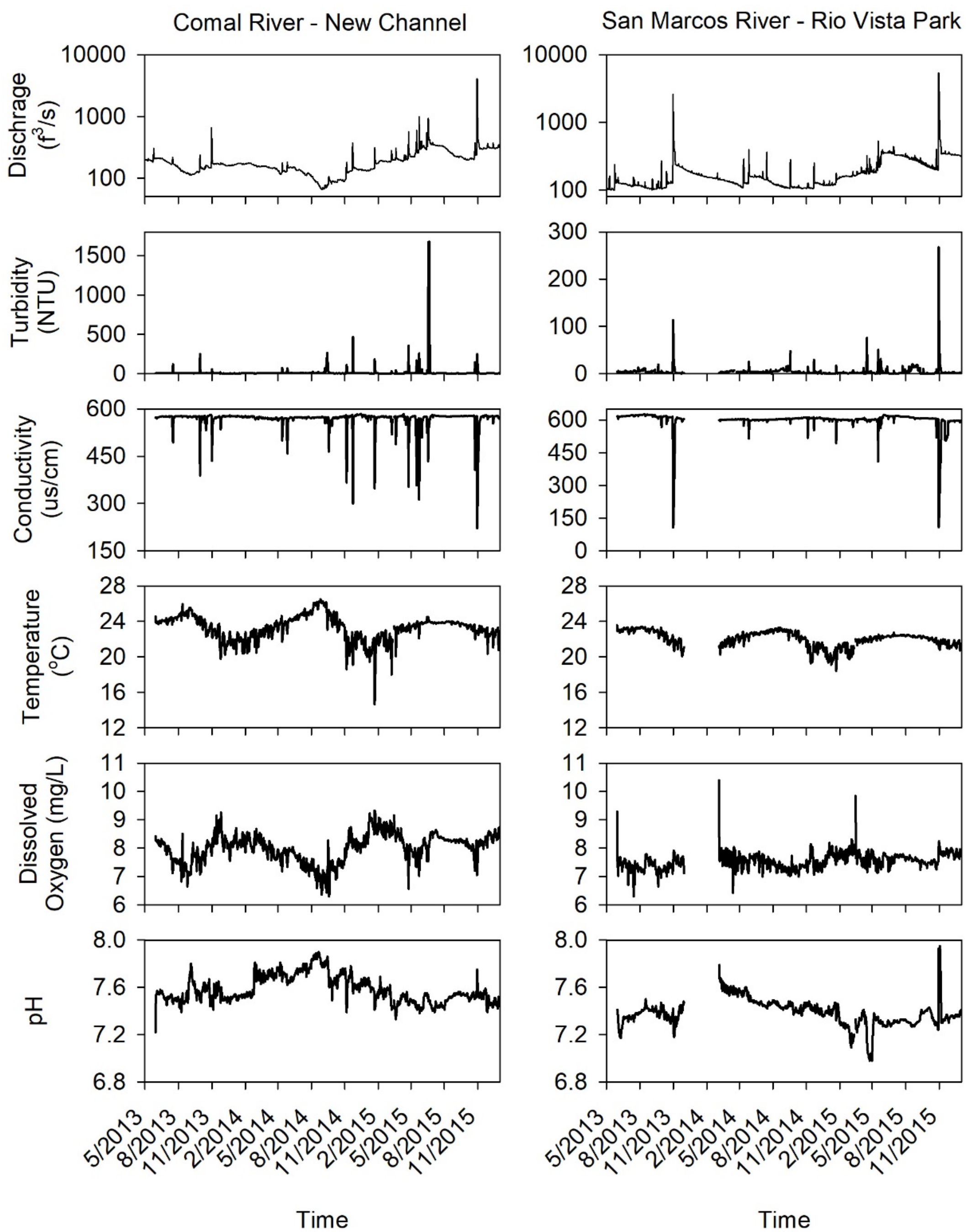


Figure 13. Daily flow data from USGS gages and water quality data from sondes deployed in the Comal (New Channel) and San Marcos (Rio Vista Park) river systems. Flow data for the Comal River are from USGS gage 08168932 and the San Marcos River are from USGS gage 08170500.

Surface Water, Storm Water and Sediment Sampling

Specific to the HCP, surface and storm water monitoring was conducted independent of the biological monitoring program during 2013-2015 at multiple sites in Comal Springs (Figure 14A) and San Marcos Springs (Figure 14B). Standard water quality parameters analyzed from these samples include turbidity (nephlometric turbidity units; NTU), alkalinity (mg/L), Temperature (^{o}C), dissolved oxygen (mg/L), conductivity (uS/cm), pH, and *E. coli* (#/100 ml), and samples were tested for a number of environmental contaminants (Tables 7 and 8). Median and range values for these parameters (except *E. coli*) during surface (non-storm) and storm event sampling are given in Table 6. These data indicate storm events tend to temporarily increase turbidity, reduce alkalinity, slightly lower temperature, slightly lower dissolved oxygen, reduce conductivity, slightly lower pH. Despite the slight reduction in dissolved oxygen during storm events, mean values remain consistently above the Texas Commission on Environmental Quality (TCEQ) standard of 5.0 mg/L (Texas Administrative Code [TAC] 2017) for the Upper San Marcos, Lower San Marcos, and Comal Segments. Storm events consistently increased *E. coli* and resulted in values greater than the TCEQ standard of 126 per 100 ml (TAC 2017) in Comal Springs (Figure 15) and San Marcos Springs (Figure 16).

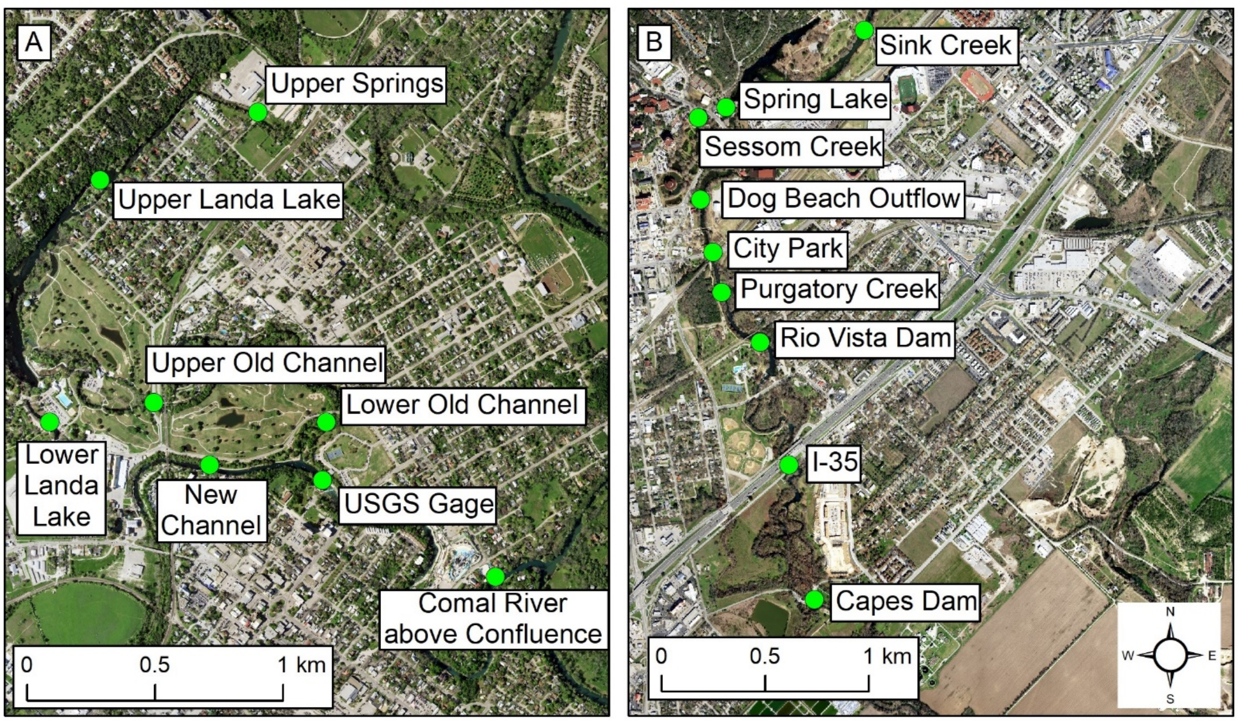


Figure 14. Locations of surface and storm water monitoring sites for (A) Comal Springs and (B) San Marcos Springs.

Table 6. Water quality parameter median, range, and sample size (N) recorded during routine (Surface) and storm-event (Storm) sampling. See Table 6 for descriptions of sampling locations.

			Turbidity (NTU)			Alkalinity (mg/L)		
System	Location	Sample	Median	Range	N	Median	Range	N
Comal	Upper Springs	Surface	3.9	(0.0-13.2)	5	201	(189-235)	6
Comal	Upper Landa Lake	Surface	0	(-4.8-1.5)	4	235	(221-255)	6
Comal	Lower Landa Lake	Surface	0	(-3.70-1.20)	5	231.5	(186-238)	6
Comal	Lower Landa Lake	Surface	0	(-2.60-2.40)	5	232	(210-244)	6
Comal	USGS Gage	Surface	0	(-3.50-1.20)	5	229	(210-235)	5
Comal	Upper Springs	Storm	31.1	(0.00-302.0)	16	93.5	(40.6-188)	16
Comal	Upper Old Channel	Storm	23.65	(0-70.6)	16	226	(154-237)	16
Comal	Lower Old Channel	Storm	20.65	(0-102)	16	212	(174-237)	17
Comal	New Channel	Storm	9.3	(3.9-610)	15	216	(110-231)	21
Comal	Comal River	Storm	13.9	(0-689)	16	206	(122-229)	20
San Marcos	Sink Creek	Surface	1.1	(-1.90-1.60)	7	245.5	(239-271)	8
San Marcos	Spring Lake	Surface	0	(-4.40-0.0)	5	249	(243-267)	5
San Marcos	Sessom Creek	Surface	0	(0.00-3.90)	5	264	(248-273)	5
San Marcos	City Park	Surface	0	(-3.70-2.30)	5	260.4	(251-268)	5
San Marcos	Rio Vista Dam	Surface	0	(-3.80-050)	6	259.33	(247-265)	6
San Marcos	I-35 Reach	Surface	0	(-3.30-0.60)	5	260.5	(245-264)	6
San Marcos	Capes Dam	Surface	0.3	(0.00-1.70)	5	258	(230-265)	5
San Marcos	Sink Creek	Storm	8.25	(0-38.1)	14	228	(94-259)	19
San Marcos	Sessom Creek	Storm	64.6	(4.4-402)	13	180	(69-248)	19
San Marcos	Dog Beach Outflow	Storm	15.4	(0-245)	15	230	(125-255)	15
San Marcos	City Park	Storm	9.6	(-2.4-368)	14	221	(140-251)	12
San Marcos	Purgatory Creek	Storm	19	(-2-321)	15	234	(78-249)	15
San Marcos	I-35 Reach	Storm	16.9	(0.6-424)	13	217	(101-253)	15
San Marcos	Capes Dam	Storm	38.3	(6.2-391)	15	202	(91-251)	15

Table 6 continued.

			Temperature (^oC)			Dissolved Oxygen (mg/L)		
System	Location	Sample	Median	Range	N	Median	Range	N
Comal	Upper Springs	Surface	20.87	(15.47-25.74)	5	10	(5.28-102.8)	5
Comal	Upper Landa Lake	Surface	23.71	(23.31-24.48)	4	7.99	(5.55-107.7)	4
Comal	Lower Landa Lake	Surface	22.98	(22.80-23.67)	5	8.1	(5.50-52.52)	5
Comal	Lower Landa Lake	Surface	23.56	(22.10-24.63)	5	9.3	(6.75-111.80)	5
Comal	USGS Gage	Surface	22.9	(22.14-24.17)	5	8.63	(6.32-122.10)	5
Comal	Upper Springs	Storm	20.55	(7.98-24.46)	16	6.75	(4.61-8.67)	16
Comal	Upper Old Channel	Storm	22.57	(19.69-24.45)	16	6.76	(4.69-9.7)	16
Comal	Lower Old Channel	Storm	22.34	(18.53-23.25)	16	6.72	(4.07-8.4)	16
Comal	New Channel	Storm	22.32	(12.81-24.15)	16	6.08	(3.83-9.16)	16
Comal	Comal River	Storm	22	(13.83-23.33)	16	6.48	(4.49-8.8)	16
San Marcos	Sink Creek	Surface	20.14	(18.53-22.54)	7	6.12	(3.17-44.70)	7
San Marcos	Spring Lake	Surface	21.84	(20.72-22.54)	5	9.32	(8.21-10.83)	4
San Marcos	Sessom Creek	Surface	21.83	(20.91-22.94)	5	6.5	(5.54-9.43)	4
San Marcos	City Park	Surface	22.04	(21.10-22.68)	5	8.58	(7.77-11.49)	4
San Marcos	Rio Vista Dam	Surface	21.89	(21.35-22.57)	6	8.09	(6.57-11.89)	5
San Marcos	I-35 Reach	Surface	22.31	(21.03-22.67)	5	9.25	(7.18-112.10)	5
San Marcos	Capes Dam	Surface	22.18	(21.26-22.43)	5	8.73	(6.94-100.60)	5
San Marcos	Sink Creek	Storm	22.7	(19.72-24.9)	14	4.57	(3.42-8.43)	14
San Marcos	Sessom Creek	Storm	21.94	(18.85-25.78)	13	6.35	(5.08-8.74)	13
San Marcos	Dog Beach Outflow	Storm	21.26	(20.54-24.18)	15	7.42	(4.5-8.74)	14
San Marcos	City Park	Storm	22.16	(20.75-27.18)	14	6.71	(4.76-9.26)	14
San Marcos	Purgatory Creek	Storm	21.88	(20.42-32.79)	15	6.9	(4.5-11.25)	15
San Marcos	I-35 Reach	Storm	21.46	(20.07-24.4)	13	6.85	(4.12-8)	13
San Marcos	Capes Dam	Storm	21.66	(19.81-24.46)	15	7.4	(4.13-8.13)	15

Table 6 continued.

			Conductivity (uS/cm)			pH		
System	Location	Sample	Median	Range	N	Median	Range	N
Comal	Upper Springs	Surface	483	(453-627)	5	7.42	(6.95-7.76)	5
Comal	Upper Landa Lake	Surface	568.5	(515-636)	4	7.16	(6.80-7.31)	4
Comal	Lower Landa Lake	Surface	577	(536-645)	5	7.26	(6.32-7.37)	5
Comal	Lower Landa Lake	Surface	569	(552-638)	5	7.58	(7.13-7.80)	5
Comal	USGS Gage	Surface	578	(552-645)	5	7.73	(7.32-7.86)	5
Comal	Upper Springs	Storm	270.5	(103-604)	16	7.26	(6.15-7.72)	16
Comal	Upper Old Channel	Storm	549	(202-750)	16	7.04	(6.66-7.31)	16
Comal	Lower Old Channel	Storm	520.5	(6-659)	16	7.24	(7-7.52)	16
Comal	New Channel	Storm	578	(289-739)	16	7.28	(6.72-7.49)	16
Comal	Comal River	Storm	541	(320-787)	16	7.41	(7-7.69)	16
San Marcos	Sink Creek	Surface	595	(214-722)	7	7.15	(6.71-7.89)	7
San Marcos	Spring Lake	Surface	611	(215-666)	5	7.22	(6.87-7.35)	5
San Marcos	Sessom Creek	Surface	632	(227-666)	5	7.21	(6.97-7.42)	5
San Marcos	City Park	Surface	599	(209-674)	5	7.47	(7.04-7.61)	5
San Marcos	Rio Vista Dam	Surface	613	(212-674)	6	7.47	(7.00-7.69)	6
San Marcos	I-35 Reach	Surface	607	(212-671)	5	7.72	(7.22-7.77)	5
San Marcos	Capes Dam	Surface	607	(214-673)	5	7.8	(7.46-7.84)	5
San Marcos	Sink Creek	Storm	601	(0.6-678)	14	7.21	(6.2-7.57)	14
San Marcos	Sessom Creek	Storm	366	(135-538)	13	7.2	(6.6-7.71)	13
San Marcos	Dog Beach Outflow	Storm	567	(237-654)	15	7.01	(6.47-7.76)	15
San Marcos	City Park	Storm	546	(258-675)	14	7.32	(6.86-8.06)	14
San Marcos	Purgatory Creek	Storm	549	(178-617)	15	7.33	(6.39-7.9)	15
San Marcos	I-35 Reach	Storm	542	(222-609)	13	7.33	(6.64-7.85)	13
San Marcos	Capes Dam	Storm	515	(211-603)	15	7.56	(6.69-7.97)	15

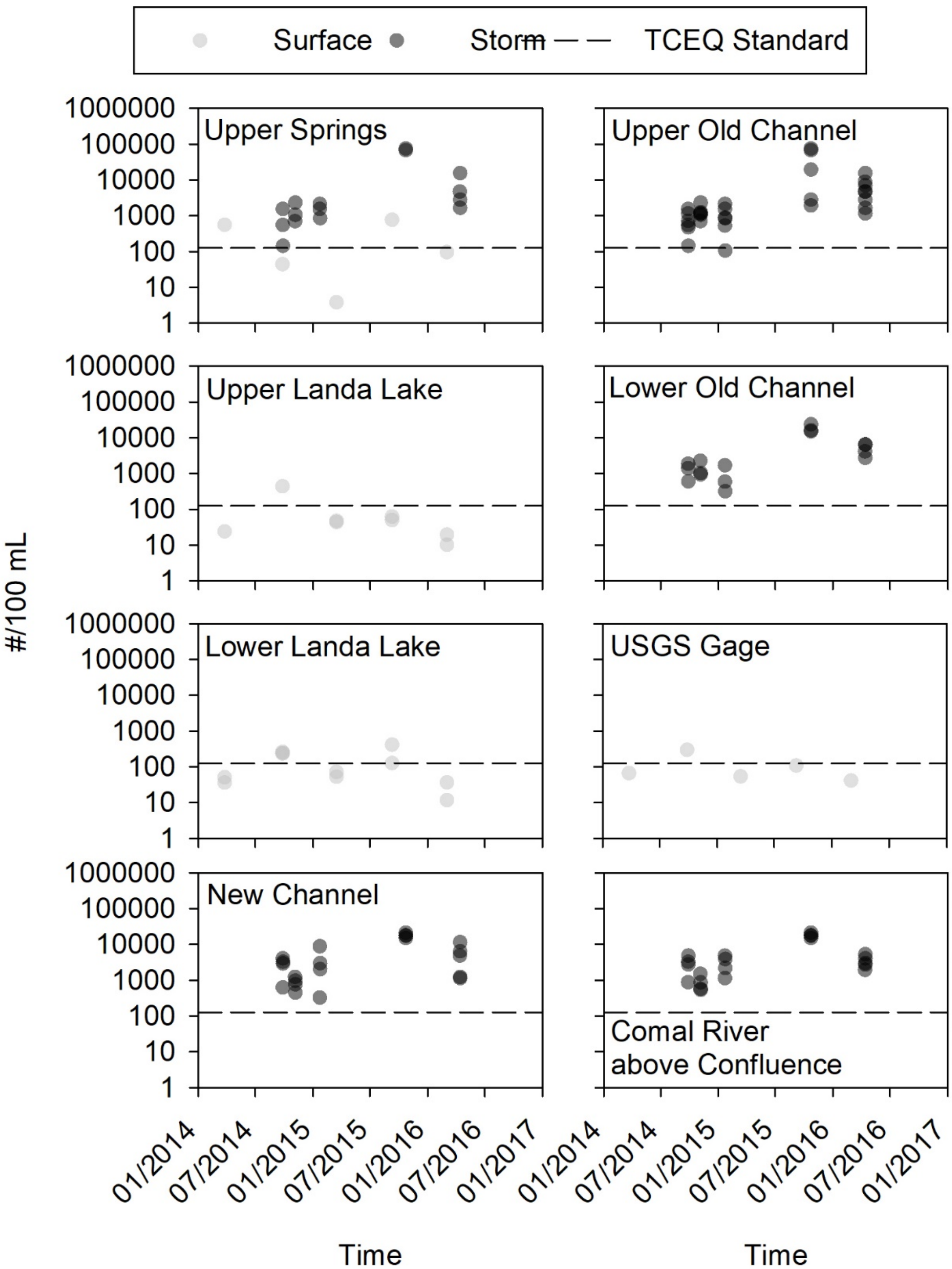


Figure 15. Results for *E. coli* counts during non-storm (Surface; light gray points) and storm (Storm; dark gray points) event sampling with reference to the TCEQ standard (dashed line) at eight locations in Comal Springs (see Figure 14 for locations of sites).

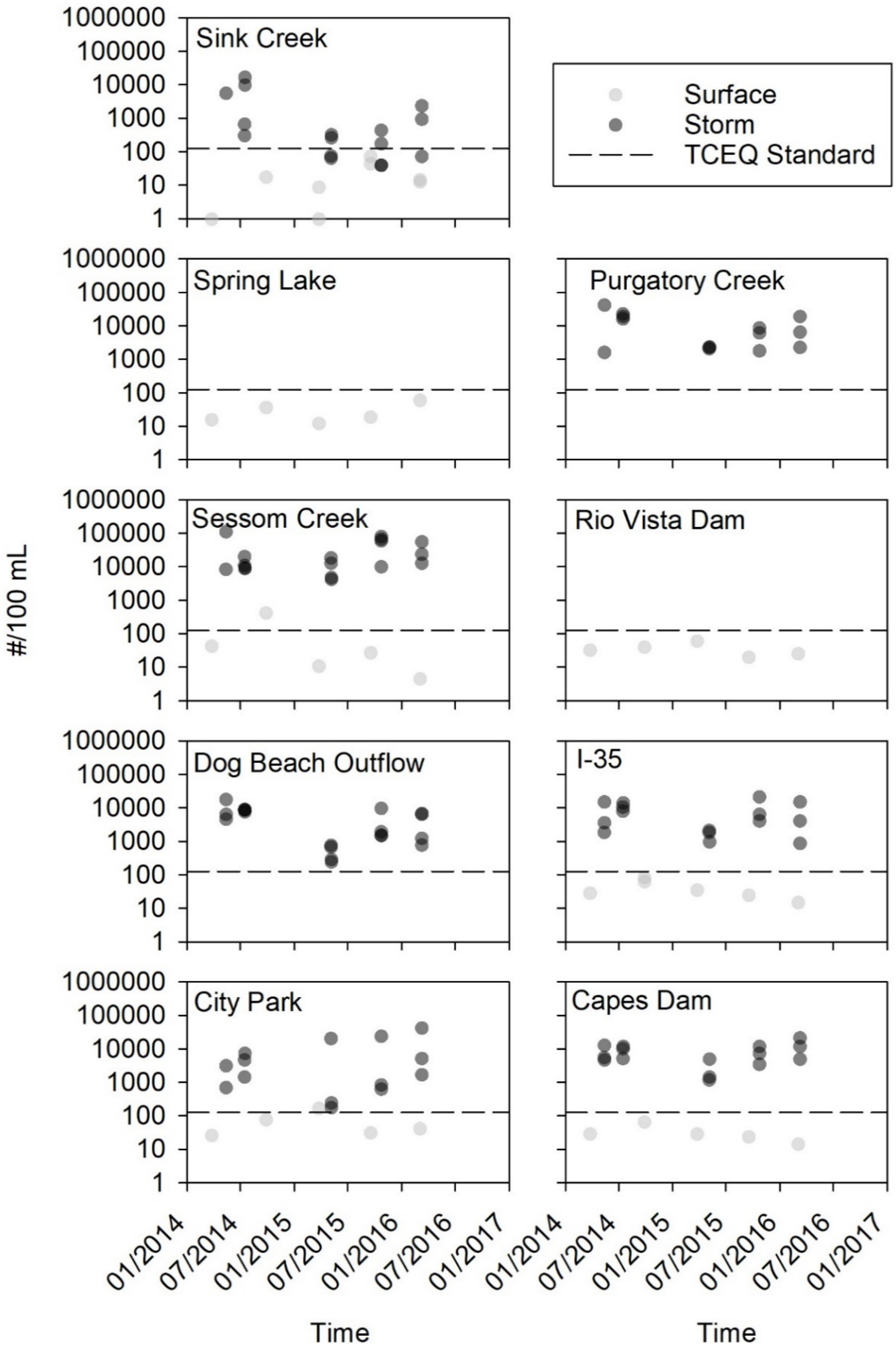


Figure 16. Results for *E. coli* counts during non-storm (Surface; light gray points) and storm (Storm; dark gray points) event sampling with reference to the TCEQ standard (dashed line) at nine locations in San Marcos Springs (see Figure 14 for locations of sites).

Contaminant Sampling

Passive Diffuser samples were tested for 59 contaminants. In the Comal Springs system, 76 samples were collected, and the total number of tests conducted was 4,484 (Table 7). Contaminants were not detected in 4,290 (96%) of tests, and of the 4% of tests that did detect contaminants, median values and ranges were generally <1 microgram per kilogram (Table 8). In the San Marcos Springs system, 104 samples were collected and the total number of test conducted was 6,136 (104 samples x 59 contaminants). Contaminants were not detected in 5,886 (96%) of tests, and of the 4% of tests that did detect contaminants, median values and ranges were generally <1 microgram per kilogram (Table 8).

Additionally, testing for 370 compounds was conducted in sediment, surface water, and storm water samples during 2014-2015. No compounds were detected in 89% (14,165 of 15,840) of tests in the Comal Springs system and 89% (18,631 of 20,925) of tests in the San Marcos Springs system. In sediment samples, 55 compounds in the Comal Springs system and 71 compounds in the San Marcos Springs system were detected at least once and in low concentrations (Table 9). In the Comal Springs system, 36 compounds in surface and 49 compounds in storm water sampling were detected in low concentrations (Table 10). In the San Marcos Springs system, 41 compounds in surface and 46 compounds in storm water sampling were detected in low concentrations (Table 11).

Table 7. Contaminants (detection limit; mass in µg) and number of detections in Comal Springs (CS; 76 samples) and San Macros Springs (SMS; 104 samples) during 2013-2015 sampling.

Contaminant	CS	SMS	Contaminant	CS	SMS
1,1,1,2Tetrachloroethane (0.02)	0	0	Endosulfan I (0.05)	0	0
1,1,1 Trichloroethane (0.02)	11	20	Endosulfan II (0.05)	0	0
1,1,2,2 Tetrachloroethane (0.02)	0	0	Endosulfan Sulfate (0.05)	0	1
1,1,2 Trichloroethane (0.02)	0	0	Endrin (0.05)	0	0
1,1 Dichloroethane (0.02)	0	0	Endrin Aldehyde (0.05)	0	0
1,1 Dichloroethene (0.02)	0	0	Endrin ketone (0.05)	0	2
1,2,4 Trimethylbenzene (0.02)	3	0	Ethylbenzene (0.02)	2	1
1,2 Dichlorobenzene (0.02)	0	0	Fluoranthene (0.05)	2	8
1,2 Dichloroethane (0.02)	0	0	Fluorene (0.05)	5	5
1,3,5 Trimethylbenzene (0.02)	1	1	gamma BHC (0.05)	0	0
1,3 Dichlorobenzene (0.02)	0	0	Heptachlor (0.05)	0	0
1,4 Dichlorobenzene (0.02)	0	0	Heptachlor Epoxide (0.05)	0	0
2 Methylnaphthalene (0.05)	0	0	m p Xylene (0.02)	6	4
4,4 DDD (0.05)	0	2	Methoxychlor Result (0.02)	0	2
4,4 DDE (0.05)	0	2	Methyl tert butyl ether (0.02)	0	0
4,4 DDT (0.05)	0	1	Naphthalene (0.05)	0	1

Table 7 continued.

Contaminant	CS	SMS	Contaminant	CS	SMS
Acenaphthene (0.05)	3	3	o Xylene (0.02)	3	0
Acenaphthylene (0.05)	2	3	Octane (0.02)	0	0
Aldrin (0.05)	0	0	Pentadecane (0.05)	2	4
alpha BHC (0.05)	0	0	Phenanthrene (0.05)	8	12
Anthracene (0.05)	2	3	Pyrene (0.05)	2	8
Benzene (0.02)	0	1	Tetrachloroethene (0.02)	76	90
beta BHC (0.05)	0	0	Toluene (0.02)	4	5
BTEX (0.02)	10	8	TPH (0.05)	42	55
Carbon Tetrachloride (0.02)	0	0	trans 1 2 Dichloroethene (0.02)	0	0
Chlorobenzene (0.02)	0	0	Trichloroethene (0.02)	0	0
Chloroform (0.02)	9	4	Tridecane (0.05)	0	0
cis 1 2 Dichloroethene (0.02)	0	1	Undecane (0.05)	1	1
delta BHC (0.05)	0	0	Vinyl Chloride (0.02)	0	0
Dieldrin (0.05)	0	2			

Table 8. Medan (mass in micrograms, µg), range, and number of detections for contaminants detected at least once in the Comal Springs and San Marcos Springs systems during 2013-2015 sampling.

	Comal Springs System			San Marcos Springs System		
Contaminant	Median	Range	N	Median	Range	N
1,1,1 Trichloroethane	0.02	(0.02-0.02)	11	0.02	(0.02-0.02)	20
1,2,4 Trimethylbenzene	0.02	(0.02-0.03)	3	-	-	-
1,3,5 Trimethylbenzene	0.02	(0.02-0.02)	1	0.02	(0.02-0.02)	1
4,4 DDD	-	-	-	0.23	(0.09-0.37)	2
4,4 DDE	-	-	-	0.24	(0.07-0.40)	2
4,4 DDT	-	-	-	0.26	(0.26-0.26)	1
Acenaphthene	1.09	(0.25-1.37)	3	0.75	(0.46-4.26)	3
Acenaphthylene	0.07	(0.05-0.09)	2	0.15	(0.08-0.72)	3
Anthracene	0.10	(0.08-0.11)	2	0.28	(0.14-1.42)	3
Benzene	-	-	-	0.28	(0.28-0.28)	1
BTEX	0.04	(0.02-0.29)	10	0.05	(0.02-0.37)	8
Chloroform	0.03	(0.02-0.10)	9	0.03	(0.02-0.03)	4
cis 1 2 Dichloroethene	-	-	-	0.14	(0.14-0.14)	1
Dieldrin	-	-	-	0.19	(0.08-0.30)	2
Endosulfan Sulfate	-	-	-	0.30	(0.30-0.30)	1
Endrin ketone	-	-	-	0.38	(0.21-0.55)	2
Ethylbenzene	0.03	(0.02-0.03)	2	0.03	(0.03-0.03)	1
Fluoranthene	0.12	(0.08-0.16)	2	1.74	(0.11-4.74)	8
Fluorene	0.25	(0.06-0.76)	5	1.29	(0.07-9.44)	5
m p Xylene	0.06	(0.03-0.18)	6	0.03	(0.02-0.09)	4
Methoxychlor	-	-	-	0.17	(0.10-0.23)	2
Naphthalene	-	-	-	0.17	(0.10-0.23)	1
o Xylene	0.07	(0.04-0.08)	3	-	-	-
Pentadecane	0.19	(0.15-0.22)	2	0.15	(0.05-0.23)	4
Phenanthrene	0.51	(0.05-3.72)	8	2.27	(0.06-8.70)	12
Pyrene	0.10	(0.06-0.14)	2	1.08	(0.09-2.21)	8
Tetrachloroethene	0.3	(0.02-0.60)	76	0.11	(0.04-2.35)	90
Toluene	0.03	(0.02-0.11)	4	0.05	(0.02-0.07)	5
TPH	1.49	(0.50-6.33)	42	1.27	(0.52-103.02)	55
Undecane	0.16	(0.16-0.16)	1	0.08	(0.08-0.08)	1

Table 9. Compounds, units of measure, median, range, and number of observations (N) for sediments sampled in the Comal Sprints and San Marcos Springs systems during 2014-2015.

		Comal - Sediment			San Marcos - Sediment		
COMPOUND_NAME	Units	Median	Range	N	Median	Range	N
2-Butanone (MEK)	µg/kg	38.7	(38.7-38.7)	1	37.8	(35.2-40.4)	2
2-Methylnaphthalene	µg/kg	-	-	-	0.15	(0.15-0.15)	1
3 & 4 Methylphenol	µg/kg	473	(374-572)	2	289	(289-289)	1
4-Isopropyltoluene	µg/kg	46.3	(46.3-46.3)	1	15.7	(15.7-15.7)	1
4,4'-DDE	µg/kg	21	(21-21)	1	31.5	(17-103)	3
Acetone	µg/kg	131.7	(9.15-224)	4	13.1	(10.4-226)	7
Alkalinity	µg/kg	303.5	(124-386)	4	183	(107-404)	5
Alkalinity, Total (as CaCO3)	µg/kg	580	(340-670)	5	395	(300-440)	6
alpha-Chlordane	µg/kg	-	-	-	7.54	(7.54-7.54)	1
Aluminum	mg/kg	5645	(952-10800)	12	4430	(1200-9790)	14
Anthracene	µg/kg	-	-	-	43.1	(0.68-232)	4
Antimony	mg/kg	1.67	(1.65-1.69)	2	0.8405	(0.82-0.861)	2
Aroclor-1260	µg/kg	-	-	-	52	(52-52)	1
Aroclor 1262	µg/kg	16.5	(16.5-16.5)	1	-	-	-
Arsenic	mg/kg	2.85	(1.25-3.83)	7	5.99	(1.67-11)	13
Barium	µg/kg	54.5	(16-80.1)	8	43.95	(24.6-118)	8
Benzo (a) Anthracene	µg/kg	1.1	(1.1-1.1)	1	0.51	(0.15-7.9)	3
Benzo (a) Pyrene	µg/kg	-	-	-	3.15	(0.57-4.6)	4
Benzo (b) Fluoranthene	µg/kg	1.1	(1.1-1.1)	1	4.405	(0.21-8.6)	2
Benzo (g,h,i) Perylene	µg/kg	-	-	-	2.4	(0.49-3.6)	4
Benzo (k) Fluoranthene	µg/kg	0.88	(0.88-0.88)	1	3.525	(0.15-6.9)	2
Benzo[a]anthracene	µg/kg	148	(140-156)	2	1070	(210-1930)	2
Benzo[a]pyrene	µg/kg	-	-	-	307	(57.8-2090)	3
Benzo[b]fluoranthene	µg/kg	376	(23.3-437)	3	511	(49.8-2690)	3
Benzo[g,h,i]perylene	µg/kg	-	-	-	827.5	(105-1550)	2

Table 9 continued.

		Comal - Sediment			San Marcos - Sediment		
COMPOUND_NAME	Units	Median	Range	N	Median	Range	N
Benzo[k]fluoranthene	µg/kg	168.5	(152-185)	2	636	(172-1100)	2
Beryllium	mg/kg	0.446	(0.075-0.9)	5	0.5145	(0.172-1.19)	12
Bicarbonate (as CaCO3)	µg/kg	580	(580-580)	1	580	(520-640)	2
Bicarbonate Alkalinity as CaCO3	mg/kg	253.5	(212-295)	2	238	(162-582)	3
Bis(2-ethylhexyl) phthalate	µg/kg	368.5	(163-531)	4	333.8	(67.1-668)	4
Bis(2-Ethylhexyl) Phthalate	µg/kg	5.07	(0.94-9.2)	2	1.22	(0.24-2.2)	2
Butyl benzyl phthalate	µg/kg	-	-	-	55.4	(55.4-55.4)	1
Cadmium	mg/kg	0.5115	(0.397-0.626)	2	0.788	(0.596-1.17)	5
Calcium	mg/kg	185500	(120000-330000)	4	212500	(71700-394000)	8
Carbon, Total Organic	mg/kg	32000	(11000-53000)	2	59000	(37000-110000)	5
Chlordane	µg/kg	110	(110-110)	1	-	-	-
Chloride	mg/kg	22	(4.73-25.1)	5	27	(4.71-84)	6
Chromium	µg/kg	10.65	(2.77-63.8)	12	11.05	(5.96-25.2)	14
Chrysene	µg/kg	269	(244-294)	2	5.2	(0.21-2080)	7
Copper	mg/kg	9.92	(3.55-16)	14	9.04	(5.39-58.3)	8
Dalapon	µg/kg	9.72	(9.72-9.72)	1	-	-	-
Dibenz (a,h) Anthracene	µg/kg	-	-	-	0.515	(0.15-0.88)	2
Dibenz(a,h)anthracene	µg/kg	-	-	-	352.3	(98.6-606)	2
Dibenzofuran	µg/kg	-	-	-	314	(314-314)	1
Fluoranthene	µg/kg	3	(0.65-61.9)	3	17.5	(0.11-4380)	7
Fluorene	µg/kg	-	-	-	613	(613-613)	1
Fluoride	mg/kg	2.5	(1.6-3.42)	6	2	(0.92-6.19)	8
Indeno (1,2,3-c,d) Pyrene	µg/kg	-	-	-	2.7	(0.43-3.5)	3
Indeno[1,2,3-cd]pyrene	µg/kg	-	-	-	207	(114-1640)	3
Iron	mg/kg	6155	(1640-12600)	16	6205	(4710-24500)	10
Lead	mg/kg	11.45	(5.92-23.9)	4	28.6	(11.5-260)	12

Table 9 continued.

		Comal - Sediment			San Marcos - Sediment		
COMPOUND_NAME	Units	Median	Range	N	Median	Range	N
Magnesium	mg/kg	2520	(1480-3860)	5	2890	(1460-5780)	11
Manganese	mg/kg	91.5	(22.3-216)	10	342.5	(137-710)	12
Mercury	mg/kg	0.0279	(0.0279-0.0279)	1	0.0558	(0.0201-0.0735)	5
Methylene Chloride	µg/kg	-	-	-	56.6	(56.6-56.6)	1
Nickel	mg/kg	12.7	(2.1-21.1)	11	8.87	(5.03-20.5)	9
Nitrate (as N)	mg/kg	-	-	-	2	(2-2)	1
Nitrate as N	µg/kg	2.23	(2.23-2.23)	1	1.82	(1.62-3.65)	5
PCB-1260	µg/kg	-	-	-	26.3	(26.3-26.3)	1
pH	pH units	7.68	(7.41-8.12)	7	7.71	(7.46-8.14)	7
Phenanthrene	µg/kg	111.5	(104-119)	2	182	(2.1-4430)	3
Phosphorus	µg/kg	342	(342-342)	1	592	(415-618)	3
Phosphorus, Total	mg/kg	4.3	(2.5-5.8)	5	3.1	(0.84-23)	6
Potassium	mg/kg	907	(212-2580)	10	626	(270-4540)	7
Pyrene	µg/kg	256	(1.8-295)	3	21.65	(0.66-3220)	6
Selenium	mg/kg	3.14	(3.14-3.14)	1	1.12	(0.386-2.15)	6
Silicon	mg/kg	2930	(1410-3100)	3	1955	(965-5270)	8
Silver	mg/kg	-	-	-	0.758	(0.384-0.822)	3
Sodium	mg/kg	198.5	(190-207)	2	183	(88.8-422)	7
Solids, Total Dissolved	mg/kg	3460	(3460-3460)	1	2175	(2090-2260)	2
Strontium	mg/kg	228.5	(108-447)	10	171	(93.5-342)	7
Styrene	µg/kg	0.641	(0.641-0.641)	1	-	-	-
Sulfate	mg/kg	51.8	(26-68)	7	38	(11-420)	9
Toluene	µg/kg	-	-	-	65.6	(2.2-129)	2
Zinc	mg/kg	30.15	(13.1-160)	12	48.8	(25.9-253)	9

Table 10. Compounds, unites of measurement, and median, range, and number of observations (N) for surface (non-storm) and storm event sampling in the Comal Springs system during 2014-2015.

		Comal - Surface			Comal -Storm		
Compound	Units	Median	Range	N	Median	Range	N
1,2-Dichloroethane	μg/L	-	-	-	0.84	(0.84-0.84)	1
2-Butanone	μg/L	-	-	-	3.7	(3.7-3.7)	1
2-Methylnaphthalene	μg/L	-	-	-	8.3	(8.3-8.3)	1
2,4-D	μg/L	-	-	-	0.16135	(0.0677-0.255)	2
Acetone	μg/L	-	-	-	10	(10-11)	2
Aluminum	mg/L	0.00862	(0.00459-0.0163)	8	0.0101	(0.00338-98)	33
Antimony	mg/L	0.0001125	(0.000103-0.000166)	4	0.0001415	(0.00011-0.000288)	9
Arsenic	mg/L	0.000511	(0.000505-0.000517)	2	0.000678	(0.000391-0.00763)	17
Barium	mg/L	0.05635	(0.0371-44.5)	16	0.0535	(0.00942-57.5)	39
Beta-BHC	μg/L	-	-	-	0.0695	(0.039-0.1)	2
Bicarbonate (as CaCO3)	mg/L	235	(200-244)	9	208	(41-233)	27
Bicarbonate Alkalinity as CaCO3	mg/L	-	-	-	211	(118-216)	8
Bromide	mg/L	0.1	(0.057-0.549)	10	0.105	(0.062-0.59)	21
Caffeine	ng/L	31	(30-33)	3	110	(11-600)	21
Calcium	mg/L	84.55	(65.6-90.2)	17	75.25	(0.0127-90.4)	36
Carbon Disulfide	μg/L	-	-	-	0.41	(0.41-0.41)	1
Carbon, Dissolved Organic	mg/L	7.6	(2.1-11)	9	4.85	(2.4-10)	25
Carbon, Total Organic	mg/L	8.2	(5.1-12)	8	5.4	(0.73-13)	20
Chloride	mg/L	18	(16-19)	6	17	(1.8-21)	19
Chromium	mg/L	0.000583	(0.000474-0.000794)	7	0.000574	(0.000417-0.000939)	22
Copper	mg/L	0.00078	(0.000234-0.0066)	11	0.00141	(0.000267-2.82)	27
Dissolved Organic Carbon	mg/L	-	-	-	0.947	(0.39-5.84)	8

Table 10 continued.

		Comal - Surface			Comal -Storm		
Compound	Units	Median	Range	N	Median	Range	N
Fluoride	mg/L	0.22	(0.2-0.3)	7	0.2	(0.059-0.94)	20
Iron	mg/L	0.0488	(0.0172-0.0761)	12	0.05185	(0.0168-1.25)	18
Lead	mg/L	0.000117	(0.000117-0.000117)	1	0.0009185	(0.0000928-2.76)	6
Magnesium	mg/L	16.8	(15.4-19.7)	14	15.3	(0.101-17.9)	33
Manganese	mg/L	0.00269	(0.000615-0.00991)	9	0.00264	(0.000998-0.00837)	30
Mercury	mg/L	-	-	-	0.000205	(0.000205-0.000205)	1
Naphthalene	µg/L	-	-	-	8.7	(8.7-8.7)	1
Nickel	mg/L	0.0019	(0.00125-0.00358)	16	0.00156	(0.00067-0.0027)	24
Nitrate (as N)	mg/L	1.6	(0.33-1.8)	6	1.5	(0.44-1.8)	12
Nitrate as N	mg/L	1.775	(1.76-1.79)	2	1.67	(0.867-1.77)	7
pH	pH units	7.31	(7.18-7.81)	9	7.705	(7.31-7.94)	28
Phosphorus	mg/L	-	-	-	0.0851	(0.0446-0.239)	5
Phosphorus, Total	mg/L	0.085	(0.085-0.085)	1	0.046	(0.02-0.36)	19
Potassium	mg/L	1.47	(1.29-2.3)	15	1.915	(1.43-3.86)	35
Selenium	mg/L	0.000503	(0.000295-1.57)	13	0.000377	(0.000171-3.45)	21
Silicon	mg/L	5.48	(0.655-6.62)	18	5.35	(0.126-6.56)	39
Sodium	mg/L	12.5	(12-14.5)	12	12.05	(0.624-15.7)	30
Solids, Total Dissolved	mg/L	340	(280-357)	8	320	(125-490)	22
Solids, Total Suspended	mg/L	15.1	(1.6-43)	4	7.5	(1.5-409)	30
Strontium	mg/L	0.681	(0.613-0.738)	18	0.595	(0.0408-0.709)	39
Sulfate	mg/L	28.5	(24.3-32)	10	26.15	(0.864-39.9)	26
Thallium	mg/L	0.000121	(0.000121-0.000121)	1	-	-	-
Toluene	µg/L	-	-	-	0.77	(0.77-0.77)	1
Total Dissolved Solids	mg/L	345	(331-349)	4	302	(71-332)	9
Total Kjeldahl Nitrogen	mg/L	0.42	(0.28-1)	6	0.56	(0.28-1.8)	10
Total Organic Carbon	mg/L	1.32	(1.07-4.79)	3	0.89	(0.599-5.18)	7

Table 10 continued.

		Comal - Surface			Comal -Storm		
Compound	Units	Median	Range	N	Median	Range	N
Total Suspended Solids	mg/L	-	-	-	5.5	(4.6-24)	7
Zinc	µg/L	0.0124	(0.00413-0.0372)	15	0.0185	(0.00195-5.06)	35

Table 11. Compounds, units of measurement, and median, range, and number of observations (N) for surface (non-storm) and storm event sampling in the San Marcos Springs system during 2014-2015.

		San Marcos - Surface			San Marcos - Storm		
Compound	Units	Median	Range	N	Median	Range	N
1,4-Dichlorobenzene	µg/L	-	-	-	0.57	(0.57-0.57)	1
Acetone	µg/L	-	-	-	7.08	(5.45-18)	8
Aluminum	mg/L	0.0105	(0.00552-0.0364)	7	0.0085	(0.00347-0.16)	37
Antimony	mg/L	0.000187	(0.000187-0.000187)	1	0.000269	(0.000111-0.000796)	27
Arsenic	mg/L	0.0005305	(0.000422-0.000639)	2	0.0007985	(0.000472-0.00199)	12
Barium	mg/L	0.0394	(0.0331-35.1)	21	0.0376	(0.0112-36.2)	53
Bicarbonate (as CaCO3)	mg/L	261	(248-268)	11	199	(69-257)	39
Bicarbonate Alkalinity as CaCO3	mg/L	245	(243-251)	3	222	(113-247)	12
Bis(2-ethylhexyl) phthalate	mg/L	12.5	(12.5-12.5)	1	-	-	-
Bromide	mg/L	0.11	(0.077-0.548)	10	0.1	(0.045-0.592)	29
Caffeine	ng/L	33.5	(17-70)	4	130	(6.9-4800)	33
Calcium	mg/L	93.1	(85.2-101)	17	82.15	(21.4-98.7)	54
Carbon, Dissolved Organic	mg/L	3.75	(1.1-7.7)	7	5	(1.6-18)	29
Carbon, Total Organic	mg/L	6.3	(2.2-9.4)	6	6.3	(0.3-50)	30
Chloride	mg/L	19	(18-29)	9	17.35	(4.4-27)	32
Chromium	mg/L	0.000544	(0.00053-4.14)	5	0.000644	(0.00062-0.00131)	5
Copper	mg/L	0.000758	(0.000208-6.77)	18	0.00125	(0.000179-0.00565)	40
Diethyl phthalate	mg/L	3.16	(3.16-3.16)	1	-	-	-
Dissolved Organic Carbon	mg/L	-	-	-	1.28	(0.394-7.19)	11
Endosulfan I	mg/L	-	-	-	0.0465	(0.045-0.048)	2
Fluoride	mg/L	0.23	(0.185-0.28)	13	0.18	(0.054-0.35)	32
Iron	mg/L	0.0448	(0.0167-0.116)	14	0.0771	(0.049-0.184)	34
Lead	mg/L	0.368597	(0.000194-0.737)	2	0.000202	(0.0000953-0.00149)	22
Magnesium	mg/L	18.1	(13.6-20.2)	18	16.55	(1.53-20.6)	48

Table 11 continued.

		San Marcos - Surface			San Marcos - Storm		
Compound	Units	Median	Range	N	Median	Range	N
Manganese	mg/L	0.00273	(0.000616-0.283)	13	0.00682	(0.000472-162)	27
Mercury	mg/L	-	-	-	0.000235	(0.000235-0.000235)	1
Nickel	mg/L	0.00193	(0.00146-0.00385)	15	0.00225	(0.00125-0.00421)	41
Nitrate (as N)	mg/L	1.2	(0.18-1.5)	7	1.1	(0.18-1.6)	24
Nitrate as N	mg/L	1.24	(1.24-1.25)	2	1.15	(0.687-1.27)	12
Nitrogen, Kjeldahl	mg/L	0.451	(0.451-0.451)	1	1.73	(1.73-1.73)	1
p-Isopropyltoluene	mg/L	-	-	-	0.18	(0.18-0.18)	1
pH	pH units	7.33	(7.03-7.82)	16	7.465	(7.13-7.7)	26
Phosphorus	mg/L	-	-	-	0.05125	(0.0423-0.141)	9
Phosphorus, Total	mg/L	0.028	(0.022-0.039)	7	0.042	(0.02-0.3)	34
Potassium	mg/L	1.48	(1.21-1.85)	20	1.865	(1.16-4.72)	52
Selenium	mg/L	0.000388	(0.000232-0.000444)	11	0.0003065	(0.00017-1.67)	31
Silicon	mg/L	5.415	(3.15-6.05)	25	5.035	(1.41-6.32)	53
Sodium	mg/L	12.7	(10.1-17.8)	22	11.45	(1.56-17.3)	49
Solids, Total Dissolved	mg/L	355	(285-450)	11	295	(115-390)	31
Solids, Total Suspended	mg/L	3.05	(1.1-9)	12	8.2	(1.1-377)	32
Strontium	mg/L	0.5365	(0.495-0.684)	22	0.4865	(0.0296-0.742)	52
Sulfate	mg/L	27	(24.5-32.6)	8	25	(12-45)	29
Thallium	mg/L	0.000135	(0.000135-0.000135)	1	-	-	-
Total Dissolved Solids	mg/L	360	(349-369)	5	322	(179-366)	13
Total Kjeldahl Nitrogen	mg/L	0.42	(0.28-0.56)	4	0.84	(0.28-2.4)	18
Total Organic Carbon	mg/L	0.525	(0.508-0.564)	3	1.465	(0.381-2.76)	10
Total Suspended Solids	mg/L	3.4	(3.4-3.4)	1	14	(2.4-78.8)	13
Trichloroethene	mg/L	-	-	-	0.74	(0.38-1.1)	2
Zinc	µg/L	0.011355	(0.000711-0.0559)	14	0.0209	(0.00791-8.48)	45

Submerged Aquatic Vegetation

Submerged aquatic vegetation areal coverage (m^2) has been surveyed since 2000 at four sites in Comal Springs and three sites in San Marcos Springs (Figure 17), at least twice annually as well as in response to flow-related triggers. The species classifications included in the EAA database and the native status according to Lemke (1989) are given in Table 12. Species-specific temporal trajectories in Comal Springs were summarized for the Upper Spring Run (Table 13), Landa Lake (Table 14), New Channel (Table 15), and Old Channel (Table 16), and for San Marcos Springs at Spring Lake (Table 17), City Park (Table 18), and the I-35 reach (Table 19). For Comal Springs, these data collectively show an historical (2000-2003) increase in native species coverage and a long-term decline of non-native species in the Upper Spring Run, a long-term increase in native species and long-term decline for non-native species in Landa Lake, a recent (2011-2015) increase in native species and serial (2005, 2010, 2015) decreases in non-native species in the New Channel, and long-term stability in native species coverage and historical (2000-2005) increase in non-native coverage in the Old Channel (Figure 18). The recent increase in native and concomitant decrease in non-native vegetation in the Comal system has been strongly influenced by HCP vegetation restoration efforts. For San Marcos Springs, native and non-native species coverage correlated with consistently greater coverage for native species at Spring Lake Dam, long-term stability for native and non-native species but recent (2013-2015) increase in native and decrease in non-native coverage at City Park, and long-term stability with recent (2013-2015) increases for both native and non-native species at the I-35 reach (Figure 18) caused by the expansion of the I-35 reach sampling area in 2014. This expansion was made with the explicit intent of including more vegetative habitat in the sampled area. Changes at City Park have been influenced by HCP vegetation restoration efforts similar to areas of Comal Springs, however this did not influence the I-35 reach. The total area of recent (2013-2016) planting were summarized for Comal Springs (Table 20) and San Marcos Springs (Table 21).

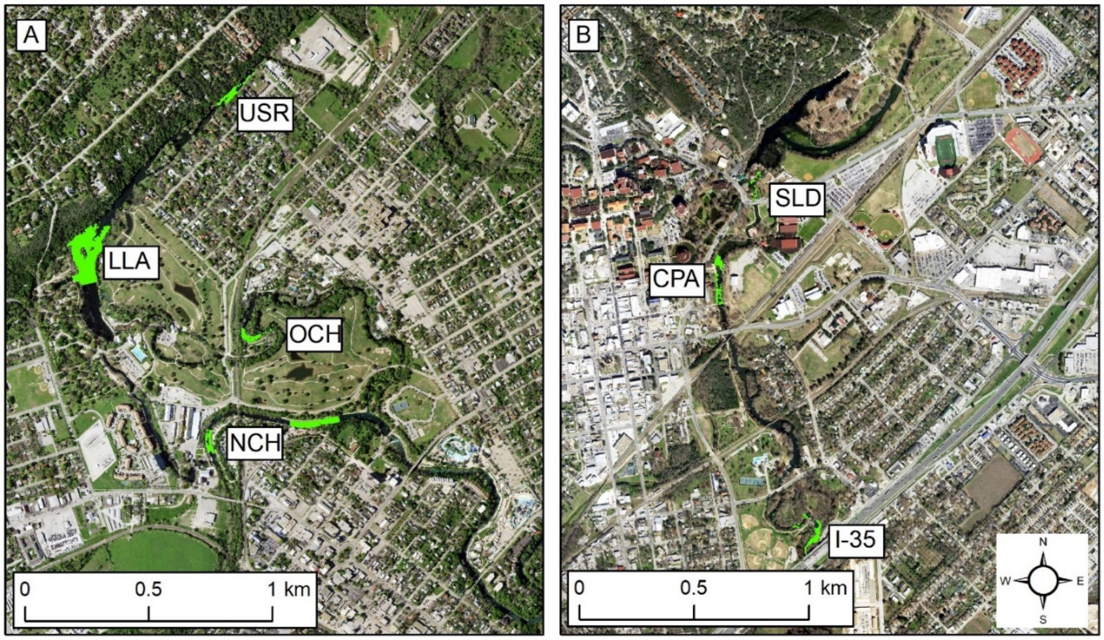


Figure 17. Mapping reaches for submerged aquatic vegetation in (A) Comal Springs and (B) San Marcos Springs. Sites are Upper Spring Run (USR), Landa Lake (LLA), Old Channel (OCH), New Channel (NCH), Spring Lake Dam (SLD), City Park (CPA), and I-35 crossing (I-35).

Table 12. Submerged aquatic plant species from the Comal and San Marcos River systems with native status.

Submerged Aquatic Vegetation	Status
Algae	Native
Bryophytes	Native
Cabomba	Native
Ceratophyllum	Native
Ceratopteris	Introduced
Chara	Native
Colocasia	Introduced
Eichhornia	Introduced
Heteranthera	Native
Hydrilla	Introduced
Hydrocotyle	Native
Hygrophila	Introduced
Justicia	Native
Limnophila	Introduced
Ludwigia	Native
M. heterophyllum	Native
Nasturtium	Introduced
Nuphar	Native
Potamogeton	Native
Rorippa	Native
Sagittaria	Native
Vallisneria	Native
Zizania	Native
Zizaniopsis	Native
Zizania	Native
Zizaniopsis	Native

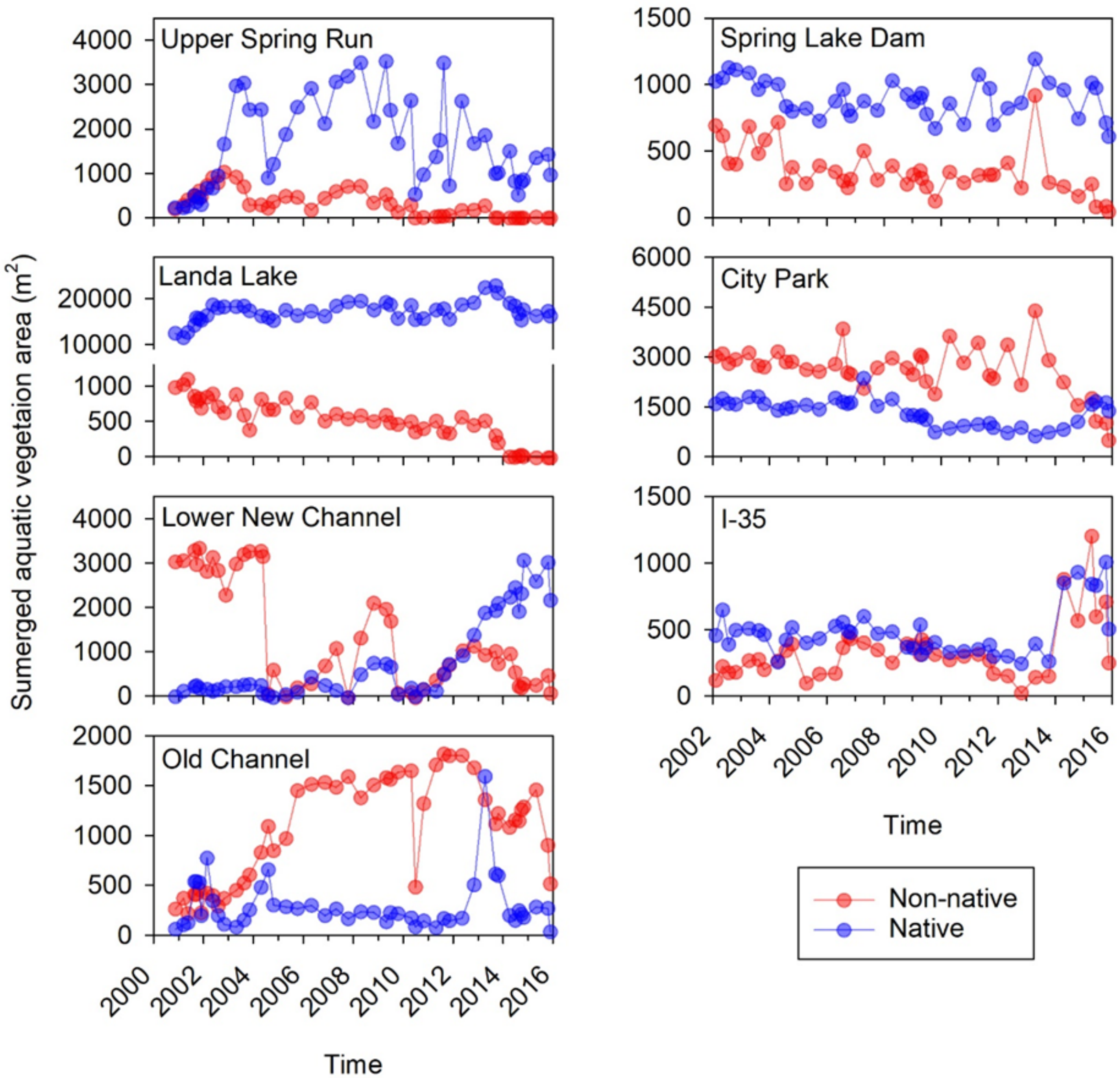


Figure 18. Change in submerged aquatic vegetation area of coverage (m^2) for native and non-native species in the Comal (left column) and San Marcos (right column).

Table 13. Temporal change area (m^2) of submerged aquatic vegetation in the Upper Spring Run mapping site in the Comal River system.

TIME	Algae	Bryophytes	Cabomba	Chara	Hygrophila	Limnophila	Ludwigia	Nuphar	Sagittaria
11/8/2000	0	0	13.4	6.1	204.3	0	1.3	2.3	210.6
3/12/2001	0	0	11	14.5	289.8	0	0.4	0	210.2
5/14/2001	0	0	13.2	13.8	412.7	0	1.8	0	236.5
8/20/2001	0	268.2	17.9	12	489.8	11.5	5.9	0	208.7
9/18/2001	0	119.3	16.2	5	479.8	6.6	7.1	0	215.7
10/31/2001	0	202.1	13.5	9.9	597.4	15.8	4.7	0	226.9
11/26/2001	0	87.3	14.6	9.1	460.3	15.8	4.2	0	187.7
2/20/2002	10.2	384.1	18.3	0	708.2	25	12.1	0.3	249.1
5/14/2002	24.1	328.15	22.5	0	881.1	31.7	18	2	285.6
7/31/2002	9.3	639.85	26.9	0	774.44	26.2	13.8	0	265.8
10/28/2002	1	1281.45	26.9	0	992.1	41.3	39.3	0	320.4
4/22/2003	0	2605.1	19.5	0	902.8	22.2	40.1	0	319.5
8/13/2003	0	2666.7	10.9	0	689.9	18.2	13.3	0	354.1
11/3/2003	0	2132	13.5	0	291.4	7	5	0	293.8
4/22/2004	0	2149.6	9.2	0	293.9	3.2	3.3	0	284.3
8/3/2004	12.3	502.7	12.3	0	223.9	2.1	7.3	0	370.8
10/19/2004	0	792.4	14.1	0	361	6.2	10.9	0	399.8
4/15/2005	0	1510.9	10.4	0	487.8	1.8	22.3	0	342.8
10/3/2005	0	1967.2	12.4	0	468.1	0	26.1	0	494.2
4/24/2006	0	2344.5	0.6	0	185.9	0	25.1	0	551.9
11/7/2006	0	1445.4	0.8	0	446.8	0	42	0	639.4
4/23/2007	0	2456.6	0	0	597.6	0	26.7	0	587.4
10/11/2007	0	2482.4	0	0	713.9	0	25.7	0	685.1
4/17/2008	0	2797.8	0	0	717.3	0	10.2	0	692.7
10/23/2008	50.1	1395.9	0	0	342.5	0	2.1	0	729.3
4/22/2009	784.5	2006.9	0	0	529	0	7	0	734.9
6/25/2009	54	1636.4	0	0	314.3	0	6.8	0	733.7
10/13/2009	0	937.3	0	0	131	0	2.8	0	747.9
4/23/2010	0	1904.1	0	0	296.9	0	8.1	0	739.9
6/23/2010	0	0	0	0	0	0	0	0	537.6
10/22/2010	449.1	15.6	0	0	14.4	0	0	0	517.6
4/25/2011	65	666.7	0	0	29.5	0	0	0	648.8
6/20/2011	1041.6	25.9	0	0	41.7	0	0	0	688.4
8/15/2011	2793.2	0	0	0	41.4	0	0.5	0	704
11/4/2011	0	30.6	0	0	60.2	0	1.2	0	697.4
5/5/2012	20.8	1841.65	0	0	175	0	3	0	772.1
10/31/2012	515.5	356.5	0	0	180.3	0	8.2	0	803
4/10/2013	0	907.7	0	0	277.3	0	14.9	0	942.9
9/11/2013	0	74.6	0	0	10.3	0	4.5	0	920.7
10/18/2013	0	184.5	0	0	0	0	3.8	0	832
4/7/2014	0	735.8	0.4	0	0	0	5.4	0	769.6
6/20/2014	0	154.7	0	2.1	0	0	0.7	0	670.9
8/9/2014	0	32.3	0	0	0	0	0.2	0	490
9/22/2014	0	49.8	0	0.5	0	0	0	0	760.9
10/24/2014	0	74.7	0	0	0	0	0	0	786
4/27/2015	0	243.7	5	276.24	23.14	0	5.227	0	827.77
10/19/2015	0	280.9	9.914	241.37	0	0	6.19	0	897.8
11/22/2015	0	35.8	2	109.9	0	0	0.8	0	825.3

Table 14. Temporal change area (m^2) of submerged aquatic vegetation in the Landa Lake mapping site in the Comal River system.

TIME	Algae	Bryophytes	Cabomba	Hygrophila	Ludwigia	Nuphar	Sagittaria	Vallisneria
11/13/2000	0	0	685.2	995.4	125.1	376	935.7	10525.6
3/13/2001	0	0	471.1	1037	148.8	0	913.1	10171.8
5/15/2001	0	0	316.6	1111.2	142.3	416.4	996.3	10988.7
8/21/2001	0	0	373.1	872	204.9	450.8	863.3	12516.7
9/18/2001	0.85	1834.5	377.4	806	177.5	415.4	791.45	12401.9
10/30/2001	56.35	2128.8	429.5	836.8	168.4	434	692.85	11894.9
11/26/2001	41.3	2142.4	417.3	704.8	139.9	396.3	678.7	11664.6
2/21/2002	0	2818	302.6	858	156.6	484.9	798.9	11955.7
5/16/2002	219.9	4368.3	316.3	904.3	259.1	486.4	872.65	12289.9
8/2/2002	0	3976.85	312	721.6	136.1	491.4	799.7	12443.7
10/29/2002	0	4238.95	348.8	638.1	99.5	444.1	914.2	12349.05
4/23/2003	0	4338.2	119	895.9	99.5	466	889.2	12543
8/15/2003	0	4114.8	233.1	605.8	91.5	462.8	986.9	12678
11/4/2003	0	3637.4	218.6	393.5	44.6	302.2	643.9	12705.7
4/25/2004	0	2465.9	76.8	830	99.1	451.7	806.6	12511.2
8/5/2004	1394.4	516	127.5	684.1	48.6	451	924.6	12626.9
10/20/2004	0	841.2	233.1	684.8	98.1	461.3	1045.7	12738.3
4/15/2005	110.8	2860	249.5	846.3	72.5	460.1	1114.5	12828.2
10/4/2005	337.2	1260.6	300.2	578.6	35.8	360.2	1174.8	13043.5
4/26/2006	617.7	2368.2	231.9	785.9	38.1	462.4	1100.4	12629.7
11/13/2006	0	1142.5	332.4	520.3	41	457.1	1123.1	13253.2
4/26/2007	245	2931.3	181	620.4	21.9	558.1	1223.8	13417.9
10/15/2007	914.7	3133.9	272.5	549.4	37.1	474.9	1008.5	13607
4/22/2008	351	3388.9	158.7	598.8	27.1	503.7	1445.8	13784.8
10/28/2008	908.2	970	178.4	515.2	17.4	431.6	1506.9	13690.6
4/24/2009	307.5	2870.4	173.8	605.3	23.4	483.5	1552.1	13931.5
6/24/2009	330.3	2348.9	180	497.2	17.1	485.9	1558.3	13941.8
10/14/2009	0	385.6	181	474.5	17.8	521.8	1296.9	13452.1
4/26/2010	222.5	2586.9	229.3	511.6	29	524	1458.1	13671.2
6/24/2010	0	348	217.1	367.1	18.5	454.2	1340.3	13259.1
10/25/2010	288.6	411.7	238.9	411.6	27.9	470.2	1483.9	12923.2
4/26/2011	537.75	1531	359.7	520.6	48.4	426.2	1804.1	13013
8/15/2011	2166.4	93.2	439.6	362.8	10.2	373.3	1875	12998.7
11/7/2011	21.3	115.7	481.4	346.8	11.8	362.8	1874.7	12855.8
5/6/2012	87.5	2404.4	645.75	575.5	24.7	483.1	1988	13227.65
10/29/2012	0	2695.05	555.6	459.6	31.4	452.1	1890.15	13651.2
4/11/2013	0	4614	262.9	522.9	46.7	431.6	2427.7	14785.9
9/12/2013	0	4428.1	213.3	314.9	379.4	419.8	2720.6	14838.6
10/18/2013	0	3948.1	295.4	212.1	354.5	349.9	2639.2	13795.3
4/8/2014	0	3324.8	197.6	14.9	247.4	352	2165.8	12930.1
6/19/2014	0	2074.9	187.5	8.8	288.4	379.4	2321	13362.3
8/18/2014	0	1533.8	212	23.5	283.5	427.8	2552.6	11929.9
9/23/2014	0	949	240	38.6	192.7	428.2	2300.9	11407
10/23/2014	0	1563.1	295.3	19.6	182.3	306.8	2686.2	12705.2
4/29/2015	0	1395.3	261.4	0	577.8	178.4	2269.9	11713.1
10/19/2015	0	1691.6	287	0	436.7	139.6	2621	12255
11/20/2015	0	728.6	239.5	0	473.6	166.9	2758.8	12011.9

Table 15. Temporal change area (m^2) of submerged aquatic vegetation in the New Channel mapping site in the Comal River system.

TIME	Algae	Bryophytes	Cabomba	Hydrilla	Hygrophila	Ludwigia	Nuphar	Sagittaria
11/15/2000	0	0	24.4	0	3057.9	0	0	0
3/14/2001	0	0	146	0	3083.2	0	0	0
8/22/2001	0	0	246.1	0	3310.7	0	0	0
9/19/2001	0	0	267.7	0	2998.1	0	0	0
10/31/2001	0	0	210.7	0	3363.1	0	0	0
2/19/2002	0	0	188.4	0	2842.7	0	0	0
5/15/2002	0	0	146.2	0	3157.9	0	0	0
8/1/2002	0	0	180.9	0	2862.4	0	0	0
11/21/2002	0	0	244.7	0	2309.7	0.8	0	0
4/22/2003	0	0	247.5	0	3011.5	0.4	0	0
8/14/2003	0	2.4	281.2	0	3228.8	0.3	0	0
11/5/2003	0	0	293.9	0	3291.3	2.6	0	0
4/21/2004	0	0	272.1	0	3300.3	3.9	0	0
5/16/2004	0	0	95.9	0	3176.9	0	0	0
8/3/2004	47.4	0	0.9	0	77.2	0.4	0	0
10/19/2004	0	0	3.1	0	619.6	0.5	0	0
4/21/2005	70.2	0	0	0	18.1	0	0	0
10/3/2005	123.3	0	0	0	219.8	0	0	0
4/25/2006	0	446	3.8	0	310.1	11.3	0	0
11/16/2006	0	121	144.1	0	715.4	9.6	0	0
4/27/2007	0	49.9	106.9	0	1107.6	8.4	0	0
10/18/2007	0	0.1	0.3	0	0.8	0	0	0
4/18/2008	0	295.3	218.2	0	1340.4	7.6	0	0
10/24/2008	4	6	751.2	0	2130.8	13.3	0	0
4/22/2009	0	54.4	680.3	0	1991.1	23.1	0	0
7/3/2009	0	0	682.8	0	1722.1	6.9	0	0
10/15/2009	0	0	73	0	99.6	0	0	0
4/28/2010	0	96.1	108.6	0	113.3	8	0	0
6/28/2010	0	0	24.4	0	1.9	0	0	0
10/22/2010	0	0	51.5	0	180.6	0	131	0
4/27/2011	0	0	143.2	0	392.1	2.8	0	0
8/17/2011	0	0	510	0	544.5	6.6	0	4.2
11/4/2011	0	0	743.1	0	733.1	8	0	0
5/21/2012	0	0	930.7	0	1054.9	13	0	0
10/31/2012	0	1.7	1409.06	0	1159.7	0	0	0
4/12/2013	0	272.3	1635.6	0	959.9	0	0	0
9/13/2013	0	174.7	1785.4	0	1043.1	0	0	0
10/22/2013	0	33.1	2089.3	0	758.3	0	0	0
4/15/2014	0	233.4	2029.9	0	986	0	0	0
6/24/2014	0	76.6	2400.6	0.3	566.7	0	0	0
8/21/2014	0	53.3	1884.6	0	248.7	0	0	0
9/26/2014	0	116.1	2230.6	0	220.9	0	0	0
10/28/2014	0	53.5	3040.3	0	306.1	0	0	0
4/28/2015	0	0	2618.18	0	279.93	0	0	0
10/20/2015	0	0	3045.48	0	495.79	0	0	0
11/23/2015	0	0	2194.1	0	94.3	0	0	0

Table 16. Temporal change area (m^2) of submerged aquatic vegetation in the Old Channel mapping site in the Comal River system.

TIME	Algae	Bryophytes	Ceratopteris	Hygrophila	Ludwigia	Nuphar
11/14/2000	0	0	281.8	0	0	81.15
3/15/2001	0	0	389.7	0	0	125.8
5/17/2001	0	0	238	0	0	146.05
8/23/2001	493.4	0	428.8	1.4	0	64.6
9/16/2001	493.4	0	428.8	1.4	0	64.6
10/31/2001	479	0	476.5	0	0	68.85
11/27/2001	101.3	0	243.9	0	0	115.2
2/20/2002	696	0	439.5	1.7	0	96.3
5/15/2002	274	0	415.5	2.6	0	90.6
8/1/2002	110.3	0	308.8	2	0	108.65
10/28/2002	32.7	0	386	2.4	0	97.55
4/24/2003	18.5	0	449.3	20.5	2.7	81.7
8/14/2003	31.4	3.4	452.7	90.2	53.6	83.9
11/5/2003	24.6	2.7	405.9	219.5	169.4	77.8
4/21/2004	24.8	98.8	327.4	521.4	249.4	127.3
8/4/2004	230.1	0	248.2	862	335.4	111.3
10/21/2004	8.6	6.6	218.5	647.7	209	97.5
4/20/2005	0	1	21.4	966.4	145.4	157.7
10/5/2005	3.1	0	110.8	1357.1	201.9	82.6
4/27/2006	6.6	0	34.3	1495.8	202.9	109.8
11/13/2006	1.1	5.9	146.2	1403.6	146.8	63.8
4/24/2007	12.2	0	91.3	1410.7	152.6	119
10/18/2007	12.1	11.8	78.2	1529.4	29.3	132.1
4/18/2008	3.6	59.6	45.4	1350	29	163.1
10/24/2008	29.2	95.5	173.1	1350.4	43.8	79.9
4/27/2009	12.6	7.9	70.8	1526.1	23.3	111.2
6/26/2009	39.1	36.2	73.7	1508.7	48.4	122.2
10/15/2009	19.2	48.2	84.4	1569.1	39.2	130
4/27/2010	0.9	18.2	79.2	1587	8.7	167
6/28/2010	0	0	3.4	498.1	1.7	103.6
10/26/2010	7	0	0	1338.3	21.7	135.3
4/25/2011	4.3	2.4	0	1725.1	26.7	62
8/17/2011	0	37.9	0	1834.9	27.7	122.8
11/8/2011	0	28.4	0	1816.8	30	107.1
5/9/2012	0	68.8	0	1820.2	29.2	92.7
10/31/2012	0	280.8	0	1696.6	20.4	222.4
4/11/2013	0	1458.8	0	1376.1	0	151.3
9/10/2013	0	476.7	0	1133.4	0	157.8
10/21/2013	0	454.4	0	1239.8	0	162.1
4/4/2014	0	70.1	0	1100.5	0	148
6/24/2014	0	69.4	0	1173.5	0	98.8
8/19/2014	0	112.2	0	1164.7	0	153.8
9/25/2014	0	123.9	0	1276.8	0	107.3
10/27/2014	0	129.1	0	1304.8	0	68.4
4/27/2015	0	180.69	0	1474.25	0	122.86
10/18/2015	0	214.39	0	920.27	25.59	49.43
11/22/2015	0	3.4	0	535.8	7.1	43.5

Table 17. Temporal change area (m^2) of submerged aquatic vegetation in the Spring Lake Dam mapping site in the San Marcos River system.

TIME	Algae	Bryophytes	Cabomba	Ceratophyllum	Ceratopteris	Eichhornia	Heteranthera	Nasturtium
2/6/2002	0	0	0	0	0	21.3	0	0
5/8/2002	0	0	0	0	0	10	0	0
7/23/2002	0	0	0	0	0	12.5	0	0
10/23/2002	0	0	0	0	0	13.6	0	0
4/11/2003	0	0	0	0	0	75.83	0	0
8/8/2003	0	0	0	0	0	124.85	0	0
10/30/2003	0	0	0	0	0	82.67	0	0
4/15/2004	0	0	0	0	0	117.07	0	0
7/28/2004	0	3.78	0	0	0	6.74	0	0
10/15/2004	0	0	0	0	0	0	0	0
4/11/2005	0	0	0	0	0	0	0	0
9/28/2005	0	0	2.6	0	0	0	0	0
4/19/2006	0	0	0	0	0	9.3	0	0
7/28/2006	0	0	1.2	0	0	0	0	0
9/27/2006	0	0	7.5	0	0	0	0	0
11/3/2006	0	0	0	0	0.3	0	0	0
4/18/2007	0	0	0	0	0	0	0	0
10/10/2007	0	0	0	0	0	0	0	0
4/16/2008	0	0	0	0	0	0	0	0
10/22/2008	0	0	0	0	0	0	0	0
1/9/2009	0	0	0	0	0	0	0	0
4/8/2009	0	0	0	0	0	0	0	0
4/28/2009	0	0	0	0	0	0	0	0
6/29/2009	0	0	1.4	0	0	0	0	0
10/16/2009	0	0	0.8	0	0	0	0	0
4/22/2010	0	0	0	0	0	0	0	0
10/20/2010	0	0	5.1	0	0	0	0	0
4/28/2011	0	0	5.2	0	0	0	0	0
9/15/2011	0	0	0	0	0	0	0	0
11/2/2011	30.4	0	0	0	0	0	0	0
5/3/2012	0	0	0	0	0	0	0	0
10/24/2012	0	0	0	3.1	0	0	0	0
4/17/2013	0	52.7	0	0	0	0	0	0
10/14/2013	0	0	0	0	0	0	0	0
4/21/2014	0	0	0	32.6	0	0	0.5	8.1
10/26/2014	0	0	0	4.6	0	0	0	1.2
4/14/2015	0	0	2.97	0	0	0	0	0
6/5/2015	0	0	0	0	0	0	0	0
10/12/2015	0	0	0	0	0	0	0	0
11/16/2015	0	0	0	0	0	0	0	0

Table 17 continued.

TIME	Hydrilla	Hydrocotyle	Hygrophila	Ludwigia	Potamogeton	Sagittaria	Vallisneria	Zizania
2/6/2002	642.5	65.2	31.65	11.4	791.15	56.4	2.2	101.1
5/8/2002	546.98	135.98	62.6	10.68	675.73	76.55	2.3	152
7/23/2002	347.3	129.1	51.6	19.2	791.6	59	0	131
10/23/2002	326.27	100.22	64.6	34.85	781.87	48	0	149.4
4/11/2003	502.62	152.77	109.28	3.74	662.43	39.25	1.04	230.69
8/8/2003	321.34	144.37	39.54	7.07	556.34	34.88	0.94	222.1
10/30/2003	434.63	134.75	70.65	5.24	582.95	39.93	1.22	266.7
4/15/2004	448.19	141.12	154.46	3.79	527.58	26.49	1.5	304.42
7/28/2004	212.85	54.92	37.23	3.37	521.17	21.18	0.23	235.07
10/15/2004	284.4	73.6	97.2	2.8	537.3	24.9	1.6	162
4/11/2005	201.7	68.2	58.6	2.1	462.1	18.6	1.2	271.9
9/28/2005	352.2	78.1	40.5	0.4	400.7	31.4	0.6	216.4
4/19/2006	246.8	148.6	90.9	0	360	34.3	13.7	321
7/28/2006	203.2	96.5	76.7	1.2	488.9	34	21	323.3
9/27/2006	176.1	112.9	52.1	4.4	556.9	22.3	19.7	86.3
11/3/2006	221.4	104.1	71.4	4.1	515.2	24.5	12.4	107.2
4/18/2007	435.3	90.1	70.2	3.7	463.3	8.5	30.2	283.2
10/10/2007	257.5	37.5	30.2	1.4	427.7	17.2	27.6	298.4
4/16/2008	364.6	104	28.7	12.2	465.1	17.7	48.8	384.8
10/22/2008	203	94.1	51.6	0	447	27.2	63.6	295.7
1/9/2009	274.7	89.3	52.7	1.5	336.7	23.6	92.6	329.5
4/8/2009	295.5	91.2	61.5	0	354.8	18.3	106.6	331.9
4/28/2009	249.6	106.8	48	0	375.8	19.3	91.2	344.9
6/29/2009	194.1	77.7	41	0	284.4	17.9	88	310.8
10/16/2009	98.8	32.5	29.1	0	335.4	14.3	30.3	260.7
4/22/2010	344.3	50.7	0.5	0	399.8	12.1	50	347.8
10/20/2010	200.9	46.8	64.8	4.3	272.5	5.5	31.5	339.4
4/28/2011	315.05	74.8	7.6	0	359.4	10.4	150.45	477.2
9/15/2011	249.4	74.2	74.9	0	304.2	10.5	107.4	477.9
11/2/2011	241.1	28.9	85.8	0	268.05	14.4	42.45	317.7
5/3/2012	301.6	33.5	113.55	0	294.95	9.1	59.4	427.6
10/24/2012	143.5	56.05	83.3	0	327.25	19.2	61.8	397.2
4/17/2013	835.6	22.2	85.2	0	281.1	59.7	142.6	637.3
10/14/2013	207.7	174.4	59.1	0	188.3	33.4	100.4	519.6
4/21/2014	150.9	82.9	77.4	0.8	179.8	60	110.3	494.9
10/26/2014	124.2	72.9	38.6	0	92.3	40.1	32.2	505.1
4/14/2015	194.06	81.09	61.86	0	107.85	12.33	63.13	748.36
6/5/2015	21.33	142.45	63.49	3.13	63.69	20.04	17	730.58
10/12/2015	30.47	28.11	58.1	1.41	6.04	21.14	2.74	656.51
11/16/2015	8.5	7.2	38.3	0	0	7	0	598.4

Table 18. Temporal change area (m^2) of submerged aquatic vegetation in the City Park mapping site in the San Marcos River system.

TIME	Algae	Cabomba	Ceratophyllum	Ceratopteris	Eichhornia	Heteranthera	Hydrilla	Hydrocotyle
2/6/2002	0	0	0	0	0	0	1856.2	0
5/7/2002	0	0	0	0	0	1.5	2003.7	0
7/23/2002	0	0	0	0	0	2.5	1781	0
10/21/2002	0	0	0	0	0	0	1913.7	0
4/9/2003	0	0	0	0	0	0.5	2086.7	0
8/6/2003	0	0	0	0	0	0	1952.3	0
10/20/2003	0	0	0	0	196.3	0.9	1622.2	3.4
4/13/2004	0	0	0	0	0	0	2268.7	1.9
7/26/2004	0	0	0	0	0	0	1999.9	0
10/11/2004	0	0	0	0	108.1	0.4	1874.4	1.9
4/13/2005	0	0	0	0	0	0	1792.6	0
9/26/2005	0	0	0	0	0	0	1752.4	3.7
4/17/2006	0	0	0	0	0	0	2000	4.9
7/25/2006	0	0	0	0	0	0	3021.1	0
9/22/2006	0	0	0	0	0	0.8	1487.8	0
11/2/2006	0	0	0	0	0	2.3	1586.3	0
4/17/2007	929.8	0	0	0	0	0	857.9	0
10/8/2007	0	0	0	0	0	0.3	1938.7	0
4/14/2008	15.5	0	0	0	0	0.7	2306.5	0
10/20/2008	0	0	0	0	0	0	1870.1	0
1/7/2009	0	0	0	0	0	0	1751.2	0
4/8/2009	0	0	0	0	0	0.3	2352.7	0
4/29/2009	0	0	0	0	0	0.3	2265.3	0
6/22/2009	0	0	0	0	0	0	1422.4	0
10/12/2009	0	34.6	34.2	0	0	0	993.9	0
4/21/2010	0	0	0	0	0	0	2557.6	0
10/19/2010	0	0	0	0	0	0	1758.3	0
4/21/2011	0	0	0	0	0	0	2423.6	0
9/14/2011	143.4	0	0	0	0	0	1533	0
11/1/2011	251.1	4.6	0	0	0	0	1443.8	0
5/1/2012	0	19.6	0	0.7	0	0	2231.2	0
10/23/2012	0	32	0	0	0	0	1385	0
4/20/2013	0	0	0	0	0	0	3200	0
10/10/2013	0	23.8	53.9	0	0	3.4	1588.8	0
4/17/2014	0	0	0	0	0	5.2	1747.7	0
10/17/2014	0	0	56.4	0	0	32.7	997.5	0
4/15/2015	0	0	0	0	0	15.9	1097.7	0
6/4/2015	0	0	0	0	0	34.2	640.4	0
10/14/2015	0	0	6.4	0	0	8.9	750.9	0
11/16/2015	0	0	0	0	0	6.5	227.8	0

Table 18 continued.

TIME	Hygrophila	Ludwigia	M. heterophyllum	Nasturtium	Potamo-geton	Rorippa	Sagittaria	Vallisneria	Zizania
2/6/2002	1187.5	0	0	0	1462	0	81.5	13.4	66.8
5/7/2002	1124.5	0	0	0	1522.7	0	156.8	13.9	81.7
7/23/2002	1053.8	0	0	0	1456.2	0	109.3	8.2	59.4
10/21/2002	1040.7	0	0	0	1470.4	0	67.2	9.5	64.4
4/9/2003	1063	0	0	0	1691.2	0	41.7	6.6	86.7
8/6/2003	822	0	0	0	1637.7	0	114.8	7.7	74.4
10/20/2003	910.1	0	0	0	1471.3	0	71.1	1.4	74.7
4/13/2004	921.8	0	0	0	1191.5	0	95.3	3.8	137
7/26/2004	884.3	0	0	0	1223	0	123.7	3.1	131.8
10/11/2004	903.4	0	0	0	1281.5	0	101.5	1.5	140.3
4/13/2005	860.3	0	0	0	1337.1	0	85.8	1.3	166
9/26/2005	842.1	0	0	0	1249.1	0	88.6	0.6	118.3
4/17/2006	817.2	0	0	0	1485.8	0	137.8	2.8	168.4
7/25/2006	854.5	0	0	0	1369.4	0	156.5	0.2	150.9
9/22/2006	1074.3	0	0	0	1222.7	0	249.2	0	154.5
11/2/2006	921	0	0	0	1240.3	0	252.6	0	168
4/17/2007	1234.8	0	0	0	1131.6	0	91.4	0	238.6
10/8/2007	764	0	0	0	1191.7	0	88.7	2.1	272.6
4/14/2008	689.8	0	0	0	1348.5	0	53.8	5.1	343.5
10/20/2008	831.6	0	0	0	933	0	61.2	7.2	288.6
1/7/2009	750.7	0	0	0	874.5	0	95.7	3	307.5
4/8/2009	736.1	0	0	0	747.7	0	141.2	4.1	335
4/29/2009	763.3	0	0	0	786.1	0	143.5	3.7	345.2
6/22/2009	872.7	0	0	0	698.1	0	145.5	5.3	300
10/12/2009	922.2	0	0	0	335.7	0	198.2	1.3	169.9
4/21/2010	1099.1	0	0	0	503.4	0	106.4	2.3	276.2
10/19/2010	1095.4	0	0	0	561.9	0	113.5	0	287.1
4/21/2011	1028.2	0	0	0	464.8	0	194.9	3.6	342.4
9/14/2011	940.8	0	0	0	374.2	0	194.7	5.8	323.2
11/1/2011	945.7	0	0	0	222	0	207.3	4.2	222.2
5/1/2012	1163.9	0	0	0	245.1	0	80.9	9.3	397.5
10/23/2012	808	0	0	0	362.5	0	115.4	0	400.5
4/20/2013	1217.4	0	0	0	160.3	0	90.4	6.1	400
10/10/2013	1345.8	0	0	0	104.6	0	204.8	13	360.6
4/17/2014	507.4	7.2	0.6	16.7	158.9	0	122.6	5.1	551.5
10/17/2014	573.3	10.2	0	5.2	58.5	0	109.3	2.6	817.6
4/15/2015	640	5.3	0	43.3	107.3	0	128.5	4.7	1344.8
6/4/2015	448.6	10.8	0	7	58.1	0	120.4	0	1470.2
10/14/2015	294.6	1.7	0	0	59	0	129.1	3	1449
11/16/2015	297.4	0.8	0	0	53.5	0	91.6	0	1260.7

Table 19. Temporal change area (m^2) of submerged aquatic vegetation in the I-35 mapping site in the San Marcos River system.

TIME	Algae	Bryophytes	Cabomba	Cerato-phyllum	Colocasia	Heteranthera	Hydrilla	Hydrocotyle	Hygrophila
2/7/2002	0	0	155.3	0	0	6.7	101	0	29.1
5/6/2002	0	0	196.7	0	0	27.3	194.4	0	39.2
7/24/2002	0	0	120	0	0	23.1	132.1	0	54.4
10/22/2002	11.8	0	157	0	0	36.5	133.9	0	55.7
4/10/2003	0	0	159.4	0	0	39.3	199.6	2.1	78.3
8/11/2003	0	0	171.8	0	0	29.4	192.9	0	93.8
10/21/2003	0	0	168.7	0	0	12.7	138	0	72.7
4/14/2004	0.7	0	0.7	0	0	6.9	193.6	0	82.6
7/27/2004	24.8	0	156.4	0	0	13.3	292	0	61.4
10/12/2004	29.7	0	182.5	0	0	6.2	309.2	0.2	93.8
4/12/2005	118.3	0	86.8	0	0	28.9	57.1	0	51.4
9/27/2005	65.5	0	130.7	0	0	42.6	65.5	1.1	110.9
4/18/2006	244.4	0	129.5	0	0	18.3	107.2	0	74.3
7/25/2006	180.1	0	225.8	0	0	5.3	316	0	59.3
10/3/2006	45.2	0	248	0	0	0	381.5	0	75.8
11/2/2006	29.2	0	253.1	0	0	5.2	357.8	0	84.7
4/19/2007	120.3	0	208.5	0	0	15	284.3	19.2	128.3
10/11/2007	0	0	204.7	0	0	43.4	282.4	0	76.2
4/17/2008	148.9	0	150.6	0	0	18.8	165.6	0	96.4
10/21/2008	0	0	205.1	0	0	3.1	291.6	0	112.4
1/8/2009	14.6	0	161.5	0	0	0.6	289.9	0	100.1
4/9/2009	241.5	0	88.3	0	0	0.2	239.6	0	84.1
4/29/2009	0	0	110.7	0	0	1.8	358.6	0	77.3
6/23/2009	0	0	158.4	0	0	0	329.6	0	71.7
10/12/2009	0	0	231.2	0	0	0	161.5	0	162.5
4/20/2010	0	0	147.6	0	0	0	169	0	114.7
10/21/2010	6.1	0	142.4	0	0	0	185.3	0	126.1
4/22/2011	0	0	126.9	0	0	3.3	300.1	0	25.9
9/14/2011	7.9	0	140.9	0	0	1.9	185	0	93.3
11/3/2011	0	0	113.2	0	0	0	64.4	0	114.4
5/4/2012	0	0	125	0	0	0	59.8	0	102.6
10/25/2012	0	0	92.4	0	0	0	24.4	0	10.4
4/24/2013	0	61.4	153.2	0	0	9.8	137.4	0	15.7
10/11/2013	0	28.9	0	87.1	0	0	113.6	0	46.5
4/23/2014	0	0	134	7.3	78.4	1.3	295.5	0	511.8
10/18/2014	0	1.5	225.1	39.7	12.3	0.9	159.7	0	405.7
4/13/2015	0	4.8	161.9	0.8	35.5	2	781.5	0	349.1
6/8/2015	0	0	183.2	0	55.2	0	312.4	1.2	239.6
10/13/2015	0	16	251.8	10.3	15.5	1.2	180.7	0	522.8
11/19/2015	0	0	33.4	0	0	0	123.5	0	136.8

Table 19 continued.

TIME	Justicia	Ludwigia	Nasturtium	Nuphar	Potamo-geton	Rorippa	Sagittaria	Vallis-neria	Zizania	Zizaniopsis
2/7/2002	164.6	2	0	0	2.1	0	37.5	0	98.6	0
5/6/2002	116.6	125.2	0	0	0	0	56.2	0	135.6	0
7/24/2002	112	1.4	0	0	0	0	40.5	0	103.4	0
10/22/2002	133.6	2.2	0	0	0	0	47.3	0	118.4	0
4/10/2003	143.3	4.7	0	0	0	1	49.8	0	119.2	0
8/11/2003	123.5	5.2	0	0.7	0	0	51.2	0	121.3	0
10/21/2003	107.7	15.4	0	0	0	0	49.7	0	119.5	0
4/14/2004	52.4	11.3	0	0	0	0	65.1	0.3	129.8	0
7/27/2004	71	8.6	0	0	0	0	41.9	0	118	0
10/12/2004	102.7	10.9	0	0	0	0	64.3	0	130.6	0
4/12/2005	55.9	0	0	0	0	0	32.3	0	88.7	0
9/27/2005	47.8	0.7	0	0	0	0	33.8	0	122.8	0
4/18/2006	13.5	4.5	0	0	0	0	38.4	0	88.7	0
7/25/2006	21.6	9.2	0	0	0	0	41.9	0	83.1	0
10/3/2006	22.2	8.7	0	0	0	0	53	0	121.4	0
11/2/2006	20.9	11.6	0	0	0	0	48.7	0	120.1	0
4/19/2007	24.1	12.7	0	0	0	0	71.6	0	139.3	0
10/11/2007	24.6	5	0	0	0	0	56.9	0	146.4	0
4/17/2008	0	1.3	0	0	0	0	55.9	0	119.4	0
10/21/2008	0	10	0	0	0	0	38.6	0	123.5	0
1/8/2009	0	15.9	0	0	0	0	46.7	0	132.8	0
4/9/2009	0	21.7	0	0	0	0	54.3	0	141.7	0
4/29/2009	0	17.2	0	0	0	0	52.8	0	140.9	0
6/23/2009	0	10	0	0	0	0	64.5	0	141	0
10/12/2009	0	12.7	0	0	0	0	46.7	0	124.1	0
4/20/2010	1.1	8.1	0	0	0	0	36.5	0	149.3	0
10/21/2010	0	14.1	0	0	0	0	18.9	0	166.1	0
4/22/2011	0	9.9	0	0	0	0	67.5	0	154.4	0
9/14/2011	0	9.8	0	0	0	0	80	0	155	0
11/3/2011	0	16	0	0	0	0	42.3	0	138.1	0
5/4/2012	0	2.9	0	0	0	0	54.4	0	129.2	0
10/25/2012	0	29.7	0	0	0	0	7.2	0	125.3	0
4/24/2013	1.9	0	0	0	0	0	10.9	0	166	0
10/11/2013	0	0	0	0	0	0	15.1	0	140.1	0
4/23/2014	0	67.3	2.8	25.8	0	0	257.3	0	363.8	3.7
10/18/2014	0	45.5	0	44.3	0	0	218.6	0	365.2	0
4/13/2015	0	19	46.1	22.7	0	0	212.5	0	424	5.3
6/8/2015	4.1	74.5	0	12.8	0	0	241.9	0	324.6	0
10/13/2015	0	73.3	0	17.9	0	0	271.2	0	374.2	2.7
11/19/2015	0	8.1	0	11.5	0	0	376.5	0	81.7	3.2

Table 20. Location, year, species, and number of plants planted in the Comal River system.

Location	Year	Vegetation	Plants
Landa Lake	2013	Cabomba	869
Landa Lake	2014	Cabomba	2038
Landa Lake	2015	Cabomba	2000
Landa Lake	2016	Cabomba	430
Landa Lake	2013	Ludwigia	2107
Landa Lake	2014	Ludwigia	1418
Landa Lake	2015	Ludwigia	4889
Landa Lake	2016	Ludwigia	1056
Landa Lake	2016	Potamogeton	150
Landa Lake	2014	Sagittaria	72
Landa Lake	2015	Sagittaria	1875
Landa Lake	2015	Vallisneria	1225
New Channel Mill Race (below Landa Lake LCRA weir)	2016	Ludwigia	515
New Channel Mill Race (below Landa Lake LCRA weir)	2016	Sagittaria	350
Old Channel	2016	Cabomba	50
Old Channel	2015	Ludwigia	2312
Old Channel	2016	Ludwigia	300
Old Channel	2016	Sagittaria	960
Old Channel ERPA	2013	Cabomba	1067
Old Channel ERPA	2014	Cabomba	646
Old Channel ERPA	2015	Cabomba	1747
Old Channel ERPA	2016	Cabomba	50
Old Channel ERPA	2014	Justicia	20
Old Channel ERPA	2013	Ludwigia	6853
Old Channel ERPA	2014	Ludwigia	1232
Old Channel ERPA	2015	Ludwigia	1762
Old Channel ERPA	2016	Ludwigia	870
Old Channel ERPA	2014	Potamogeton	27
Old Channel ERPA	2015	Potamogeton	25
Old Channel ERPA	2016	Potamogeton	60
Old Channel ERPA	2013	Sagittaria	611
Old Channel ERPA	2014	Sagittaria	470
Old Channel ERPA	2015	Sagittaria	4967
Old Channel ERPA	2014	Vallisneria	1350
Old Channel ERPA	2015	Vallisneria	650
Upper Spring Run	2016	Ludwigia	622
Upper Spring Run (Upstream of USR HCP study reach)	2016	Ludwigia	530

Table 21. Location, year, species, and number of plants planted in the San Marcos River system.

Location	Year	Vegetation	Plants	Location	Year	Vegetation	Plants
Below Sewell Park AboveCityPark	2013	Heteranthera	63	Cypress Island	2016	Cabomba	968
Below Sewell Park AboveCityPark	2014	Heteranthera	269	Cypress Island	2015	Heteranthera	2544
Below Sewell Park AboveCityPark	2013	Hydrocotyle	27	Cypress Island	2016	Heteranthera	1643
Below Sewell Park AboveCityPark	2013	Ludwigia	780	Cypress Island	2015	Ludwigia	768
Below Sewell Park AboveCityPark	2014	Ludwigia	631	Cypress Island	2016	Ludwigia	3
Below Sewell Park AboveCityPark	2013	Potamogeton	124	Cypress Island	2016	Potamogeton	1438
Below Sewell Park AboveCityPark	2013	Sagittaria	204	Cypress Island	2015	Sagittaria	305
Below Sewell Park AboveCityPark	2014	Sagittaria	66	Cypress Island	2016	Sagittaria	129
Below Sewell Park AboveCityPark	2013	Zizania texana	1443	Cypress Island	2015	Zizania texana	7752
Below Sewell Park AboveCityPark	2014	Zizania texana	1292	Cypress Island	2016	Zizania texana	965
Bicentennial Park	2014	Sagittaria	133	IH-35	2016	Cabomba	975
Bicentennial Park	2014	Zizania texana	384	IH-35	2016	Hydrocotyle	3505
City Park	2014	Heteranthera	1344	IH-35	2016	Ludwigia	3847
City Park	2015	Heteranthera	825	IH-35	2016	Potamogeton	2070
City Park	2016	Heteranthera	24	IH-35	2016	Sagittaria	612
City Park	2016	Hydrocotyle	6	IH-35	2016	Zizania texana	1375
City Park	2014	Ludwigia	4112	Sewell Park	2013	Heteranthera	62
City Park	2015	Ludwigia	2349	Sewell Park	2014	Heteranthera	460
City Park	2016	Ludwigia	100	Sewell Park	2013	Ludwigia	407
City Park	2015	Potamogeton	54	Sewell Park	2014	Ludwigia	777
City Park	2016	Potamogeton	324	Sewell Park	2014	Sagittaria	351
City Park	2014	Sagittaria	552	Sewell Park	2013	Zizania texana	343
City Park	2015	Sagittaria	569	Sewell Park	2016	Zizania texana	3762
City Park	2014	Zizania texana	7293	Veterans Park	2016	Heteranthera	108
City Park	2015	Zizania texana	8847	Veterans Park	2016	Potamogeton	450
City Park	2016	Zizania texana	348	Veterans Park	2016	Sagittaria	306
				Veterans Park	2016	Zizania texana	869

Covered Species

Fountain Darter

Drop Net Sampling – Fountain Darter populations are monitored within designated study reaches using drop nets in Comal Springs (Figure 19A) and San Marcos Springs (Figure 19B). Drop netting was conducted quarterly (2000-2002), three times annually (2003-2004), or twice annually (2000-2015) at three sites in Comal Springs, and one site (New Channel) was sampled during 2000-2004 and 2014-2015. In the San Macros Springs system, two sites were sampled quarterly (2000-2002), three times annually (2003-2004), or twice annually (2000-2015) and one additional site (Spring Lake Dam) during 2012-2015 (Table 22). In addition to routine sampling periods, drop net sampling was also conducted in response to flow-related triggers. Drop netting consists of using a rectangular plot of known dimensions (2 m^2) with 2-mm mesh along four sides and dip-netting (50 cm by 50 cm net with 2-mm mesh) from above for a minimum of 15 passes within the enclosed area to capture and enumerate all aquatic organisms present (Table 23).

Relationships between environmental variables and Fountain Darter abundances measured during drop netting were assessed using random forest models. Random forest (RF) models are a tree-based ensemble learning process based on construction of multiple decision trees, and can be used in regression (continuous response) or classification (nominal response; Breiman 2001). Here, the term ensemble refers to RF models repeatedly fitting trees so that many “weak learners” (individual trees) combine to function as a “strong learner” (forest of trees). The predictive power of the ensemble of learns can be assessed using cross-validation and either the percent of variance explained (if used in regression) or the area under the curve (AUC; if used in classification). These models can incorporate continuous and nominal predictor variables and provide the ability to rank predictor variables by their importance. This is done by measuring the percent increase in mean square error when a predictor is permuted (when used in regression) or by measuring increase node purity (high inter node variance and low intra node variance) when classifications are split by a predictor (when used in classification). Another benefit of RF models is the use of partial dependence plots in which relationships between a response variable and any predictor variable can be assessed by essentially “averaging over” all other predictor variables and assessing the directionality (partial dependence) of the response variable. The ‘randomForest’ function from the ‘randomForst’ Package in the R Statistical Environment was used to fit models using 500 trees and default settings (Liaw and Wiener 2002).

Fountain Darter abundance (number of fish collected) in drop nets was predicted using RF models at multiple scales. The broadest scale was a “global” model in which all sites and all sample events (routine and flow-triggered) from the Comal Springs and San Marcos Springs systems were combined. Predictor variables in the global model included river (binomial: CS or SMS), site (nominal with 7 classes corresponding to sites shown in Figure 19), dominant vegetation within the drop net (nominal), water depth (continuous), the year collections were made (continuous), the water velocity at 15 cm above the substrate (continuous), and the substrate present (nominal). The global model explained 43% of variance in Fountain Darter abundance and the most important predictor variables were dominant vegetation, site location, water depth, and year of collections (Figure 20). Given the importance of site location in the global model, RF models were then fit to each sampling site location independently using the same predictor variables as the global model (with the omission of river and site). In the Comal Springs system, site-specific models explained 20-36% of variance in Fountain Darter abundance, and in the San Marcos River site-specific models explained 14-40% (Figure 20A). Across all site-specific models, dominant vegetation type, depth, and year were consistently ranked as important variables (Figure 20B), though overall importance was low for models with poor predictive ability. Partial dependence plots illustrated increased abundance of Fountain Darter across a variety of dominant vegetation types, but no strong consistency for a single species across all sites (Figure 21). The partial effect of time on predicted abundance of Fountain Darters was largely consistent across years, as evidenced by generally flat lines in partial dependence plots. Finally, Fountain Darter abundance was slightly higher at greater depths across sites, though the New Channel site in the Comal Springs system and the Spring Lake Dam site in the San Marcos Springs system were exceptions to this pattern.

In summary, only a minority of variance (14-43% across models) in Fountain Darter abundance could be explained using local-scale environmental predictors collected during drop net sampling. Incorporating variables representing water quantity (e.g., flow regime components), water quality (e.g., temperature regimes), could moderately, perhaps, improve modeling, though the observed range of conditions for these data are limited and unlikely to produce directly observable biological effects. However, potential exists for including broad-scale (i.e., entire mapped areas) submerged aquatic vegetation data with drop net data to assess factors correlated with abundances (see Future Research and Hypothesis Development section). It is also suggested that applying models that include adjustment of predictions for “nuisance parameters” such as heterogeneous detection may also elucidate variance components in these data and improve the ability to accurately assess population trends in these systems with drop net data as well as quantify uncertainty in the data.

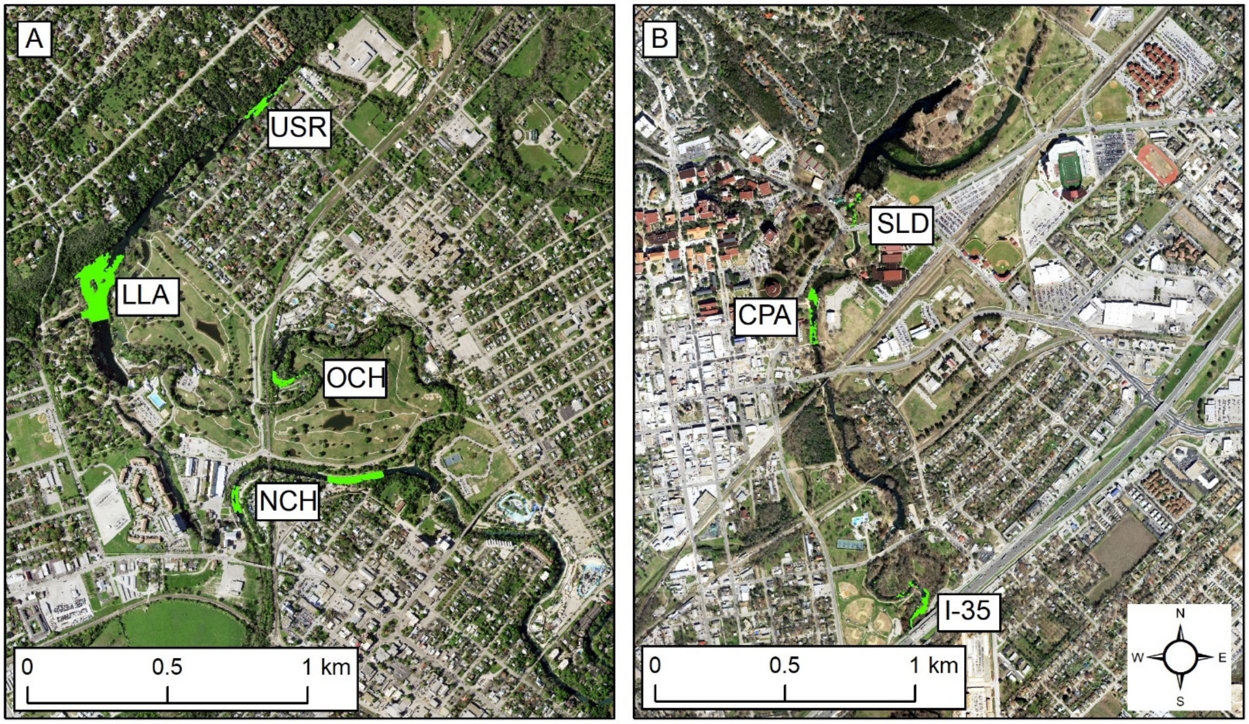


Figure 19. Locations of Fountain Darter drop netting and random and fixed dip netting in (A) Comal Springs and (B) San Marcos Springs ecosystems. Visual observations are also conducted in Landa Lake.

Table 22. Sampling reaches on the Comal and San Marcos rivers, years included during sampling, and number of drop nets set during routine monitoring, low-flow conditions, and high-flow conditions.

River and Reach		Years	Routine	Low-Flow	High-Flow	Not Defined
Comal River						
	Upper Spring Run	2000-2015	222	36	22	8
	Landa Lake	2000-2015	361	56	42	18
	Old Channel	2000-2015	195	32	18	38
	New Channel	2000-2004; 2014-2015	54	19	9	1
San Marcos River						
	Spring Lake Dam	2012-2015	45	0	13	0
	City Park	2000-2015	288	48	25	0
	IH-35	2000-2015	285	47	25	1

Table 23. Scientific names of organisms (fish and invertebrates) collected during drop net sampling and the total number of occurrences in the Comal and San Marcos river systems.

Organism	Occurrence	Organism	Occurrence
Ambloplites rupestris	741	*Marisa cornuarietis*	475
Ameiurus melas	2	*Marisa cornuarietis* egg mass	6
Ameiurus natalis	266	*Micropterus punctulatus*	3
Astyanax mexicanus	604	*Micropterus salmoides*	998
Campostoma anomalum	4	*Micropterus sp.*	4
Centrarchid sp.	4	*Moxostoma congestum*	2
Cyprinella venusta	6	*Notropis amabilis*	617
Dionda nigrotaeniata	1229	*Notropis chalybaeus*	131
Etheostoma fonticola	30515	*Notropis sp.*	6
Etheostoma lepidum	70	*Notropis volucellus*	38
Gambusia sp.	178243	*Noturus sp.*	4
Herichthys cyanoguttatus	924	*Oreochromis aureus*	93
Hypostomus plecostomus	141	*Palaemonetes sp.*	1162
Lepisosteus sp.	1	*Percina apristis*	24
Lepomis auritus	237	*Percina carbonaria*	1
Lepomis cyanellus	39	*Pimephales vigilax*	4
Lepomis gulosus	85	*Poecilia latipinna*	4535
Lepomis macrochirus	317	*Poecilia sp.*	516

Lepomis megalotis	286	*Procambarus sp.*	1582
Lepomis microlophus	4		
Lepomis miniatus	3816		
Lepomis sp.	1235		

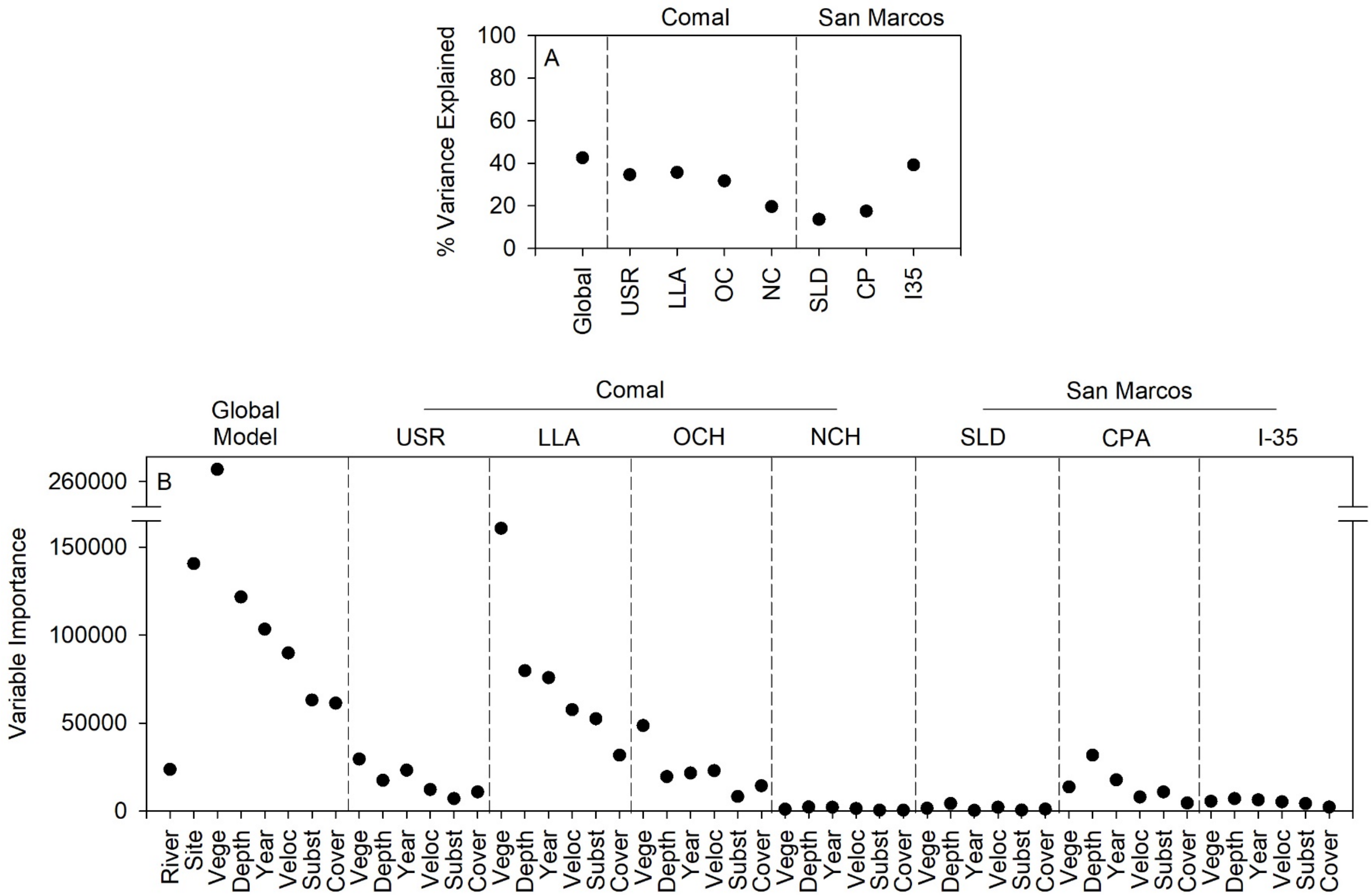


Figure 20. (A) Random forest model performance and (B) variable importance for Fountain Darter abundance in drop net sampling based on all sites combined (global) and individual site models in the Comal Springs and San Marcos Springs systems. See Figure 19 for locations of sampling sites.

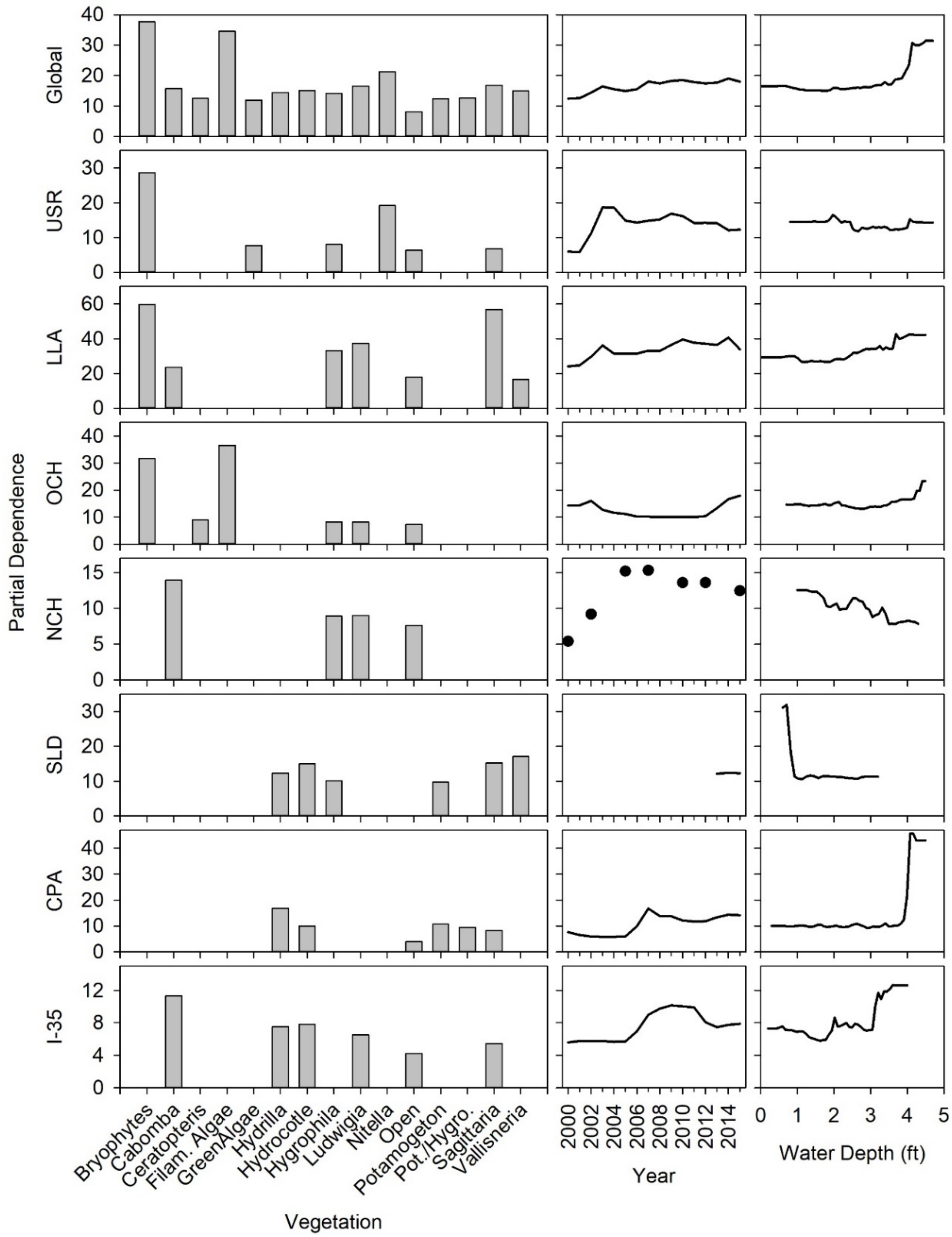


Figure 21. Partial dependence plots illustrating the effect of dominant vegetation type, year of sampling, and water depth within drop nets on the predicted abundance of Fountain Darter in the Comal Springs and San Marcos Springs systems. Data were collected discontinuously through time at the NCH site; see Figure 19 for locations of sampling sites.

Dip Net Sampling – Over the years, Fountain Darter dip net sampling has been conducted within random, fixed, and timed categories. The random dip netting protocol consisted of randomly choosing up to 50 sample points within the same sites used for drop net sampling and repeating four dips per location using a 40 cm by 40 cm net with 1.6 mm mesh. The random dip netting dataset contains 3,221 observations with 15 measured variables (Table 24), and samples were collected at seven locations during 2006-2015, and an eighth site was added in 2013 (Table 25). Dip net sampling was temporally distributed consistent with drop net sampling. Fixed dip netting was initiated in 2014 at the same sampling sites as drop netting and random dip netting resulting in 899 observations. The same environmental variables are measured for fixed and random dip netting, but substrate is classified for fixed dip net sampling.

Random Forest models were fit to random and fixed dip net sampling dataset using the same protocol as described for drop net sampling, except that here classification (i.e., Fountain Darter present or absent) was used instead of regression. The switch from regression to classification also meant that model performance was measured by the area under the curve (AUC) rather than percent of variance explained. The AUC metric ranges 0.5-1.0 with values near 0.5 representing essentially random explanatory power and values near 1.0 represent perfect model fit. Hosmer and Lemeshow (2000) suggest AUC values ranging 0.5-0.7 represent poorly performing models with near-random predictive ability, and models with 0.7-0.8, 0.8-0.9, and >0.9 have, "acceptable", "excellent", and "outstanding" predictive power, respectively. For random and fixed dip netting, dominant vegetation type, presence or absence of bryophytes, presence or absence of algae, and substrate classification (fixed dip net sampling only) were used as predictor variables. An initial global model including spring system and site identifiers was constructed for random and fixed datasets, and then site-specific models were fit independently. Variable importance was ranked and partial dependence plots were generated as with the drop net sampling analysis.

Random forest models explained intermediate levels of variance in Fountain Darter occurrence in random dip net samples. The global model AUC value was 0.77, site-specific models in the Comal Springs system ranged 0.70-0.76, and site-specific models in the San Marcos Springs system ranged 0.57-0.74. All models provided acceptable predictive power with the exception of the City Park site in the San Marcos Springs system (Figure 22A). In the global model, dominant vegetation type, site location, and year of sampling were the most important predictor variables for occurrence of Fountain Darter in random dip net sampling, and in site-specific models dominant vegetation and year of sampling were typically most important (Figure 23A). Partial dependence plots illustrate relatively strong but taxonomically inconsistent partial dependence of Fountain Darter occurrence on dominant vegetation type (Figure 24). When plotted against year of collection, Fountain Darter occurrence declined after 2013 in the global model, was largely consistent in the Comal Springs system, and declined slightly through time in the San Marcos Springs system (Figure 25).

Random forest model performance ranged from excellent to essentially random in predicting the occurrence of Fountain Dater in fixed dip net sampling. The global model AUC value was 0.80, site-specific models in the Comal Springs system ranged 0.66-0.80, and site-specific models in the San Marcos Springs system ranged 0.59-0.80 (Figure 22B). In the global model, dominant vegetation, presence of bryophytes, and site location were ranked as most important in predicting Fountain Darter occurrence in fixed dip net samples, and in site-specific models dominant vegetation and substrate classes were typically most important (Figure 23B). Fountain darter abundance was much less dependent on site location than for random dip net samples, a result consistent with the underlying sampling protocols. New random dip net sample sites were chosen for each event, without respect to vegetation or any other habitat features. Fixed dip net sites were initially randomly selected, within vegetation, and the same sites were sampled continuously. Partial dependence plots illustrated relatively strong partial dependence of Fountain Darter occurrence on dominant vegetation type, but there was no apparent consistency in the effect of particular vegetation types (Figure 26). When plotted again substrate type, Fountain Darter occurrence in fixed site dip netting was negatively partially dependent on silt in the global model and at four of five site-specific models (Figure 27).

In summary, the ability of local spatial and environmental variables to predict Fountain Dater occurrence in random and fixed dip netting sampling ranged from essentially random to outstanding, depending upon the sampling site. The generally excellent or near-excellent explanatory ability of the global models suggests broader-scale factors might be involved with regulating Fountain Darter occurrence. Differential detection of Fountain Darters in different vegetation types may be an underlying factor confounding the results of within-site as well as global models of abundance, and could be investigated using open population repeated count models. As with drop net sampling, there is potential for including water quantity (e.g., flow regime components), water quality (e.g., temperature regimes), and broad-scale (i.e., entire mapped areas) submerged aquatic vegetation data with dip net sampling data to assess factors correlated with abundances (see Future Research and Hypothesis Development section).

Table 24. Variables, descriptions, and number of missing values (NA) in Fountain Darter random dip net dataset with 3,221 observations.

Variable	Description	NAs
Date	Date sample was collected	0
River	Identifier for Comal or San Marcos rivers	0
Location	Reach from which sample was collected	0
Site	Short name for river reach names	0
Biowest_identifier	Sample identifier used for linking samples to habitat maps	0
Vegetation	Vegetation type from which sample was collected	0
BryPresent	Binary for presence or absence of bryophytes	0
AlgaePresent	Binary for presence or absence of algae	0
Dip1	Binary for presence or absence of Fountain Darter in first dip	0
Dip2	Binary for presence or absence of Fountain Darter in second dip	0
Dip3	Binary for presence or absence of Fountain Darter in third dip	0
Dip4	Binary for presence or absence of Fountain Darter in fourth dip	0
Overall	Binary for presence or absence of Fountain Darter across all dips	0
Time	Time (HH:MM) of survey	2336
Notes	Comment on sampling	2362

Table 25. River system, site, and number of random dip net collections during each year of sampling during 2006-2015. The Spring Lake site was added in 2013.

Location	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Comal River										
Upper Spring Run	12	12	12	18	18	24	12	26	25	25
Landa Lake	44	44	44	66	66	66	44	102	100	100
New Channel	12	12	12	18	18	18	12	26	25	25
Old Channel	32	32	32	48	48	48	32	96	100	100
San Marcos River										
Spring Lake	-	-	-	-	-	-	-	20	30	50
Spring Lake Dam	57	28	28	70	28	42	28	44	65	76
City Park	88	44	44	88	44	66	44	62	40	100
IH-35 Crossing	56	28	28	70	28	42	28	44	30	75

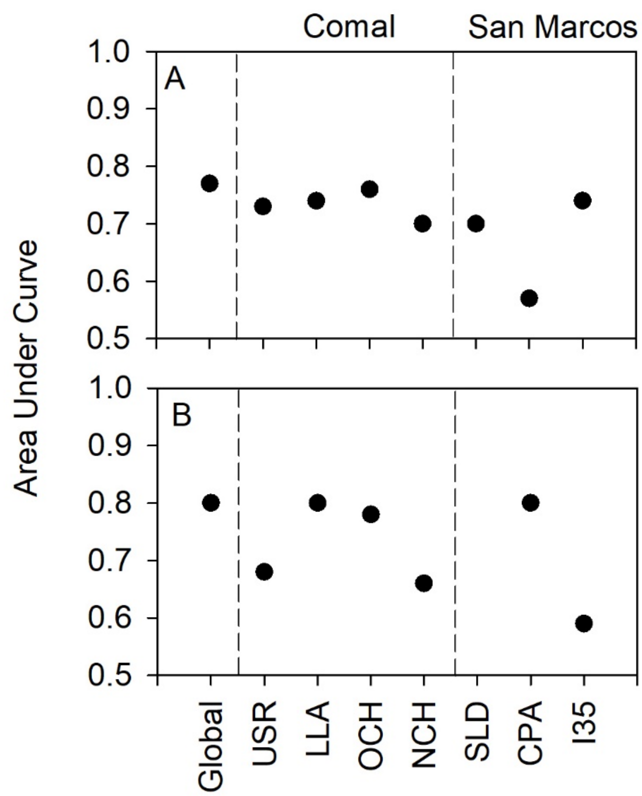


Figure 22. Random forest model performance (measured as area under the curve) for Fountain Darter occurrence (presence or absence) in (A) random and (B) fixed dip net sampling for models fit to all sites combined (global) and sites independently in the Comal and San Marcos river systems. Fixed dip net sampling was not conducted at SLD in the San Marcos River system. See Figure 19 for locations of sampling sites.

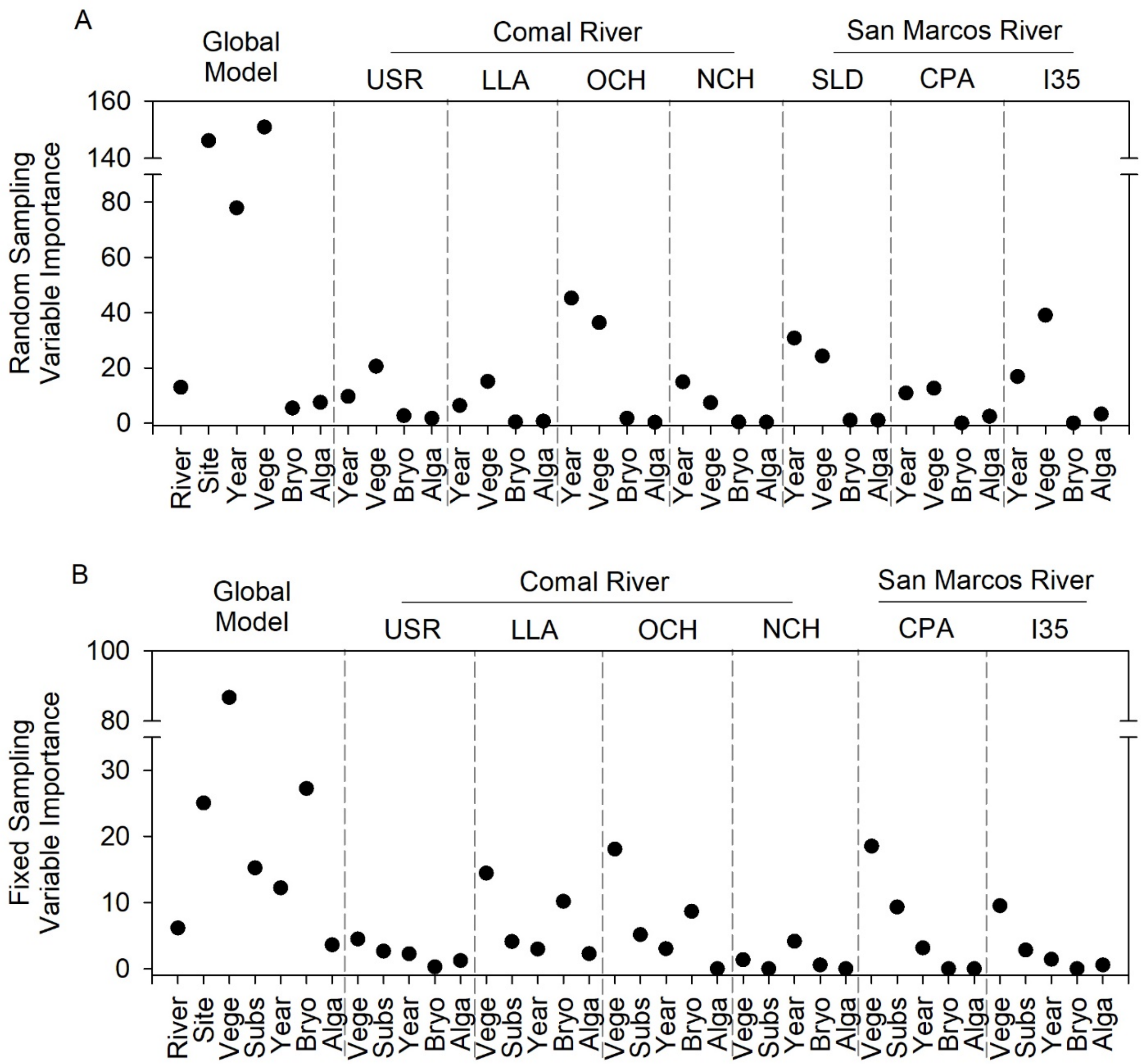


Figure 23. Variable importance for environmental parameters used to predict occurrence of Fountain Darter in (A) random and (B) fixed dip net sampling in the Comal and San Marcos River systems. Models were fit to all sites combined (global) and then each site independently. See Figure 19 for locations of sampling sites.

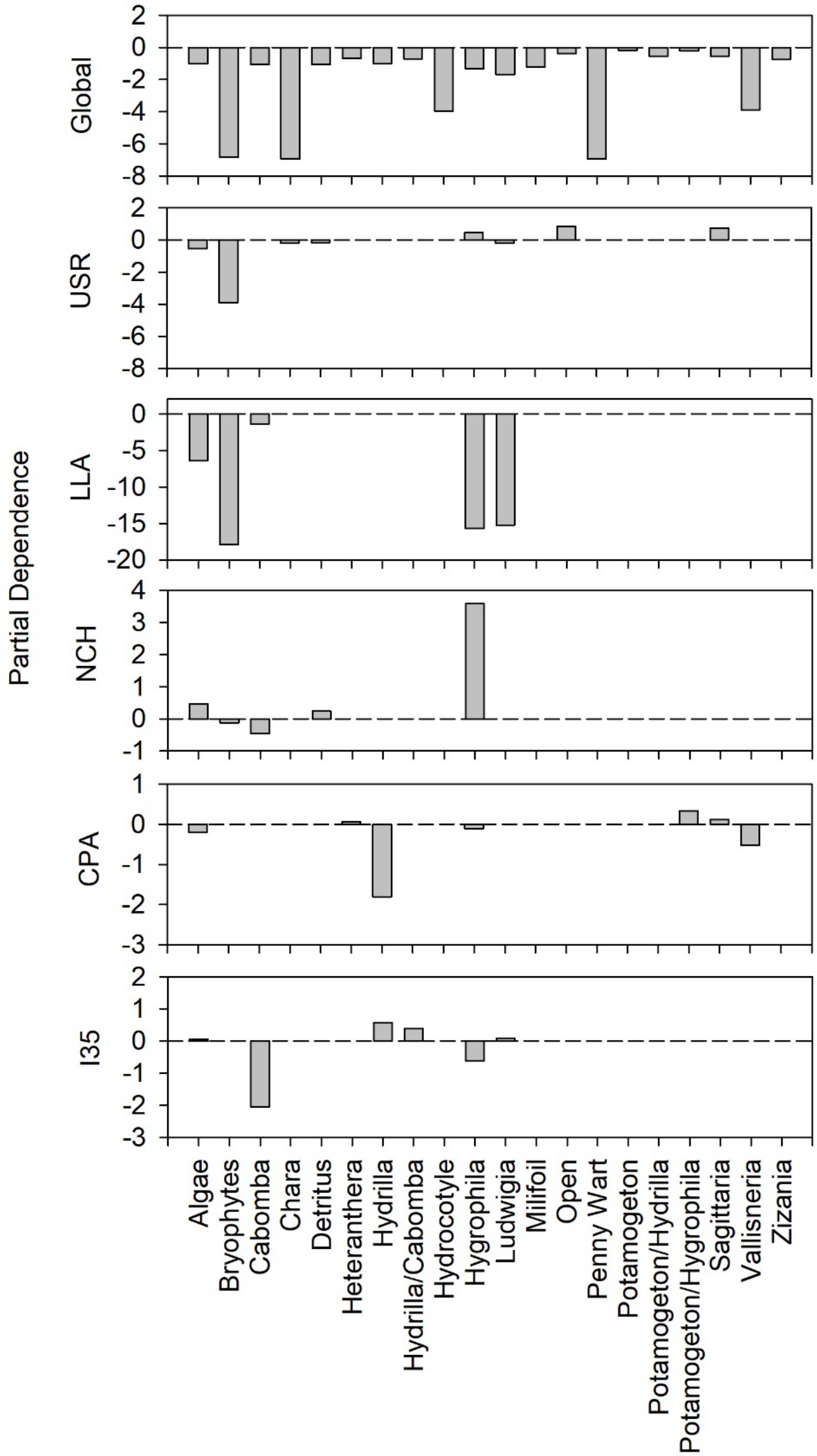


Figure 24. Partial dependence of Fountain Darter occurrence on vegetation type in random dip netting surveys for random forest models fit to all sites combined (global) and each site independently. Positive partial dependence values denote increased occurrence. See Figure 19 for locations of sampling sites.

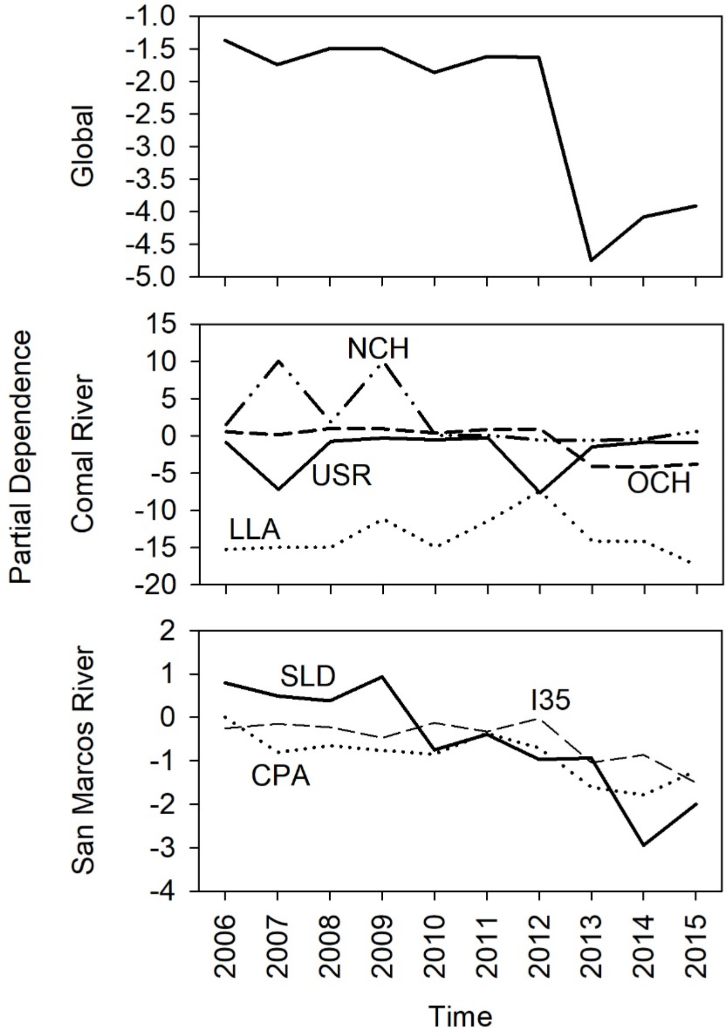


Figure 25. Partial dependence of Fountain Darter occurrence on time in random dip netting surveys for random forest models fit to all sites combined (global) and each site independently. Positive partial dependence values denote increased occurrence. See Figure 19 for locations of sampling sites.

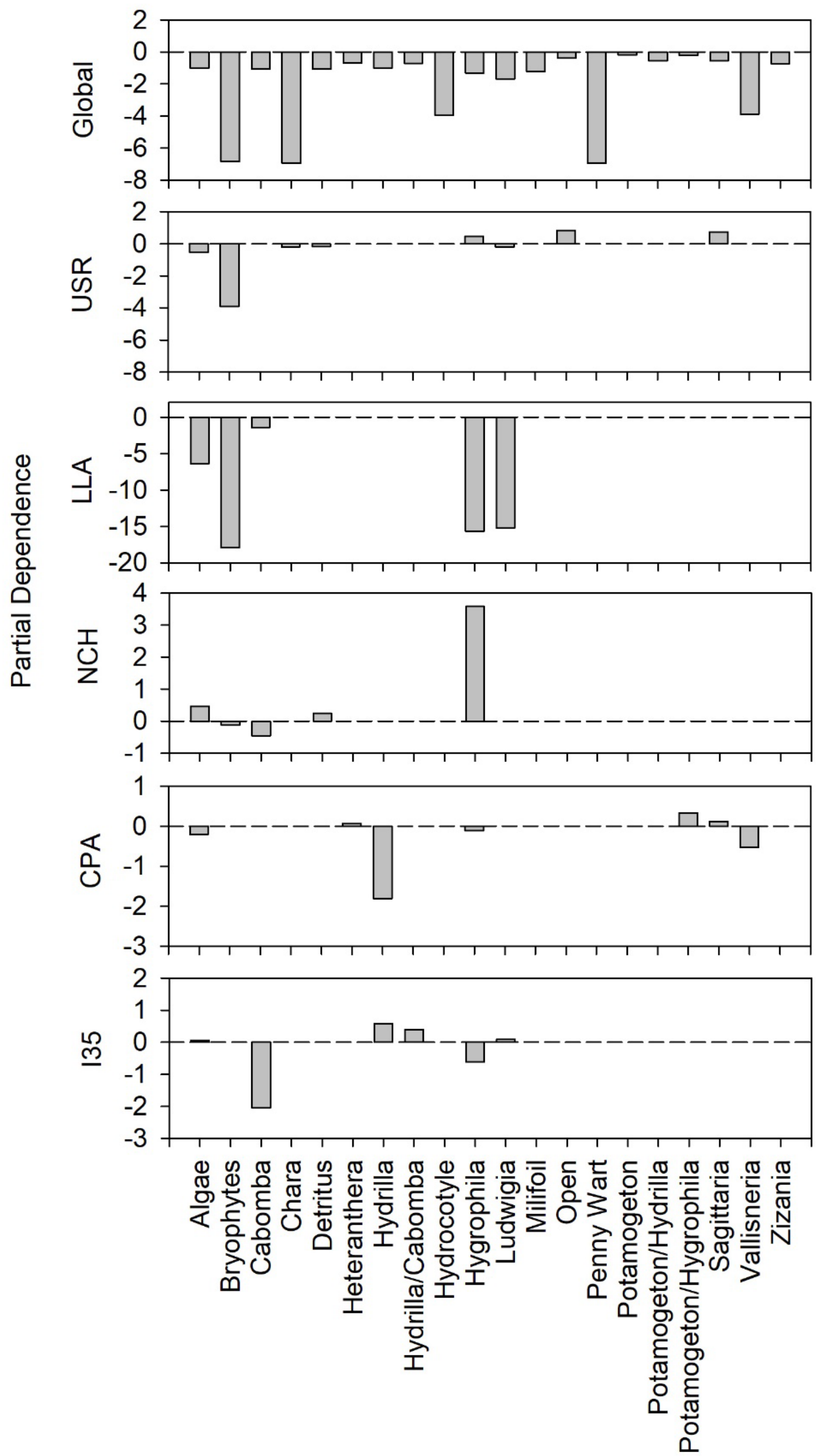


Figure 26. Partial dependence of Fountain Darter occurrence on vegetation type in fixed dip netting surveys for random forest models fit to all sites combined (global) and each site independently. Positive partial dependence values denote increased occurrence. See Figure 19 for locations of sampling sites.

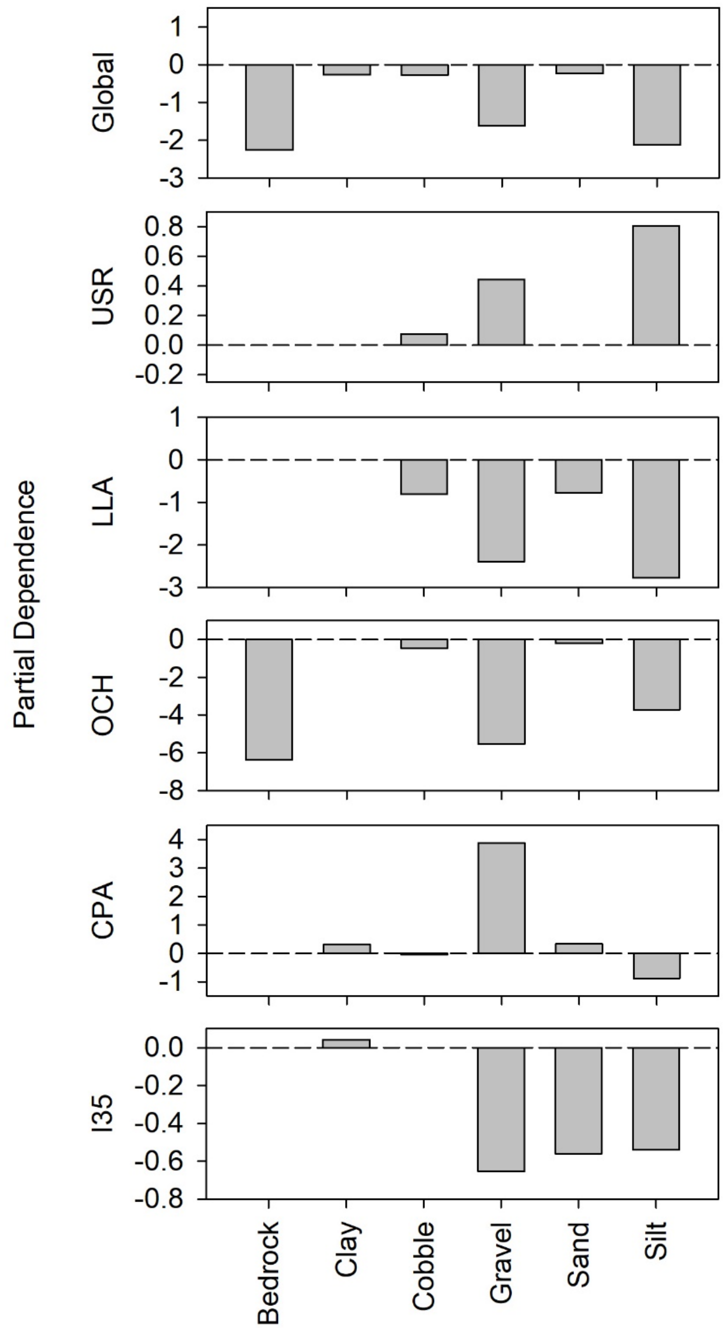


Figure 27. Partial dependence of Fountain Darter occurrence on substrate type in fixed dip netting surveys for random forest models fit to all sites combined (global) and each site independently. Positive partial dependence values denote increased occurrence. See Figure 19 for locations of sampling sites.

Time Dip Netting-Timed dip net surveys were conducted at 10 sites total, six in Comal Springs system and four in San Marcos Springs system (Figure 28) All sites were sampled at least twice annually during 2000-2015, except for Todd Island in the San Marcos Springs system that was added in 2009. Each Fountain Darter that is encountered is measured for total length (mm) as a means of tracking size distributions over space and time. Ridgeline plots were fit to length data using the function ‘ggridges’ enabled through the ‘ggplot2’ Package in Program R (Wickham 2009). These plots were used to compare length frequency distributions expressed as density functions among sites and through time for each site.

Ridgeline plots fit to sites within each spring system illustrated longitudinal variation in the size structure of Fountain Darters. In the Comal Springs system, the proportion of larger fish relative to smaller fish increased in a downstream direction as a unimodal distribution transformed into a bimodal distribution (Figure 29). In the San Marcos Springs system, a similar longitudinal pattern in size structure was apparent, including a unimodal distribution of sizes in Spring Lake but an increasingly bimodal distribution at down sites (Figure 30). Across years at individual sites in the Comal Springs system, ridgeline plots illustrated inter-annual fluctuations in size distributions through time for the Upper Spring Run (Figure 31), Spring Island (Figure 32), Landa Lake (Figure 33), Old Channel (Figure 34), New Channel (Figure 35), and Other Place (Figure 36). Across years at individual sites in the San Marcos Springs system, ridgeline plots illustrated inter-annual fluctuations in size structure at Hotel (Figure 37), City Park (Figure 38), I35 (Figure 39) and Todd Island (Figure 40) sites.

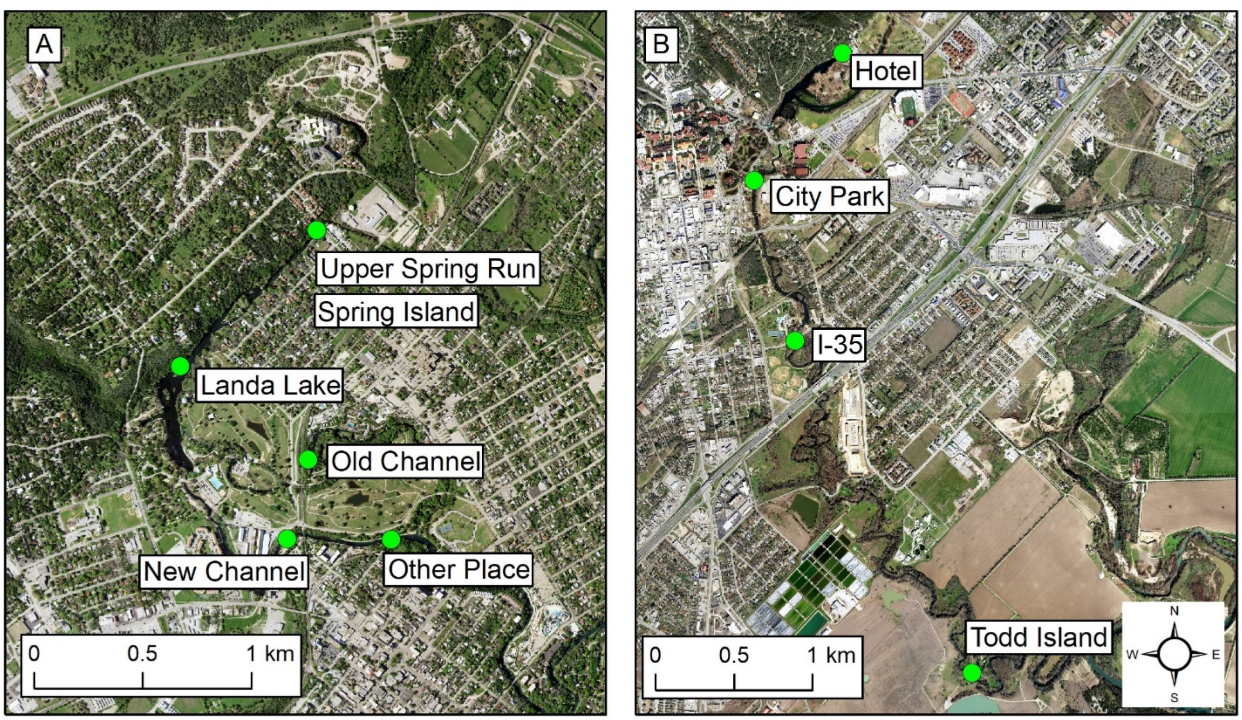


Figure 28. Locations of timed dip net sampling in (A) Comal Springs and (B) San Marcos Springs systems.

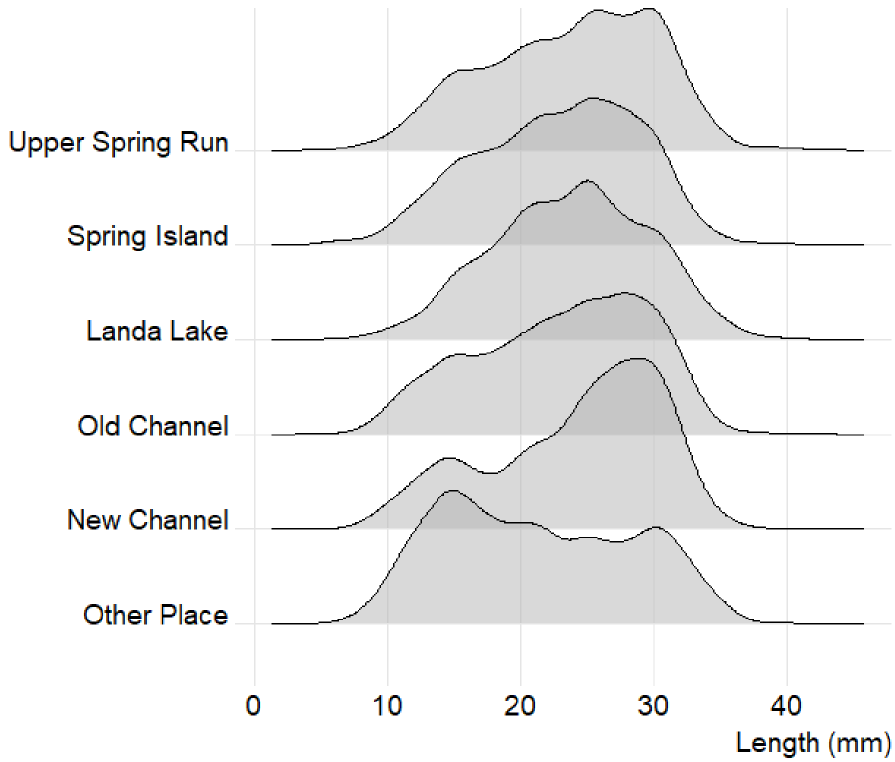


Figure 29. Ridgeline plot of longitudinal changes in Fountain Darter total length measured during timed dip net surveys at sites in the Comal Springs system. See figure 28 for site locations.

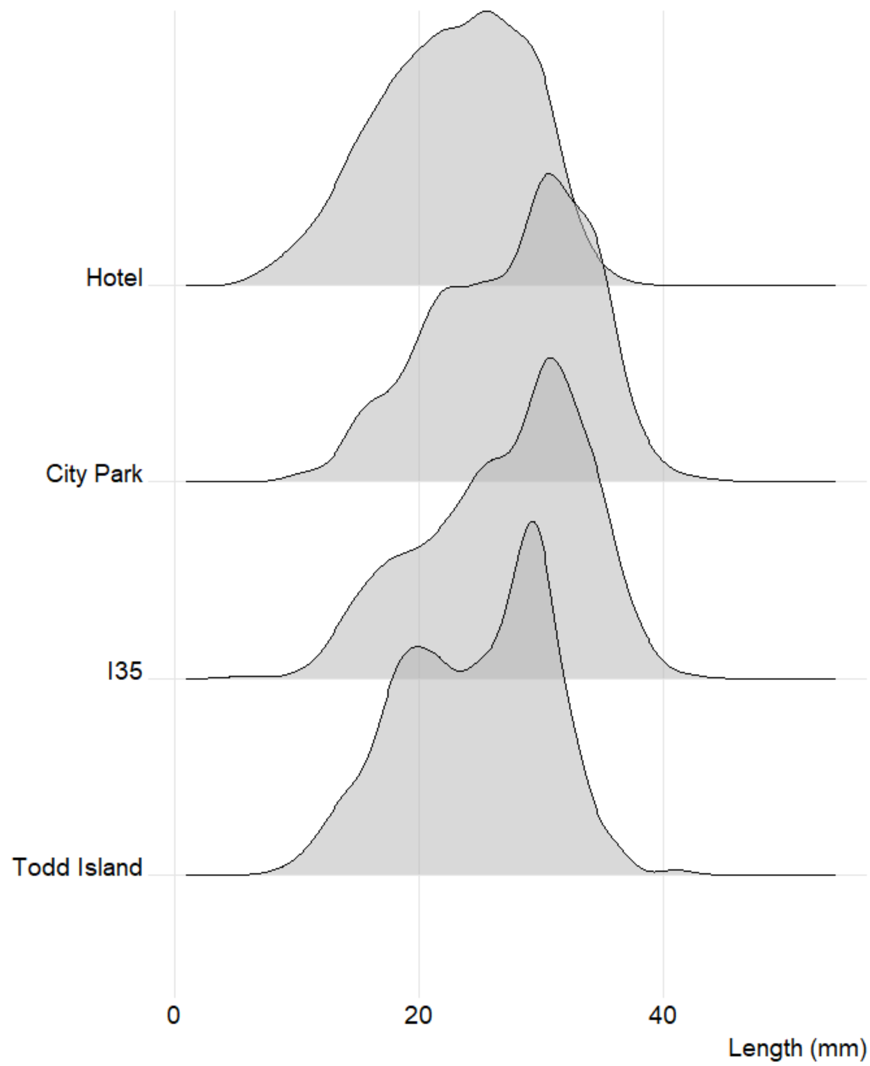


Figure 30. Ridgeline plot of longitudinal changes in Fountain Darter total length measured during timed dip net surveys at sites in the San Marcos Springs system. See figure 28 for site locations.

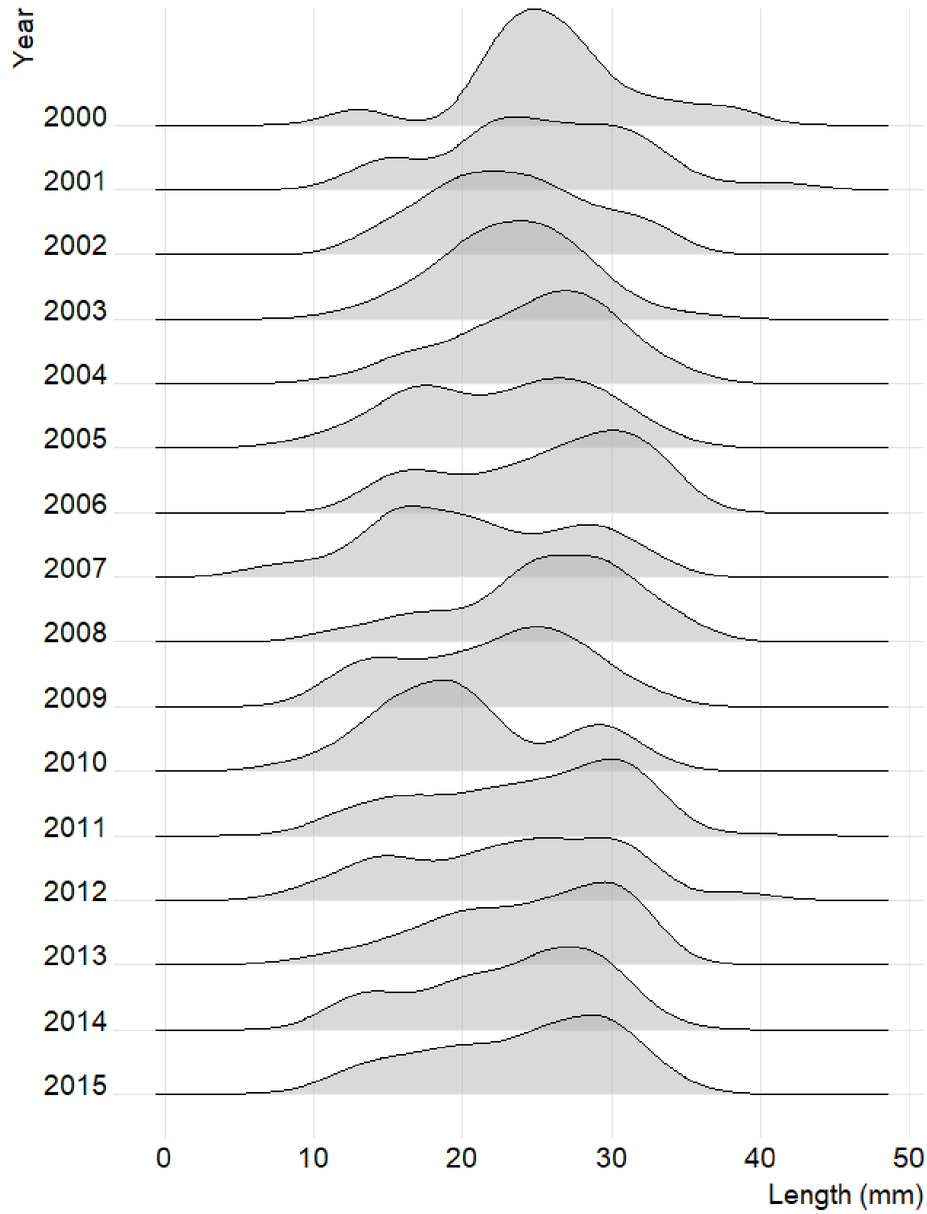


Figure 31. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Upper Spring Run site in the Comal Springs system during 2000-2015.

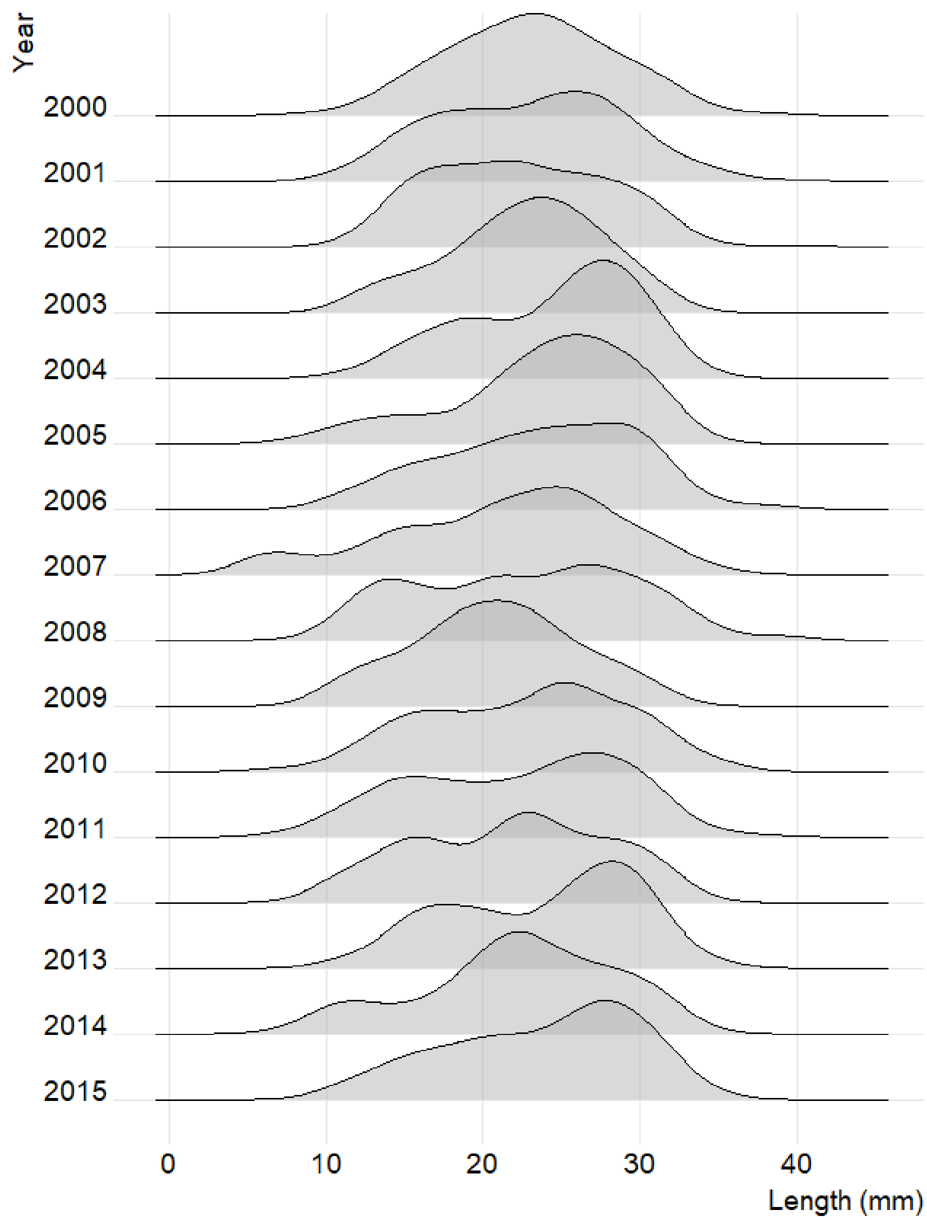


Figure 32. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Spring Island site in the Comal Springs system during 2000-2015.

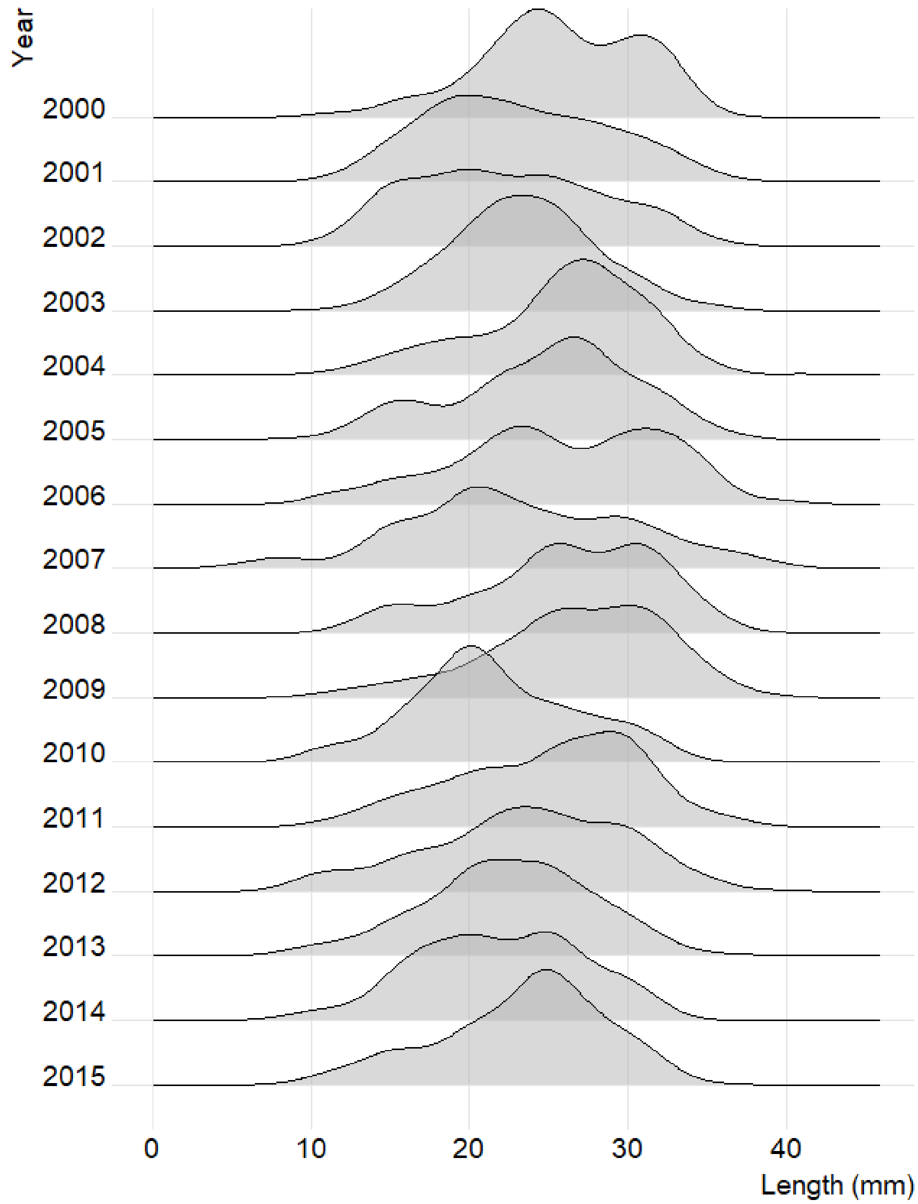


Figure 33. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Landa Lake site in the Comal Springs system during 2000-2015.

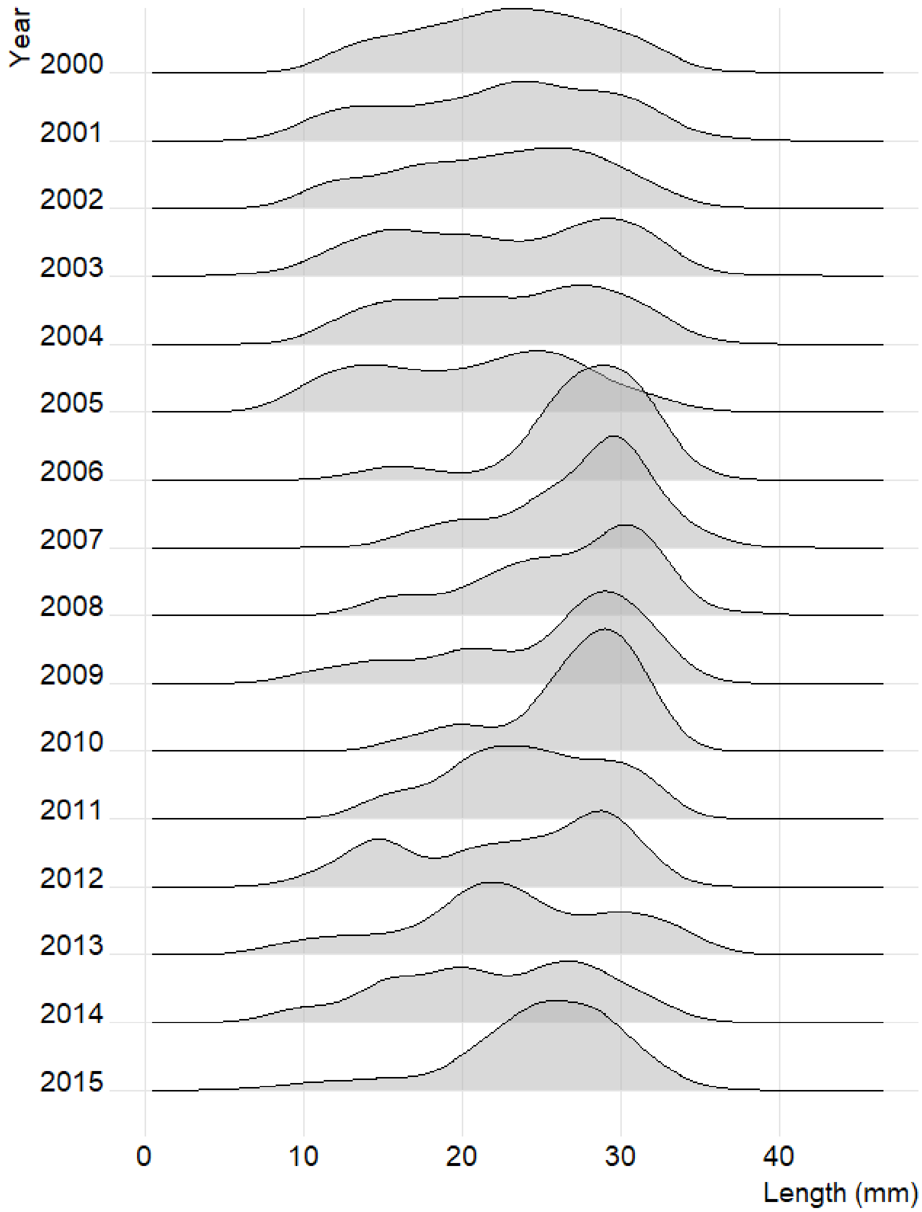


Figure 34. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Old Channel site in the Comal Springs system during 2000-2015.

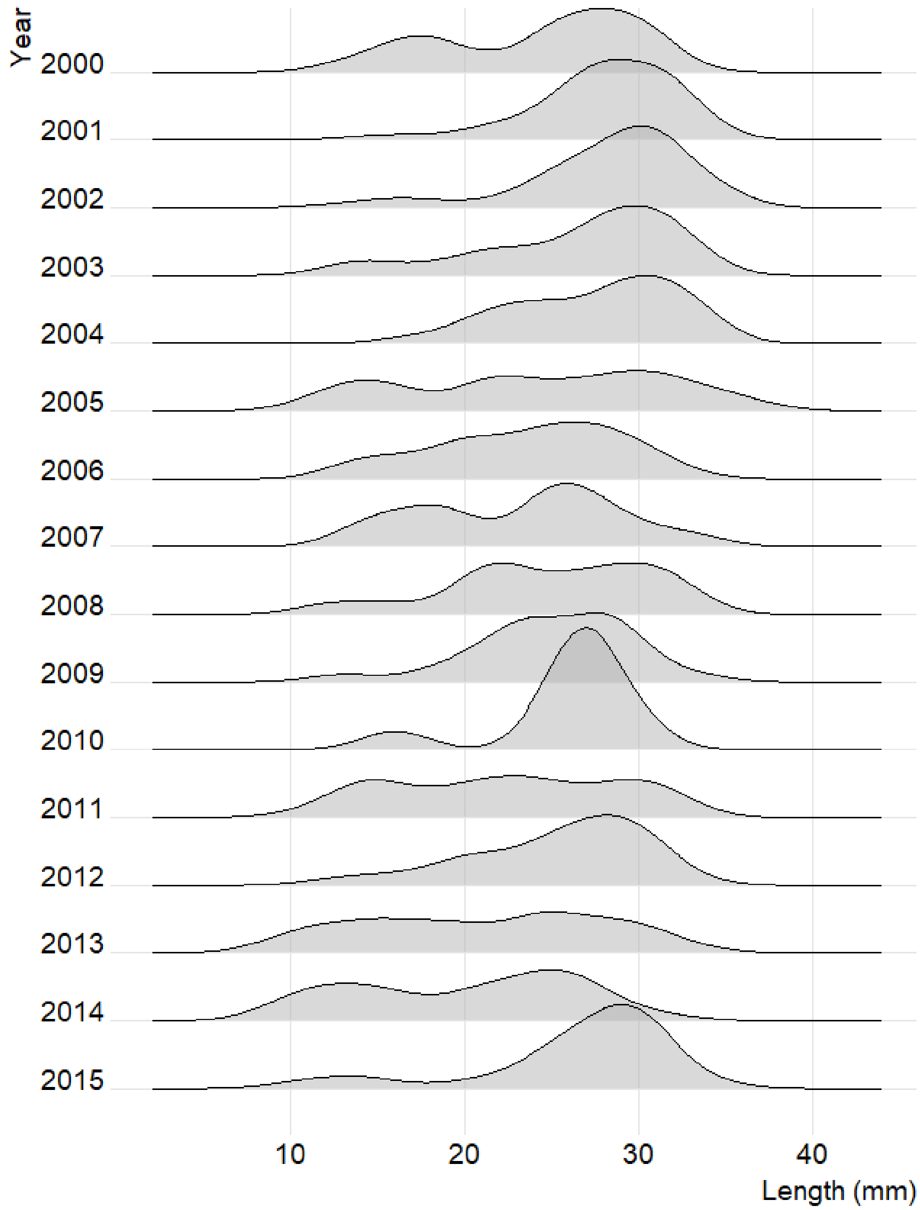


Figure 35. Ridgeline plot of temporal changes in Fountain Darter length distributions at the New Channel site in the Comal Springs system during 2000-2015.

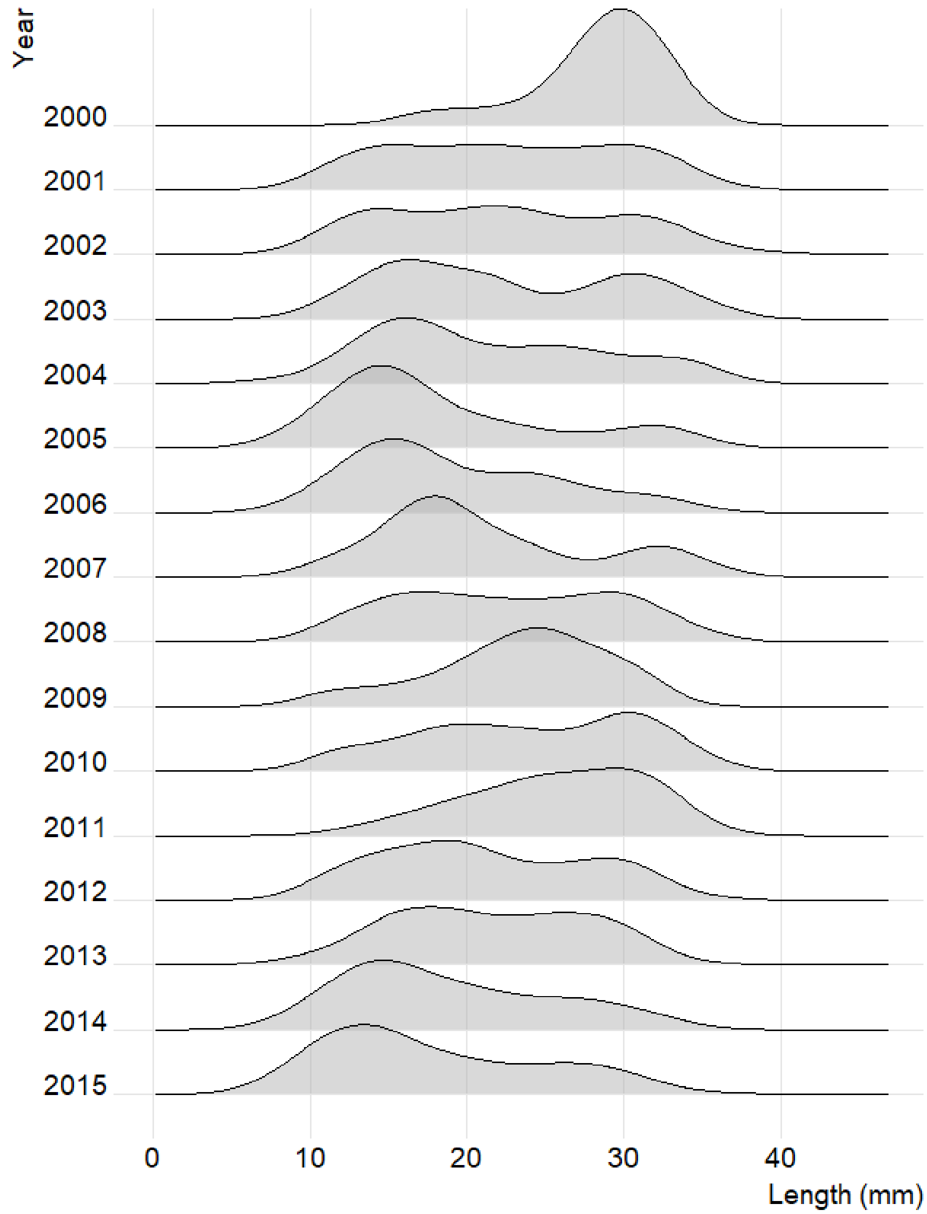


Figure 36. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Other Place site in the Comal Springs system during 2000-2015.

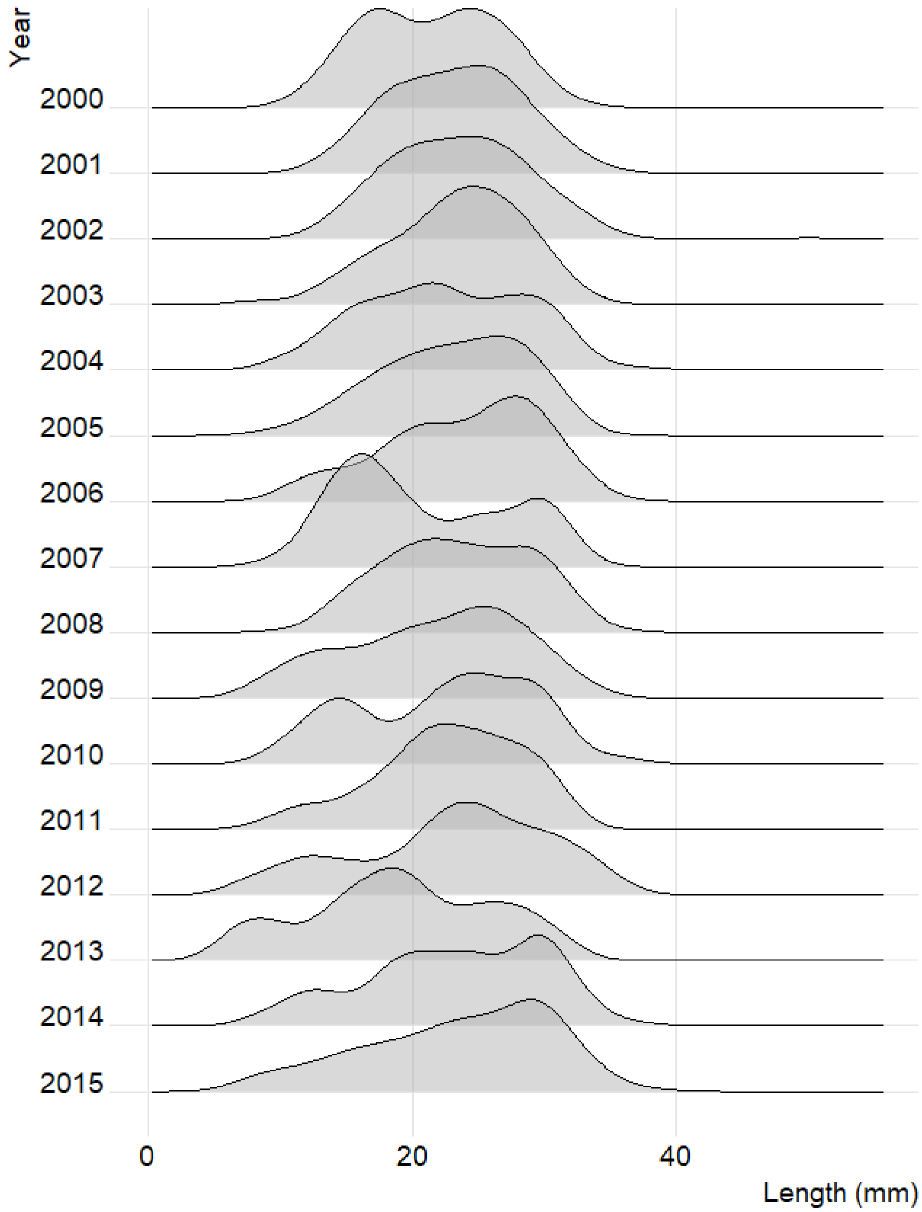


Figure 37. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Hotel site in the San Marcos Springs system during 2000-2015.

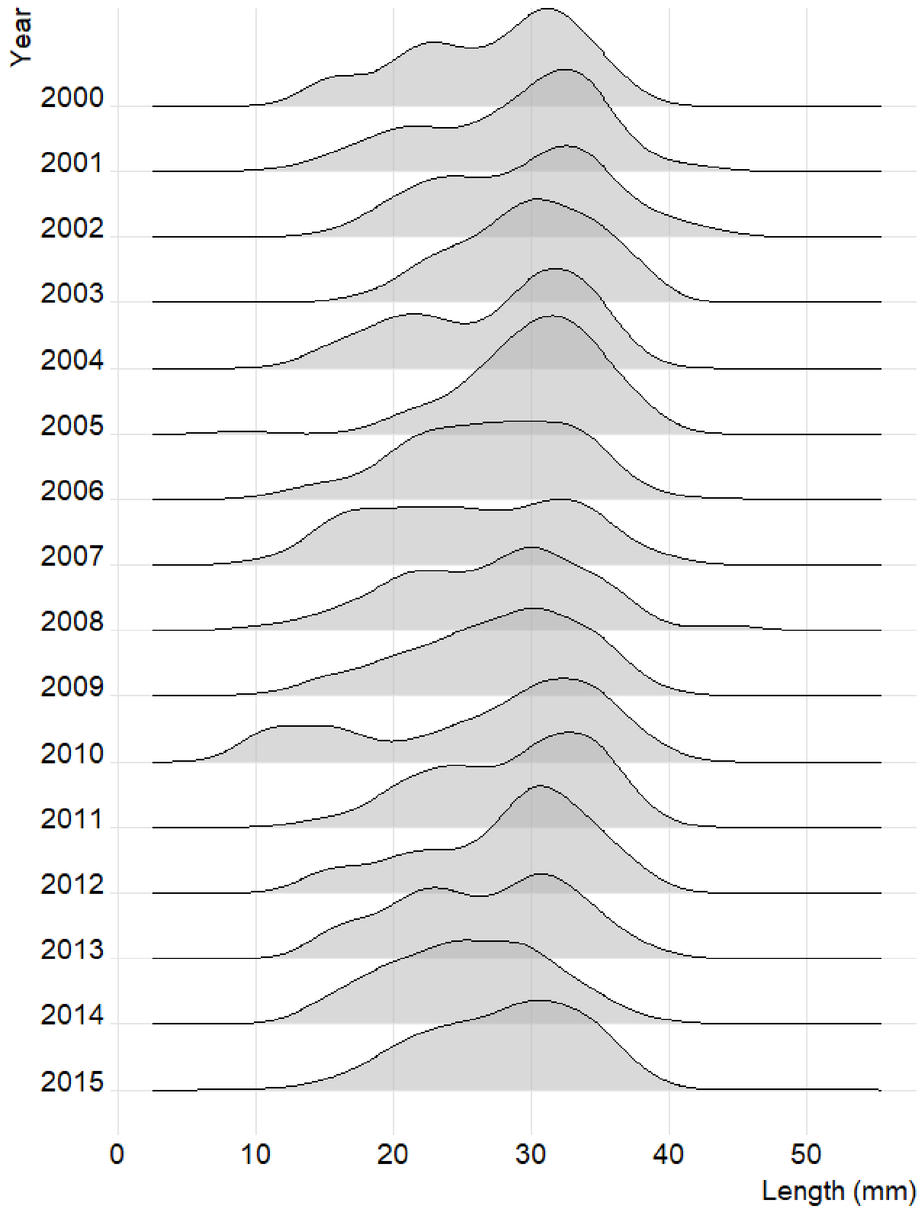


Figure 38. Ridgeline plot of temporal changes in Fountain Darter length distributions at the City Park site in the San Marcos Springs system during 2000-2015.

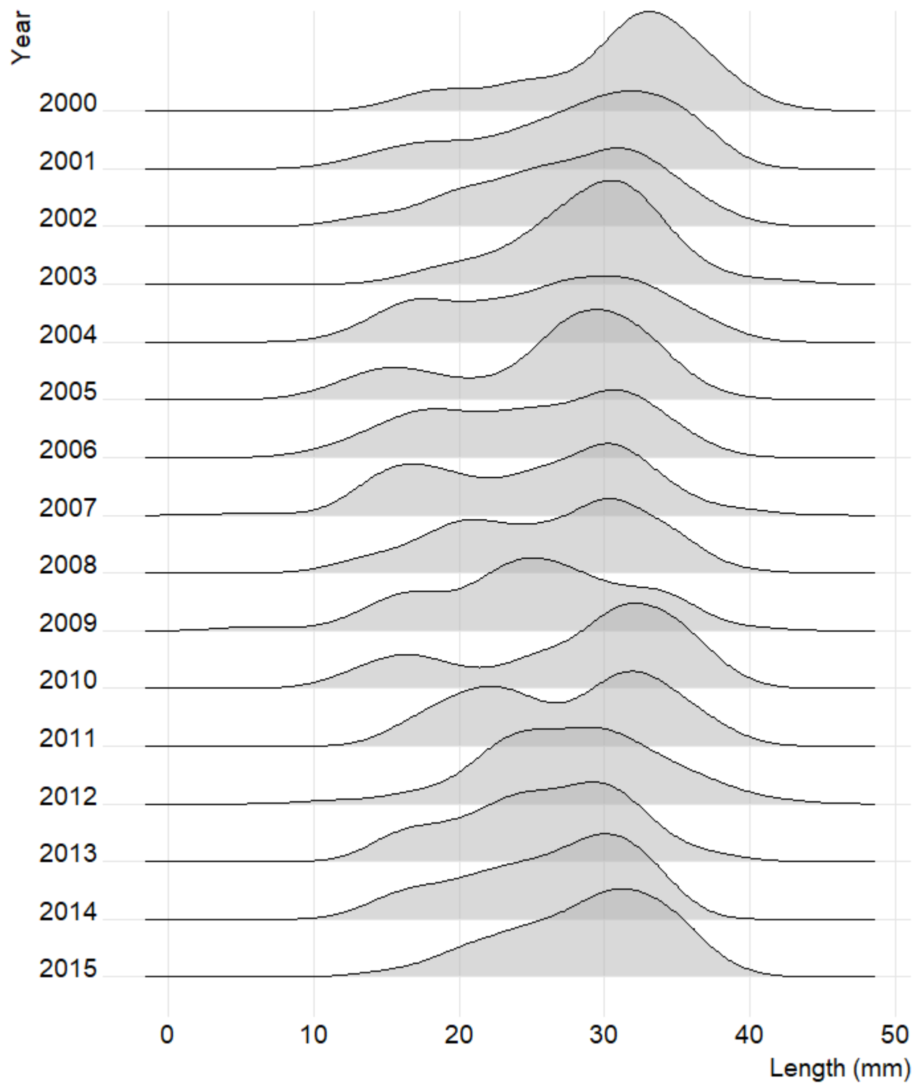


Figure 39. Ridgeline plot of temporal changes in Fountain Darter length distributions at the I-35 site in the San Marcos Springs system during 2000-2015.

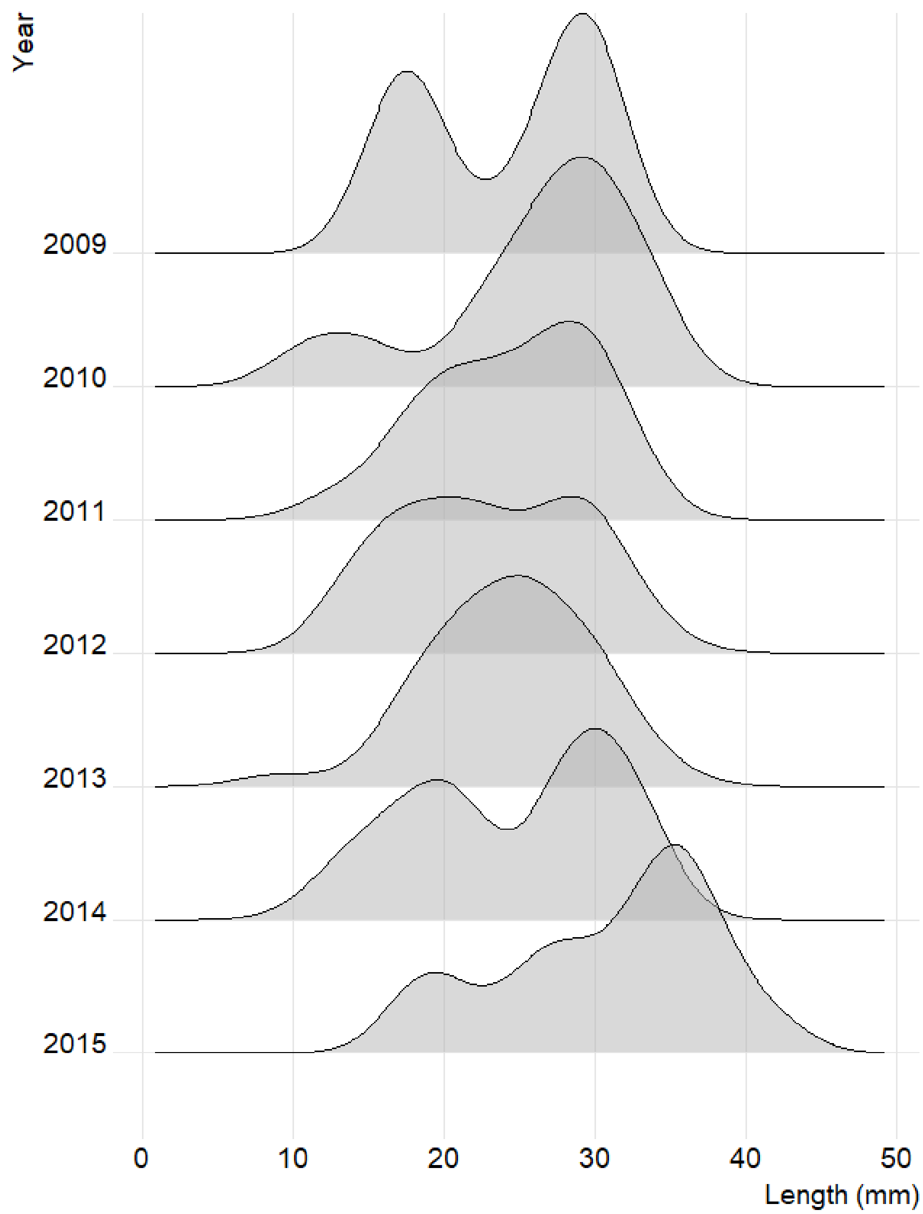


Figure 40. Ridgeline plot of temporal changes in Fountain Darter length distributions at the Todd Island site in the San Marcos Springs system during 2009-2015.

Visual Observations – Visual observations of Fountain Darter abundance were conducted during the same periods as drop and dip net surveys in 2001-2015 using self-contained underwater breathing apparatus (SCUBA) gear in Landa Lake. A transect from the upper to the lower reaches of Landa Lake was followed and the number of darters observed was noted. Additionally, the total percent of the area surveyed that was covered by submerged aquatic vegetation was visually estimated. Figure 41A illustrates a time series of darter numbers (black points and line) and percent vegetative coverage (gray points and line), and Figure 41B illustrates the correlation between vegetative coverage and darter observations made through time.

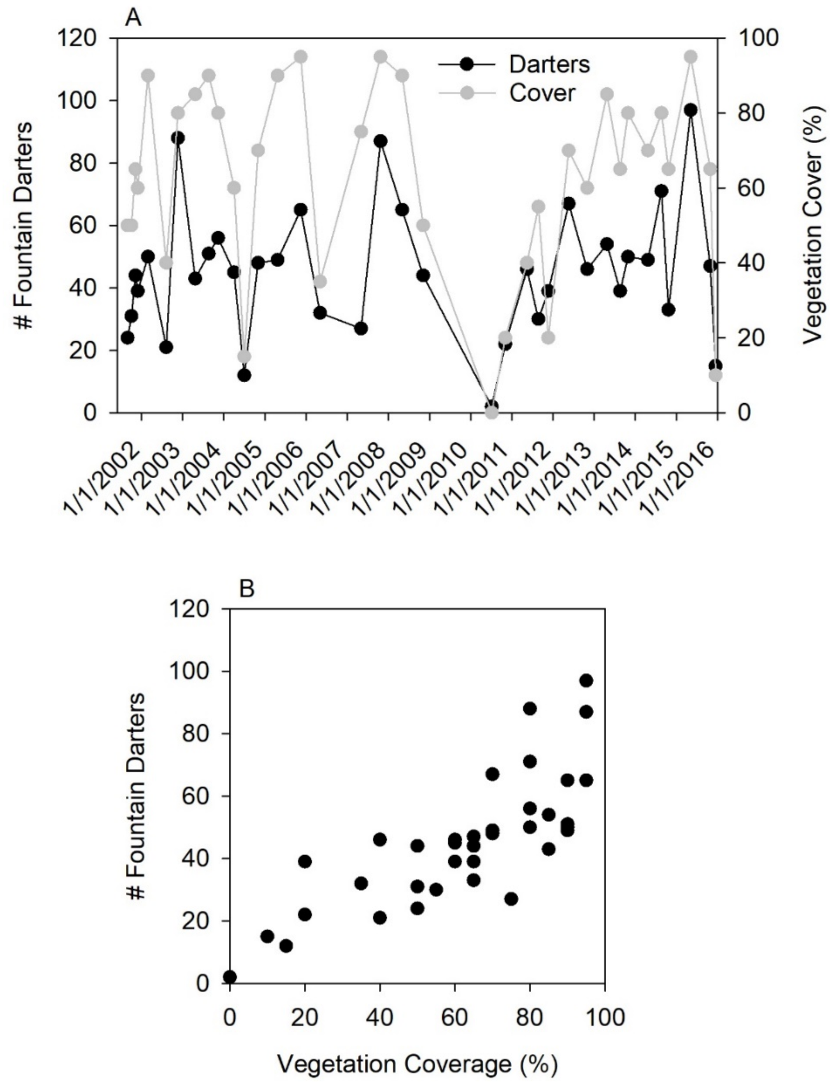


Figure 41. (A) Relationship between time and number of Fountain Darters observed (black line) and percent of the observation area covered by vegetation (gray line) in Landa Lake during 2001-2015. (B) Relationship between percent of vegetation coverage and number of Fountain Darters observed.

Macroinvertebrate Sampling

Macroinvertebrates are one of the most used groups of taxa for biological monitoring (Metcalfe 1989, Barbour et al. 1999, Wright et al. 2000) because they are ubiquitous, diverse, and there is an accepted working knowledge of their taxonomy with adaptable life-history information (Poff et al. 2006, Merritt et al. 2008). Macroinvertebrate sampling was originally incorporated into biomonitoring under the HCP as an investigation of prey abundance for Fountain Darters in different Fountain Darter habitat types. Samples were taken in spring and fall from 2013-2014 from 3 sites in the San Marcos River (City Park, I35, and Spring Lake Dam) and 4 from the Comal River (Landa Lake, New Channel, Old Channel, and Upper Spring Run). An ekman grab sample was taken from available vegetation at each site. Vegetation types included: Bryophytes, Cabomba, Hydrilla, Hygrophila, Ludwigia, Potamogeton, Sagittaria, and Vallisneria. Not all vegetation types existed at each site and sometimes only for various seasons or years. Although The objective of this analysis was to take a more detailed examination of these data to investigate macroinvertebrate community structures at San Marcos and Comal Springs.

Because sampling targeted vegetation types representative of Fountain Darter habitat at the same sites, analyses were performed to compare among sites by using vegetation types as replicates and among vegetation types using sites as replicates, separately. Non-metric multidimensional scaling (NMDS) was performed to see if there were any clustering patterns based on macroinvertebrate community structure. Because of seasonal effects on assemblage structure (Wiens 2002, Kosnicki and Sites 2011) years and seasons were kept separate.

One hundred ninety-one samples were processed for analysis across all three years, represented by 42 unique taxa. Stress for the NMDS results expressed as 2 dimensions ranged from 0.201-0.238 across all year and season combinations, separately, indicating that the ordinations may be somewhat arbitrary and that 3 dimensions may be more appropriate; however, we kept these analyses at 2 dimensions so that interpretations would be easier. Inspection of the NMDS pots did not show any clear associations of community assemblages within vegetation type, though the community structures of Hydrilla tended to be different than those in Bryophytes (Figs. 42-47). Inspection of sites ordinated within macroinvertebrate space showed more consistency within river system along the first dimension (x-axis), though there were some exceptions.

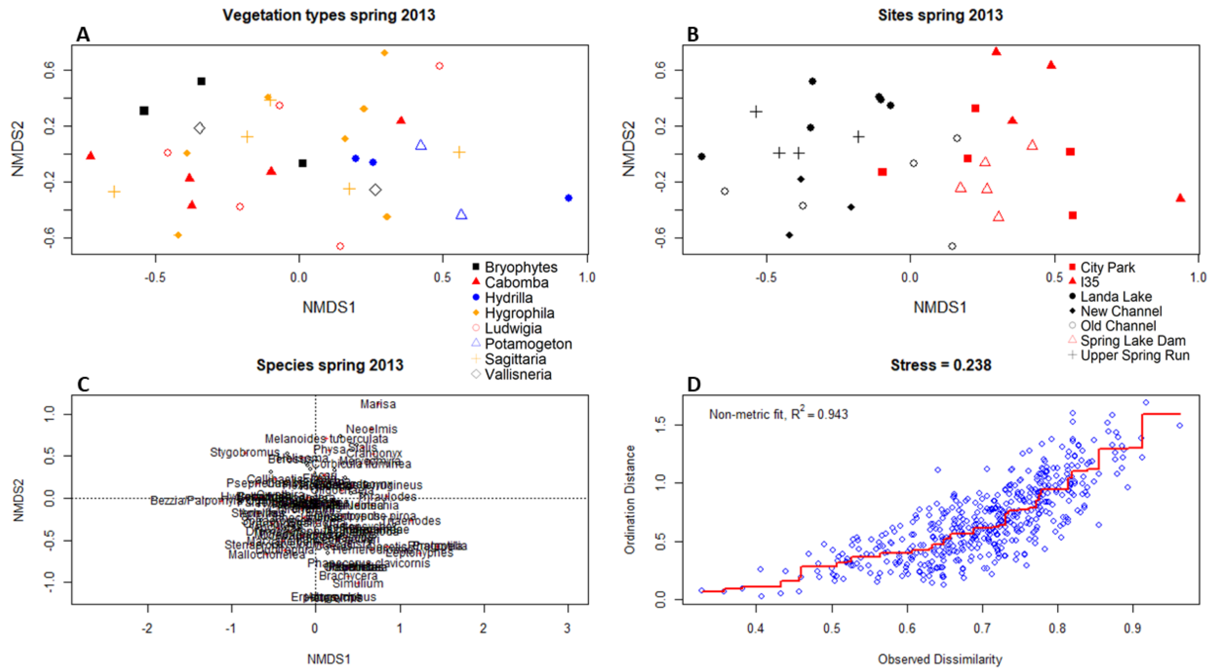


Figure 42. Non-metric multidimensional scaling for macroinvertebrate samples taken in spring of 2013 from sites on the San Marcos and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

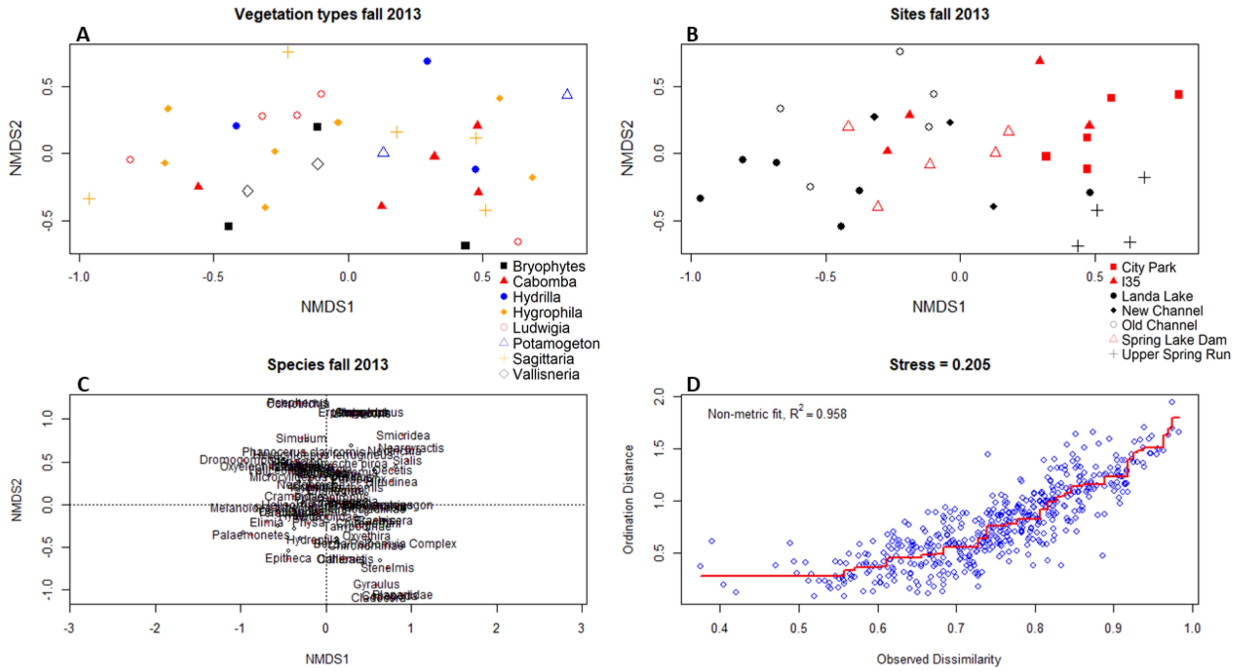


Figure 43. Non-metric multidimensional scaling for macroinvertebrate samples taken in fall of 2013 from sites on the San Marcos and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

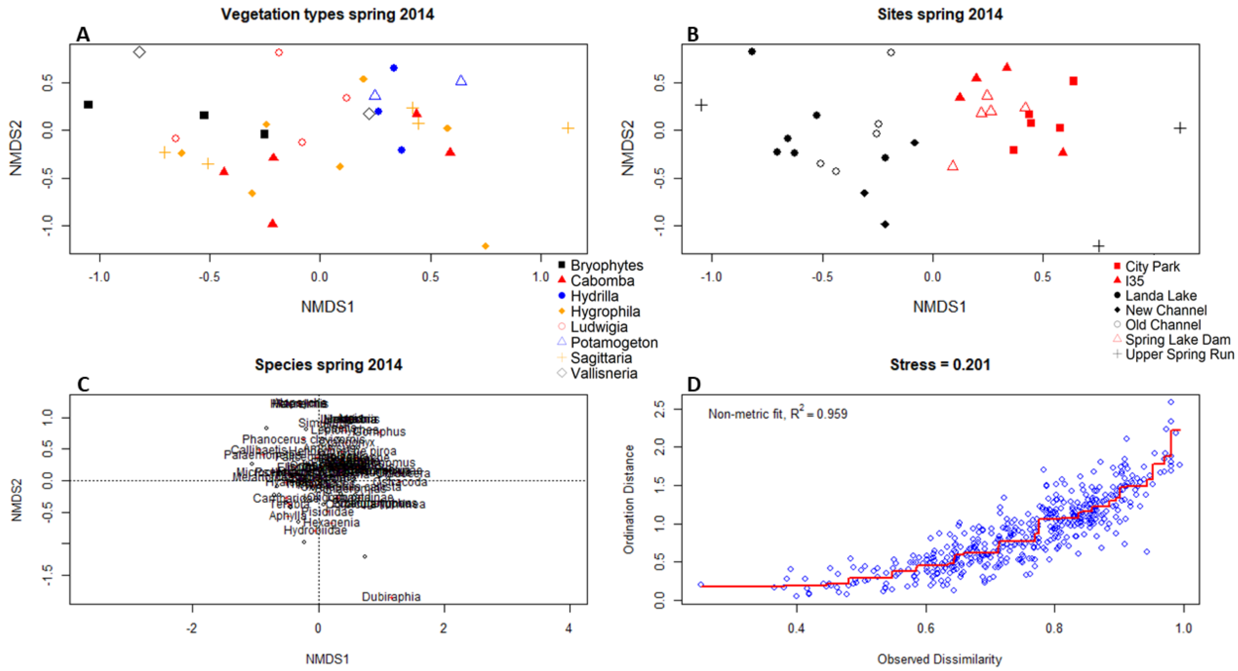


Figure 44. Non-metric multidimensional scaling for macroinvertebrate samples taken in spring of 2014 from sites on the San Marcos and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

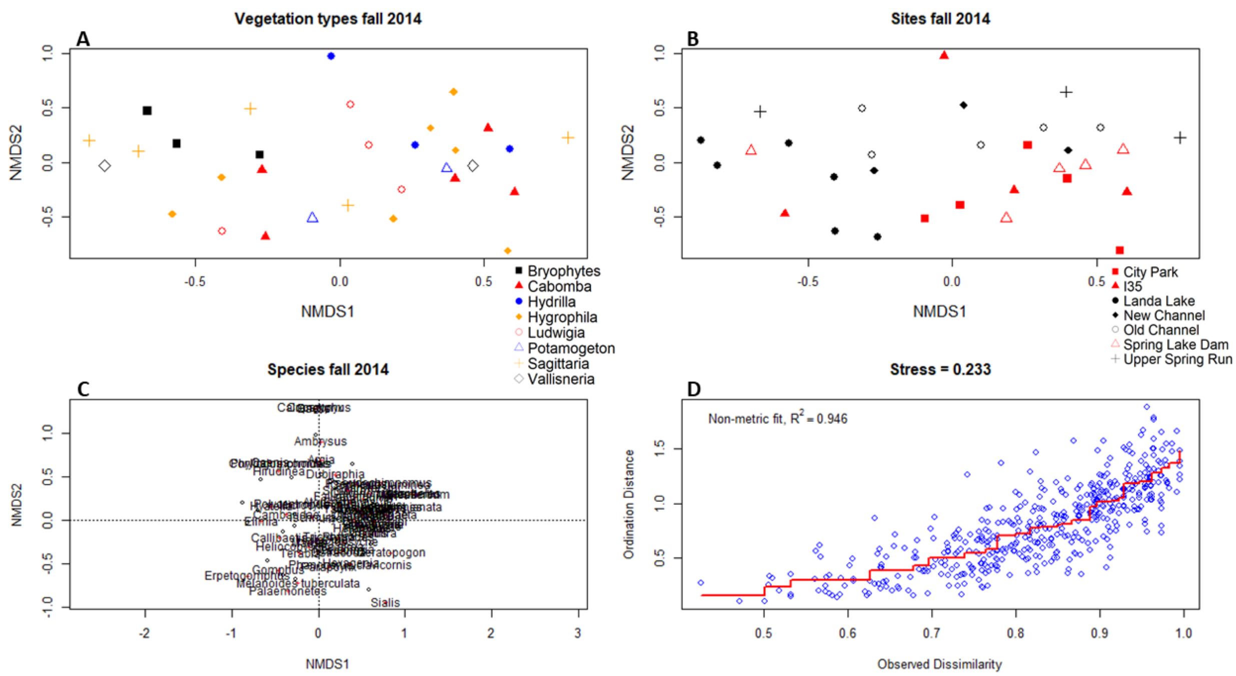


Figure 45. Non-metric multidimensional scaling for macroinvertebrate samples taken in fall of 2014 from sites on the San Marcos and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

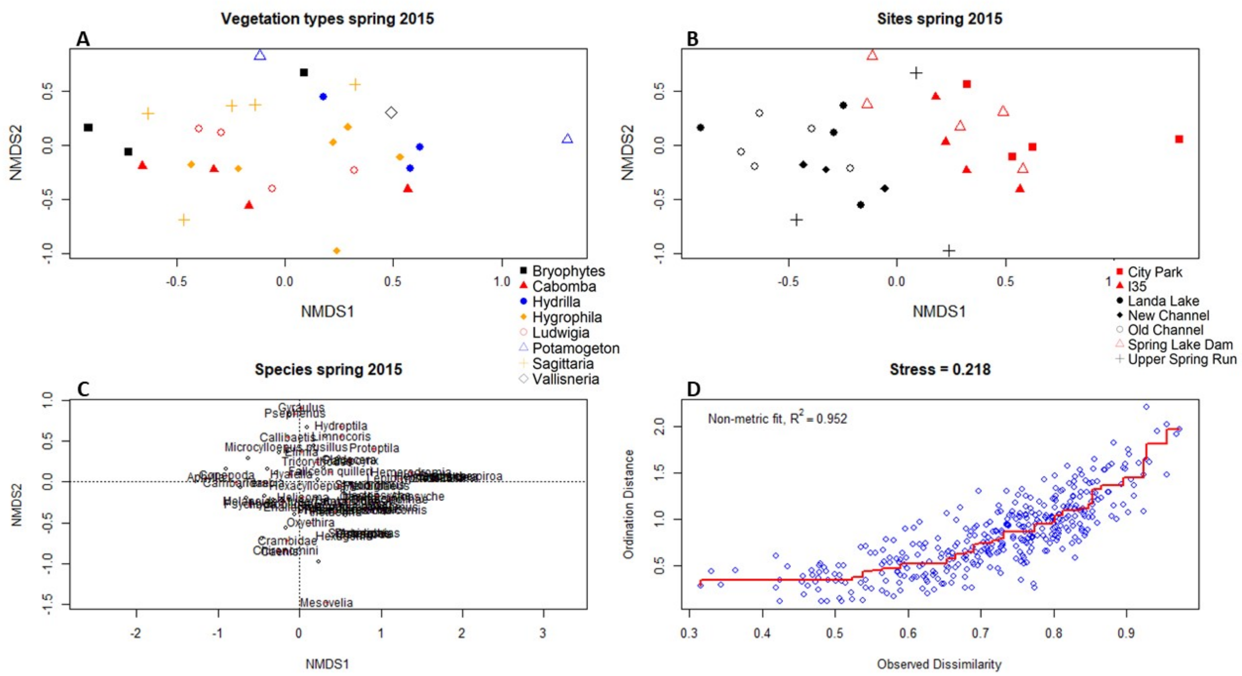


Figure 46. Non-metric multidimensional scaling for macroinvertebrate samples taken in spring of 2015 from sites on the San Marcos and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

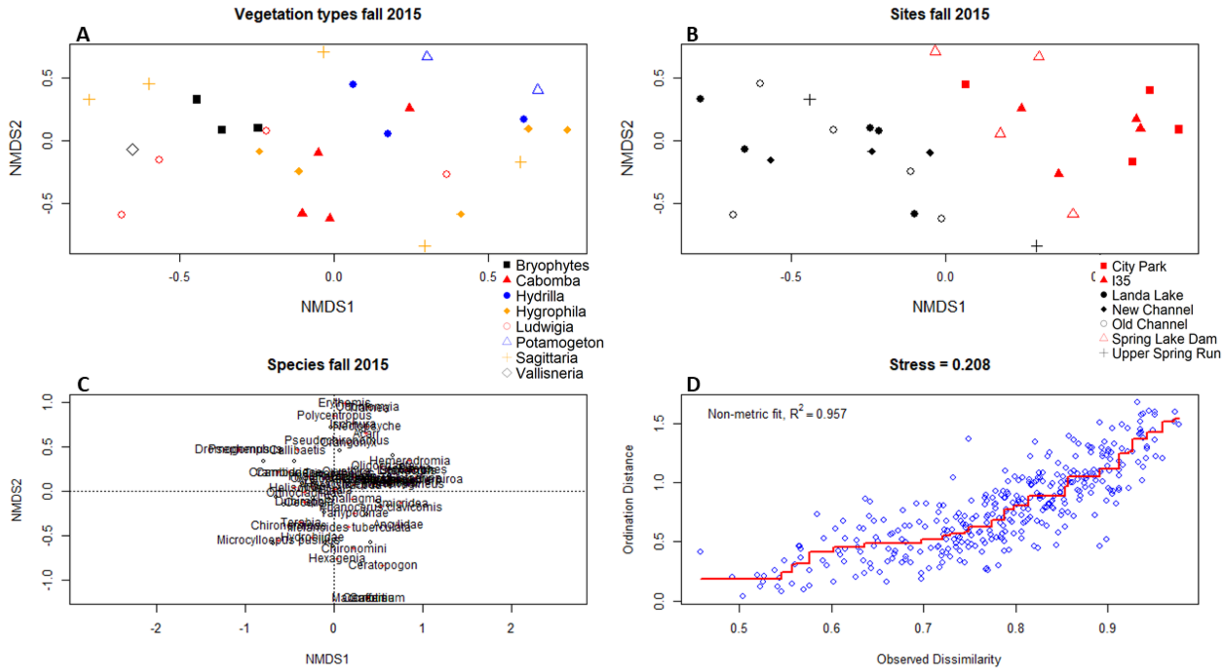


Figure 47. Non-metric multidimensional scaling for macroinvertebrate samples taken in fall of 2015 from sites on the San Marco and Comal Rivers. Ordination of samples based on vegetation (A) and sites (B) are shown on top. Sites from the San Marcos River are colored in red while sites of the Comal River are colored in black. Species (C) and stress plots (D) are shown on the bottom.

Results from the NMDS indicate that Comal and San Marcos Rivers share many of the same taxa; however, there appeared to be some sets of taxa that were more exclusive to one river system or the other, and possibly more specific to certain sites. For instance, Sials, Protoptila, Leptohyphes, Baetis, and Crangonyx were only found in the San Marcos River while Mccaffertium and Dromogomphus were only found in Comal River samples. Although vegetation may be thought of as different types of Fountain Darter habitat, it is possible that many of the vegetation types are similar in characteristics with regard to macroinvertebrate preferences.

Comal Springs and San Marcos Salamanders

Salamander abundances were monitored during spring and fall for the period 2002-2015 at four repeated sites and one additional exploratory site that was not consistently sampled (SRP) in the Comal Springs system for Comal Springs salamanders (*Eurycea* sp.) utilizing timed surveys with dive mask and snorkel. In the San Marcos Springs system, (Figure 48; Table 26) San Marcos salamander (*E. nana*) abundances were counted using SCUBA and snorkeling to conduct visual observations using a quantitative approach (Nelson 1993). In addition to routine surveys, identical surveys were conducted following specific low flow or high flow triggers. Environmental variables are collected per sampling design.

Random forest models were fit the Comal Springs and San Marcos salamander data following the methods described above for Fountain Darter. The number of salamanders observed at a site and date was the response variable, and a series of hierarchical models for each site independently were fitSite-specific RF models predicting salamander abundance explained 6-58% of variance in abundances across sites. Models fit to sites in the San Marcos Springs system did not consistently out-perform models in the Comal Springs system (although they performed more consistently). Time (year) was consistently the most important variable for predicting salamander abundance in the Comal Springs system across all sites (Figure 51A). Partial dependence of predicted abundance of salamander on time illustrated little consistency in temporal trends among sites (Figure 51B-F), although Spring Run 1 and Spring Run 3 both illustrated threshold changes during 2014 (Figure 51D, F), and Spring Run 1, Spring Run Pool, and Spring Run 3 all indicated an increase and then decrease in abundances during 2009-2014 (Figure 51D-F). Replicated sampling sites in the San Marcos Springs system illustrated consistent relative importance of time, number of rocks turned (effort), and survey type in predicting salamander abundance (Figure 52A). Partial dependence plots illustrated consistent temporal trajectories across sites, characterized by increasing abundances during 2002-2012, followed by leveling-off or reductions in abundance (Figure 52B-D). The number of rocks moved during surveys was negatively correlated with salamander abundance at the two upstream sites (Figure 52D-F) and positively correlated with abundance at the downstream site (Figure 52G). The predicted number of observed salamanders was consistently highest in routine surveys across all sites (Figure 52H-J).

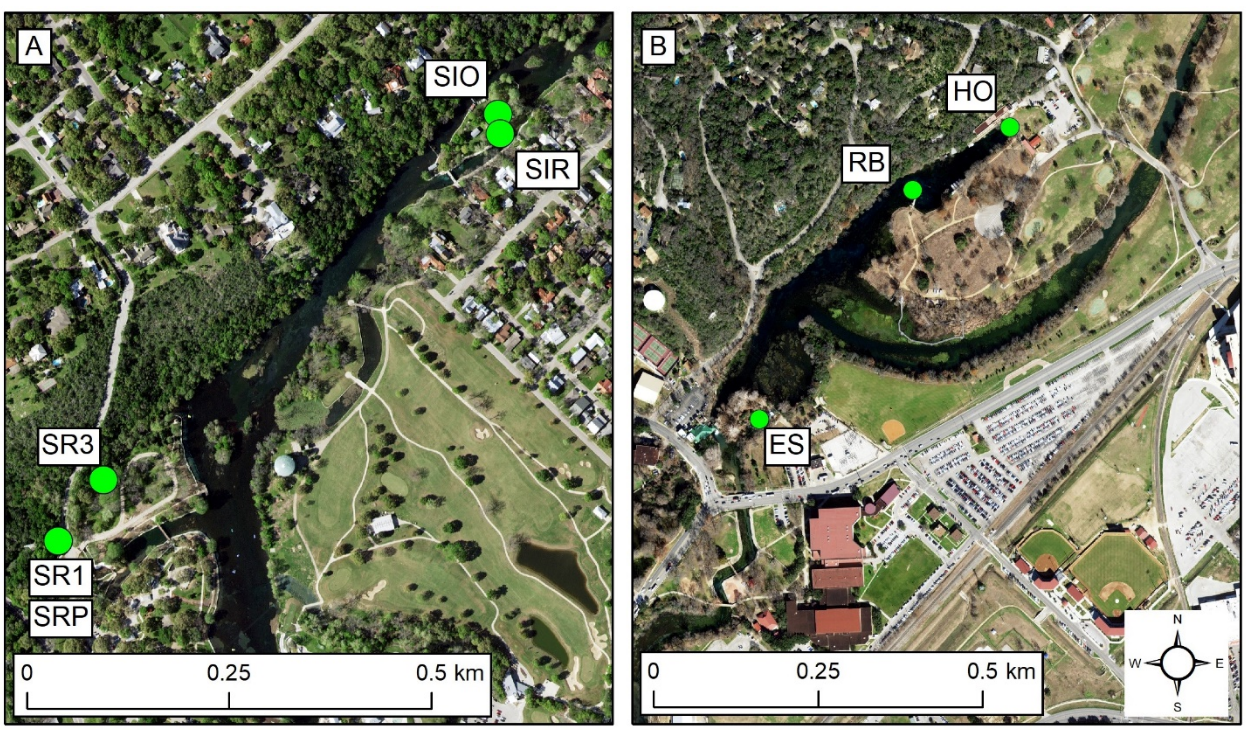


Figure 48. Locations of salamander monitoring sites in (A) Comal Springs system and (B) San Marcos Springs system. See Table 26 for descriptions of location abbreviations.

Table 26. Descriptions, abbreviations, and locations of monitoring sites for salamanders in the Comal and San Marcos river systems.

River System	Site	Code	US Northing	US Easting	DS Northing	DS Easting
Comal	Spring Island Run	SIR	3287817	583967	3287826	583980
Comal	Spring Island East Outfall	SIO	3287793	583970	3287806	583997
Comal	Spring Run 1	SR1	3287289	583423	3287209	583431
Comal	Spring Run 1 Pool	SRP	3287289	583423	3287209	583431
Comal	Spring Run 3	SR3	3287365	583479	3287419	583526
San Marcos	Hotel	HO	3307533	603327	3307487	603266
San Marcos	River Bed	RB	3306724	602766	3306522	602765
San Marcos	Eastern Spillway	ES	3303591	604153	3303466	603158

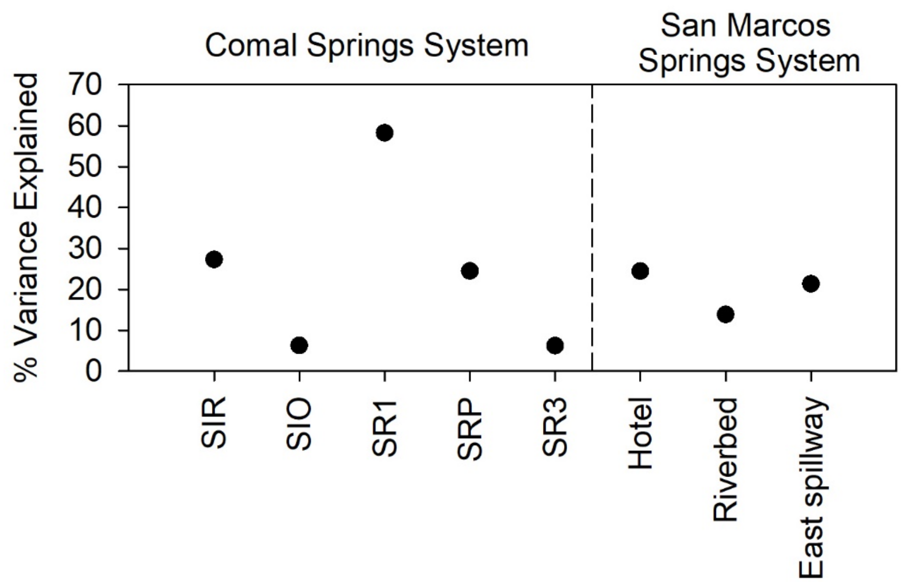


Figure 49. Percent variation in salamander counts explained by random forest models fit to individual sites in the Comal and San Marcos river systems. See Table 26 and Figure 48 for descriptions and locations of sites.

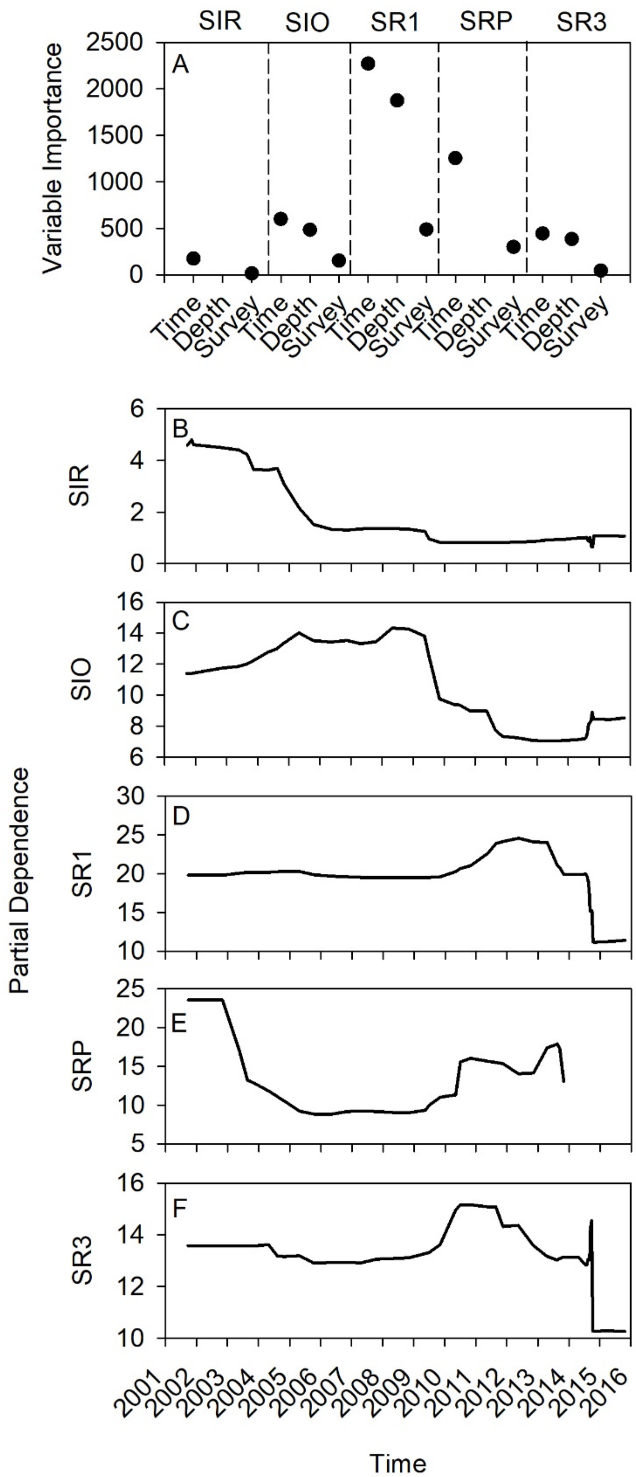


Figure 51. (A) Relative importance of variables (measured as increased node purity) for random forest models of Comal Springs salamander abundance fit to each sampling site independently, (B-F) site-specific temporal trajectories for salamander count partial dependence on time. See Table 26 and Figure 48 for descriptions and locations of sampling sites.

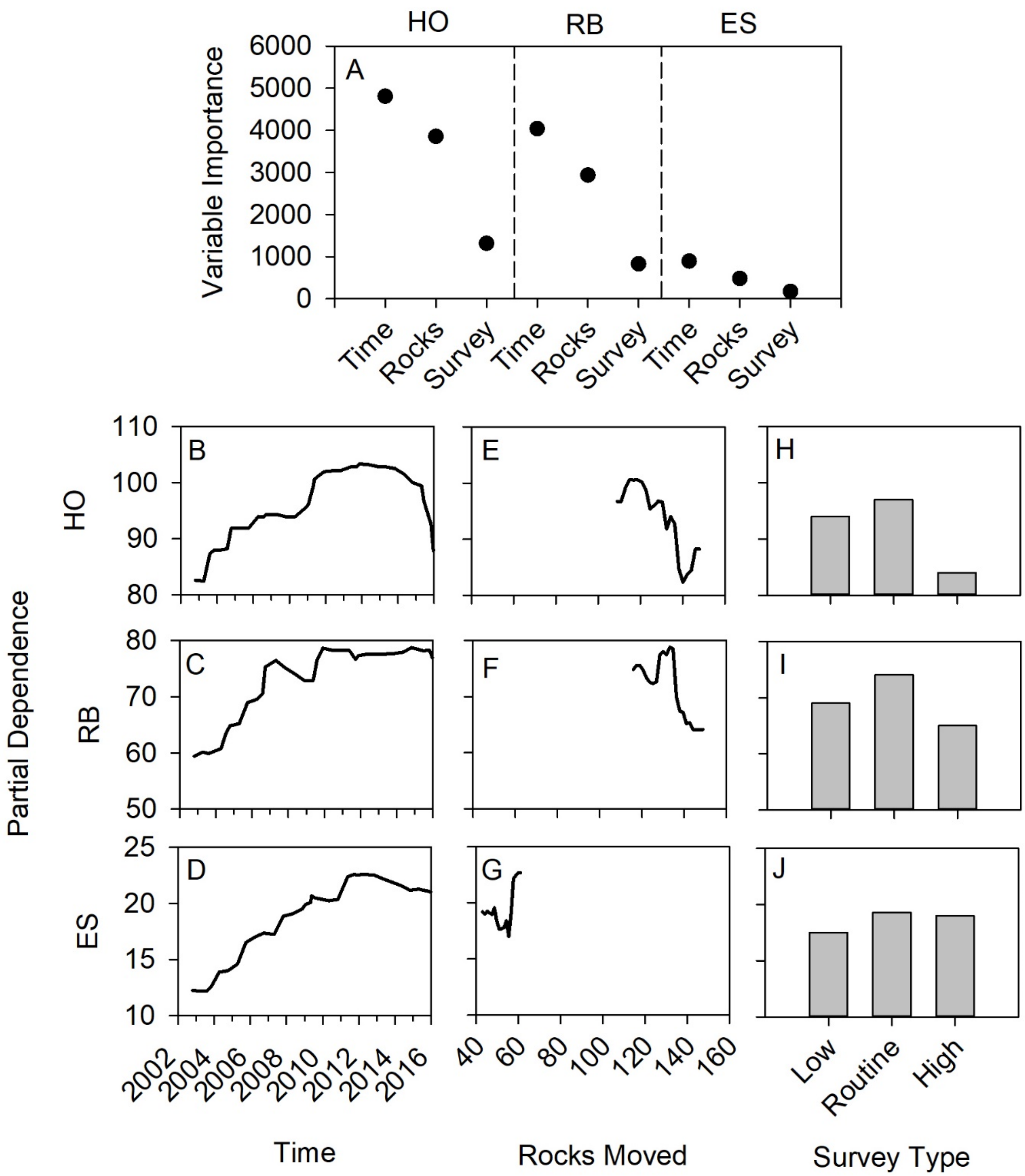


Figure 52. (A) Relative importance of variables (measured as increased node purity) for random forest models of San Marcos salamander abundance fit to each sampling site independently, and partial dependence of salamander abundance on (B-D) time, (E-G) number of rock moved during surveys, and (H-J) survey type (low flow, routine flow, high flow). See Table 26 and Figure 48 for descriptions and locations of sampling sites.

Comal Springs Riffle Beetle

Comal Springs riffle beetle (CSRB) abundances were monitored during 2004-2015 using cotton lures distributed across 10 locations within three sites (Figure 53). Cotton lure traps are 15 cm by 15 cm folded cotton squares that are placed in spring openings with rocks loosely stacked on top of them and left in place for approximately 30 days. Lures are then collected and the number of adult and larva CSRB are counted. Depth and velocity (termed “flow”) of water in which the lure was set was recorded until 2012.

Random forest models were fit using a hierarchical approach in which all sites were combined (global model) and then each site was modeled independently. Models were fit to adult versus larva life stages, and to all observations versus only data with complete depth and flow records (truncated at 2012). Abundance of CSRB in lure traps was the response variable, and predictor variables included year of collection, water depth (complete data only), flow (complete data only), collection site (global model only) and lure replicate at each site (site models only). Model performance was generally pool (explained variance < 25%) across life stage, dataset completeness, and sites (Table 27). The global models explained <6% of variance in CSRB abundance across life stages and datasets, the best performing model was for Spring Island (10-23% explained), and model performance was weakest for Western Shoreline (all % variance negative; no predictive power).

The remainder of the CSRB analysis focuses on the adult life stage and the complete dataset because these cases provided the most information. The global model including all sites explained 5% of variance (Figure 54A), and the most important predictors were depth, flow, and year of collection (Figure 54B). Site-specific models explained 23% for the Spring Island site,15% for the Spring Run 3 site, and -1% for the Western Shoreline site (Figure 54A). Lure identity and water depth were consistently the most important variables among site-specific models, and flow and year the least important (Figure 54B). Partial dependence plots for the global model illustrated highest abundances were at Spring Run 1, CSRB abundances were greatest in shallower depths with faster velocities, and abundances varied little during 2004-2012 when depth and velocity data were collected (Figure 55). Partial dependence plots for site-specific models illustrated changing abundances across lure replicates, indicated some suite of unmeasured variables were associated with lure-specific abundances. CSRB abundance partial dependence on remaining predictor variables in site-specific models followed the same general pattern as the global model, although the range of depths at the Western Shoreline was truncated compared to other sites (Figure 55).

In summary, CSRB abundance was poorly modeled by the available environmental variables measured for lure traps. Site identity was of low importance in the global RF model, and abundance responses to depth and flow were consistent across all models. This suggests responses might be linked across sites and that system-scale processes might be driving consistent responses among sites. However, the high importance of lure identity within site-specific models suggests other predictors of CSRB abundance not routinely measured might be correlated with counts. This point is echoed in the generally low predictive power of all models fit to CSRB abundance data and suggests alternative predictors should be included to measure changes in abundances (see Future Research and Hypothesis Development section).

Figure 53. Locations of Comal Springs Riffle Beetle monitoring sites in Comal Springs system.

Table 27. Sample sizes (italicized text) and random forest model performance (% variance explained) for Comal Springs riffle beetle abundances at adult and larva life stages for cotton lure trap datasets with all observations (all data) and only complete cases for which water depth and flow were measured (complete only). Negative % variance explained values represent poor model performances in which predictor variables performed as well as random chance (i.e., no predictive ability).

Data	Life stage	Global	Spring Island	Spring Run 3	Western Shoreline
All data		*1120*	*361*	*366*	*393*
	Adult	2.67	15.38	14.2	-10.1
	Larva	3.35	13.21	-0.99	-7.48
Complete only		*538*	*184*	*174*	*180*
	Adult	5.41	22.67	14.74	-0.9
	Larva	0.71	10.24	8.94	-7.03

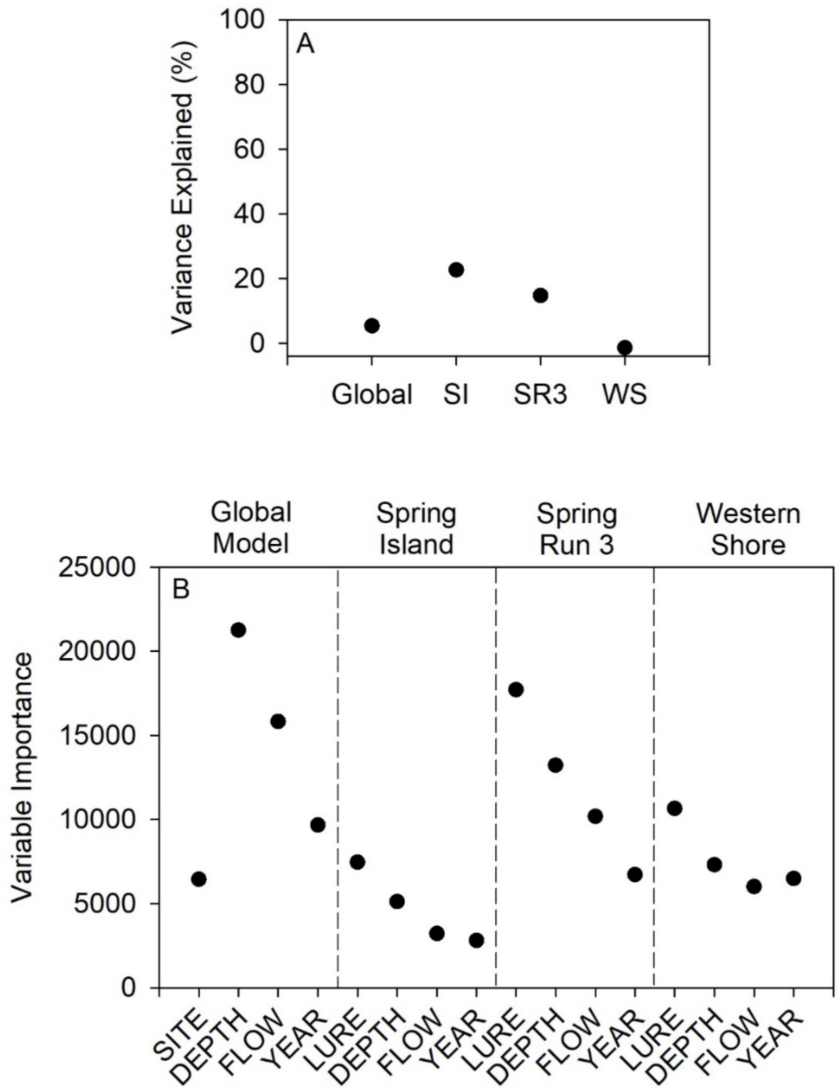


Figure 54. (A) Random forest model performance and (B) variable importance for Comal Springs riffle beetle abundance in cotton lure traps based on all sites combined (global) and individual site models in the Comal Springs system. See Figure 34 for locations of sampling sites.

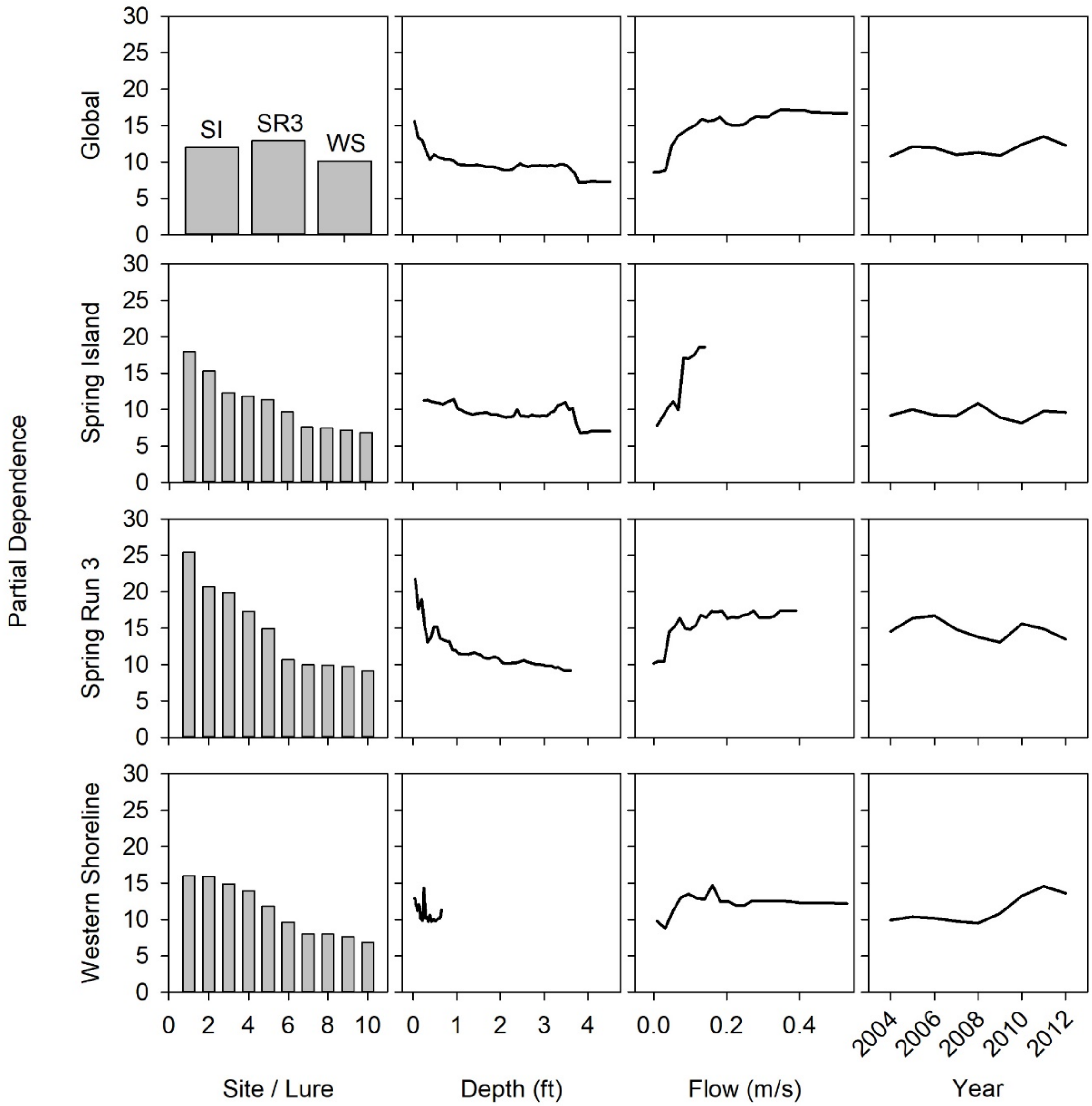


Figure 55. Partial dependence plots illustrating the effect of location (sampling site or lure replicate), water depth, water current velocity (flow), and year of collection on the predicted abundance of Comal Springs riffle beetle in the Comal Springs system. See Figure 34 for locations of sampling sites.

Peck's Cave amphipod, Comal Springs dryopid beetle and Invertebrate Drift Sampling

Hydrology has been shown to be a major driver of aquatic communities (Power et al. 1995, Feminella 1996, Hart and Finelli 1999, Lake 2000, Bunn and Arthington 2002). Furthermore, due to the nature of spring systems, it is expected that other water parameters such as temperature and pH will be fairly consistent within and among years. Therefore, more attention was given to inspecting numbers of Peck's Cave amphipod and Comal Springs dryopid beetle individuals collected during varying levels of flow; do more endangered troglobitic species enter the surface water during higher flow regimes?

Drift data was structured around collections of specific invertebrates, mainly of hypogean origin, in drift over about a 24-hour period once in the spring and once in the fall from 2003-2015 for three locations of the Comal Springs (Spring Run 1, Spring Run 3, and West Upwelling [Spring Run 7]). Considering only 16 "taxa types" were included for this dataset, six of these designations were "targeted" for this analytical investigation: *Stygobromus pecki*, immature *Stygobromus* spp., *Lirceolus*, *Haideoporous*, *Heterelmis comalensis*, and *Stygoparnus comalensis*. Each of these taxa are listed as endangered under the Endangered Species Act or are petitioned to be listed.

Discharge (Q) was calculated by multiplying the area of the net opening with the velocity measured at the time the net was placed within the stream. Considering that velocity was measured in the center of the net, and presumably the net was fully submerged, Q was estimated consistently for all samples. Scatter plots were made for each species and water quality measures and inspected for trends that could be related to numbers of individuals in drift for each species and each location, separately. Regression analysis of each target species with Q was performed for each collection site, separately.

Inspection of dissolved oxygen, temperature, conductivity, and pH levels and numbers of target invertebrates collected, did not show any trend with number of individuals collected in drift. This was expected since the Comal Springs system receives stable year-round water quality at spring orifices even during the extreme drought conditions experienced during 2013-2014.

Mean and associated variance of federally listed species is given in Table 28 for the entire sample period from 2003-2015. The number of individuals found in drift for most groups was quite high, indicating that other factors related to discharge may be more influential with regard to numbers of individuals found in drift. Immature *Stygobromus* were most prevalent, followed by *Lirceolus*. *Heterelmis comalensis*, *Stygoparnus comalensis*, and *Haideoporous* were rarely encountered during 12-year period. The West Upwelling tended to have the highest concentrations of amphipods compared to the spring runs, but conversely, less representation of other invertebrates compared to the other sites.

Table 28. Mean and standard deviation (Stdev) of target species over the entire sample period from 2003-2015.

	Spring Run 1		Spring Run 3		West Upwelling	
Species	Mean	Stdev	Mean	Stdev	Mean	Stdev
Stygobromus pecki	6.6	6.0	9.1	5.3	18.7	14.0
Immature *Stygobromus*	54.5	22.9	79.0	30.7	170.5	71.6
Heterelmis comalensis	1.9	1.8	1.0	2.2	0.2	0.5
Haideoporous	0.0	0.2	0.8	1.4	0.0	0.0
Lirceolus	29.9	19.2	20.4	15.0	5.5	5.1
Stygoparnus comalensis	1.0	1.4	0.7	1.2	0.0	0.2

Figure 56 shows scatter plots of target species over Q for each sample station. Regression statistics are given in Table 29. Nine-hundred fifty-six mature *S. pecki* were found to have weak positive relationships with Q at Spring Run 1 and 3. Immature stages of *Stygobromus* were considered to be primarily *S. pecki*, but also low numbers of *S. russeli* (40 adults total) were caught in drift at these locations. Immature *Stygobromus* were found to be positively related to Q at Spring Run 1. *Stygoparnus comalensis* was found to be positively related to Q at Spring Run 1. Forty-four specimens of *S. comalensis* were collected during the time period of this study and most of these (26) from Spring Run 1. None of the other target taxa showed a relationship with Q.

Only 20 specimens of the predaceous diving beetle *Haideoporous* were collected during this 13-year period of time and almost all from Spring Run 3, indicating that they may be low number or they rarely come to the surface compared to other hypogean species. One the other hand, 1514 *Lirceolus* were collected, representing the second most abundant taxon among target species, next to *Stygobromus* (probably *S. pecki*). *Heterelmis comalensis* were collected from all locations, represented by 86 individuals.

It is unclear from this analysis if hydrology influenced the presence of some of our target species to drift in surface waters. It is likely that some species, such as *Lirceolus,* are probably distributed at varying levels beneath the surface and will venture to the epigean domain by chance, regardless to flow conditions. However, it stands to reason that hypogean organisms are more likely to be pushed to the surface and drift during higher flows.

Table 29. Results of regressions analysis for target species in drift related to Q from 3 localities in Comal Springs, collected from 2003-2015.

	Spring Run 1		Spring Run 3		West Upwelling	
Species	R-squared	p-value	R-squared	p-value	R-squared	p-value
Stygobromus pecki	**0.16**	**0.017**	**0.16**	**0.022**	0.05	0.131
Immature *Stygobromus*	**0.25**	**0.004**	-0.001	0.3361	0	0.331
Heterelmis comalensis	0.03	0.196	-0.03	0.591	0.07	0.0927
Haideoporous	-0.04	0.692	0.01	0.28	-	-
Lirceolus	-0.03	0.579	-0.04	0.963	-0.01	0.36
Stygoparnus comalensis	**0.21**	**0.008**	0.01	0.274	-0.01	0.433

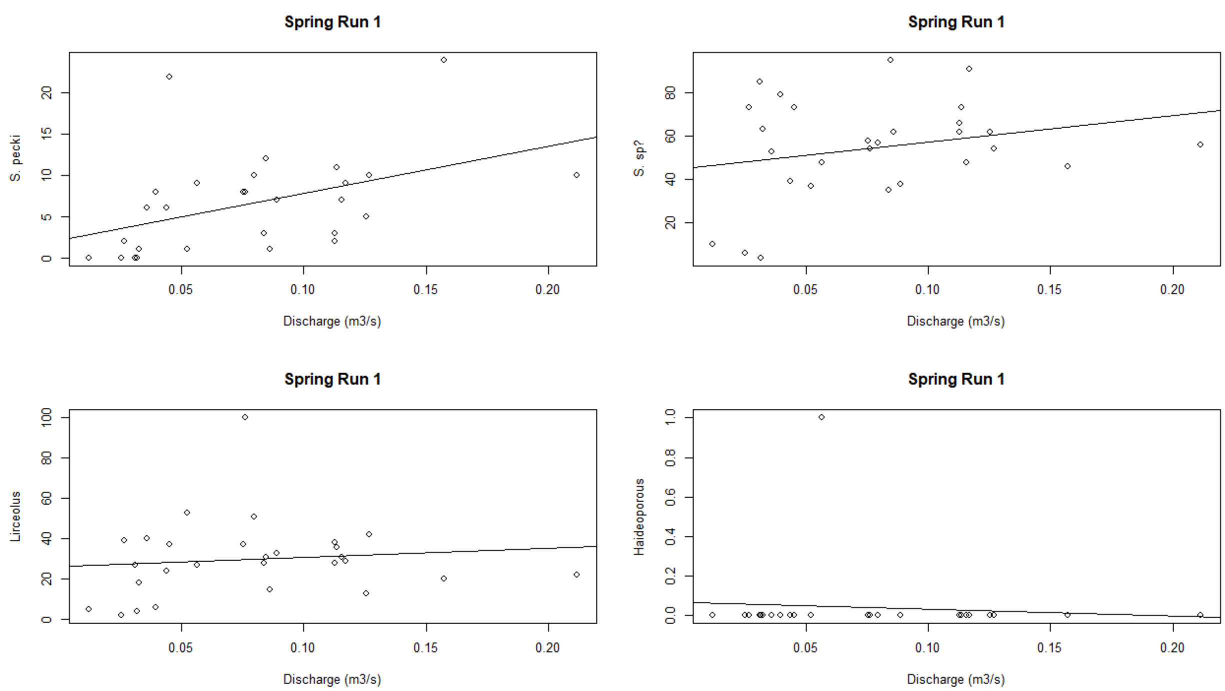


Figure 56. Scatter plots of numbers of target species per discharge (Q) for Spring Run 1, Spring Run 3, and West Upwelling localities from 2003-2015.

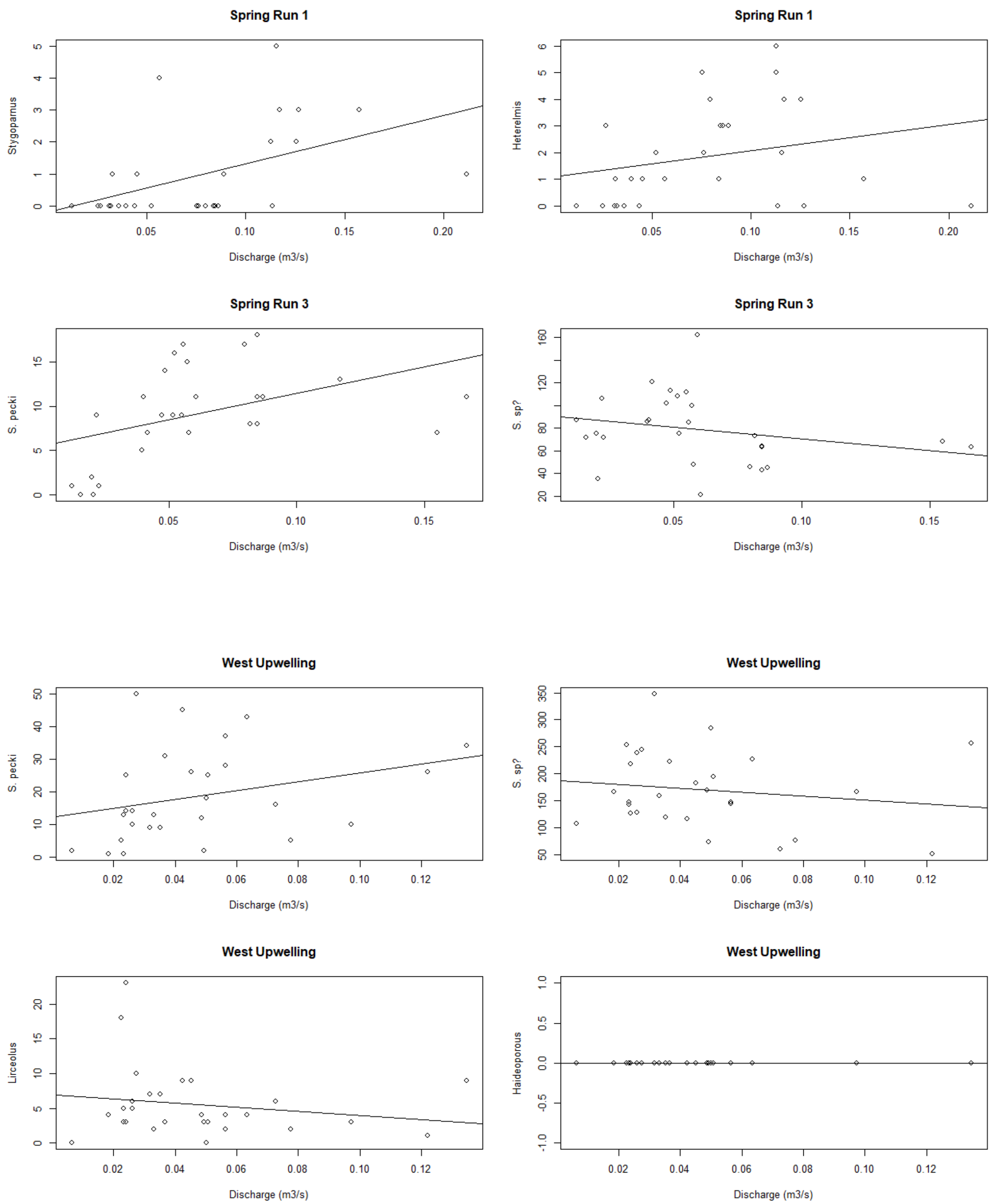
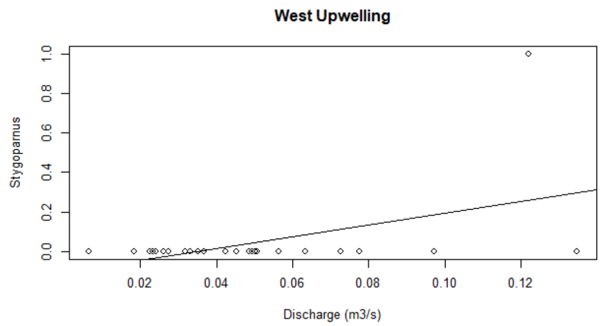


Figure 56 (Continued). Scatter plots of numbers of target species per discharge (Q) for Spring Run 1, Spring Run 3, and West Upwelling localities from 2003-2015.



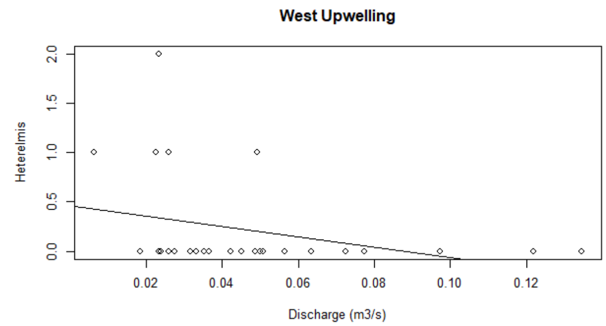


Figure 56 (Continued). Scatter plots of numbers of target species per discharge (Q) for Spring Run 1, Spring Run 3, and West Upwelling localities from 2003-2015.

Predation gillnet

The abundance and prey items consumed by predatory fishes was assessed using gill nets and rod/reel sampling in Landa Lake in the Comal Springs system and Spring Lake in the San Marcos Springs system. This sampling occurred during 2001 and 2002 in both systems, and then again during 2006 in the San Marcos Springs system and 2014 in the Comal Springs system. All fish were measured for total length (mm) and the proportion of individuals examined containing specific prey categories was calculated. Prey categories included Fountain Darter, San Marcos Salamander, empty stomachs, algae, other fish species, crayfish and/or shrimp, aquatic invertebrates, and other prey categories.

Table 30 gives a summary of findings for the Comal Springs system and Table 31 give a summary of findings for the San Marcos Springs system.   In the Comal Springs system, 231 fish were retained for diet examination during 2001, 2002, and 2014. Of these fishes, two Largemouth Bass (*Micropterus salmoides*) had consumed Fountain Darter in 2001. No other Fountain Darter consumption was detected, and salamanders were not detected in fish diets. In the San Marcos Springs system, 200 fish were retained for diet examination during 2001, 2002, and 2006. Of these fishes, one Warmouth (*Lepomis gulosus*) had consumed a Fountain Darter in 2001, one Largemouth Bass has consumed a salamander in 2001, and one Warmouth had consumed a salamander in 2002.

Table 30. Gill net sampling and diet analysis results from the Comal Springs system including sampling year, fish tax captured, number of individuals captured/examined, mean length of all individuals, and the percent of individuals with various prey categories present in their gut contents. Prey categories are: FD (Fountain Darter), SA (Salamander), E (Empty), A (Algae), OF (Other Fish), CS (Crayfish/Shrimp), AI (Aquatic Invertebrates), and O (Other). All collections used gill nets and rod/reel sampling except for 2014 when only gill nets were used.

Year	Taxa	#	Length	FD	SA	E	A	OF	CS	AI	O
2001	*Herichthys cyanoguttatus*	(23/23)	153	0.00	0.00	34.78	39.13	0.00	13.04	30.43	0.00
2001	*Lepomis auritus*	(9/9)	138	0.00	0.00	22.22	0.00	11.11	11.11	77.78	0.00
2001	*Lepomis cyanellus*	(1/1)	105	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00
2001	*Lepomis gulosus*	(4/4)	103	0.00	0.00	25.00	0.00	0.00	0.00	50.00	25.00
2001	*Lepomis megalotis*	(17/17)	128	0.00	0.00	29.41	0.00	0.00	17.65	58.82	11.76
2001	*Lepomis miniatus*	(42/42)	126	0.00	0.00	26.19	2.38	0.00	11.90	52.38	7.14
2001	*Micropterus salmoides*	(29/29)	290	6.90	0.00	27.59	0.00	24.14	51.72	3.45	3.45
2001	*Hypostomus plecostomus*	(1/1)	440	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2001	*Oreochromis aureus*	(40/27)	367	0.00	0.00	35.48	54.84	0.00	0.00	25.81	0.00
2002	*Herichthys cyanoguttatus*	(2/2)	157	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
2002	*Ameiurus natalis*	(1/1)	191	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2002	*Ambloplites rupestris*	(1/1)	111	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2002	*Lepomis gulosus*	(2/2)	184	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
2002	*Lepomis megalotis*	(6/6)	126	0.00	0.00	0.00	0.00	0.00	16.67	83.33	0.00
2002	*Lepomis miniatus*	(37/37)	127	0.00	0.00	24.32	16.22	8.11	51.35	21.62	8.11
2002	*Micropterus salmoides*	(21/21)	287	0.00	0.00	14.29	0.00	52.38	61.90	4.76	4.76
2002	*Hypostomus plecostomus*	(1/0)	380	-	-	-	-	-	-	-	-
2002	*Oreochromis aureus*	(19/1)	369	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2014	*Micropterus salmoides*	(3/3)	289	0.00	0.00	66.67	0.00	0.00	33.33	0.00	0.00
2014	*Oreochromis aureus*	(13/3)	350	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
2014	*Pterygoplichthys sp.*	(1/1)	371	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00

Table 31. Gill net sampling and diet analysis results from the San Marcos Springs system including sampling year, fish tax captured, number of individuals captured/examined, mean length of all individuals, and the percent of individuals with various prey categories present in their gut contents. Prey categories are: FD (Fountain Darter), SA (Salamander), E (Empty), A (Algae), OF (Other Fish), CS (Crayfish/Shrimp), AI (Aquatic Invertebrates), and O (Other). All collections used gill nets and rod/reel sampling except for 2006 when only rod/reel was used.

Year	Taxa	#	Length	FD	SA	E	A	OF	CS	AI	O
2001	*Herichthys cyanoguttatus*	(3/2)	221	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2001	*Lepisosteus oculatus*	(14/14)	690	0.00	0.00	57.14	0.00	35.71	0.00	0.00	7.14
2001	*Lepomis auritus*	(5/5)	174	0.00	0.00	0.00	0.00	0.00	0.00	80.00	40.00
2001	*Lepomis gulosus*	(11/11)	192	9.09	0.00	54.55	0.00	0.00	45.45	0.00	0.00
2001	*Lepomis macrochirus*	(14/14)	140	0.00	0.00	21.43	7.14	0.00	7.14	78.57	21.43
2001	*Lepomis megalotis*	(11/11)	184	0.00	0.00	36.36	0.00	0.00	9.09	54.55	0.00
2001	*Lepomis microlophus*	(4/4)	171	0.00	0.00	75.00	0.00	0.00	0.00	25.00	0.00
2001	*Lepomis miniatus*	(22/22)	124	0.00	0.00	36.36	0.00	0.00	0.00	59.09	4.55
2001	*Micropterus salmoides*	(27/27)	280	0.00	3.70	18.52	0.00	25.93	22.22	3.70	29.63
2002	*Herichthys cyanoguttatus*	(3/3)	224	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00
2002	*Lepisosteus oculatus*	(3/3)	650	0.00	0.00	33.33	0.00	66.67	0.00	0.00	0.00
2002	*Lepomis auritus*	(2/2)	230	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00
2002	*Lepomis gulosus*	(3/3)	207	0.00	33.33	0.00	0.00	0.00	100.00	0.00	0.00
2002	*Lepomis macrochirus*	(8/8)	171	0.00	0.00	12.50	0.00	0.00	0.00	87.50	50.00
2002	*Lepomis miniatus*	(22/22)	129	0.00	0.00	9.09	0.00	0.00	0.00	81.82	9.09
2002	*Micropterus salmoides*	(29/29)	254	0.00	0.00	62.07	0.00	20.69	6.90	6.90	13.79
2006	*Micropterus salmoides (less than 300mm)*	(6/6)	221	0.00	0.00	0.00	17.00	67.00	33.00	17.00	17.00
2006	*Micropterus salmoides (greater than 300 mm)*	(9/9)	384	0.00	0.00	22.00	11.00	44.00	33.00	22.00	33.00
2006	*Lepomis auritus*	(5/5)	230	0.00	0.00	0.00	20.00	20.00	0.00	60.00	60.00

Future Research and Hypothesis Development

Future analyses through development and testing of hypotheses based on this summarization and initial exploration of EAA long-term monitoring database could be conducted to assess mechanisms regulating the potential to meet and maintain compliance with HCP long-term biological goals (LTBGs) as well as explore application and ecological responses of past or ongoing HCP mitigation and restoration activities.

Submerged Aquatic Vegetation

The HCP has set threshold values for goals pertaining to the areal coverage of SAV, and the data in the long-term monitoring database provide a method for measuring progress towards these goals. Two regulators of SAV area are flow regime (flow-based disturbances) and recent plantings and removals of SAV. The baseline data in the monitoring database can be used to test future hypotheses using co-integration (e.g., Zhou et al. 2016) and before-after-control-impact (BACI) designs to assess flow-mediated and removal/plating-mediated responses to management actions. Specifically, the flow regime data and long-term areal coverages of SAV can be used to assess plant community changes at replicated sites using time-series analysis such as co-integration analysis to assess interactions (competition, exclusion) between species based on measured coverages. Areal coverages can also be used to measure responses to implementation of plantings or removals before and after manipulations as well as at sites near and distant from manipulations (i.e., BACI). Tables 2 and 9-15 of this report provide the baseline data required for testing such hypotheses.

Fountain Darter

The HCP has set goals for threshold density values of Fountain Darter in specific forms of SAV (EAA 2012). The results of the current report suggest the densities of Fountain Darter predicted using random forest models meet these goals at all but one vegetation form: Bryophytes in the Comal Springs system (Figure 57). Improving density estimates by including confounding effects of abundance and detection will be critical for ensuring measurements towards LTBG target density goals are accurate. A useful series of hypotheses that might be tested involve testing the effects of sampling efficiency on density estimates. Statistical tools such as open population size estimates from repeated counts of unmarked individuals (e.g., Fiske and Chandler 2011) mean that these hypotheses can be tested using the existing EAA database and implemented in future monitoring to ensure density estimates are as accurate as possible. An added benefit of such models is their ability to include not only detection covariates, but also covariates that predict total abundances. In the context of HCP LTBGs, this means water quantity, water quality, and SAV covariates could be included and corrected for the confounding effects of heterogeneous detection in models for Fountain Darter densities.

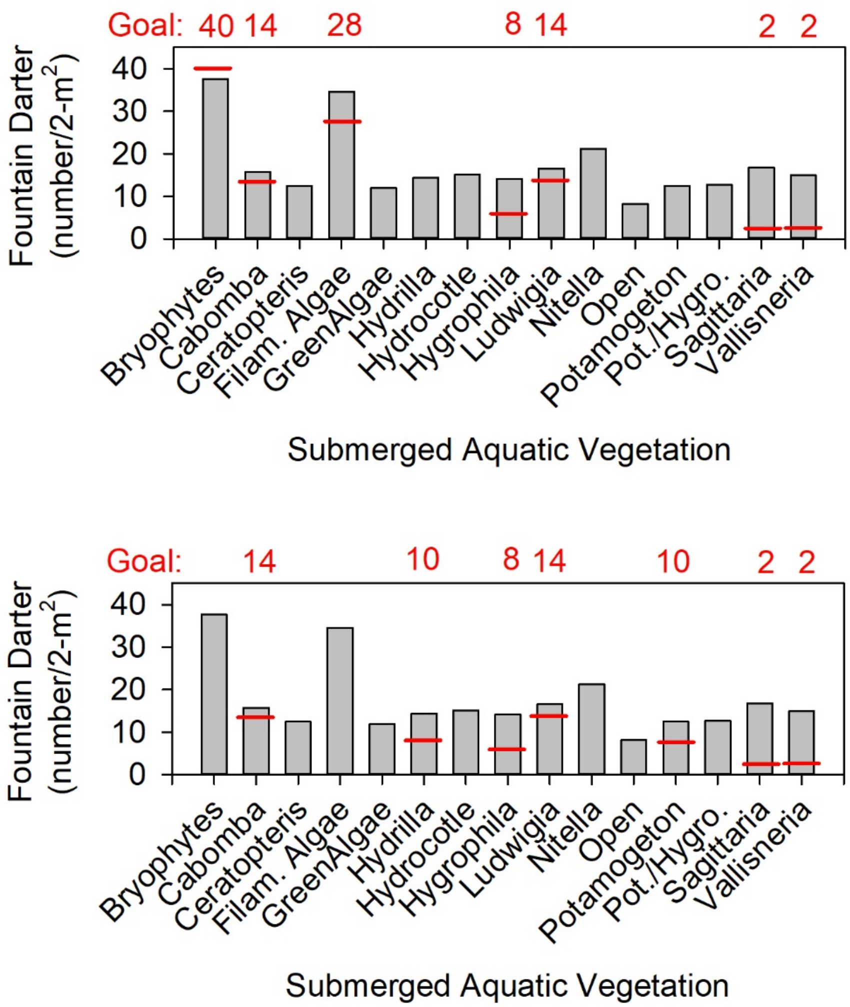


Figure 57. Partial dependence plots for modeled Fountain Darter abundance in the (A) Comal Springs and (B) San Marcos Springs systems with LTBG threshold values overlaid (red text indicates threshold values).

San Marcos Salamander

The HCP LTBGs for San Marcos Salamander were set using the long-term median abundance values recorded at sites in the San Marcos Springs system. Data presented in the current report with the most recent data from 2015 suggest recent abundances are below the long-term median for one of three sites in the San Marcos Springs system (Figure 58), however data collected since 2015 appears to return to "normal or above normal" abundances. As with the Fountain Darter, applying open population count-based population estimates to test hypotheses regarding effects of detection on abundance estimates would allow for development of more robust estimates. Potential covariates for detection that already exist in the EAA long-term monitoring database include turbidity, water temperature, discharge magnitudes (including for specific spring outflows), and coverage by SAV (at a limited number of sites).

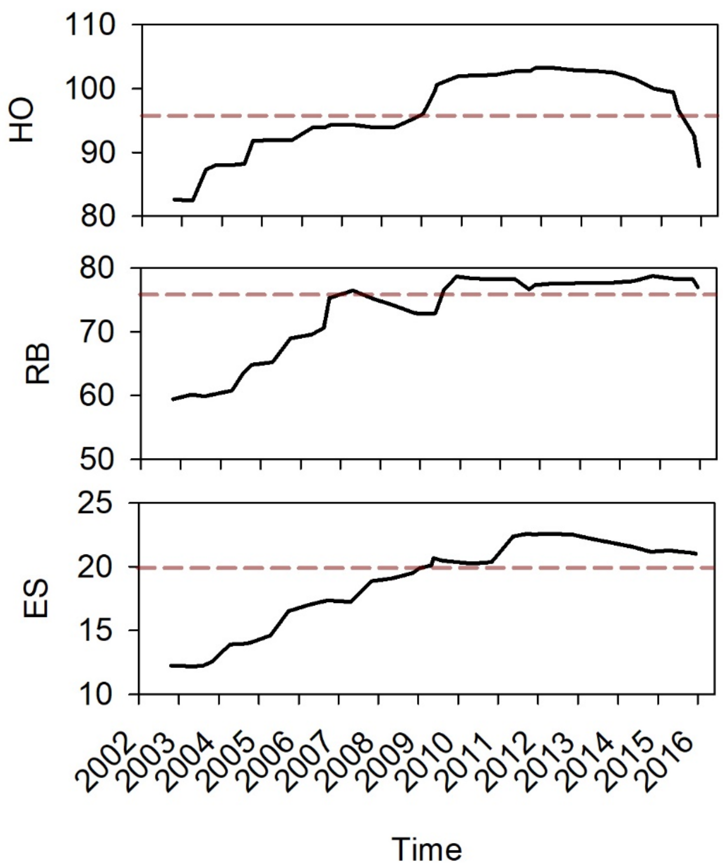


Figure 58. Partial dependence plots for San Marcos salamander. Red dashed lines represent LTBGs shown here using the long-term predicted median abundance from partial dependence plots (i.e., after adjusting for measured environmental variables).

Comal Springs riffle beetle

The HCP LTBGs for Comal Springs riffle beetle were set for each site (EAA 2012) and include 15/lure at the Spring Island site, 15/lure at the Western Shoreline site, and 20/lure at the Spring Run 3 site. Data presented in the current report illustrate modeled abundances were consistent through time and were just beneath LTBG thresholds at each site (Figure 59). Future hypotheses might be tested to determine uncertainty around these estimates and incorporate the spatially-structured nature of the data collected at lure traps. One potentially useful approach would be to develop spatially structured models that provide point count estimates and uncertainty around these estimates (e.g., Baddeley and Turner 2005). These models allow for include environmental covariates that could be pulled from other portions of the EAA long-term monitoring dataset, such a flow and water temperature regimes measured near specific sites. The benefit of employing these analysis methods could be increased by minor modifications to the CSRB biomonitoring protocols, which could be tailored to evaluate the effects of other EAHCP activities (such as sediment trapping) conducted for this species.

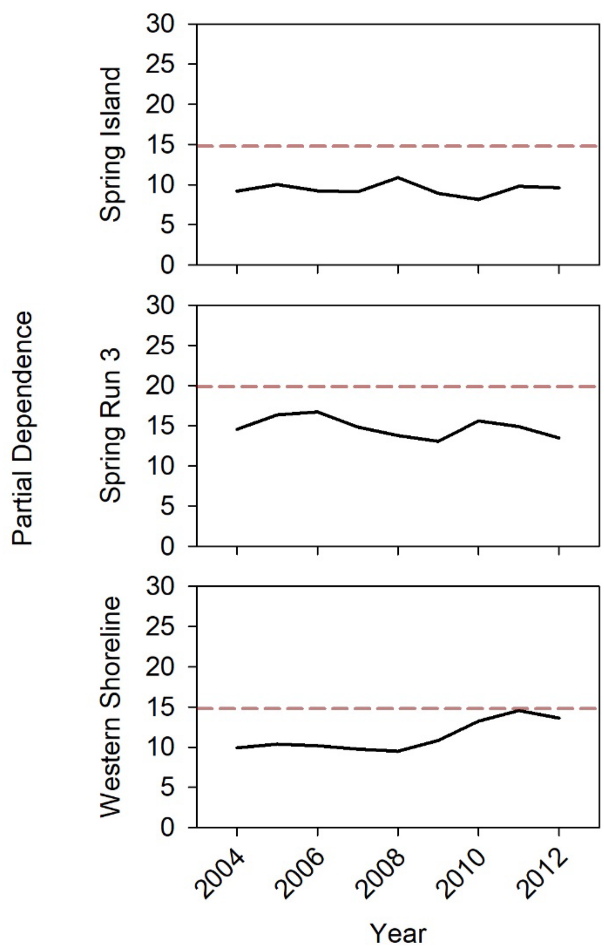


Figure 59. Partial dependence plots for Comal Springs riffle beetle at three sites in the Comal Springs system. Red lines indicate LTBGs set by the EAA and provide references for the

modeled abundances for each site after adjusting for measured habitat variables (water depth and flow).

Peck's Cave amphipod and Comal Springs dryopid beetle

There are presently no EAHCP LTBGs for these subterranean species that can be addressed with biological data. It is nonetheless of interest to determine if biological sampling can be used to develop goals that can be directly monitored by biological response. It is suggested that predictive models can be constructed with the current drift dataset. Incorporation of precipitation data and other factors that may affect subsurface-flow conditions. If numbers of drifting species can be predicted relatively consistently, then changes in drift numbers expected from input conditions may flag that something other than flow-related effects may be responsible for changes in numbers of individuals caught in drift. Furthermore, well performing models could be used to predict how changes in flow-regime, and/or climate, will affect drifting individuals. As an initial evaluation of this concept, a preliminary predictive model was developed for *S. pecki* from Spring Run 1. A random forest (RF) model was constructed with a suite of variables representing ambient and temporal conditions based on water quality, flow, and precipitation data that were readily available (Table 32). After an initial run with 500 trees it was determined by plotting the mean square residuals that 96 trees would be optimal for growing the forest. Internal validation set by the program determined that -2.58% of the variation was explained, indicating that the model prediction was poor. However, paired t-test showed that there were no statistical differences (t-value = 0.242, p-value = 0.811) among observed and predicted numbers of drifting individuals.

Ambient velocity at the drift net and discharge at the USGS Comal Springs gauge station along with the 120-day cumulative precipitation from the Fischer Store weather station were the most important variables (Figure 60). The model appeared to perform well for predicting numbers of individuals in drift for intermediate observations between 5-10 (Figure 61), but not so well for lower and higher drifting individuals. It is likely that additional factors related to groundwater more specific to the Comal Springs system would be necessary to make more accurate predictions. However, this "quick" analysis suggests that a more thorough model could be constructed with a better set of predictors that may yield more accurate predictions. These types of models could be used to predict the number of individual species drifting per year and might help give us a better idea of population sizes.

Table 32. Ambient and temporal variables used to construct a random forest model.

Predictors	Description	Condition
Temp	Temperature (degrees Celsius)	Ambient
Conductivity	Conductivity (microsiemens)	Ambient
pH	pH	Ambient
DO_mg.L	Dissolved Oxygen (milligrams per liter)	Ambient
Velocity	Velocity (meters/second)	Ambient
cfs.x	Discharge (cubic feet per second) from USGS gauge station at Comal River	Ambient
cfs.y	Discharge (cubic feet per second) from USGS gauge station at San Marcos Springs	Ambient
cfs	Discharge (cubic feet per second) from USGS gauge station at Comal Springs	Ambient
pecp_fs90	Cumulative 90 days of precipitation (inches) from Fischer Store climate station.	Temporal
pecp_fs120	Cumulative 120 days of precipitation (inches) from Fischer Store climate station.	Temporal
pecp_fs365	Cumulative 365 days of precipitation (inches) from Fischer Store climate station.	Temporal
prcp_fs30	Cumulative 30 days of precipitation (inches) from Fischer Store climate station.	Temporal
precp_sb90	Cumulative 90 days of precipitation (inches) from Spring Branch climate station.	Temporal
precp_sb120	Cumulative 120 days of precipitation (inches) from Spring Branch climate station.	Temporal
precp_sb365	Cumulative 365 days of precipitation (inches) from Spring Branch climate station.	Temporal

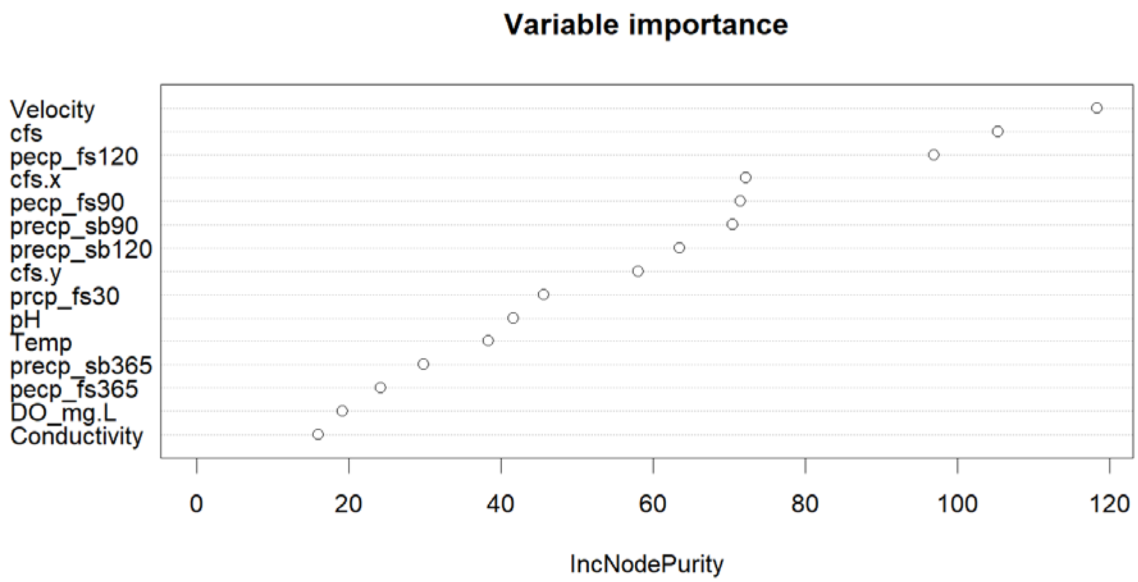


Figure 60. Variable importance for random forest prediction of number of individuals in drift at Spring Run 1 for drift samples collected from 2003-2015.

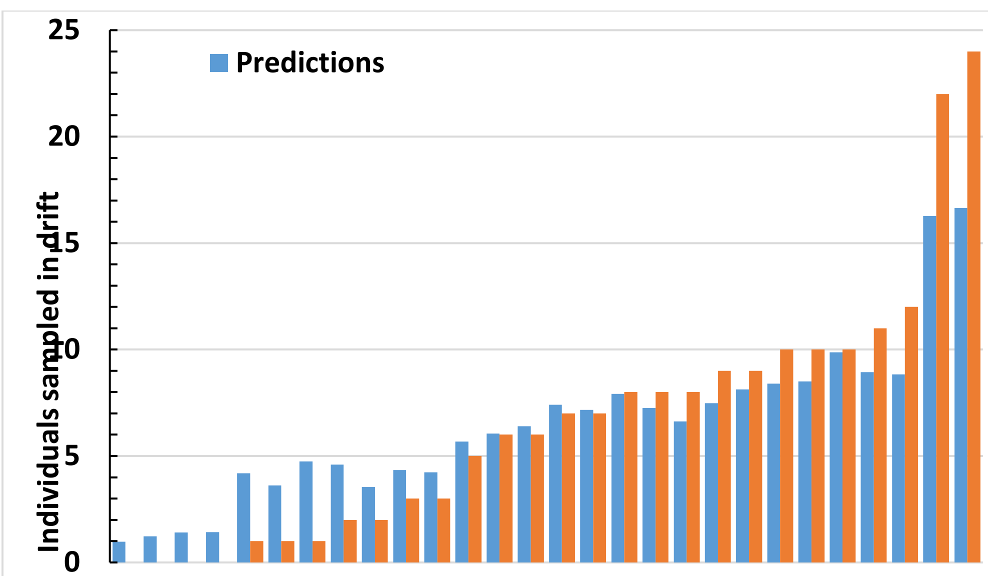


Figure 61. Paired comparisons of observed and predicted numbers of individuals in drift from Spring Run 3 from 2003-2015.

Other Species and Associated HCP Activities

Follow up research for macroinvertebrates could focus on the development of a biological monitoring tool specific to these spring-fed systems. In fact, in 2017 (per HCP biological working group recommendation) there was a change in the macroinvertebrate sampling strategy as it was switched to the Texas rapid bioassessment protocol (TCEQ 2014). Further analysis is not recommended on this existing macroinvertebrate dataset specifically in terms of biological integrity assessment; however, future collection as described above could be useful for monitoring aquatic health.

Comal Springs salamanders, while not a federally listed species at present, could warrant additional efforts for several reasons, the foremost of which is that given trends in taxonomic classification of Central Texas *Eurycea* species they could foreseeably be listed in the future and are presently a HCP covered species. Regardless of the possibility of listing, they are an important biological component of the Comal Springs ecosystem and have strong potential as a biological indicator of changes in water quality, etc. that could affect listed species sympatrically distributed (i.e. Comal Springs riffle beetles). Though taxonomically distinct, this species occupies a similar niche to the San Marcos salamander. With minor adjustments to biomonitoring protocols, Comal Springs salamander data could be collected more similarly to San Marcos Springs salamander data, such that salamander monitoring data from both systems could be compared under global models. This could be used to determine if there are effects to one system that are not present in the other.

It is possible that SAV data and Fountain Darter sampling data could be mined and integrated to gain insight into the effects of native vegetation restoration on both covered species and communities as a whole. With carefully designed modifications or additions to biomonitoring practices, explicit evaluation of restoration effects can be conducted in future years.

Finally, additional data has and continues to be collected that was outside the scope of this contract. To achieve the highest benefit from future analyses of biomonitoring data, we feel that it may be of interest to consider including some of these data. For instance, there are a number of additional water quality sondes in these systems that were not considered here that could be included if it is determined that water quality is of interest in future analyses. Fish Community data has also been collected since the implementation of the HCP in 2013. One example of utility for this data set would be assessment of the effectiveness of exotic fish removal under the HCP. Fountain Darter sampling methods here are not appropriate for assessing trends in abundance of armored catfish or tilapia, however the Fish Community data is likely a reasonable tool to begin evaluating the effects of these efforts. This data could also be employed to gain additional insight into the effectiveness of vegetation restoration on various fish species in certain areas.

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