Summary of Jollyville Plateau Salamander Data (1997 – 2006) and Status

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Abstract

The Jollyville Plateau salamander (Eurycea tonkawae) occurs in springs, spring-runs, and caves associated with the northern segment of the Edwards Aquifer, from West Bull Creek north to Brushy Creek, in Travis and Williamson counties, Texas. Examination of 10 years of data shows that salamander counts are declining at four of nine long-term monitoring sites. The long-term monitoring sites that still have large numbers of salamanders are on the LCRA Wheless tract in the Long Hollow watershed and the Bull Creek above Tributary 7 site on the Balcones Canyonlands Preserve. Salamanders with deformities have been documented at two long-term monitoring sites. Salamanders may be extirpated from a more recent monitoring site at the only known location in the Walnut Creek watershed. The declining counts and deformities appear to be related to water quality and habitat degradation due to urban development. Thirty-eight salamanders from 15 sites in six watersheds tested positive for the chytrid fungus in May and June 2006. The chytrid fungus is a worldwide pathogen that mainly affects adult frogs and toads, but the Jollyville Plateau salamander does not show any obvious adverse effects. The Jollyville Plateau salamander continues to exhibit seasonal reproductive cycles, which may be regulated by rainfall and flow.

Introduction

The Jollyville Plateau salamander (JPS) has been found in springs, spring-runs, and caves of the Northern Edwards Aquifer in nine watersheds – Bull, Brushy, Buttercup, Cypress, Lake, Long Hollow, Shoal, Walnut, and West Bull creeks. A two-year intensive study in 1997 and 1998 was designed to collect baseline information about these salamanders. The results of that study are documented in the City of Austin (2001) and in Bowles et al. (2006). From 1999 to 2003, City of Austin biologists continued to conduct salamander counts at some of the original monitoring sites, but on a less frequent basis. Beginning in 2004, City of Austin biologists expanded the monitoring efforts to include all of the original sites as well as new sites in different watersheds. In this report, data from the original study has been combined with the more recent data and examined for time trends, site differences, and seasonal variations in reproduction.

While the JPS has no federal or state protection, in June 2005 the Save Our Springs Alliance submitted a petition to the U.S. Fish and Wildlife Service to list this species as threatened or endangered.

Sites

An effort has been made to continue monitoring at all nine original JPS study sites in three watersheds (Bull, Long Hollow, Shoal) and to expand surveys to include sites in other watersheds. The original study included monthly surveys. Currently, an effort is made to survey eight of the original sites quarterly. One of the long-term sites (Tanglewood) is surveyed annually. Table 1 lists the original and new sites, and Figure 1 presents a map of the sites. City staff has a draft Quality Assurance Project Plan that summarizes the current JPS monitoring program.

Figure 1. Jollyville Plateau Salamander Locations and Monitoring Sites in Travis and Williamson Counties, Texas

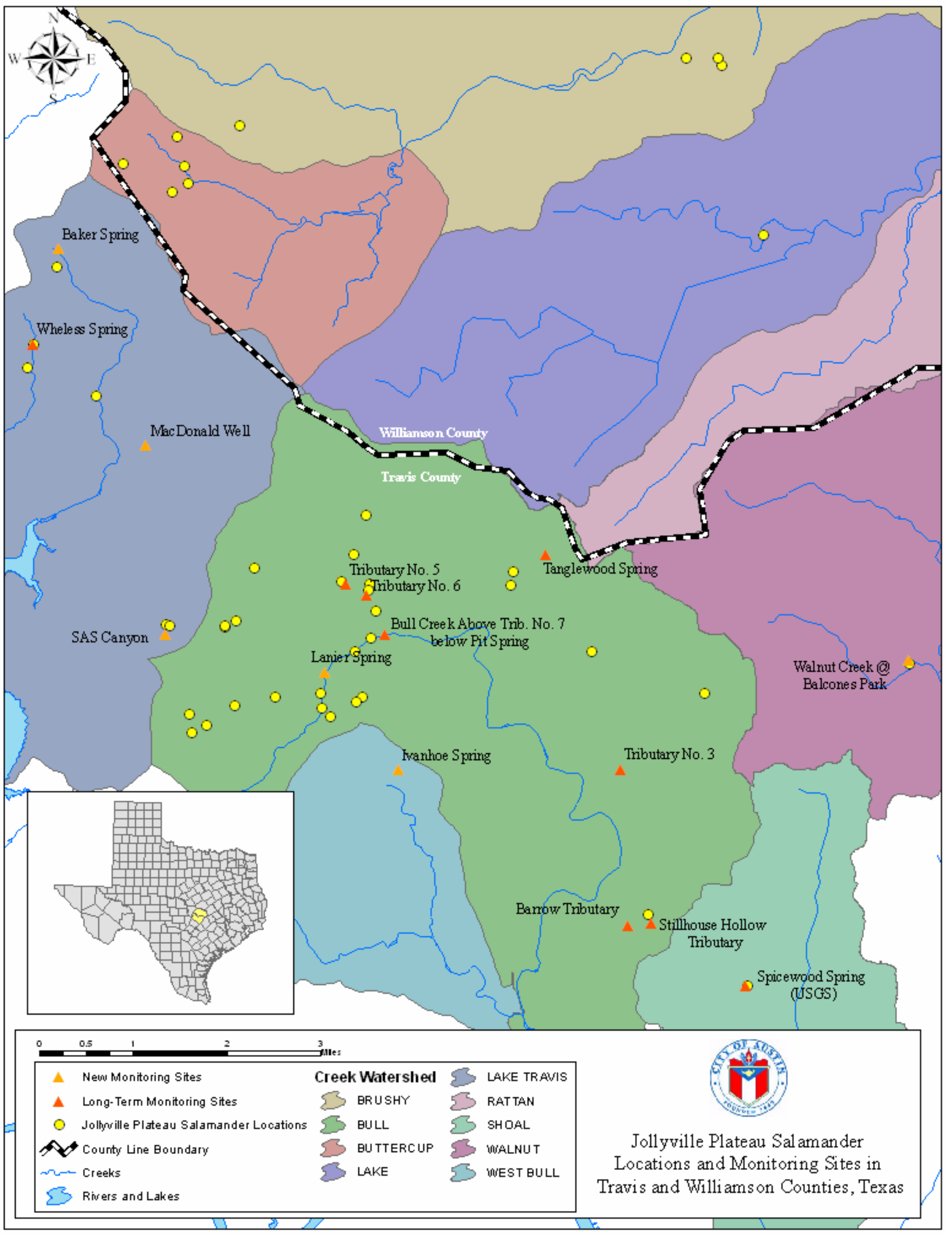


Table 1. Jollyville Plateau Salamander Monitoring Sites

Jollyville Plateau Salamander Monitoring Sites with Original Study Sites Highlighted							
Spring Site		Salamander Survey Site					
Site Number	Site Name	Site Number	Site Name	Site Characteristics	Property Ownership	Current Monitoring Frequency	Routine Water Quality/Quantity Sampling?
Bull Creek Watershed							
25	Barrow Preserve Spring	929	Barrow Preserve Tributary Below Barrow Spring	Urban - old development	City of Austin preserve	Quarterly	nutrients basic chemistry flow
24	Stillhouse Hollow Spring	927	Stillhouse Hollow Below Stillhouse Hollow Spring	Urban - old development	Private	Quarterly	nutrients basic chemistry flow
31	Tanglewood Spring	928	Tanglewood Tributary Below Tanglewood Spring	Urban - recent development	Private	Annually	basic chemistry flow
		926	Tributary 3 @ Great Hills Golf Course	Urban - recent development	Private	Quarterly	basic chemistry flow
		151	Tributary 6 @ Bull Creek (EG)	Urban - recent and continuing development	City of Austin's Hanks tract in the BCP	Quarterly	nutrients, ions basic chemistry benthic macroinvertebrates flow
		1164	Tributary 5 Below Hanks Tract Property Line	Rural at start of study - now developing	City of Austin's Hanks tract in the BCP	Quarterly	nutrients, ions basic chemistry benthic macroinvertebrates flow
34	Pit Spring	349	Bull Creek Above Tributary 7	Rural	City of Austin's Franklin tract in the BCP	Quarterly	nutrients, ions basic chemistry benthic macroinvertebrates flow
		3963	Lanier Spring	Rural	City of Austin's Lanier tract in the BCP	Quarterly	basic chemistry flow
			Cistern Spring	Rural	City of Austin's Franklin tract of the BCP	Annually	No
Cypress Creek Watershed							
	Baker Spring	3959	Tributary below Baker Spring	Rural	Travis Aubudon Sanctuary in the BCP	Quarterly	basic chemistry flow
	MacDonald Well	3958	Tributary below MacDonald Well	Rural	Travis County tract in the BCP	Quarterly	basic chemistry flow
3960	SAS Canyon Spring	3966	Tributary below SAS Canyon Spring	Some existing and ongoing development	Travis County tract in the BCP	Quarterly	basic chemistry flow
		3965	Kretschmarr Salamander Cave	Some existing and ongoing development	Private	Quarterly	No
Long Hollow Watershed							
1044	Long Hollow Creek Spring @ Wheless Tract	1045	Long Hollow Creek @ Wheless Tract	Rural	LCRA Wheless tract in the BCP	Quarterly	basic chemistry flow
Shoal Creek Watershed							
582	Spicewood Spring	930	Spicewood Tributary Below Spicewood Spring	Urban – old development	Private	Quarterly	basic chemistry flow
Walnut Creek Watershed							
		445	Balcones District Park Spring	Urban – old development	City of Austin park	Quarterly	basic chemistry flow
West Bull Creek Watershed							
		1072	Ivanhoe Spring	Rural	City of Austin's Ivanhoe tract	Quarterly	basic chemistry

Jollyville Plateau Salamander Count Data

Figures 2-10 show salamander counts and flow measurements for the nine long-term monitoring sites, from January of 1997 through the fall of 2006. Regression analysis with total count as the dependent variable and date as the independent variable found significant trends for four long-term monitoring sites (Table 2, figures 4, 7, 8, 9). Salamander counts have decreased significantly at the sites along Bull Creek tributaries 3, 5, and 6 and at the Spicewood site in the Shoal Creek watershed. There is new and ongoing development affecting Tributaries 3, 5, and 6, and old development in the Shoal Creek site. In contrast, salamander counts at the rural Long Hollow site on the LCRA Wheless tract appears to have increased, but the significance is at the 0.1 level. The Bull Creek above Tributary 7 site also continues to have large numbers of salamanders.

Counts for more recent monitoring sites are presented in Table 3. These will be presented as graphs in future reports as more data are collected to assess trends. Many JPS sites were dry during much of 2006 due to drought, which was relieved briefly following heavy rains in May and October. Only Baker Spring (Travis Audubon Society's Baker Sanctuary) did not appear to respond to the May rains and only flowed for a few days following the October rains. It is possible that nearby development activities have influenced flow at this site. Several of these new sites have recently been added to the City's database, so data have not yet been entered for all of the sites. City staff will examine trends of salamander count data for all of the new sites in the 2007 report.

One dead and several other emaciated JPS were found in Testudo Tube Cave in April and May 2006, in addition to large, robust salamanders. The cause for the emaciated condition of some of the JPS observed is unknown, but flows and wetted area were greatly diminished during this time period. This diminished flow may have contributed to the decline in the food source for the JPS. Though staff noticed a large number of stygobitic flatworms (*Sphalloplana sp)*, no stygobitic crustaceans were observed in the presence of salamanders.

The JPS population at Balcones District Park spring in the Walnut Creek watershed may be extirpated, since no JPS have been observed at this site since September 23, 2005. The same individual had been documented at this site (based on photographs) over a 14-month period from January 2004 through March 2005. City biologists were unable to capture the individual observed in July and September 2005. Although the initial counts at this site were very low, the declining trend is significant (Table 2).

Table 2. Sites with Statistically Significant Changes (0.05 and 0.10 level) in Total Salamander Counts over Time

Site	Pr>F	R^2	Direction	Average 1997 Count	Average 2006 Count
Balcones District Park Spring	0.0037	0.6264	Decreasing	2 (2004)	0
Tributary 3 @ Great Hills Golf Course	0.0368	0.1709	Decreasing	32	7
Bull Creek Tributary 6	0.0001	0.2142	Decreasing	30	5
Bull Creek Tributary 5	<0.0001	0.3824	Decreasing	42	1
Spicewood Spring and Tributary	0.0006	0.3383	Decreasing	12	1
Long Hollow Creek on Wheless tract*	0.0565	0.2216	Increasing	33	159

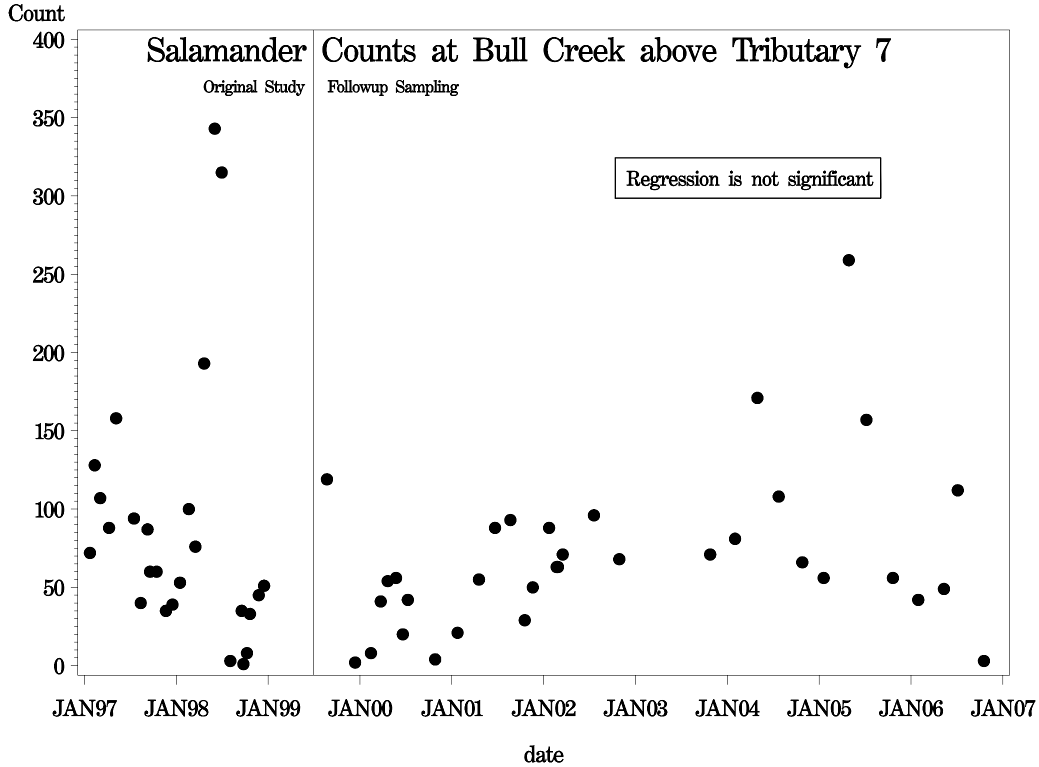
* Note: Wheless is significantly increasing at the 0.1 level; the rest are significant at the 0.05 level

Table 3. Salamander Counts at New and Other Monitoring Sites, 2004-2006

Site	Date	Salamander Count Total (<1", 1-2", >2")	Flow (cfs)	Comments
Buttercup Creek Watershed				
Testudo Tube Cave	4/14/06	5* (0, 4*, 1)		*Includes 1 dead 1-2" JPS and 4 live JPS, including 1 healthy >2" recapture (marked with elastomers) and 3 emaciated 1-2" JPS. Very little water/flow.
	4/25/06	3 (0, 3, 0)		Very cursory survey; 3 emaciated 1-2" JPS collected for captive breeding.
	5/18/06	8 (0, 1, 7*)		Cursory survey; *4 recaptures (marked with elastomers -- nnRnnn, RRnnnn, Gnnnnn, nRnRnn); 3 sampled for chytrid fungus incl. 1 recapture (nnRnnn); 1 emaciated and collected for captive breeding
	9/19/06	7 (0, 4, 3*)		Cursory survey; *2 recaptures (marked with elastomers -- GGnnnn, nnnRnn)
Bull Creek Watershed				
Lanier Spring	1/4/04	0 (0, 0, 0)		Very cursory survey; significant damage from feral hogs
	3/16/05	18 (15, 3, 0)		Prior to this survey, BCP staff installed a fence around the spring to exclude hogs
	7/1/05	50 (23, 21, 6)		
	9/23/05	30 (3, 23, 4)		Flow has diminished since the July survey
	12/12/05	17 (0, 11, 6)		Poor visibility due to dense leaf litter
	3/10/06	16 (5, 8, 3)	0.04	Poor visibility due to sediment
	5/23/06	24 (1, 8, 15)	0.01	Poor visibility due to dense leaf litter deposited from recent rains
	5/26/06			Staff removed large amounts of flood materials including sediment and organic detritus
	August-Sept. 06		0	Dry
	10/29/06			Spring flowing, found 1 healthy adult JPS during very cursory check
Cistern Spring	2/1/04	0 (0, 0, 0)		Small spring with ongoing habitat restoration by BCP staff
	7/24/04	0 (0, 0, 0)		
	11/6/04	1 (0, 0, 1)		
	4/29/05	1 (0, 1, 0)		
	10/21/05	2 (0, 0, 2)		
Moss Gully	1/4/05	2 (0, 0, 2)		
	4/4/05	2 (0, 0, 2)		
	5/10/05	3 (1, 0, 2)		
	5/23/06	3 (0, 2, 1)		
Cypress Creek Watershed				
Baker Spring	4/14/04	33 (0, 6, 27)	0.07	
	8/24/04	1 (0, 0, 1)	--	Too shallow to measure flow
	5/20/05	7 (0, 3, 4)	0.02	
	8/26/05	2 (0, 0, 2)	0.001	
	Fall 05	--	0	Dry
	4/11/06	0 (0, 0, 0)	0	A few wet pools but no obvious flow
	5/18/06	0 (0, 0, 0)	0	Dry
	8/25/06	0 (0, 0, 0)	0	Dry
	10/24/06	0 (0, 0, 0)	0	Dry; flowed briefly after 4.7 inches of rain on 10/10/06

Site	Date	Salamander Count Total (<1", 1-2", >2")	Flow (cfs)	Comments
SAS Canyon	6/13/06	13 (1, 6, 6)		Survey boundaries have not been established yet; flow too low to measure
	6/20/06			Not a survey; collected 9 salamanders (4 large juveniles, 5 adults) for captive breeding
	8/25/06	4 (1, 0, 3)		Surveyed upper spring pool and lower spring pool only
	9/20/06			Not a survey; collected tail tissue for genetic study from 9 salamanders; saw 3-4 additional salamanders but unable to collect
	11/17/06	13 (0, 4, 9)	0.007	Established survey boundaries (upper pool, lower pool/springrun) but still need to map
Krestchmarr Salamander Cave	10/1/04	4 (0, 0, 4)		
	8/25/06	3 (0, 1, 2)		
	11/9/06	5 (1, 0, 4)		
MacDonald Well	8/10/04	25 (2*, 16, 7)	0	*no flow, only small spring pools; includes 1 dead juvenile found
	Fall 05		0	Dry
	5/19/06	237 (12, 69, 156*)	0.44	*Includes 1 dead adult
	6/20/06			Not a survey; collected 8 salamanders (6 large juveniles, 2 adults) for captive breeding. Saw 27 total (14, 10, 3) during cursory search.
	8/25/06	2 (1, 1, 0)	0	Dry except for small pool in well
	10/30/06	100 (2, 38, 60)	0.25	Collected 3 adults, including 1 albino and 1 gravid female, for captive breeding
Walnut Creek Watershed				
Balcones District Park Spring	1/13/04	3 (0, 0, 3)		Photographed salamander
	4/8/04	3 (1, 1, 1*)		*Recaptured salamander from 1/13/04
	7/20/04	1 (0, 0, 1)		Unable to catch salamander
	10/25/04	0 (0, 0, 0)		
	3/16/05	1 (0, 0, 1*)		*Recaptured salamander from 1/13/04 and 4/08/04
	7/1/05	1 (0, 0, 1)		Unable to catch salamander
	9/23/05	1 (0, 0, 1)		Unable to catch salamander
	12/22/05	0 (0, 0, 0)		
	3/10/06	0 (0, 0, 0)		
	5/18/06	0 (0, 0, 0)		
	6/19/06			Trenched through pavement to create artificial springrun
	10/13/06	0 (0, 0, 0)	0.01	
West Bull Creek Watershed				
Ivanhoe Spring	1/13/04	0 (0, 0, 0)		Very little flow
	7/20/04	17 (5, 10, 2)		
	10/25/04	3 (0, 2, 1)		
	3/16/05	18 (15, 1, 2)		
	7/1/05	10 (6, 3, 1)		
	9/23/05	4 (0, 4, 0)		
	10/25/05			Installed hog proof fence to prevent hog wallowing in spring
	12/12/05	8 (0, 6, 2)		
	3/10/06	1 (0, 0, 1)		Very little flow
	5/23/06	6 (0, 1, 5)		

Figure 2. Salamander Counts and Flow at Bull Creek above Tributary 7



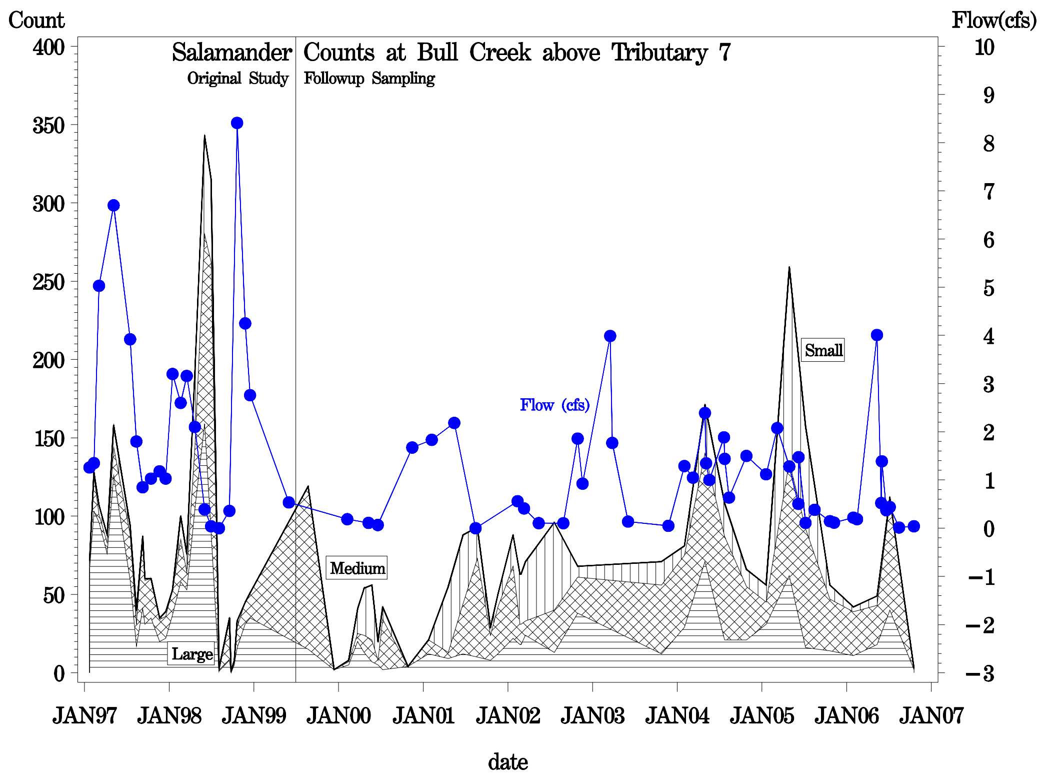
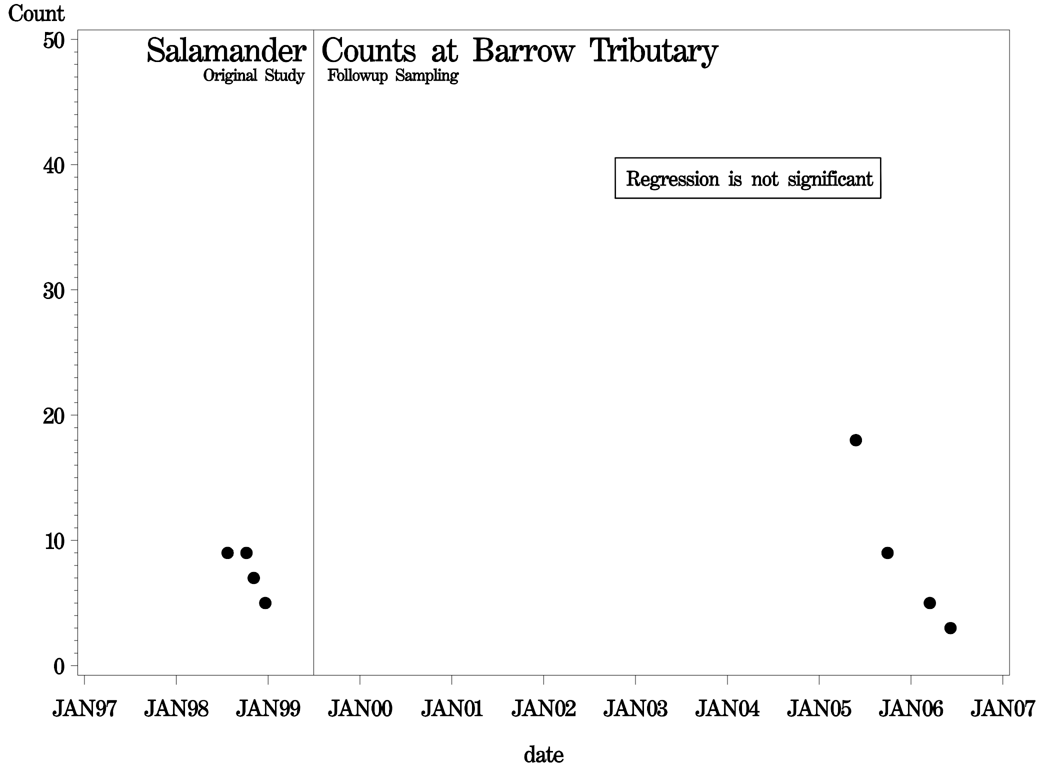


Figure 3. Salamander Counts and Flow at Barrow Tributary



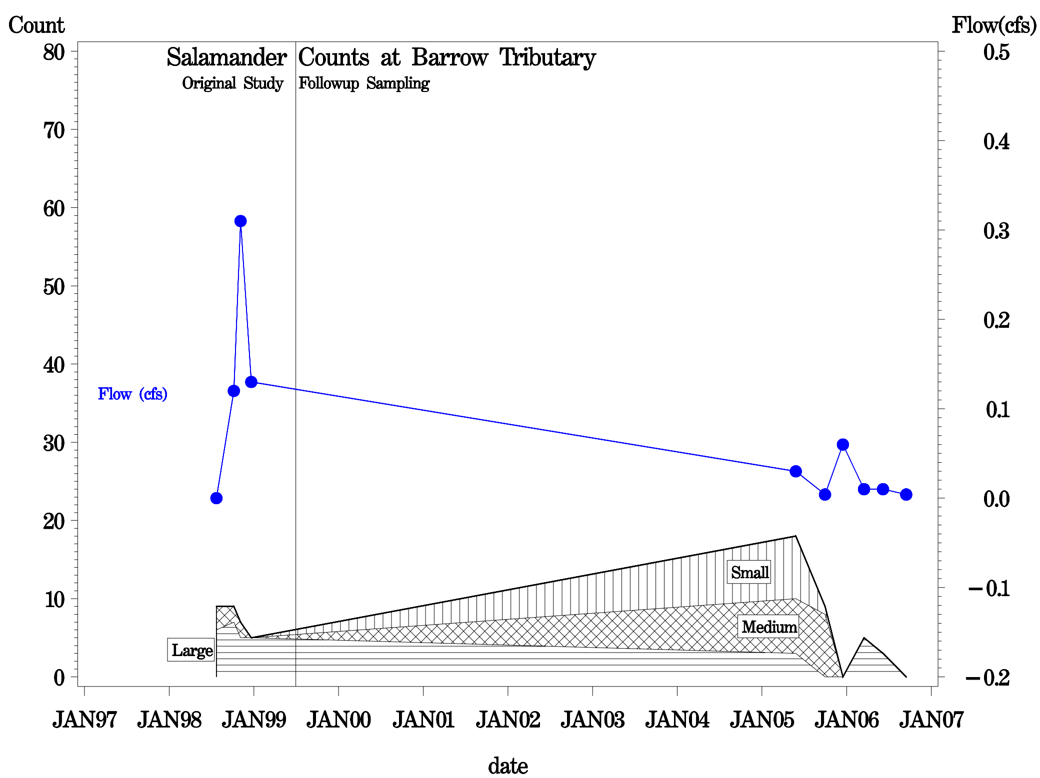
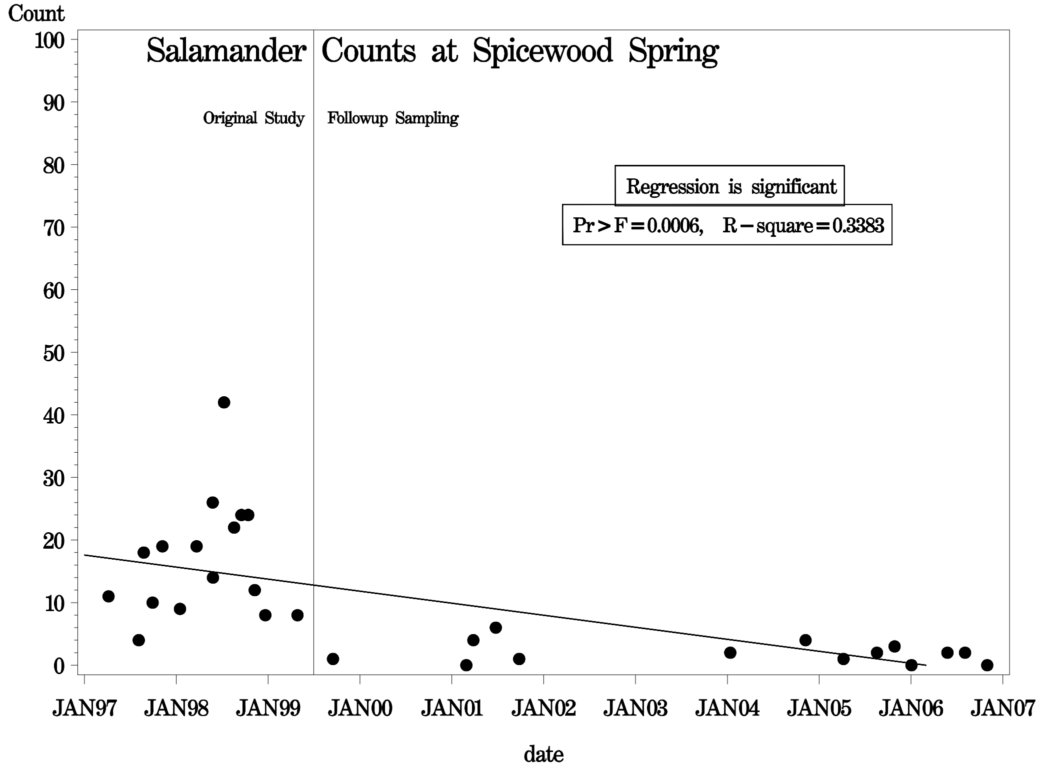


Figure 4. Salamander Counts and Flow at Spicewood Spring



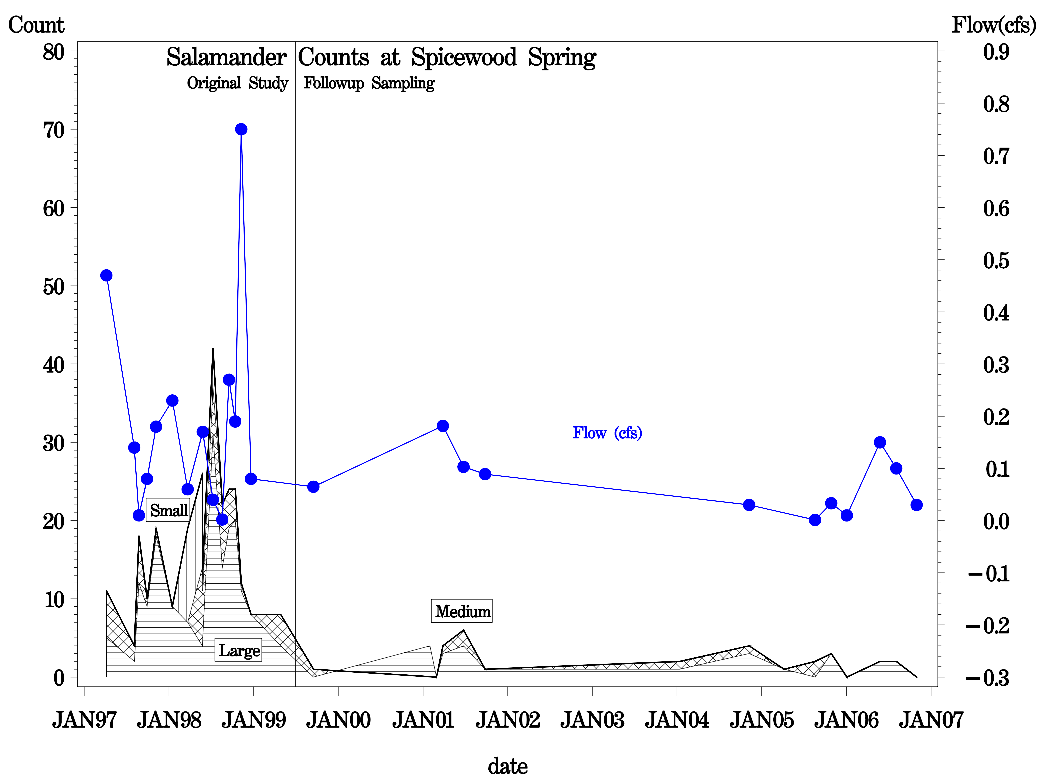
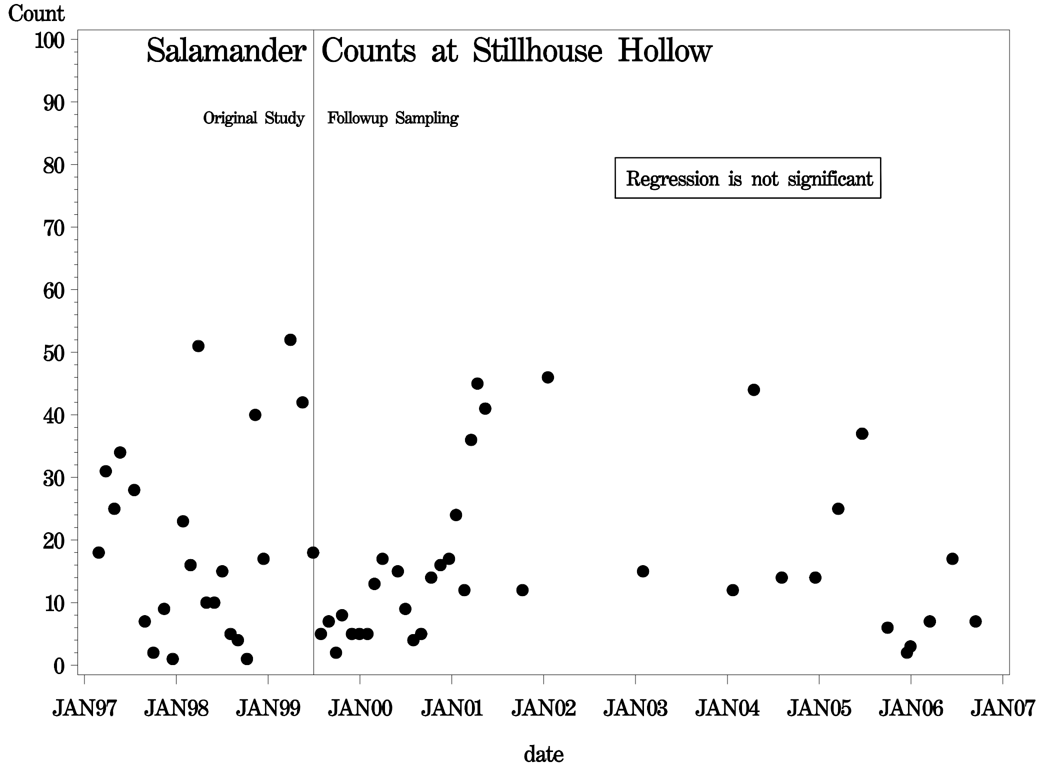


Figure 5. Salamander Counts and Flow at Stillhouse Hollow



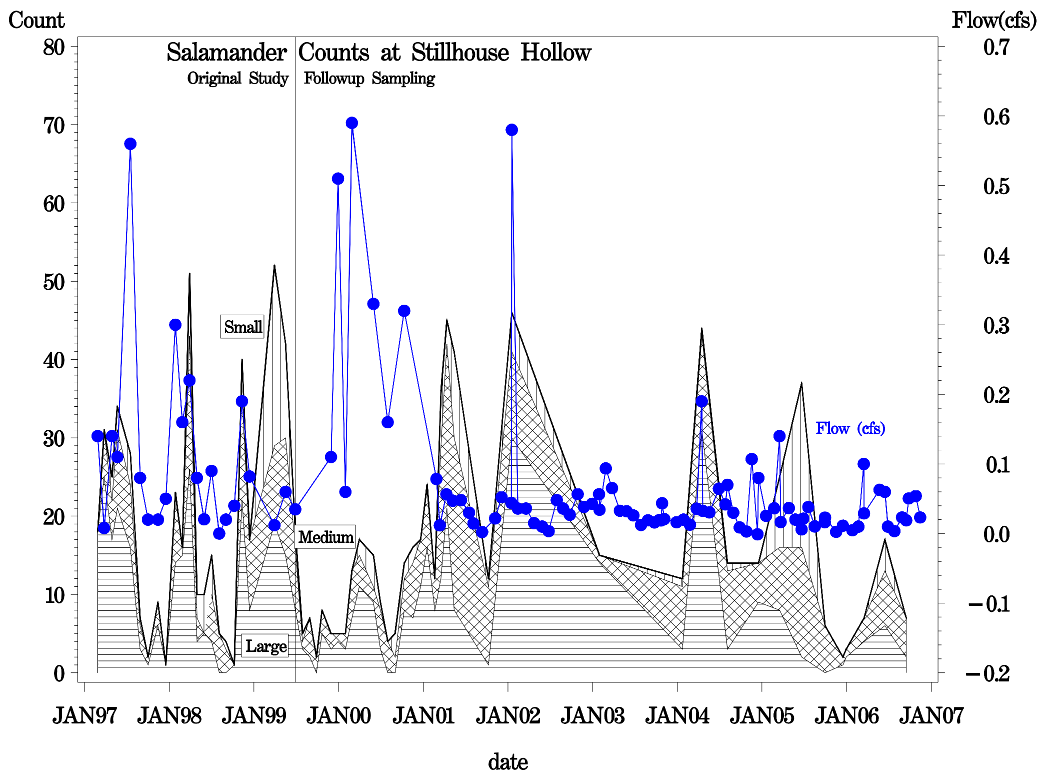
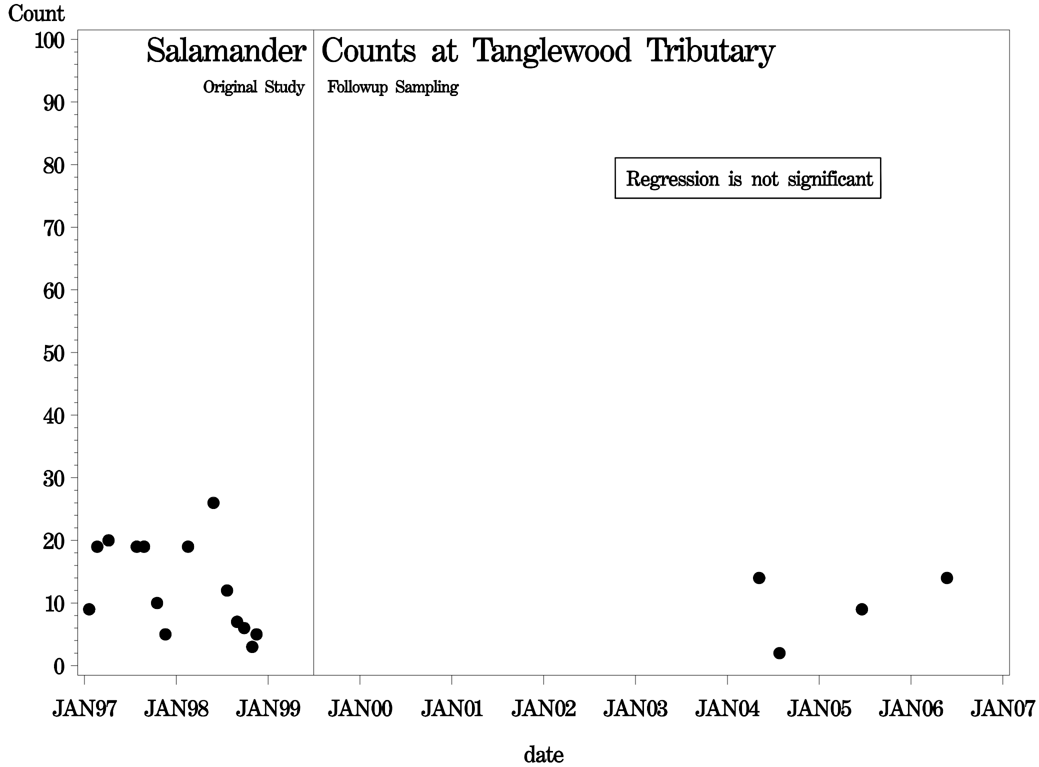


Figure 6. Salamander Counts and Flow at Tanglewood Tributary



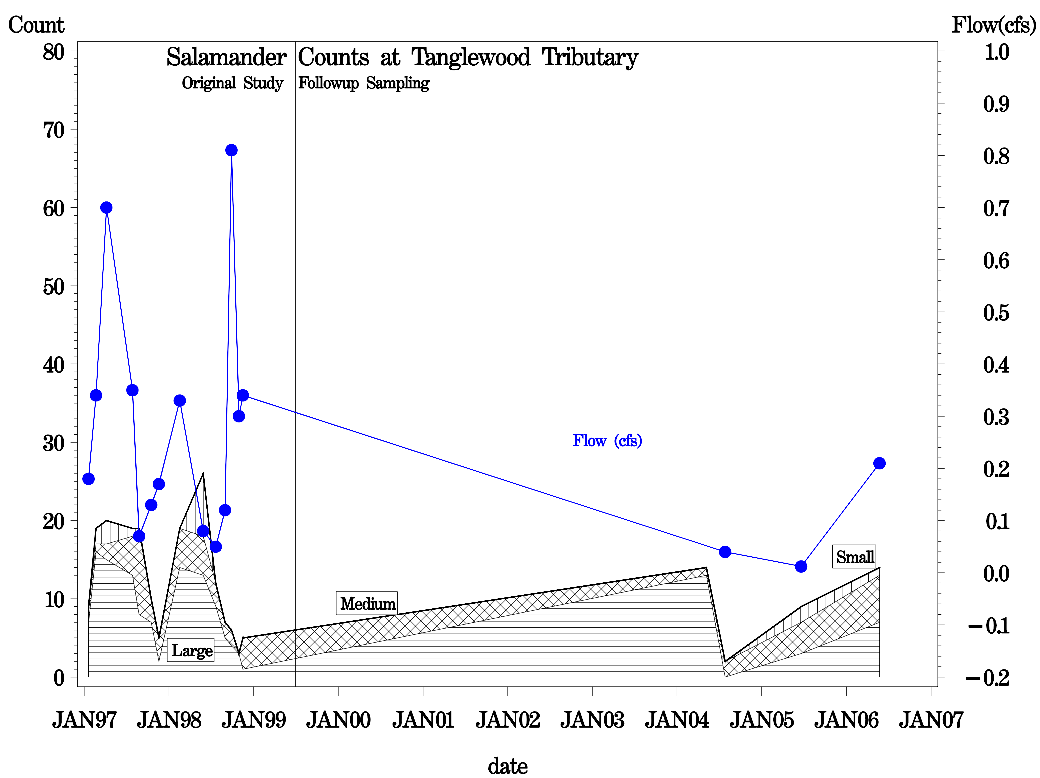
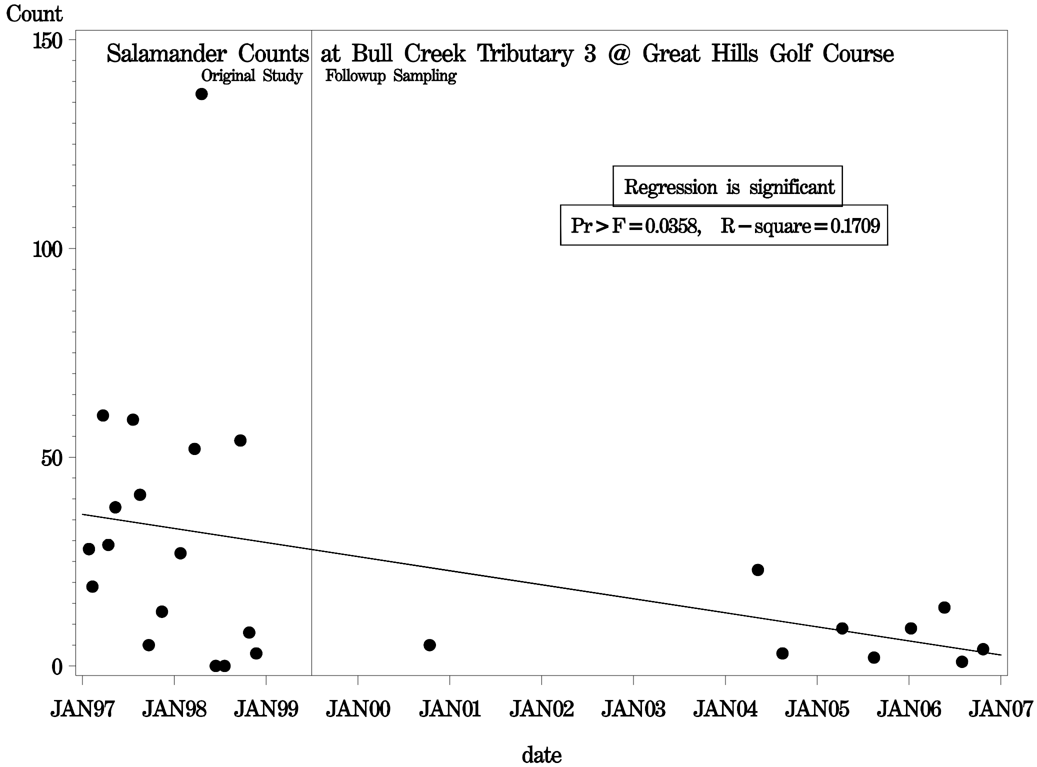


Figure 7. Salamander Counts and Flow at Bull Creek Tributary 3 @ Great Hills Golf Course



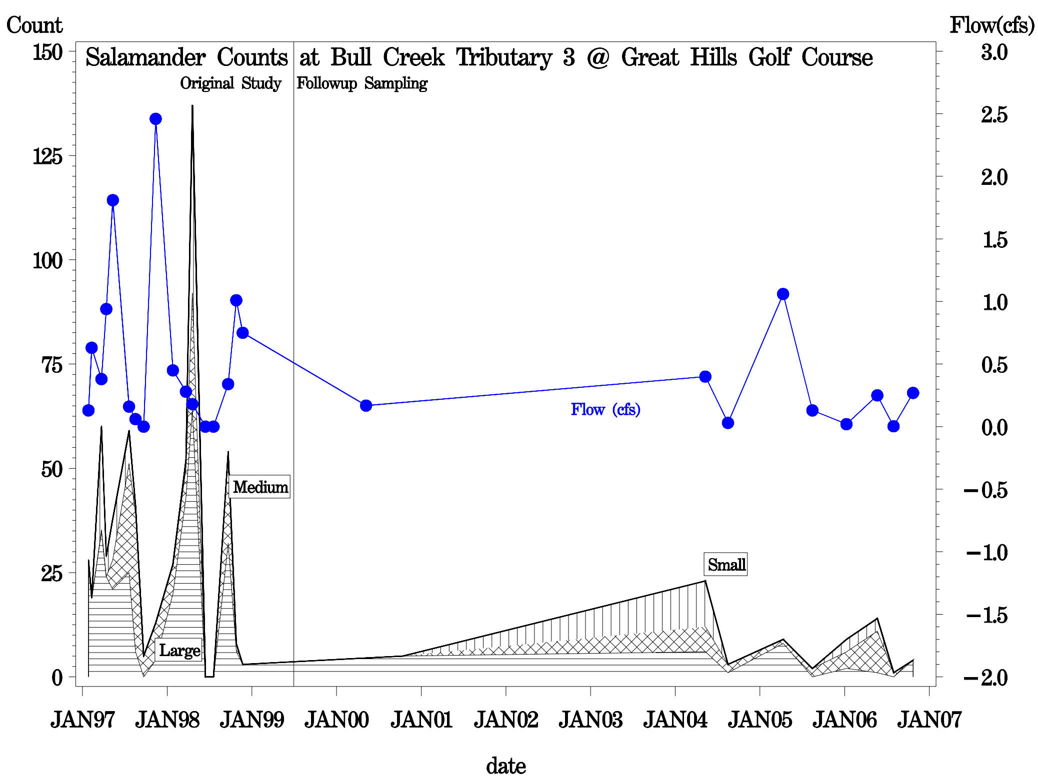
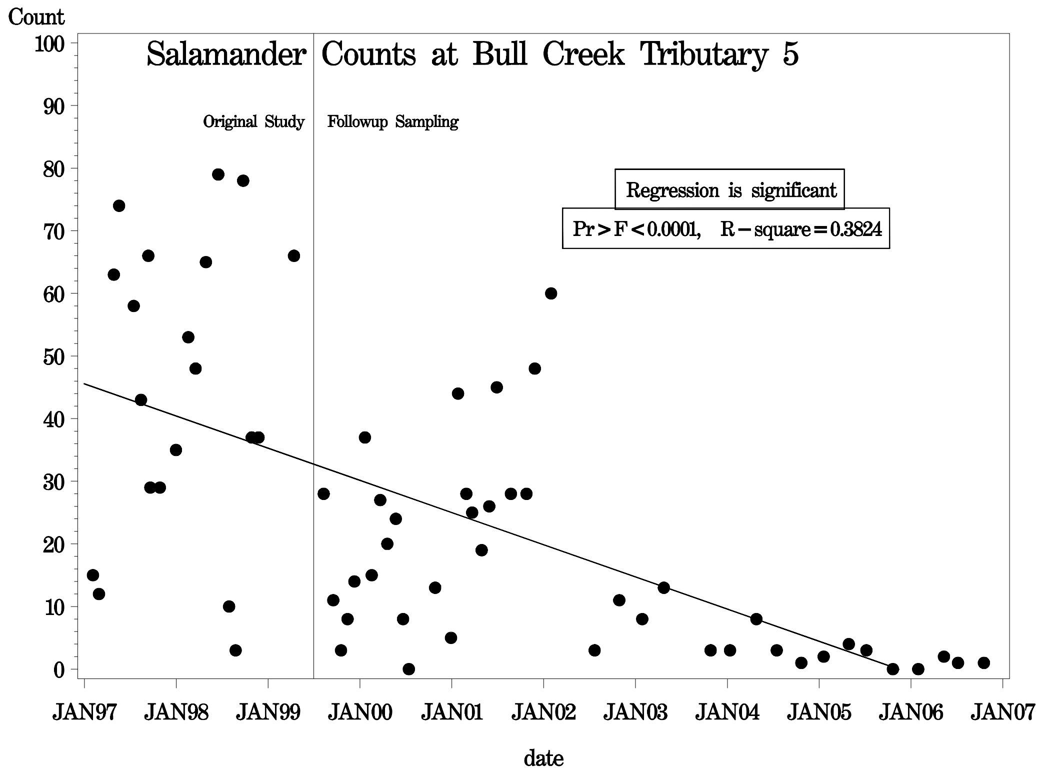


Figure 8. Salamander Counts and Flow at Bull Creek Tributary 5



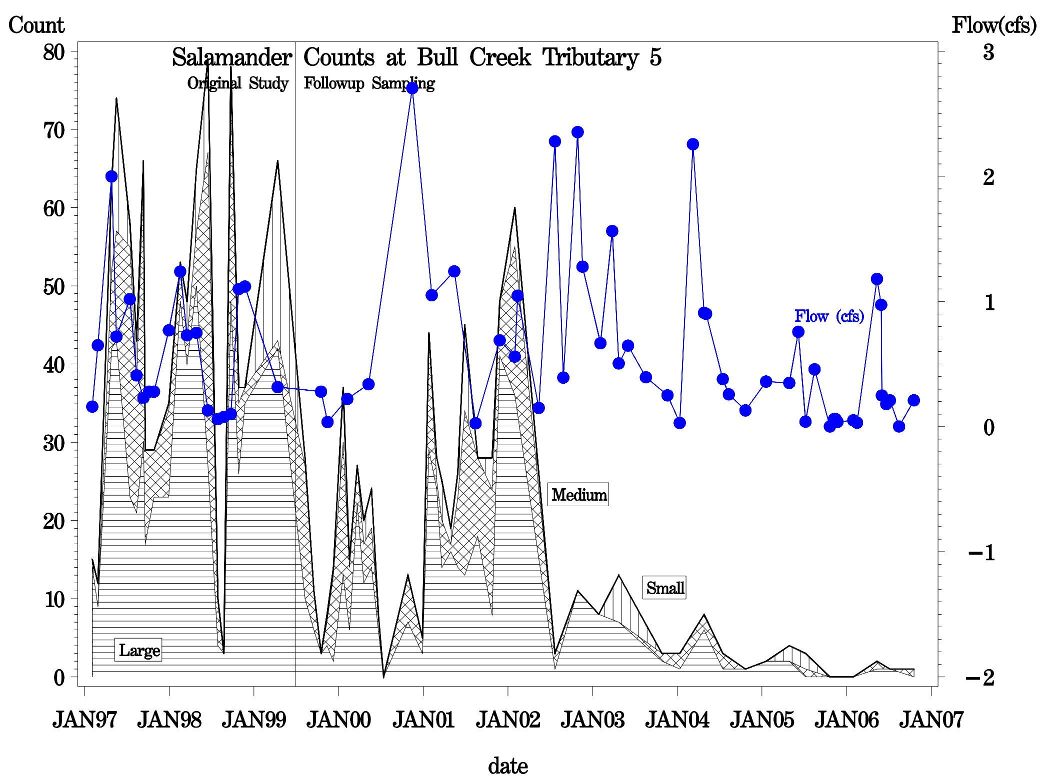
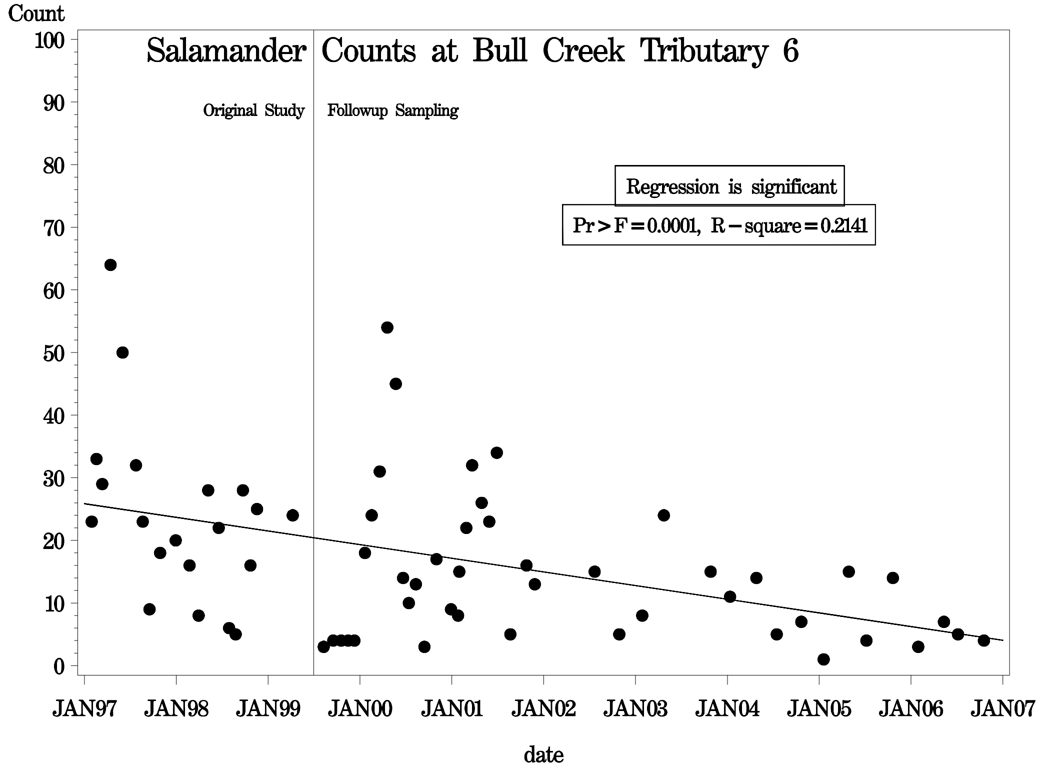


Figure 9. Salamander Counts and Flow at Bull Creek Tributary 6



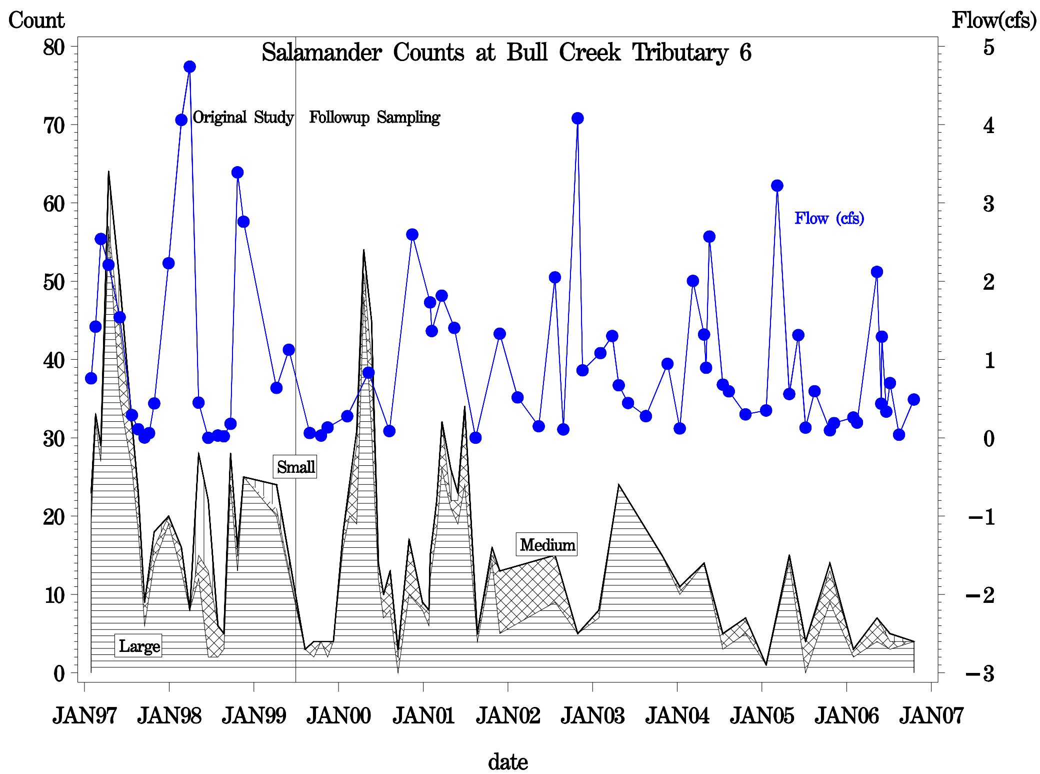
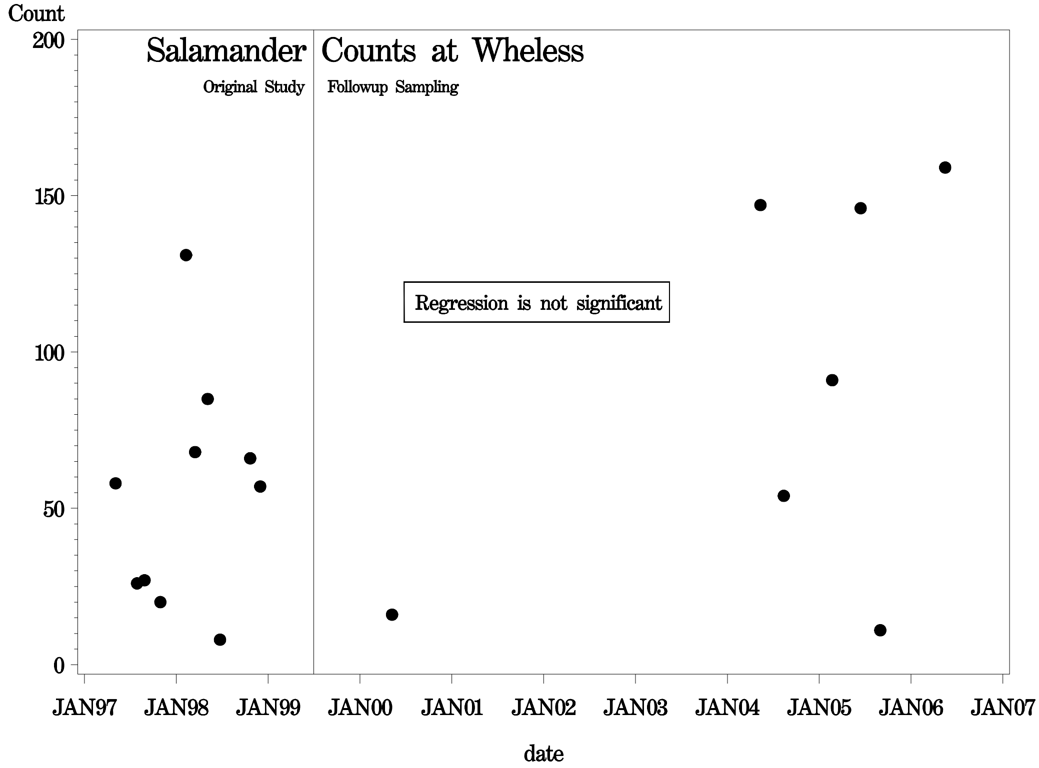
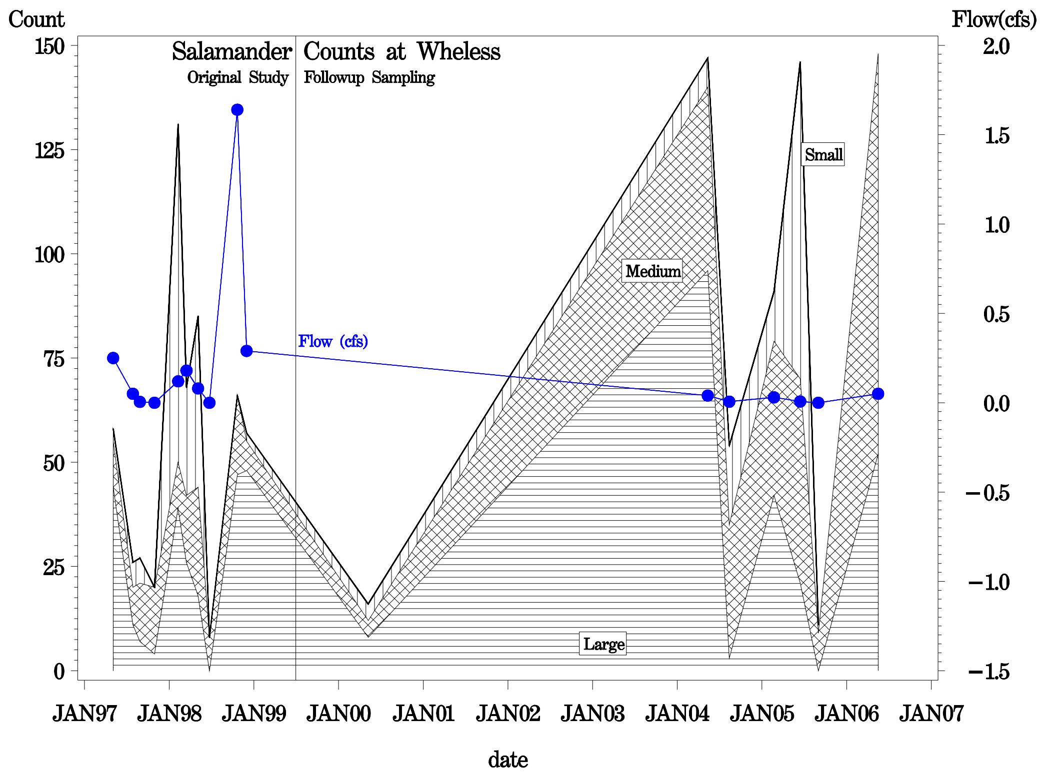


Figure 10. Salamander Counts and Flow at Wheless





New Jollyville Plateau Salamander Locations

During April and May 2006, City of Austin staff conducted creek walks along the mainstem of Bull Creek from the Water Treatment Plant 4 tract downstream to the Franklin tract, along Tributary 6 from the headwaters to the long-term monitoring site above the collapsed bridge, and along Tributary 7 from the confluence with the mainstem to the headwaters below the Wal-Mart tract. City biologists conducted spot checks along each tributary and found a few new JPS sites along the mainstem and Tributary 7. No JPS were found during the periodic spot checks along Tributary 6, although this walk was conducted shortly after a major rain event.

Jollyville Plateau Salamander Collections for Genetic Research

Samples for population and phylogenetic work were collected from JPS in the Bull Creek watershed during the summer of 2006. Samples included 18 specimens and small amounts of tissue clipped from the tips of the tail of 62 individuals (Table 4). The specimens were found stranded in small pools that were rapidly drying up at the Bull Creek above Tributary 7 site (BCP Franklin tract). One individual was found dead with a fire ant attached to the back left toes, and fire ants were attacking another. All specimens were given to the University of Texas (Dr. Paul Chippindale and Corey Roelke) and Dr. Andy Gluesenkamp, who are collaborating to help resolve the taxonomic status of the salamanders in the Buttercup Creek cave system. The tail clips were collected by Lauren Lucas, a Texas State University graduate student, as part of a population genetics study of *Eurycea* salamanders in the Edwards Aquifer. Table 4 summarizes the collections for genetic research.

Table 4. Jollyville Plateau Salamander Collections for Genetic Research

Site	Date	Number of Specimens Collected Total (<1", 1-2", >2")	Number of Tail Tips Collected Total (<1", 1-2", >2")	Comments
Bull Creek Watershed				
Stillhouse Hollow	6/15/06	--	11 (0, 7, 4)	Tail clips collected by Lauren Lucas, Texas State University
Bull Creek Above Tributary 7 (Franklin)	7/6/06	--	38 (0, 36, 2)	Tail clips collected by Lauren Lucas, Texas State University
	8/27/06	5 (0, 5, 0)	--	Stranded JPS collected by Mark Sanders and given to the University of Texas @ Arlington (Dr. Paul Chippindale, Corey Roelke) and Dr. Andy Gluesenkamp.
	9/4/06	13* (0, 12*, 1)	--	One dead individual found on an emergent rock with a fire ant on back left toes, another was being attacked by fire ants. Remaining JPS found stranded in drying pools with very little water and lots of fire ants in the area. Stranded JPS collected by Mark Sanders and given to Dr. Andy Gluesenkamp.
Tributary 5 (Hanks)	7/7/06	--	1 (0, 0, 1)	Tail clips collected by Lauren Lucas, Texas State University.
Tributary 6 (Hanks)	7/7/06	--	3 (0, 0, 3)	Tail clips collected by Lauren Lucas, Texas State University.
Cypress Creek Watershed				
SAS Canyon	9/20/06	--	9 (0, 7, 2)	Tail clips collected by Lauren Lucas, Texas State University.

Jollyville Plateau Salamander Collections for Captive Breeding

A captive breeding plan was drafted in February 2006 that provides information on the collection priorities, maintenance, and care of JPS. A new tank system was set up during the spring 2006 that houses JPS from the Lake Travis watershed (Cypress Creek, Long Hollow) and Buttercup Creek watershed. The tank system consists of three 75-gallon tanks and five 10-gallon tanks and is equipped with a chiller, filters, and CO_2 system (Figure 11). A separate 75-gallon tank was set up during the summer 2006 to house JPS collected from Bull Creek.

To date, 45 JPS have been collected for the captive breeding program (Table 5). The majority of these were collected under drought conditions as the springruns were drying up. All JPS were measured and photographed upon collection. Photographs are used to identify individuals based on dorsal pigment patterns. A few JPS were also marked with one to two visible implant elastomers (VIE) to more quickly identify them and to evaluate whether any potential negative effects occur from this type of marking. VIEs are tiny amounts of non-toxic colored polymers that are injected under the skin and quickly harden into a mark that fluoresces under UV light. VIEs have been used successfully on a variety of fish, anuran, and salamander larvae (Nauwelaerts et al. 2000, Bailey et al. 2004, Johnson and Wallace 2002), including the San Marcos salamander (*Eurycea nana*) (Joe Fries, U.S. Fish and Wildlife Service, *pers. comm.*) and JPS in Testudo Tube Cave (Andy Gluesenkamp, Zara Environmental, *pers. comm.*). In the JPS captive breeding program, marks have been injected at one or two of six different locations on the body, next to the front and back limbs and on either side of the base of the tail. Marks are recorded from left to right beginning at the front left limb, left to right on the rear limbs, then left to right on the base of the tail. To date, no obvious problems from marking captive JPS with one to two VIEs have been observed, although the procedure does require training and skill.

Table 5. Jollyville Plateau Salamander Collections for Captive Breeding

Site	Date	Number Collected Total (<1”, 1-2”, >2”)	Comments	
Bull Creek Watershed				
Bull Creek Above Tributary 7 (Franklin)	8/22/06	17 (0, 16, 1)	Survey area almost dry; collected from small pools between sections 2 and 4	First female with eggs observed 11/20/06
Tributary 8 (Lanier)	8/25/06	1 (0, 1, 0)	Spot-checked from mainstem to fenceline, found in small pool about midway along this stretch	
Tributary 6 (Hanks)	8/25/06	2 (0, 2, 0)	Collected from section 2	
Buttercup Creek Watershed				
Testudo Tube Cave	4/25/06	3 (0, 0, 3)	Emaciated	2 escaped from coolers on 6/18/06
	5/18/06	1 (0, 0, 1)	Emaciated	
Cypress Creek Watershed				
MacDonald Well	6/20/06	8 (0, 6, 2)	Marked 4 with red elastomers (Rnnnnn, nRnnnn, nnRnnn, nnnRnn)	Females beginning to develop eggs in November 2006
	10/30/06	3 (0, 0, 3)	1 albino, marked the other 2 (1 gravid female) with red elastomers (RnnnRn, nRnnnR)	
SAS Canyon	6/20/06	9 (0, 4, 5)	Marked 4 with blue elastomers (nnnBnn, nBnnnn, Bnnnnn, nnBnnn); saw 21 total salamanders (3, 6, 12) during cursory search	One female beginning to develop eggs in November 2006
Long Hollow Watershed				
Wheless	6/23/06	1 (0, 1, 0)	Collected from section 5	
Total Collected	2006	45 (0, 30, 15)		

Figure 11. Aquaria at the City of Austin's Captive Breeding Facility

Prey Items from Wild-Caught Jollyville Plateau Salamanders

Some of the JPS collected from SAS Canyon and MacDonald Well were held for 1 to 3 days in small aerated containers to obtain fecal pellets prior to release into the tank system. The contents of the fecal pellets were analyzed by Andrew Clamann (Watershed Protection and Development Review Department) and are presented in Table 6. It is interesting to note that MacDonald Well was dry for much of 2006 and had been dry shortly before the collections; all of the JPS collected seemed healthy and robust. Predominant prey items for both sites include ostracods and chironomid larvae. Like other *Eurycea* species, the JPS appears to have a general and varied diet of small macroinvertebrates.

Table 6. Contents of Fecal Pellets from Wild-Caught Jollyville Plateau Salamanders

Invertebrate Taxon	SAS Canyon, Collected 6/21/06			MacDonald Well, Collected 10/30/06		
	61 mm total length	39 mm total length	54 mm total length	46 mm total length (albino)	64 mm total length	70 mm total length (gravid)
Laccophilus						1
Orthocladiinae (chironomid)	4	7	2			
Ostracoda	2	2	7	45	35	
Physella				2		3
Tanypodinae (chironomid)	1			1		
Tanytarsini (chironomid)	1			1	1	
Midge pupa (chironomid)	1	1	1			
Copepoda				7	9	1
Chironomidae				3		
Fossaria				1		
Dasyhelea			2			
Unidentified beetle	2					
Unidentified snail	1					
Unidentified hexapod			1			1
Unidentified ?	2		1		1	

Growth Rates in Refugium

Based on a limited dataset, there does not appear to be a significant difference between growth rates (Table 7, Figure 12) or egg development (Table 7) in unmarked salamanders and the salamanders marked with one VIE. Five of the eight salamanders marked with one VIE began developing eggs within five months from the date of collection and are now gravid; four of these are in the MacDonald Well tank and one is in the SAS Canyon tank. Growth rates decrease as the salamander size increases (Table 7, Figure 12). There does not appear to be a significant difference in growth rates, adjusted for initial length, from the MacDonald Well, SAS Canyon, Testudo Tube, or Wheless sites (Table 7, Figure 13). JPS from the Bull Creek site have not been re-measured since they were collected. The salamanders collected from Testudo Tube Cave had little or no growth while maintained in small, aerated coolers, but growth rates increased considerably after transfer into the tank system.

Table 7. Growth Rates of Jollyville Plateau Salamanders in Refugium

Collection Site	Initial Date Measured	Initial Total Length (mm)	Eggs Visible ?	Most Recent Date Measured	Current Total Length (mm)	Eggs Visible ?	Growth (mm)	Days Since First Measured	Growth Rate (mm/day)	VIE Marks and Comments
MacDonald Well	6/20/06	38	No	12/1/06*	65	No	27	163	0.17	
	6/20/06	45	No	12/1/06	67	No	22	163	0.13	
	6/20/06	47	No	12/1/06	61	No	14	163	0.09	
	6/20/06	49	No	12/1/06	71	Yes	22	163	0.13	nnnRnn
	6/20/06	50	No	12/1/06	65	Yes	15	163	0.09	
	6/20/06	51	No	12/1/06	70	Yes	19	163	0.12	Rnnnnn
	6/20/06	57	No	12/1/06	70	Yes	13	163	0.08	nRnnnn
	6/20/06	65	No	12/1/06*	76	Yes	11	163	0.07	nnRnnn
	11/1/06	46	No	12/106	49	No	3	29	0.1	Albino
	11/1/06	64	No	12/106	64	No	0	29	0.0	nRnnnR
	11/1/06	70	Yes	12/106	74	Yes	4	29	0.14	RnnnRn
SAS Canyon	6/20/06	39	No	12/1/06	71	No	32	163	0.20	nnnBnn (very small mark)
	6/20/06	46	No	12/1/06	69-72	No	23-26	163	0.14-0.16	
	6/20/06	50	No	12/1/06	68	No	18	163	0.11	
	6/20/06	50	No	12/1/06*	68	No	18	163	0.11	
	6/20/06	55	No	12/1/06	79	No	24	163	0.15	Bnnnnn
	6/20/06	56	No	12/1/06	69-72	No	13-16	163	0.08-0.10	
	6/20/06	58	No	12/1/06	66	No	8	163	0.05	nBnnnn
	6/20/06	58	No	12/1/06	59	No	1	163	0	
	6/20/06	61	No	12/1/06*	69	Yes	8	163	0.05	nnBnnn (very small mark)
Wheless	6/23/06	36		10/27/06	~70		~34	126	~0.27	Observed in tank
Testudo Tube	7/5/06	67	No	12/1/06*	80	No	13	148	0.09	Emaciated when collected; little growth while kept in small cooler; moved to tank system on 7/5/06
	7/5/06	62	No	12/1/06*	71	No	9	148	0.06	Emaciated when collected; no growth while kept in small cooler; moved to tank system on 7/5/06

*Sampled (toe clips) for chytrid fungus on 12/1/06

Figure 12. Growth Rates of Marked (1 VIE) and Unmarked JPS in Refugium

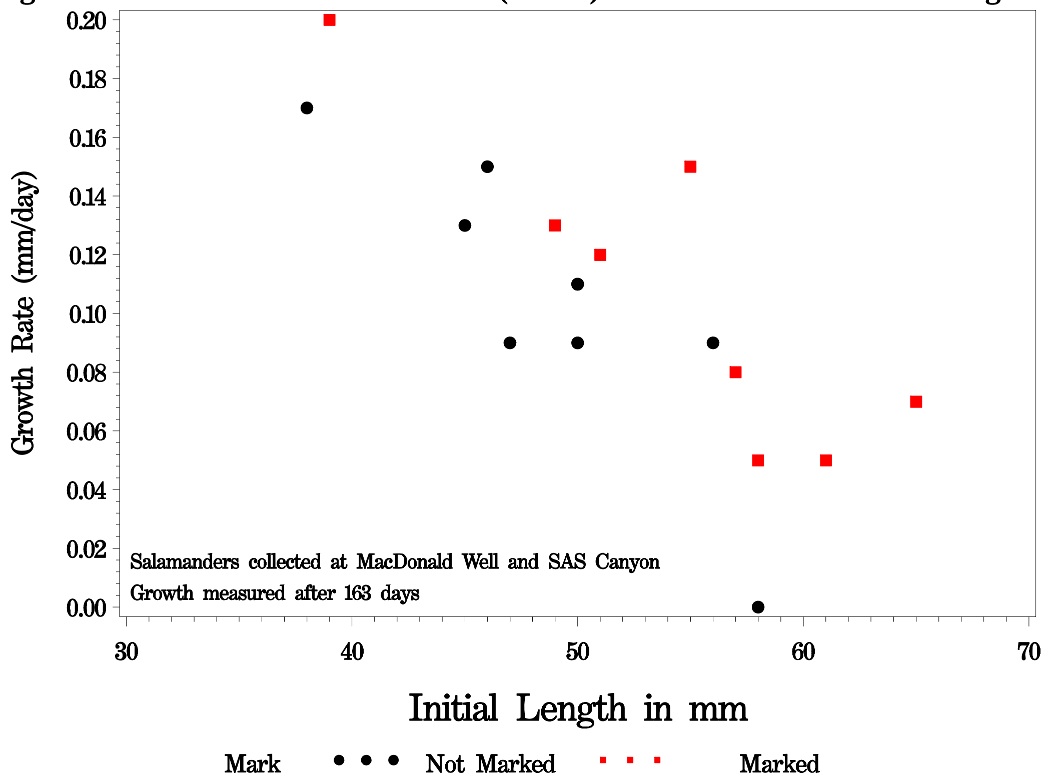
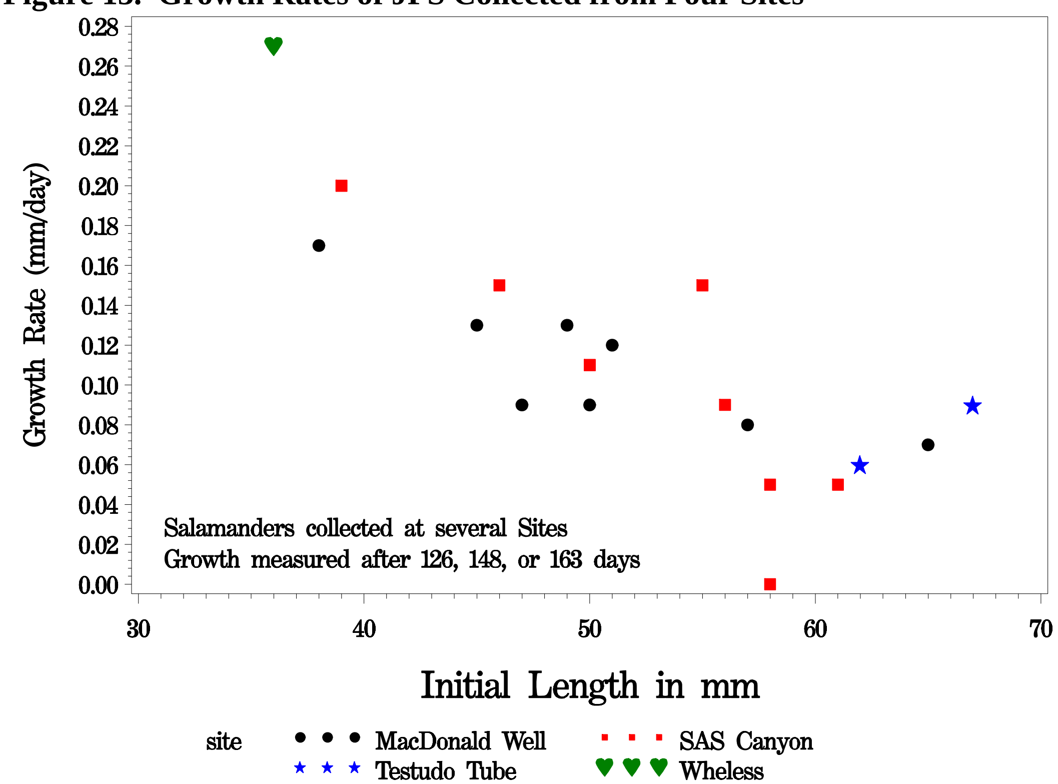


Figure 13. Growth Rates of JPS Collected from Four Sites



Chytrid Fungus

In May 2006, City biologists made a concerted effort to determine whether JPS were infected with the chytrid fungus (*Batrachochytrium dendrobatidis*). The fungus had already been confirmed in salamanders from Stillhouse Hollow and protocols to prevent its spread in the field have been developed (City of Austin 2005). Determining whether this pathogen was present in other JPS populations was included as part of the JPS captive breeding plan. The fungus infects keratinized skin, which in aquatic salamanders is believed to be limited to the toes and does not appear to cause any noticeable health effects. A few JPS at Stillhouse Hollow have exhibited some of the characteristic signs of infection found in adult anurans (emaciation, pale skin, lethargy), but this could be due to water quality or other problems.

To test for the presence of the fungus, JPS were anaesthetized in MS-222 and the tips of one to three toes were clipped from one or both back feet using sterile scissors or razor blade. City biologists were initially concerned about obtaining false negative results and were also interested in knowing whether all or a percentage of individuals are infected within a given population. Wherever possible, samples were obtained from at least three individuals across different areas at each site. Tissue samples were sent to Pisces Molecular for chytrid PCR analysis. Of the 15 sites that were sampled, all 38 individuals tested positive for the chytrid fungus (Table 8).

Table 8. Chytrid Fungus Test Results – JPS in Wild Populations

Site	Sampling Date	Size (total length)	PCR Assay Results	Comments
Bull Creek Watershed				
Tributary 5, Section 4	5/12/06	65mm	+++	
Tanglewood, Section 3	5/24/06	59mm	+++	
Tanglewood, Section 3	5/24/06	51mm	+++	
Tanglewood, Section 3	5/24/06	58mm	+++	
Franklin, Section 1	5/12/06	56mm	+++	
Franklin, between sections 3 and 4	5/12/06	48mm	++	
Franklin, Section 7	5/12/06	49mm	+++	
Trib 6, Section 3	5/12/06	48mm	+++	
Trib 6, Section 3	5/12/06	59mm	++	
Trib 6, Section 3	5/12/06	49mm	+++	
Lanier Spring	5/23/06	50mm	+++	
Lanier Spring	5/23/06	56mm	+++	
Lanier Spring	5/23/06	49mm	+++	
Trib 3, Section 4	5/22/06	29mm	+++	
Trib 3, Section 2	5/22/06	48mm	+++	
Trib 3, Section 2	5/22/06	54mm	+++	
Moss Gully	5/23/06	55mm	+++	
Moss Gully	5/23/06	48mm	+++	
Moss Gully	5/23/06	40mm	+++	
Floral Park	5/22/06	55mm	+++	
Floral Park	5/22/06	68mm	+++	
Floral Park	5/22/06	62mm	+++	
Water Treatment Plant 4	5/23/06	62mm	+++	
Water Treatment Plant 4	5/23/06	60mm	+++	
Water Treatment Plant 4	5/23/06	61mm	+++	

Site	Sampling Date	Size (total length)	PCR Assay Results	Comments
Buttercup Creek Watershed				
Testudo Tube	5/18/06	100mm	+++	Recapture (marked with elastomer -- nnnRnn)
Testudo Tube	5/18/06	80mm	++	
Testudo Tube	5/18/06	62mm	+++	Salamander emaciated
Cypress Creek Watershed				
MacDonald Well	5/19/06	68mm	+++	
MacDonald Well	5/19/06	78mm	+++	
MacDonald Well	5/19/06	70mm	+++	
SAS Canyon, Section 1	6/13/06	50mm	+++	
Long Hollow Creek (Lake Travis) Watershed				
Wheless, Section 5	5/17/06	64mm	+++	
Wheless, Section 6	5/17/06	52mm	+++	
Wheless, Section 5	5/17/06	60mm	+++	
Shoal Creek Watershed				
Spicewood, Section 2	5/26/06	73mm	+++	
West Bull Creek Watershed				
Ivanhoe	5/23/06	60mm	+++	
Ivanhoe	5/23/06	53mm	+++	

+++ = very strong positive signal; ++ = strong positive signal

In addition to sampling JPS, skin swabs were obtained from an adult and a juvenile Rio Grande leopard frog (*Rana berlandieri*) at SAS Canyon on June 13, 2006. The juvenile tested negative, while the adult tested positive (++). Tadpoles, which are abundant at most springsites in the Edwards Aquifer, are not found at some of the more urban sites, including Spicewood Spring and tributary, Stillhouse Hollow, and Tanglewood. The chytrid fungus may have contributed to the absence of tadpoles from these sites.

One possible hypothesis is that the chytrid fungus is present in the environment but stress contributes to infection to detectable levels. The fungus has been confirmed at sites that are showing obvious signs of urban effects as well as rural sites. At the more pristine sites, drought and/or other factor(s) may be contributing stressors. To help test this hypothesis, six individuals in the captive breeding program (two Testudo Tube, two MacDonald Well, and two SAS Canyon JPS) were resampled in December 2006 to assess whether the fungus is still detectable; results are all positive (Table 9).

Table 9. Chytrid Fungus Test Results – JPS in Refugium

Site	Sampling Date	Size (total length)	PCR Assay Results	Comments
SAS Canyon	12/01/06	69mm	+++	nnBnnn; gravid female
SAS Canyon	12/01/06	68mm	+++	
MacDonald Well	12/01/06	76mm	+++	nnRnnn
MacDonald Well	12/01/06	64mm	++	
Testudo Tube Cave	12/01/06	80mm	++	
Testudo Tube Cave	12/01/06	71mm	++	

Deformities at Stillhouse Hollow and Barrow Tributaries

Biologists began noticing salamanders with curved spines (scoliosis) along the Stillhouse Hollow tributary in 1995, and more recently in the adjacent Barrow tributary. The Barrow tributary flows into the Stillhouse Hollow tributary below where the JPS surveys are conducted. Because one salamander with a curved spine was documented at the Barrow site in May 2005, City staff began surveying this site on a quarterly basis in 2006. No evidence of spinal curvature was observed during the 2006 surveys at either Stillhouse Hollow or Barrow. Frequency at Stillhouse Hollow from 1995 to 2006 has ranged from 0 to 50 percent and appears to be decreasing, with a current long-term average of about 4 percent (Table 10).

Table 10. Frequency of Spinal Curvature in Salamanders at Stillhouse Hollow, 1995-2006*

Survey Date	Curved Spine (#/Size)	Total # Salamanders	Incidence Rate (%)
4/12/95	1 adult	19	5.3
5/10/95	0	35	0
8/15/95	1 adult	30	3.3
10/12/95	0	6	0
12/11/95	0	6	0
Beginning of More Standardized Monitoring			
12/23/96	0	20	0
2/27/97	0	19	0
3/27/97	0	35	0
4/30/97	0	40	0
5/23/97	0	54	0
7/18/97	2 large juveniles	44	4.5
8/29/97	1 adult	16	6.3
10/2/97	0	7	0
11/14/97	0	12	0
12/18/97	0	1	0
1/28/98	1 large juvenile	29	3.4
2/27/98	1 adult	35	2.9
3/30/98	3 large juveniles, 1 adult	89	4.5
5/1/98	1 adult	16	6.3
5/25/98	1 adult	Not surveyed	NA
6/1/98	2 small juveniles	27	7.4
7/3/98	1 large juvenile	22	4.5
8/5/98	3 large juveniles	9	33.3
9/3/98	1 adult	9	11.1
11/11/98	0	62	0
10/9/98	1 small juvenile	13	7.7
12/14/98	1 gravid adult	37	2.7
3/31/99	1 adult	51	2
5/18/99	1 large juvenile	42	2.4
6/29/99	0	20	0
7/30/99	0	5	0
8/30/99	1 large juvenile	7	14.3
9/28/99	1 large juvenile	2	50
10/22/99	0	8	0
11/30/99	0	5	0
12/30/99	0	5	0
1/31/00	1 large juvenile	5	20

Survey Date	Curved Spine (#/Size)	Total # Salamanders	Incidence Rate (%)
2/28/00	1 large juvenile	13	7.7
3/31/00	2 large juveniles	17	11.8
4/17/00	1 large juvenile	19	5.3
5/31/00	1 adult, possibly 2	15	6.6
6/30/00	1 large juvenile, 1 adult	9	22.2
8/1/00	0	4	0
9/1/00	0	5	0
10/11/00	0	14	0
11/17/00	0	16	0
12/21/00	0	17	0
1/18/01	0	24	0
2/20/01	1	12	8.3
3/19/01	0	36	0
4/13/01	1 large juvenile, 1 adult	45	4.4
5/14/01	0	41	0
10/9/01	0	12	0
1/18/02	0	46	0
1/31/03	0	15	0
1/23/04	0	12	0
4/16/04	0	44	0
8/5/04	0	14	0
12/17/04	2 large juveniles	14	14.3
Began Photographing Individuals			
3/18/05	1 large juvenile	27	3.7
6/21/05	4 small, 1 large juvenile	37	13.5
9/30/05	0	6	0
12/16/05	0	2	0
3/17/06	0	7	0
6/15/06	0	17	0
9/15/06	0	7	0
		Average Frequency	**4%**

*Note: the frequency data should be viewed as minimums, since salamanders with curved spines may have gone unnoticed or unreported during earlier surveys; since initiating photography in March 2005, other deformities/anomalies are also being observed that are not reported in the table.

Based on a literature search and review, possible causes of scoliosis in amphibians and fish include pathogens, contaminants, and inadequate nutrition. Necropsies conducted on five salamanders from Stillhouse Hollow between 1998 and 2005 at three different research laboratories have largely been inconclusive, except that the problem appears to reside in the muscle rather than the bone. Because no obvious pathogens emerged as a causative agent, environmental toxins are suspected as one of the leading causes of the spinal curvature.

One JPS collected with a curved spine from Stillhouse Hollow in early 1998 reversed this condition within a few weeks in captivity while being maintained in water from this site. This individual is still alive with no obvious problems. Two other salamanders with curved spines were collected December 17, 2004 and maintained for over a month in water from Barton Springs but did not show signs of recovering from this condition.

To document spinal curvature and other deformities or health problems, City biologists began photographing all of the salamanders observed at Stillhouse and Barrow in March 2005. Biologists enlarge the images and inspect external features of the salamanders that might otherwise be missed in the field. Using this method, other deformities, including missing eyes, limbs, and digits, can be observed. In 2006, no anomalies were reported for the few salamanders at Barrow. At Stillhouse Hollow, no problems were noticeable in the 6 salamanders that were photographed during the March 17, 2006 survey. On June 15, 2006, 6 of 14 salamanders (43%) had some form of anomaly ranging from short tail, webbed/fused or short digits, redness near the joints, and possible eye injury. One individual of the 5 (20%) that were photographed during the September 15, 2006 survey had righting problems due to what appeared to be a paralyzed or rigid front right limb. Biologists will continue to monitor the health condition of salamanders found at Barrow and Stillhouse.

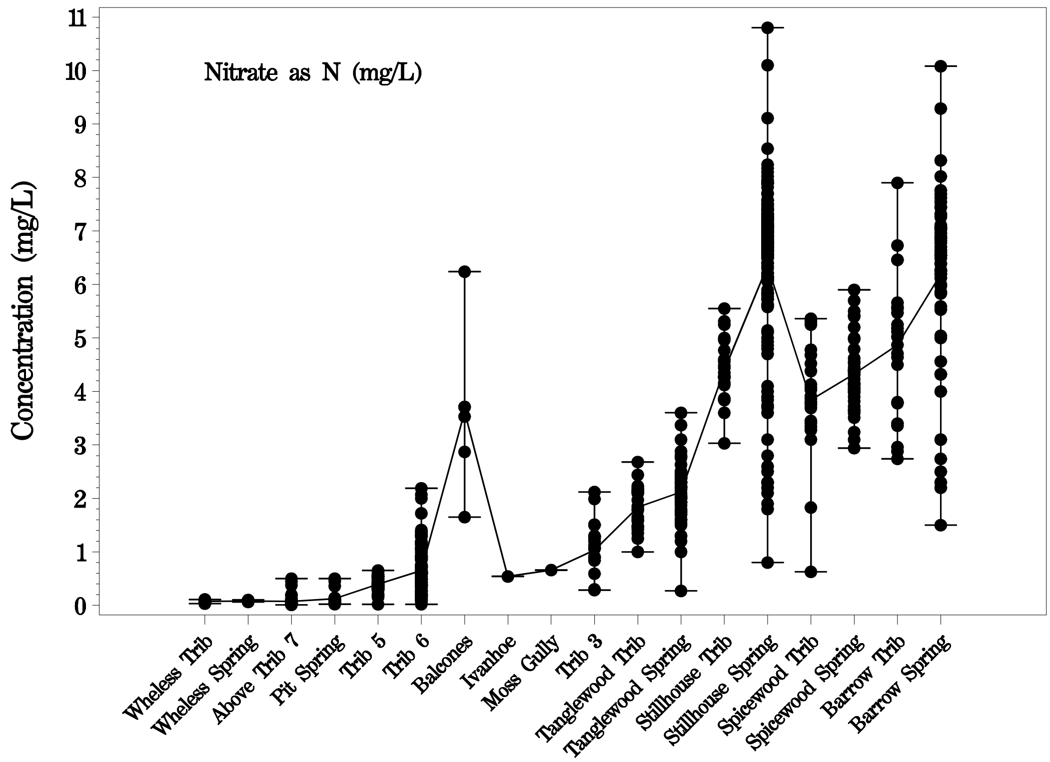
Impervious cover in the Stillhouse and Barrow drainage areas is estimated to be greater than 23% and includes residential and commercial development (City of Austin 2001). In 1992, foam was observed emerging from the main spring at Stillhouse Hollow following a rain event, but its identity and origin was not determined, except that it tested negative for surfactants. Salamanders were reportedly abundant at the main Stillhouse Hollow spring prior to this event (USFWS 1994). A gasoline station near Stillhouse Hollow had a leaking underground storage tank for several years, but groundwater tracing indicates flow under base conditions is eastward toward Spicewood Spring. It is unknown whether groundwater moves toward Stillhouse Hollow under stormflow conditions.

The most obvious chronic water quality problem that has been documented at Stillhouse Hollow and Barrow is high nitrate levels, which average over 6 mg/L, have exceeded 10 mg/L (Figure 14), and are higher than any other JPS spring site. Nitrate levels in undeveloped Edwards Aquifer springs are typically close to 1 mg/L. Samples at Barrow are collected at the head of the tributary, where JPS has not been found during the few surveys that have been conducted along this section, most recently on December 12, 2005. More frequent (currently quarterly) JPS surveys are conducted at the long-term monitoring site farther down the tributary, where nitrate levels were found in the 3 to 4 mg/L range in December 2005 and January 2006.

The Spicewood Spring site has high nitrate levels, although they are lower than those observed at Stillhouse Hollow and Barrow. JPS found at the Spicewood Spring site are also photographed, and to date no deformities have been observed, although this site has experienced a significant decline in the numbers of salamanders observed.

Very few chronic nitrate toxicity studies have been conducted on salamanders and other amphibians, which appear to be one of the most sensitive taxonomic groups (Marco et al. 1999, Scott and Crunkilton 2000). Salamander larvae and tadpoles have developed deformities, including bent tails, in response to relatively short-term ($\leq$15-day) exposures to elevated nitrate levels (Hecnar 1995, Marco et al. 1999, Schuytema and Nebeker 1999). There is not an obvious correlation between nitrate concentrations and numbers of JPS with spinal curvature observed at Stillhouse Hollow (Figure 15). However, toxicity research is recommended to determine whether nitrate is contributing to the scoliosis and other deformities observed in JPS at Stillhouse Hollow.

Figure 14. Nitrate Concentrations at JPS Sites



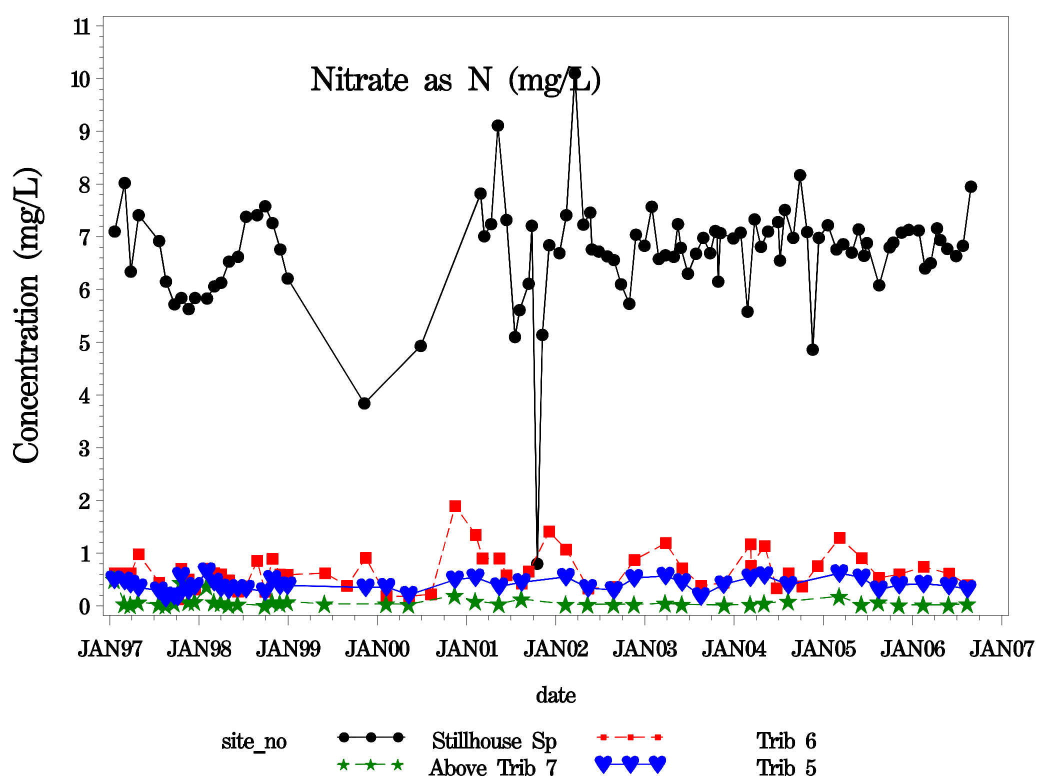
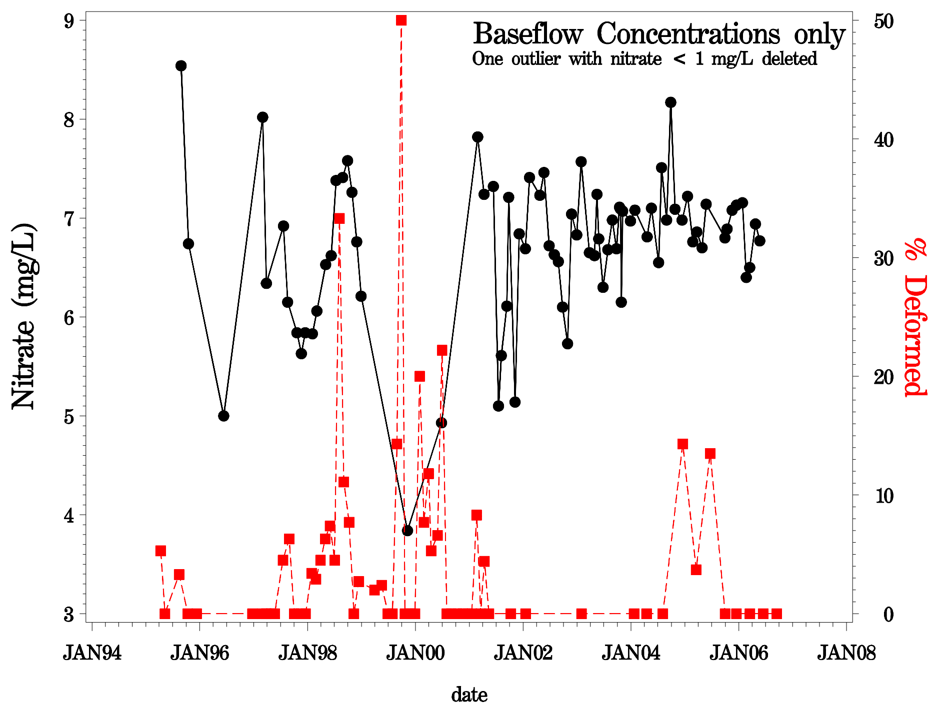
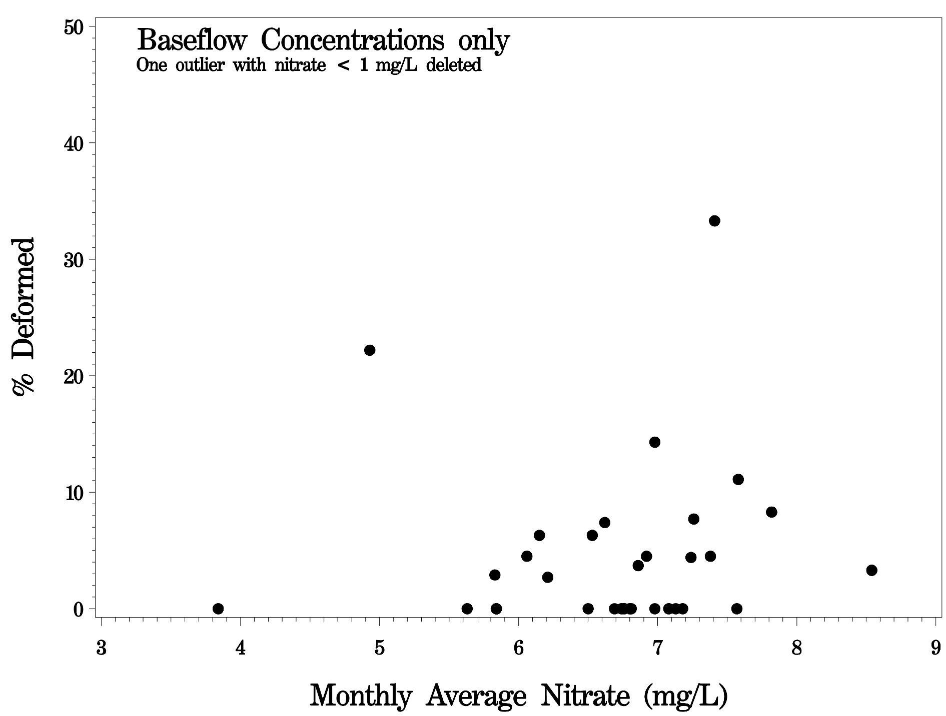


Figure 15. Nitrate and Deformities (Spinal Curvature) at Stillhouse Hollow.
Nitrate concentrations taken during baseflow were used. If the flow type was unknown or storm the concentrations were not used in these plots. Nitrate concentrations ≤ 5 are probably storm flow although they were listed in the database as baseflow. Monthly average nitrate concentrations were plotted vs. the % deformed only if a baseflow nitrate sample was taken during the same month as the salamander survey occurred.





Water Quality Measurements and Habitat Assessments

In addition to basic chemistry (temperature, dissolved oxygen, conductivity, pH), which is currently measured with a Hydrolab Minisonde at all of the monitoring sites, water quality samples are taken from five long-term monitoring sites (Table 1). Parameters at Stillhouse Hollow and Barrow include nitrate/nitrite as N (NO3), ammonia as N (NH3), total Kjedhal nitrogen, total phosphorous, and orthophosphorous. Monitoring at the other three sites (Bull Creek above Tributary 7, Tributary 5, and Tributary 6) includes these same parameters as well as total suspended solids, turbidity, volatile suspended solids, chemical oxygen demand, and inorganic ions including chloride, sulfate, calcium, magnesium, potassium and sodium. These data are presented in City of Austin reports on Bull Creek (Geismar 2001) and in Stillhouse Hollow newsletters. Since water samples are currently collected at a few JPS sites, data on these parameters for most JPS sites are limited.

Water quality parameters were investigated to see if any of them have changed over time at the long-term monitoring sites. One or all of three parameters -- conductivity, nitrate, and sodium -- have shown an increase at Bull Creek Tributary 6, Tributary 5, and Tanglewood. Figures 16-19 show the changes in these parameters over time, and Tables 11 and 12 present the parameters with statistically significant trends.

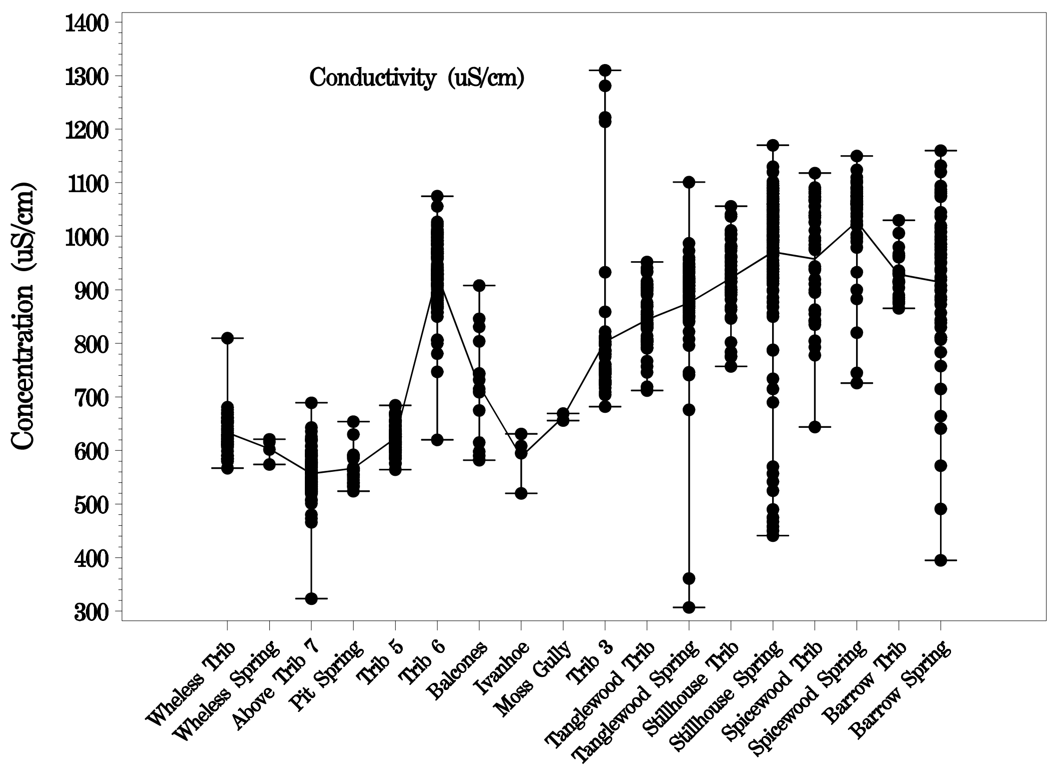
Table 11. Water Quality Parameters with Statistically Significant Changes at the 0.05 level

Site	Parameter	Pr>F	R^2	Direction	Average 1997 Value	Average 2006 Value
Bull Creek Tributary 6	Conductivity	0.0215	0.05	Increasing	912 uS/cm	920 uS/cm
Tanglewood Spring and Tributary	Conductivity	<0.001	0.28	Increasing	829 uS/cm	910 uS/cm
Bull Creek Tributary 5	Conductivity	0.0038	0.09	Increasing	617 uS/cm	630 uS/cm
	Sodium	<0.001	0.47	Increasing	7.1 mg/L	12.1 mg/L

Table 12. Water Quality Parameters with Statistically Significant Changes at the 0.10 level

Site	Parameter	Pr>F	R^2	Direction	Average 1997 Value	Average 2006 Value
Bull Creek Tributary 6	Nitrate	00823	0.02	Increasing	0.44 mg/L	0.58 mg/L
	Sodium	0.0717	0.06	Increasing	38.4 mg/L	43.2 mg/L

Figure 16. Specific Conductance Concentrations at JPS Sites



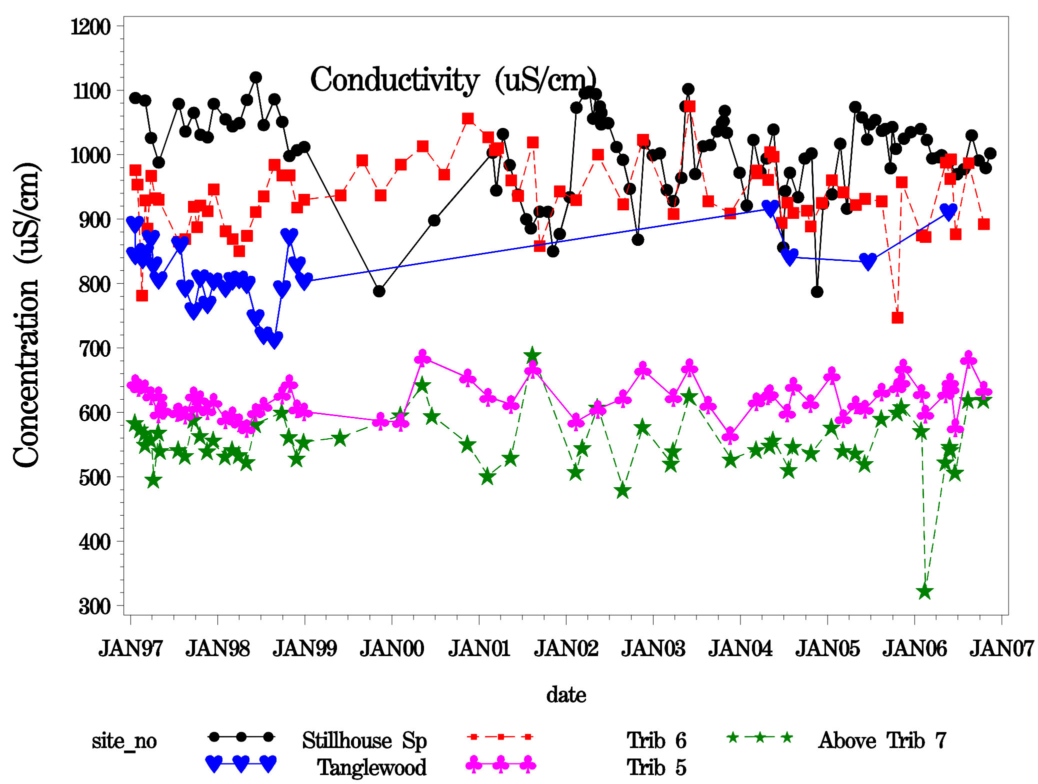
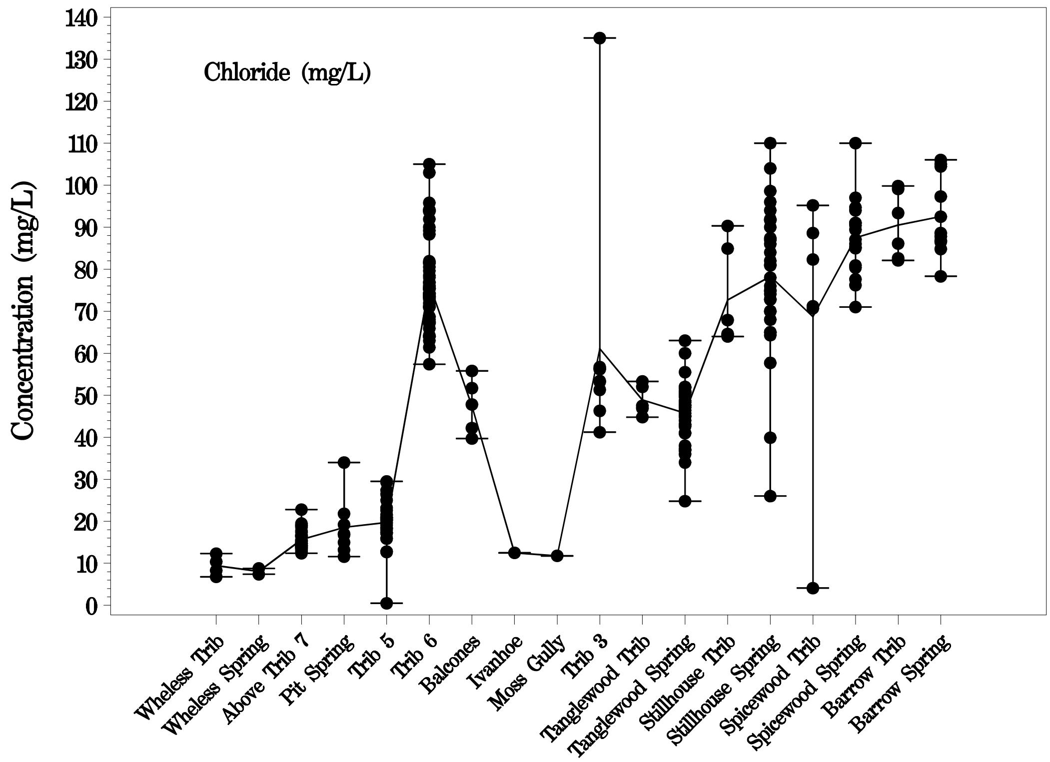


Figure 17. Chloride Concentrations at JPS Sites



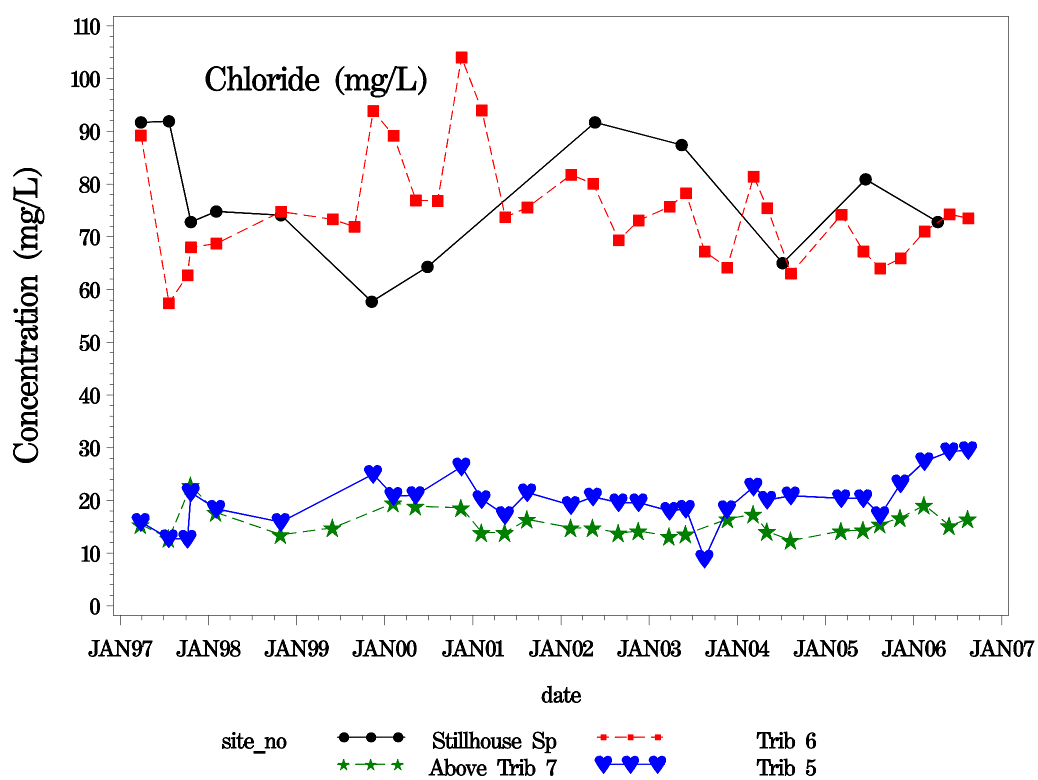
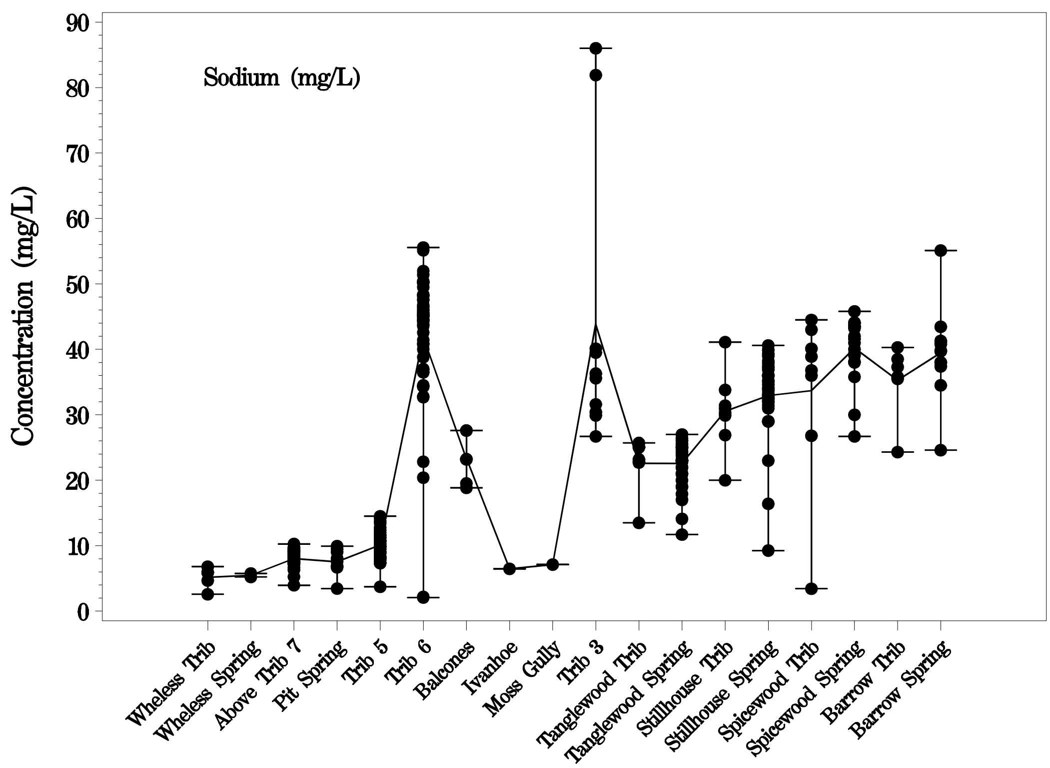


Figure 18. Sodium Concentrations at JPS Sites



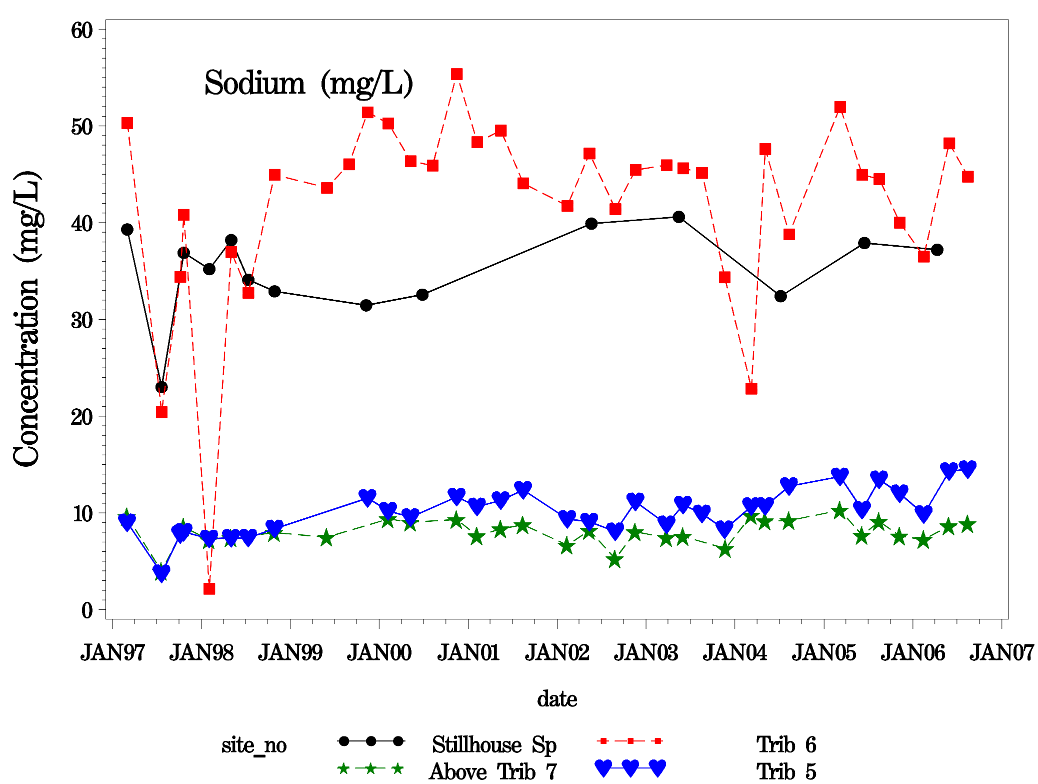
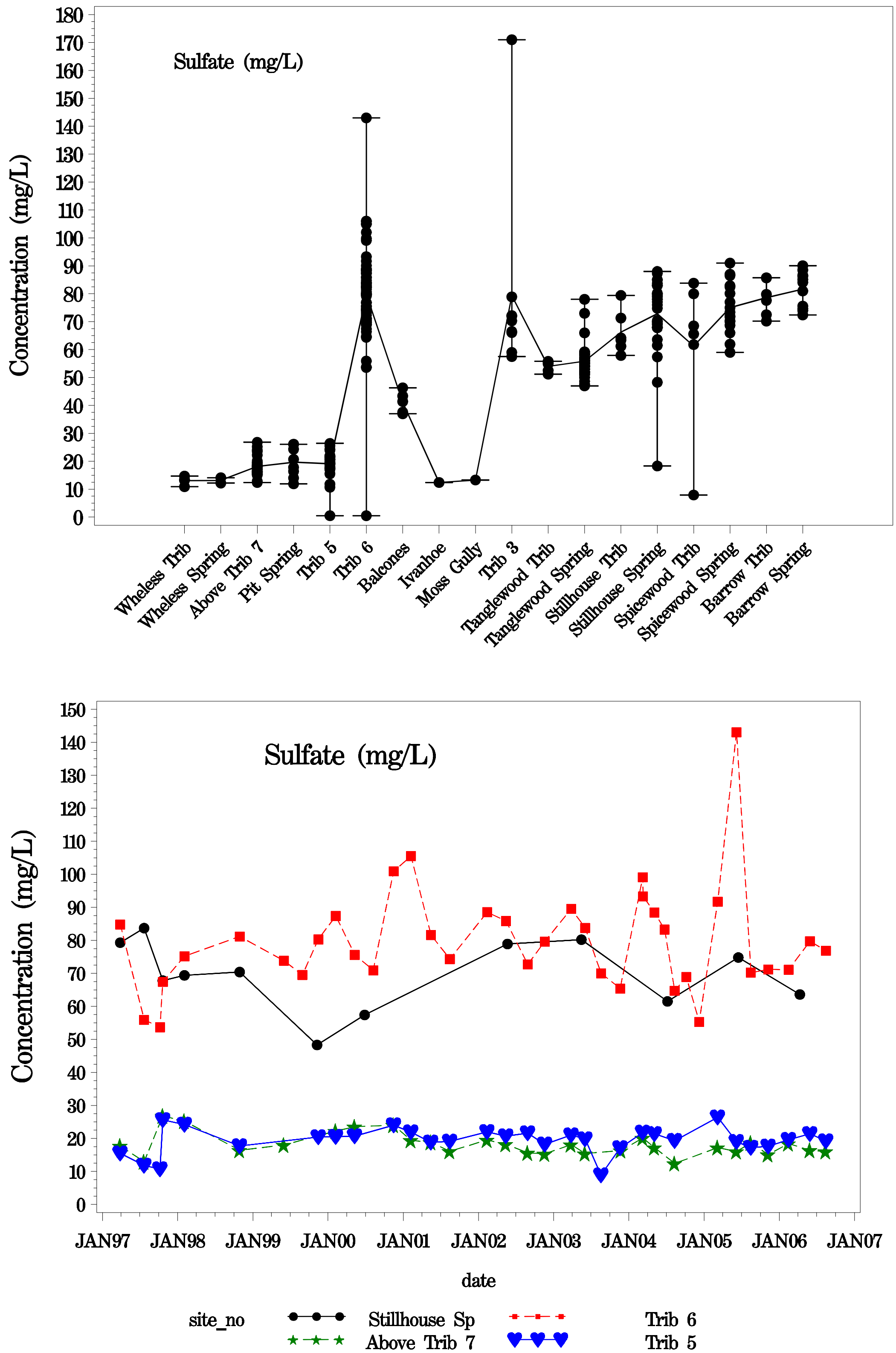


Figure 19. Sulfate Concentrations at JPS Sites



The following is a brief summary of the water quality parameters that appear to be influencing water quality and declining JPS counts.

Sediment Deposition and Embeddedness – Sediment deposition and embeddedness from recent construction activities are currently the most obvious factors contributing to the decline of salamander counts along Tributary 5 (Figures 20, 21). There have been several reports of large sediment spills occurring upstream of Tributary 5, starting in the year 2000, outside of the preserve boundaries. Inundation of the creek channel with sediment in salamander habitat has occurred periodically over the past several years and is believed to have led to declines in the salamander counts.

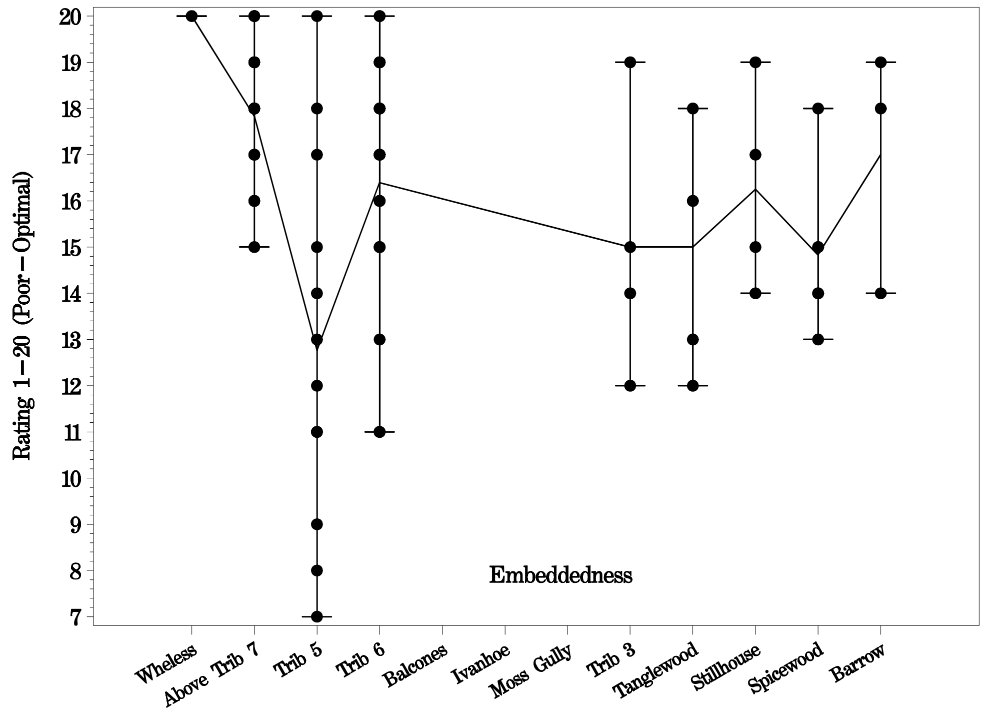
Feral hogs are also adding to sediment deposition within JPS habitat via their tendencies to "wallow" and dig up areas immediately on and adjacent to JPS habitat. City of Austin staff has noticed an increase in hog related damage throughout the Bull and Cypress creek watersheds.

Embeddedness reflects the degree to which rocks (which provide cover for salamanders) are surrounded or covered by fine sediment. Increased sedimentation from urban development is a major water quality threat to the JPS because it fills interstitial spaces where the JPS and its prey base (small aquatic invertebrates) live. Both sediment deposition and embeddedness estimates have increased significantly along Tributary 5. However, since both of these parameters are estimated visually and thus subject to error and observer bias, the data are less reliable for statistical purposes. More quantitative measurements are warranted to show how well sediment correlates with salamander abundance and distribution. City biologists are currently refining the methodology to better quantify and characterize sediment and embededdness in JPS habitat.

Bowles et al. 2006 did not find a correlation between embeddedness and JPS abundance. However, their dataset was limited to 1996-1998, prior to the increase in sediment deposition and embeddedness at the Bull Creek Tributary 5 site. They also found that embeddedness due to loose rock particles such as sand did not appear to be detrimental, which is consistent with current observations.

Bull Creek above Tributary 7 has also periodically experienced increased sediment deposition following construction activities at the headwaters near Four Points. This site appears to have recovered at this time, since no further construction activities have been initiated in this watershed. The habitat at the Bull Creek above Tributary 7 site is still considered the most pristine in the Bull Creek watershed.

Figure 20. Embeddedness (From Visual Estimates) at JPS Sites



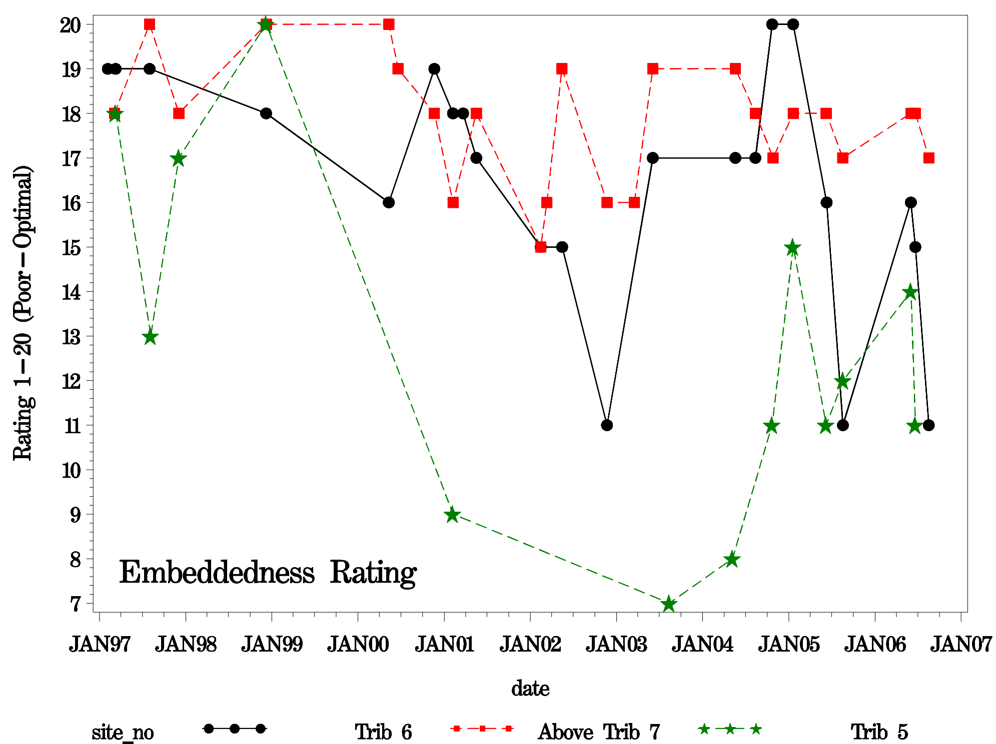
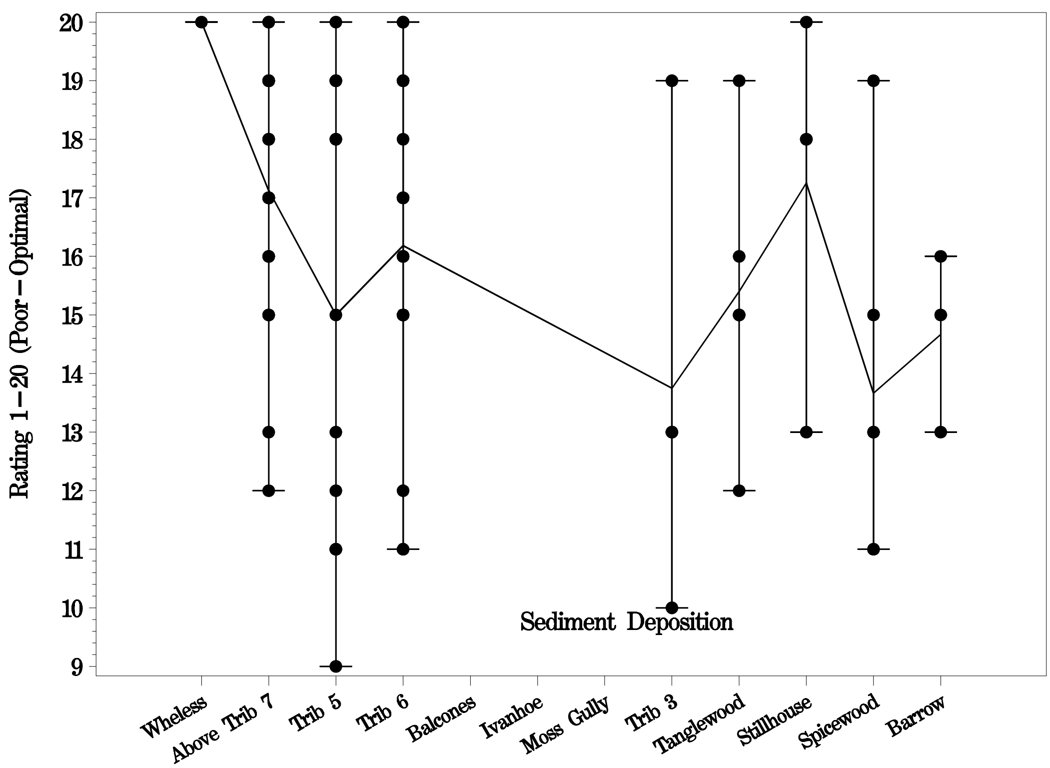
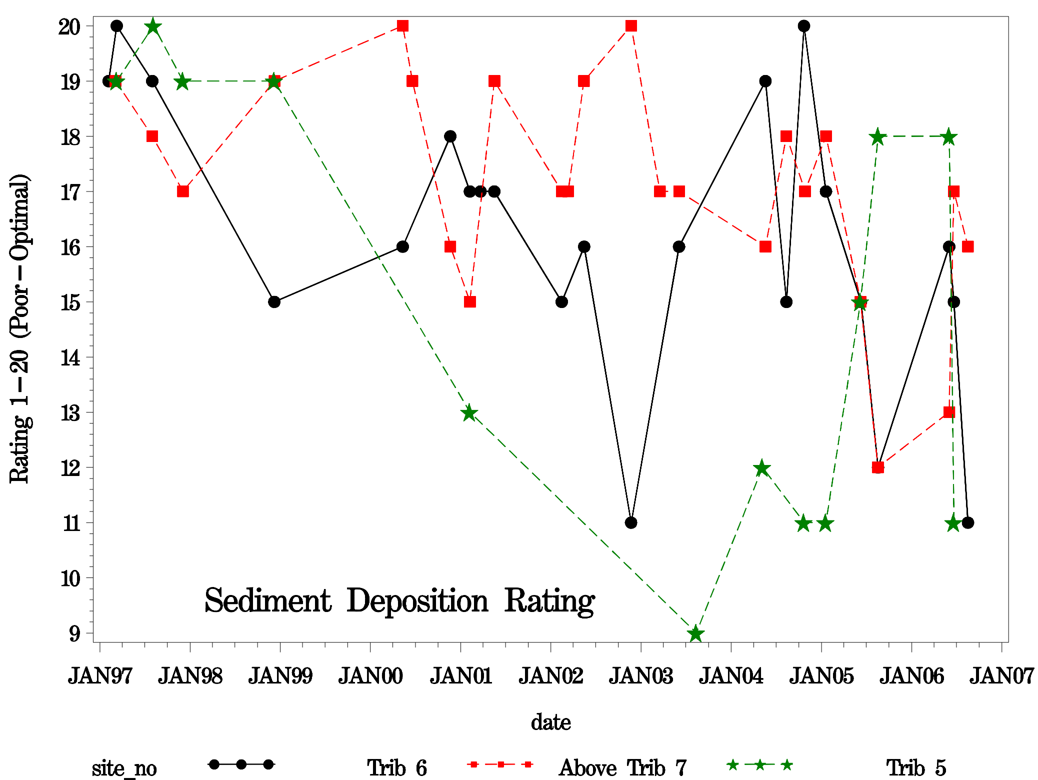


Figure 21. Sediment Deposition (From Visual Estimates) at JPS Sites



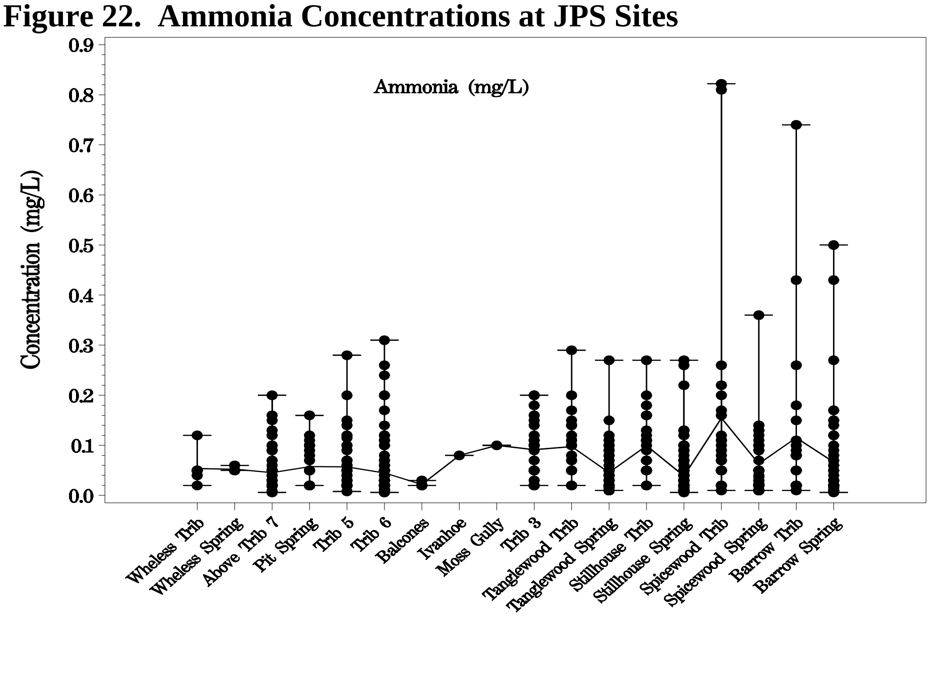


Specific Conductance, Ions, and Nutrients – Mean baseflow conductivity in rural springs in the Jollyville Plateau region generally average between 550 and 650 uS/cm. Ion concentrations (chloride, sodium, sulfate) tend to be highest at the developed sites (Barrow, Tributary 6, Spicewood, Stillhouse, Tanglewood, Tributary 3) where specific conductance typically averages between 900 and 1000 uS/cm (Figures 16-19). Nitrate, which also contributes to high specific conductance, is highest at Barrow, Stillhouse, and Spicewood (Figure 14) and is increasing at Tributary 6 (Table 12).

Whether high ion concentrations could be contributing to the declining salamander counts at Tributaries 3 and 6, where nitrate levels are relatively low compared to the other urban monitoring sites, is not clear. Sodium and sulfate appear to be slightly higher at these sites than at the other sites, while chloride is about the same or slightly lower (figures 17-19). In one study using saline well water from the "bad water zone", San Marcos salamanders (*Eurycea nana*) experienced 100 percent mortality within 24 hours in non-aerated water that had a conductivity of 1145 uS/cm and a dissolved oxygen of 6.8 to 7.5 mg/L (Edwards Aquifer Research and Data Center, in City of Austin 2001). In aerated water, 48-hour mortality was 50 percent with conductivities ranging from 1111-1240 uS/cm and higher, and 7.4 to 8.6 mg/L dissolved oxygen. However, a study being conducted by the University of Texas at Austin (funded by the Barton Springs/Edwards Aquifer Conservation District) has found no mortality, differences in activity patterns, or metabolic effects in adult San Marcos salamanders (*Eurycea nana*) from high conductivity (up to 2400 uS/cm from a well in the saline zone of the Edwards Aquifer) during a 28-day exposure period, although there was a greater decrease in body mass from the 100% saline well water treatment compared to the other treatment levels (Woods and Poteet 2006).

Nitrate levels are higher at Stillhouse Hollow and Barrow Tributary than any other JPS site, with concentrations at the springs averaging over 6 mg/L and maximum concentrations exceeding 10 mg/L (Figure 14). Springs tend to have higher nitrate concentrations than the tributaries. Nitrate concentrations at Spicewood Spring and tributary are only slightly lower than those measured in the Stillhouse and Barrow tributaries. Ammonia appears to be higher at Spicewood Spring than at other JPS sites, averaging over 0.15 mg/L and exceeding 0.80 mg/L (Figure 22), which could be contributing to the declining counts at this site.

Figure 22. Ammonia Concentrations at JPS Sites



Contaminants – Sampling to determine whether chemicals are present in sediment at levels that could cause salamander deformity at Stillhouse and Barrow was conducted by City of Austin staff during the summer and fall 2005. The results are presented in City of Austin (2005). This and previous sampling efforts have detected elevated PAHs in sediment from the Spicewood tributary, but not from Stillhouse, Barrow, or other sampling sites.

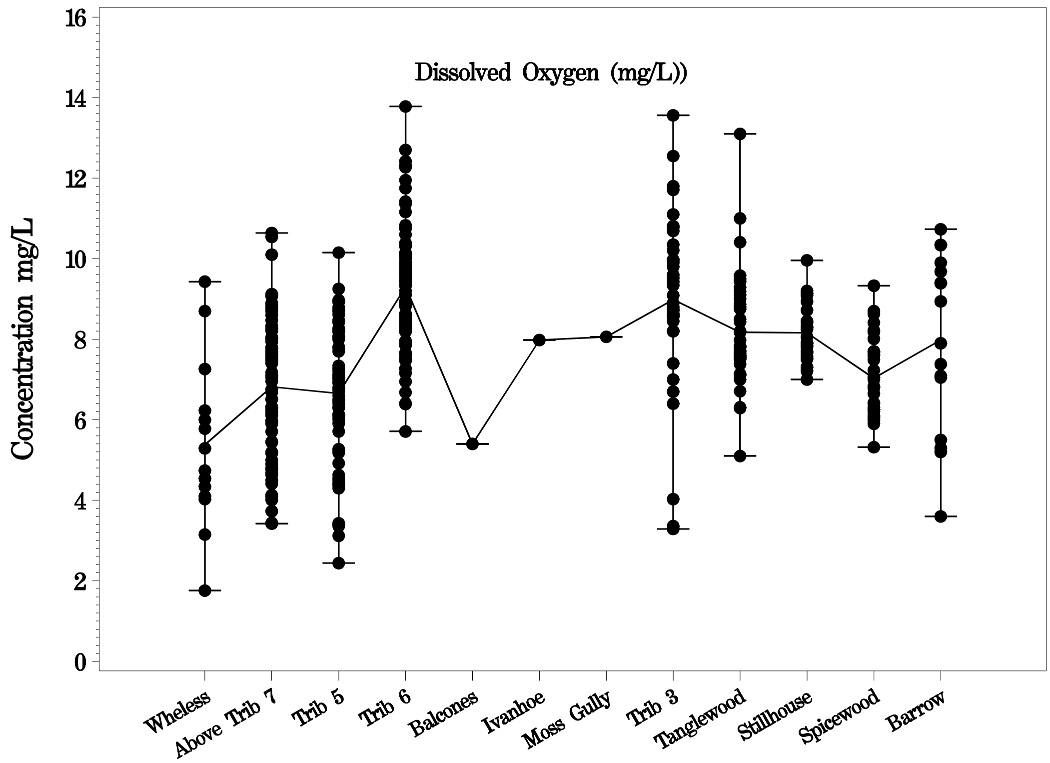
Other Water Quality and Habitat Parameters – No obvious trends or differences in dissolved oxygen among the JPS monitoring sites has been observed, except that DO appears to be somewhat higher in the more urban sites, especially Bull Creek Tributary 6 and Tributary 3 at Great Hills Golf Course (Figure 23). This may be due to increased algae production and/or increased flow from anthropogenic sources such as stormwater outfalls and golf course irrigation.

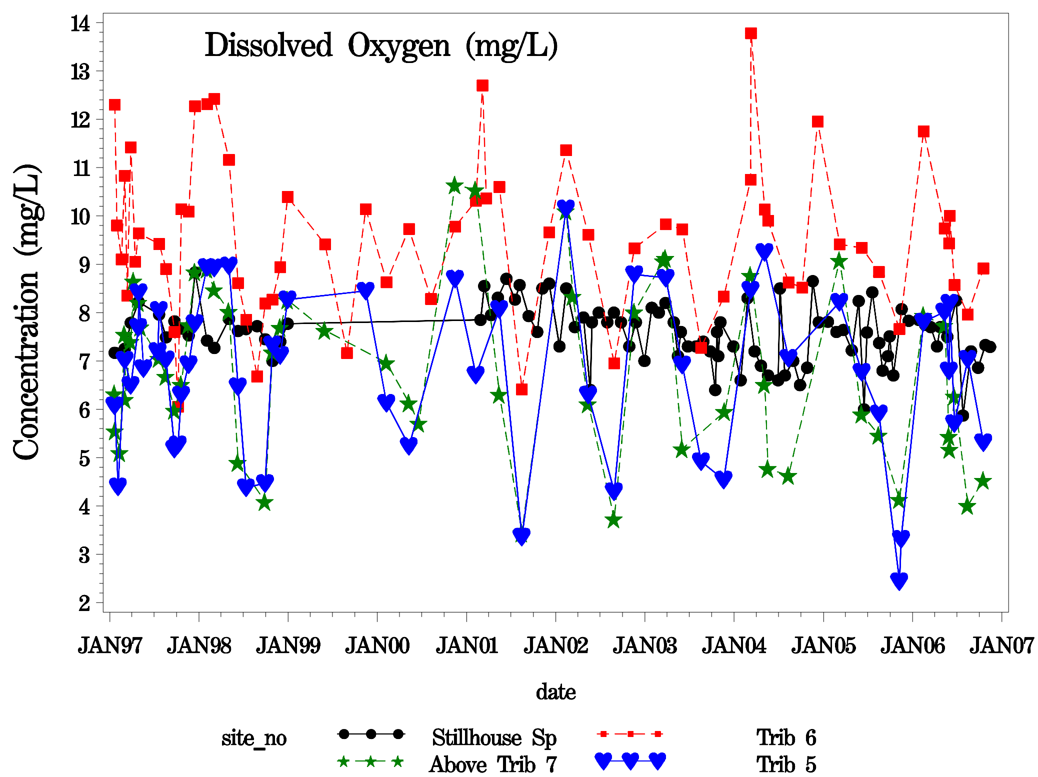
Sampling for benthic macroinvertebrates, which is conducted near three of the long-term monitoring sites in Bull Creek (Bull Creek above Tributary 7, Tributary 5, and Tributary 6), was conducted during 2006. These data are now being analyzed and will be presented in the 2007 annual report.

Flooding is problematic in urban watersheds. Following rain events, Spicewood Spring becomes inundated with leaf litter, woody debris, and trash. The Spicewood tributary also appears to be filling in with cobble, gravel, sand, and sediment. Tributary 3 has a more scoured appearance following rains, and undercutting of the banks along the monitoring site is becoming more apparent.

Bowles et al. (2006) report finding few predatory fish at the JPS sites between 1996-1998. Sunfish are now commonly observed at the Bull Creek Tributary 5 site, which could be influencing JPS numbers. A sunfish was seen capturing and eating a large juvenile JPS during the May 2006 survey. On several occasions, City staff captured predatory fish from Tributary 5 in an effort to reduce salamander predation. If time allows, staff will conduct gut content analyses of these fish in order to determine if salamander predation is occurring.

Figure 23. Dissolved Oxygen Concentrations at JPS Sites





Reproductive Cycles and Flow

As discussed in previous reports (City of Austin 2001, 2005), data continue to suggest possible seasonal reproductive cycles in the JPS. Salamander counts from all of the long-term monitoring sites, with the exception of Barrow Tributary due to the small sample size, were summed and the percent of the total count in each size class (<1 inch, 1-2 inches, >2 inches, total length) was calculated for each month. Figure 24 shows the cyclical nature of the percent in each size class. The percent of salamanders in the smallest size class peaks in April, with moderately high values from March through July. The percent in this class tends to be lowest from August through February. The middle size class, 1-2 inches in length, peaks in August, four months after the smallest size class peaks. The percent in the largest size class is highest from October through April, with a peak in December, and lower from May through September. Bowles et al. (2006) present similar results, although the size classes were divided into two categories (<1", >1") instead of three. However, because JPS spends part of its life in the aquifer, Bowles et al. (2006) avoid drawing any conclusions regarding seasonal reproduction, survival, or population size.

In addition to looking at size classes for all of the long-term monitoring sites grouped together, sites were separated according to rural (Bull Creek above Tributary 7, Wheless) and developed (Bull Creek Tributaries 3, 5, and 6, Stillhouse, Spicewood, Tanglewood) classifications (Figures 25, 26). While the two categories show similar overall trends, there are some noticeable differences. For the rural sites (Figure 25), the percentage of smaller and medium size classes is higher while the percentage of adults is lower than for the developed sites (Figure 26), indicating more reproduction and recruitment into the population. For the small size class (<1") at the rural sites, counts are highest from March through August (with a peak in June), followed by an increase in the medium size class (1-2") from July to November (with a peak in September). The largest size class (>2") peaks in December, with highest counts from October through March.

For the developed sites (Figure 26), the small and medium size classes show a similar trend to the rural sites, except the percent contribution to the total population is much lower, indicating less reproduction and recruitment. The largest size class makes up the largest percentage of the total population compared to the rural sites, is high year-round, and highest from October through April (with peak in February).

The relationship between flow, salamander counts, and reproductive cycles was investigated. While flow measurements provide a good indication of available wetted area and available surface habitat, there does not appear to be an obvious direct significant relationship between the amount of flow and counts of small juveniles. The seasonal patterns in flow were investigated by calculating the mean flow at three sites (Bull Creek above Tributary 7, Tributary 5, Tributary 6) for each year and month and summing these mean flows. The other sites were not included because their flow measurements have been too sporadic in recent years. The summed flows are plotted by month (Figure 27). Flows are lowest in the months of August and September. The rest of the year has flows that are higher but quite variable, with the highest flow in March. The percent and number of the smallest size class of salamanders are very low from August through February. It is possible that the low flows in August and September are related to the low counts of the smallest salamanders from August through February. When the time series of monthly flow and monthly percent of small salamanders were overlaid, the patterns appear similar but offset from one another (Figure 28). The lag is approximately four months. Figure 29 shows the percent of small salamanders together with the flow from four months before and the patterns are remarkably similar.

To date, only one JPS egg has ever been seen in the wild. On September 9, 1996, Mark Sanders found one egg attached to the bottom of a large rock near the spring outlet at Spicewood Spring. The egg was collected by Dr. Andy Price (Texas Parks and Wildlife Department). Because eggs are bright white and seldom if ever seen, reproduction is assumed to occur in the aquifer.

Figure 24. Percent of JPS by Size Categories, All Long-term Monitoring Sites

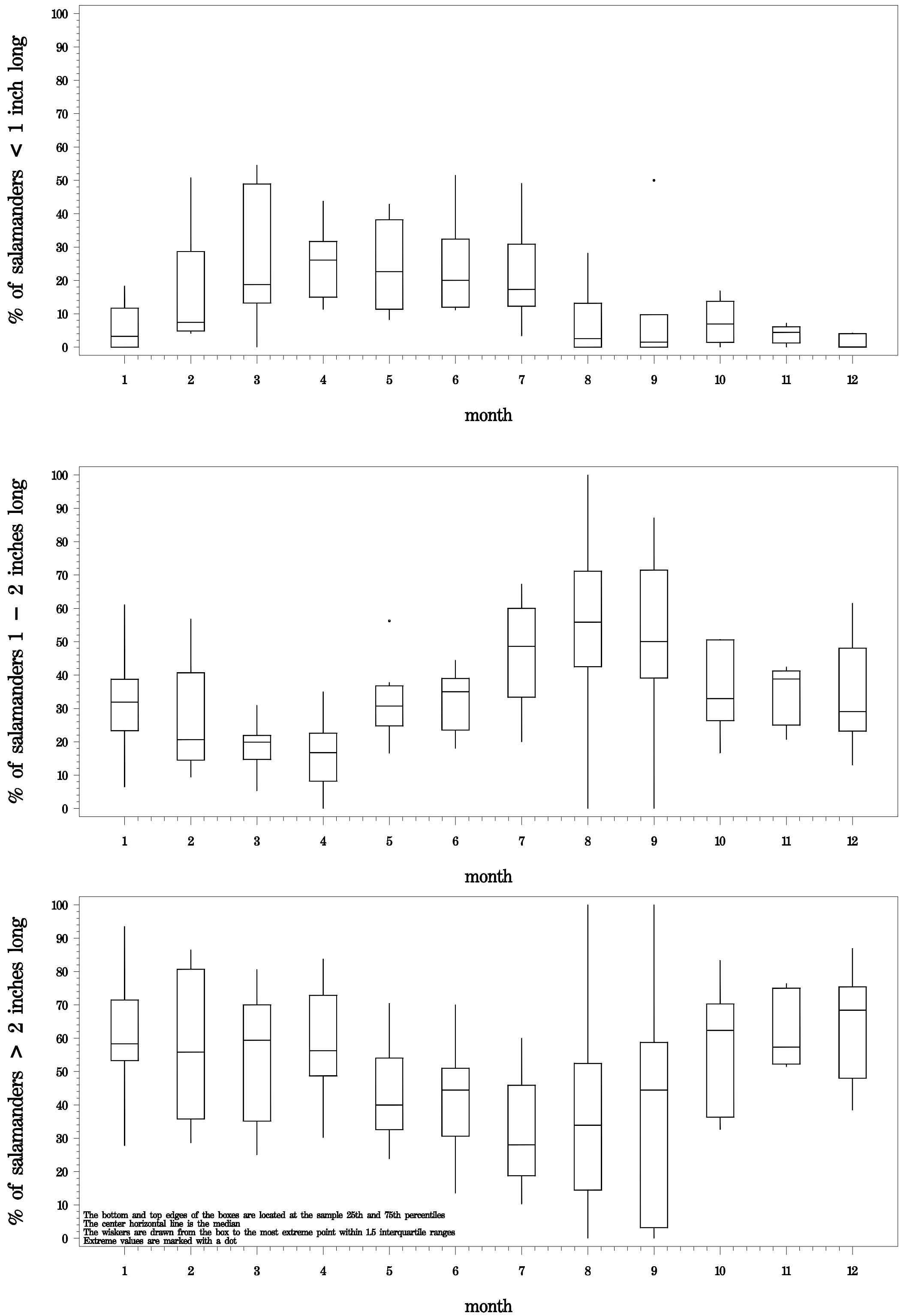


Figure 25. Percent of JPS by Size Categories, Rural Long-term Monitoring Sites

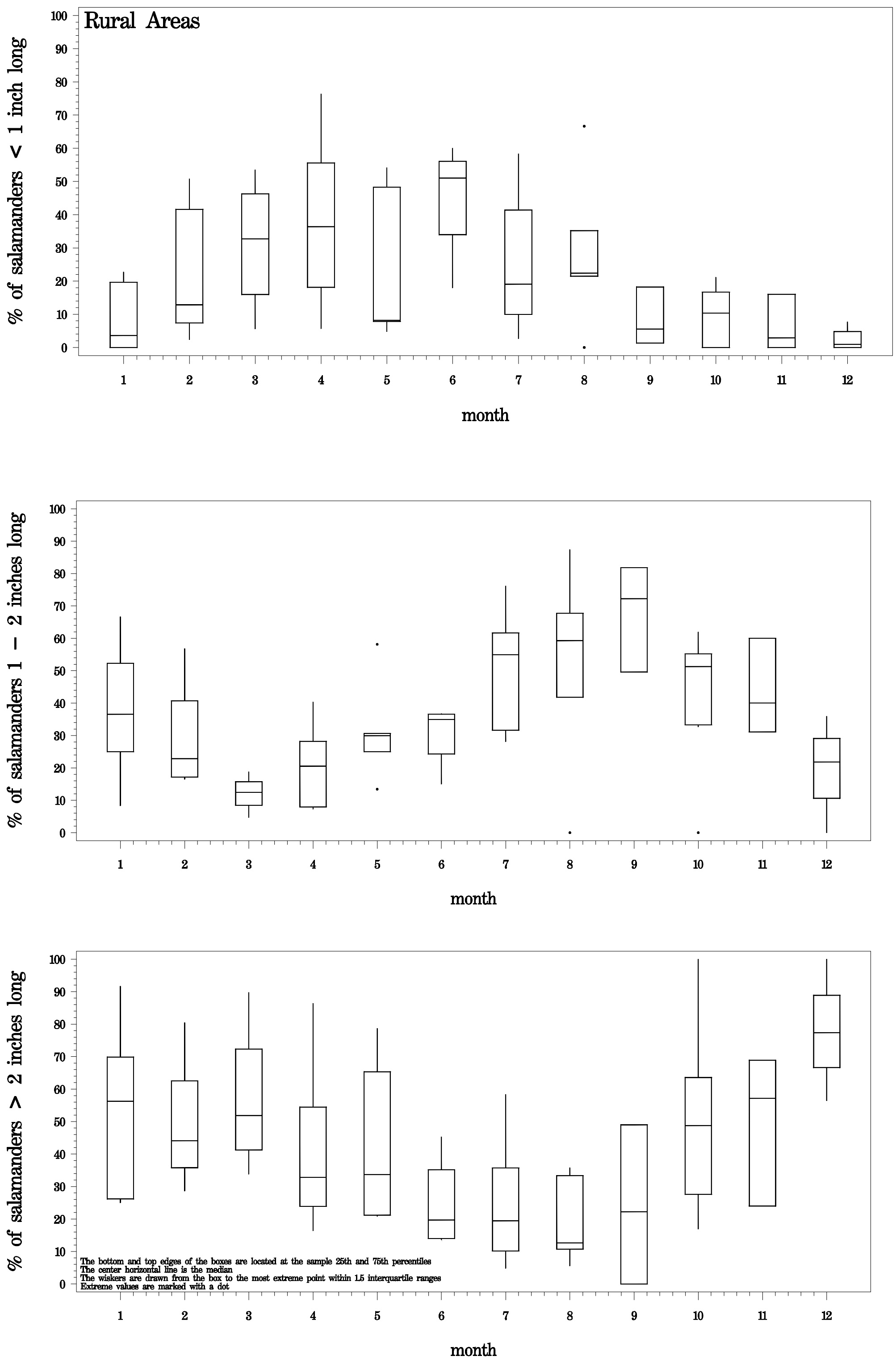


Figure 26. Percent of JPS by Size Categories, Developed Long-term Monitoring Sites

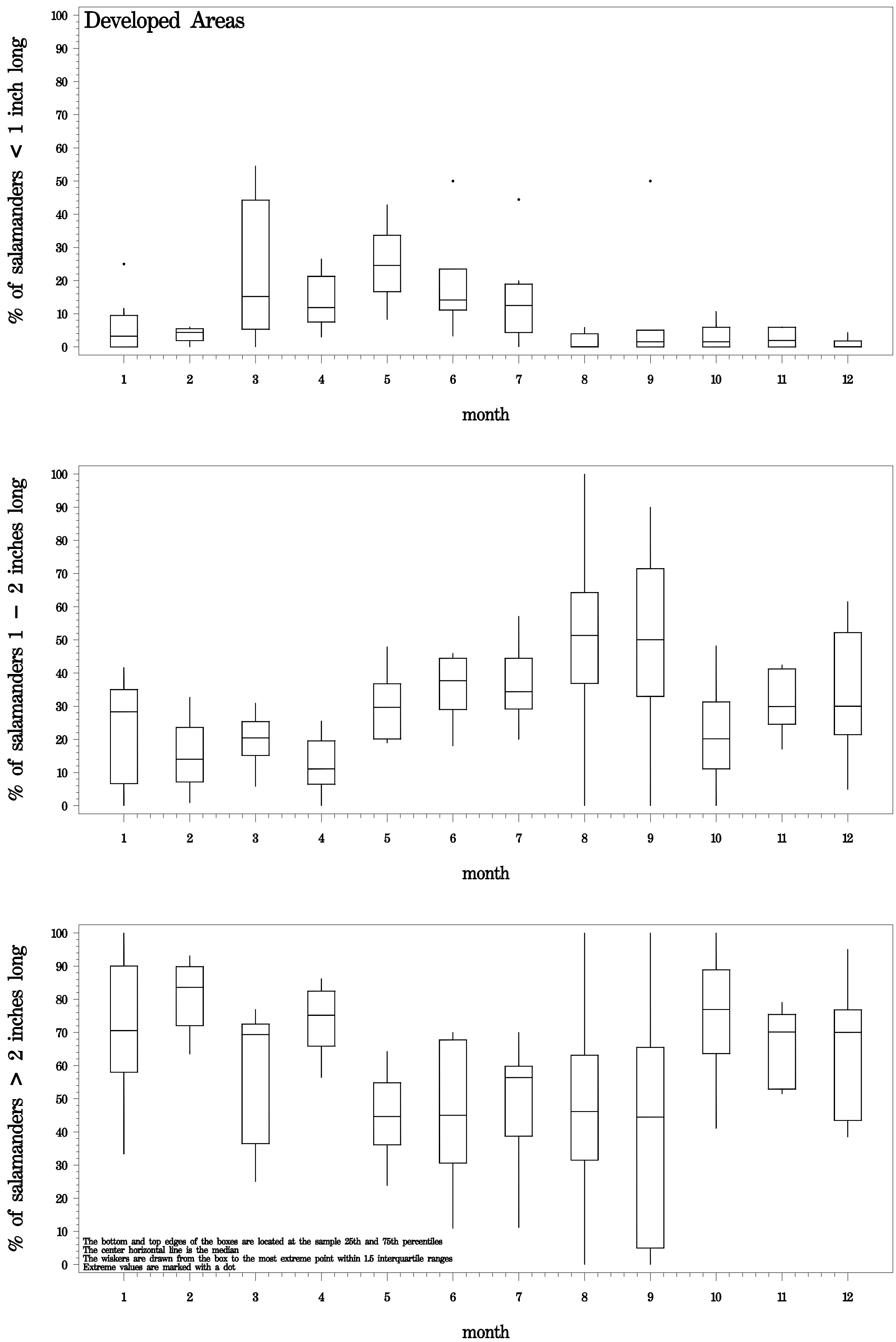


Figure 27. Flow at Bull Creek Tributaries 5, 6, and Above Trib. 7

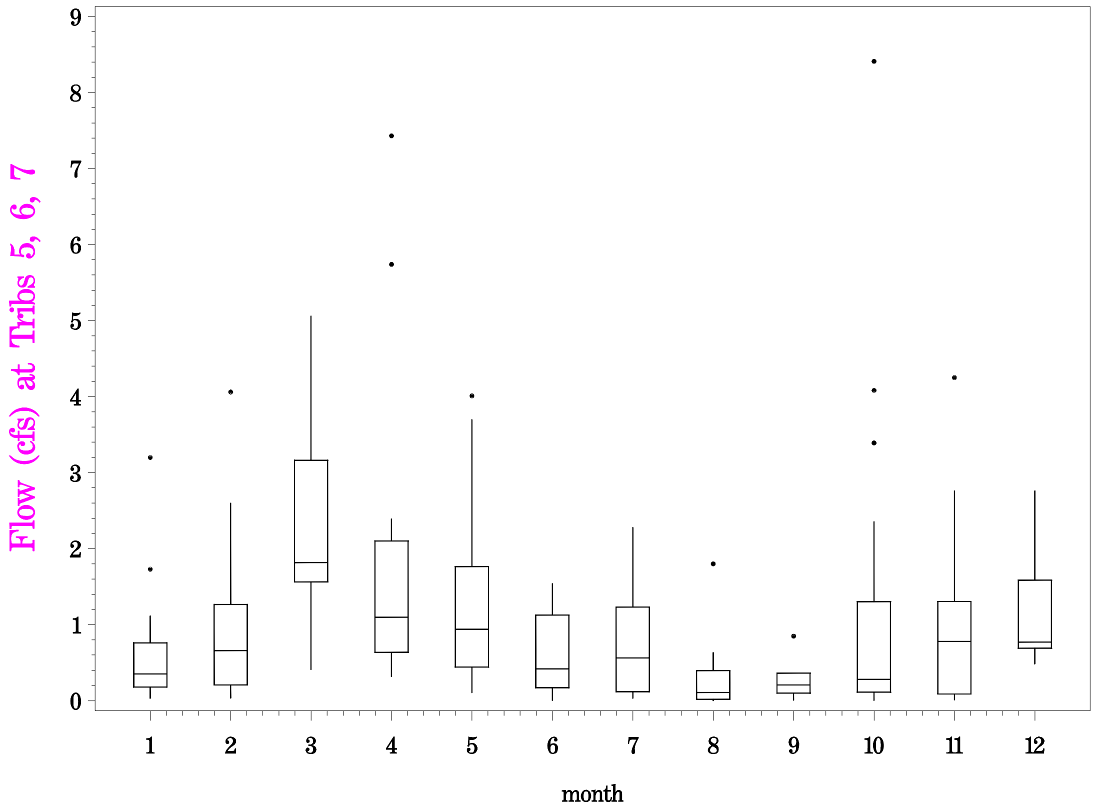


Figure 28. Percent of JPS <1 inch Long and Flow at Tribs. 5, 6, and Above Trib. 7

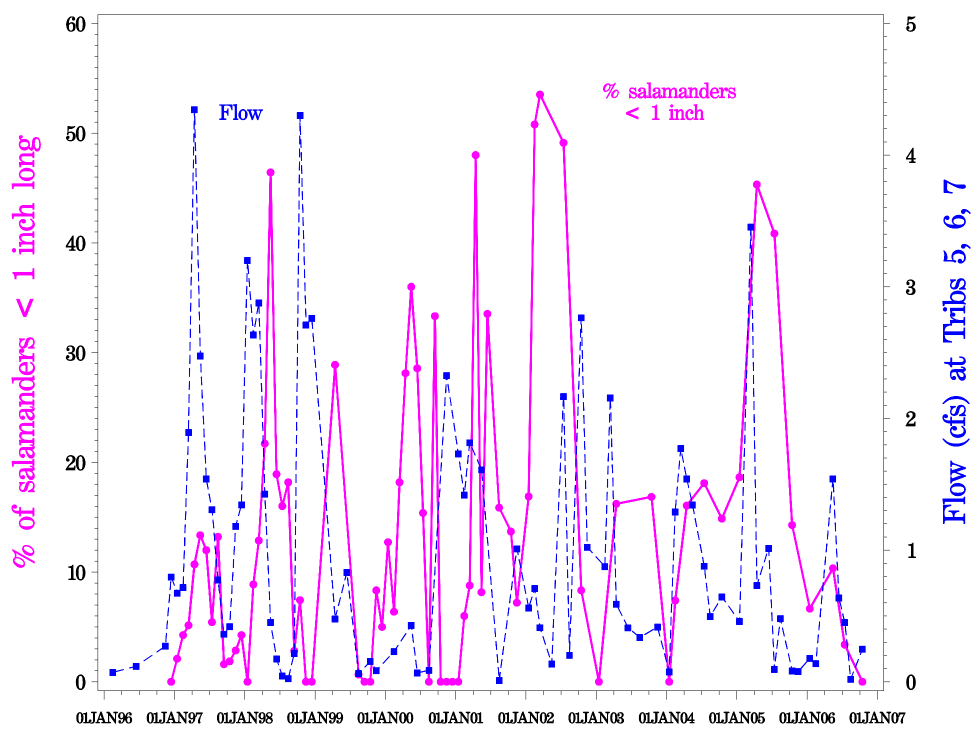
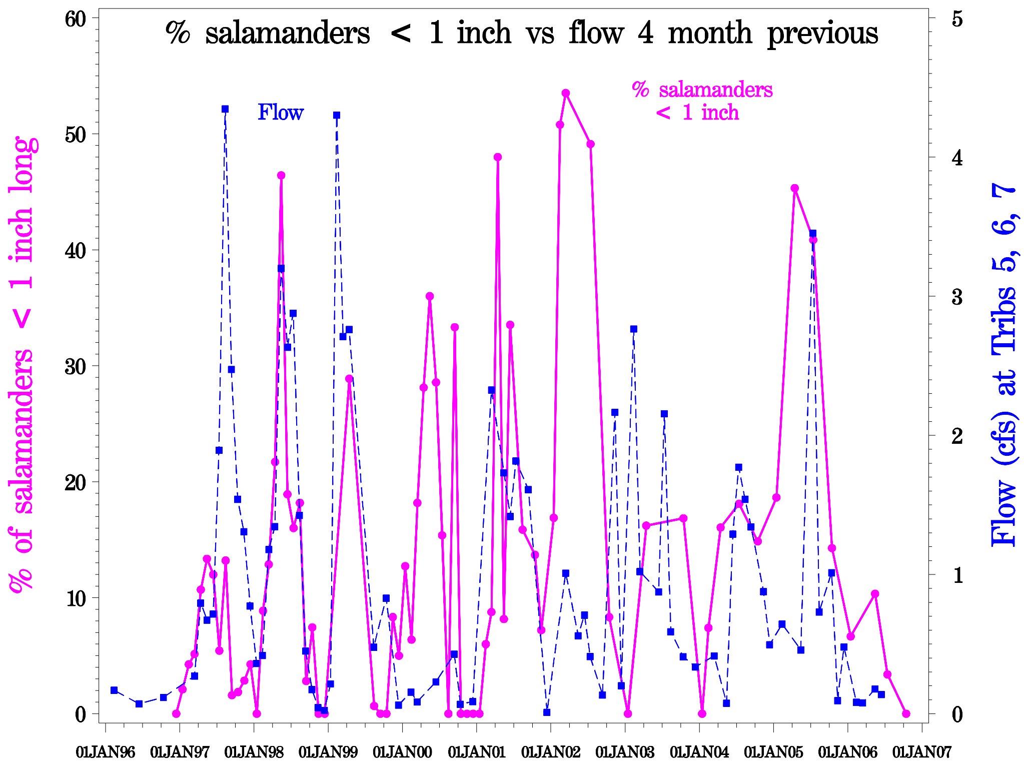


Figure 29. Percent of JPS <1 inch Long and Flow 4 Months Previous



General Status Assessment

City staff has been using GIS to look at the landscape-scale status of and threats to the JPS. Data layers include known JPS localities, Travis County Appraisal District parcels (an indication of development), watersheds, creeks, the Northern Edwards Aquifer, roads, and preserve lands. The following is a brief summary of the status of the JPS within each surface creek watershed. A quick visual analysis of these data layers show that most of the headwaters in the nine watersheds and much of the entire watersheds outside of the BCP lands are either developed or slated for future development. However, little is known of the sources and movements of groundwater to springs and caves inhabited by the JPS. Groundwater often flows through karst in patterns contrary to surface drainage, including crossing surface water drainage boundaries. The only known dye-traces in this portion of the aquifer include dye injections in Whitewater Cave and Marigold Cave in the Buttercup Creek cave system, both of which discharge from Blizzard Spring in the Cypress Creek watershed (Hauwert and Warton, 1997), injections near the Stillhouse Hollow springs in the Bull Creek watershed(City of Austin, unpubl. data), and injections near Spicewood Spring in the Shoal Creek watershed (Hauwert and Smith 1992).

Additional work is needed to understand the groundwater flowpaths from source areas to discharge from JPS springs and caves, which is critical to identifying management and conservation needs for the JPS. In 2006, the City of Austin and George Veni and Associates received a federal Section 6 grant to conduct a pilot dye-tracing study in the northern Edwards Aquifer to help delineate recharge areas and groundwater flowpaths influencing JPS distribution and habitat.

Feral hog numbers appear to be on the rise within much of the Bull and Cypress creek watersheds. The increased hog activity is negatively impacting JPS habitat. In direct response to this new threat, City of Austin BCP staff has installed hog exclosures around three springs in the Bull/West Bull Creek watershed. Other means of control include trapping and shooting; however, to date, these management measures have had very limited success. These methods are certainly reducing overall numbers, but not enough to keep up with the hogs' reproductive capabilities. BCP staff will continue to look into new methods of protecting JPS habitat from feral hogs.

Bull Creek – Most of the known JPS spring locations are found in Bull, Cypress, and Long Hollow watersheds. In Bull Creek, survey efforts are focused on continuing long-term monitoring from the original study. Most of the surveys are conducted on City of Austin lands within the Balcones Canyonlands Preserve (BCP) and other parks/preserves.

JPS appears to be most abundant along the mainstem of Bull Creek, including Bull Creek above Tributary 7 and Lanier Spring in the BCP. Both of these sites were dry or nearly dry due to drought conditions during August and September 2006. They began flowing again following rain events in October 2006.

During the 1990s, Moss Gully Spring on the proposed WTP4 tract reportedly still produced substantial flow, with more than a dozen salamanders easily found along the entire springrun. Sometime prior to 2001, springflow at Moss Gully diminished due to unknown causes so that the spring now has very little flow, and the few salamanders that are found are restricted to small pools near the head of the spring.

JPS counts along Tributaries 3, 5, and 6 are declining. The most obvious factor currently affecting Tributary 5 is high sediment levels. Noticeable problems along Tributary 6 include high specific conductance, ion concentrations, and impacts (scouring, deposition of debris) from flooding. Water quality and flooding may also be problematic at the Tributary 3 site.

As discussed above, deformities have been documented in both the Stillhouse and Barrow populations. Nitrates, specific conductance, and ions are elevated at these sites.

Brushy Creek – JPS has been found in springs on Avery Ranch, which is in a rapidly urbanizing area. Due to staff limitations, City biologists were unable to survey these sites in 2006.

Buttercup Creek – The taxonomy of the *Eurycea* salamanders found in caves in the Buttercup Creek watershed has not been fully resolved. These salamanders have been tentatively classified as *Eurycea tonkawae* (Chippindale et al. 2000).

In April 2004, Zara Environmental initiated mark-recapture surveys using visible implant elastomers to mark salamanders in Testudo Tube Cave and has been conducting follow-up monitoring for marked individuals. This study is not discussed here but will be prepared by Zara Environmental in a separate document to the Austin Water Utility's BCP staff. Several healthy and emaciated salamanders were observed at this site during the spring of 2006.

This watershed lies within a rapidly urbanizing area. Groundwater from a couple of cave streams in the Buttercup Creek watershed flow toward a spring in Cypress Creek.

Cypress Creek – This watershed contains some of the largest known concentrations of JPS, many of which are located within preserve lands. Most of the headwaters appear to be developed or slated for future development. At least one spring in the Cypress Creek watershed (Blizzard Spring) is influenced by the quality and quantity of groundwater in the Buttercup Creek watershed (Hauwert and Warton 1997).

Flow at Baker Spring in the Travis Audubon Society's Baker Sanctuary appears to have subsided considerably. The last survey when salamanders were found at Baker Spring was on August 26, 2005. It was not flowing following rains in May 2006 when springs at all other monitoring sites were flowing. It flowed briefly following rains in October and ceased again shortly thereafter. Recent development activities in this area may have influenced flows at this site.

MacDonald Well, SAS Canyon, and Kretschmarr Salamander Cave sites were surveyed two to three times in late 2005 - 2006. MacDonald Well was dry during most of 2006; however, when flowing, the MacDonald Well site has large numbers of salamanders.

<u>Lake Creek Watershed</u> – Only one spring ("PC Spring") with JPS is known from this watershed. PC Spring is located along the SH45 right-of-way and is owned by the Texas Turnpike Authority. Due to staff limitations, City of Austin biologists have not surveyed this site. The watershed lies within a rapidly urbanizing area.

<u>Long Hollow</u> – This is the only watershed within the known JPS range that has the entire headwaters within a preserve (LCRA's Wheless tract). Although the survey is conducted near the head of the watershed, occasional spot checks have found JPS along most of the creek. Because of the habitat degradation at many of the long-term monitoring sites, this is one of the few original sites that is still considered to be pristine. This is also one of the more ephemeral areas and is typically one of the first of the long-term JPS monitoring sites to go dry. It was dry during most of 2006. When flowing, large numbers of JPS are found in the survey area, and JPS has been found along much of the creek. JPS counts at this site may be increasing slightly.

<u>Shoal Creek Watershed</u> – Only one spring with JPS is known from this watershed (Spicewood Spring), which is one of the most urban of the JPS sites. PAHs have been found in sediments at this site. Elevated levels of nitrate, ammonia, and specific conductance in water have also been documented. Effects from flooding are an ongoing problem. Salamander counts at this site have declined significantly in recent years. No deformities have been observed.

<u>Walnut Creek Watershed</u> – Only one tiny spring pool with JPS is known from this watershed, which is located along the eastern edge of the Edwards Plateau in a park that is surrounded by urban development. JPS was last observed at this site in September 2005. It is possible that JPS has been extirpated from this site.

<u>West Bull Creek Watershed</u> – West Bull Creek is a large tributary joining with Bull Creek near the confluence with Lake Austin. Only one small spring has been found with JPS in this watershed. The spring lies within an existing preserve (Ivanhoe). Since surveys were intitiated at this site in 2004, 0 to 18 salamanders have been observed.

Summary

- Only 2 of the 9 long-term monitoring sites have large numbers of salamanders -- the LCRA Wheless tract in the Long Hollow watershed and the Bull Creek above Tributary 7 site on the Balcones Canyonlands Preserve.
- Salamander counts are declining at 4 of the 9 long-term monitoring sites.
- Salamanders with curved spines and other deformities have been observed at 2 of the 9 long-term monitoring sites.
- Salamanders appear to have been extirpated from a more recent monitoring site at Balcones District Park spring in the Walnut Creek watershed.
- The declining counts and health problems appear to be related to water quality and habitat degradation due to urban development.
- The chytrid fungus has been confirmed in all 38 salamanders sampled from 15 sites in 6 different watersheds.
- Although the Jollyville Plateau salamander is found in several springs and caves in nine watersheds, the majority of these sites are threatened by urban development.
- For most sites, recharge areas are unknown. A pilot dye-tracing study will be underway in 2007 to help identify groundwater source areas for springs inhabited by JPS.
- The JPS continues to exhibit seasonal reproductive cycles, which may be regulated by rainfall/flow.
- Feral hogs continue to degrade JPS habitat throughout Bull and Cypress Creek watersheds.

Sampling Plan and Research Recommendations

As time and staff allow:

- Conduct quarterly salamander counts at all of the nine long-term monitoring sites.
- Expand survey efforts to include at least one site in each watershed where JPS is known to occur.
- Continue to take flow and basic chemistry measurements with each salamander count.
- Collect water samples (e.g., nutrients, ions) where basic chemistry show potential water quality problems (e.g., high specific conductance). Coordinate with WRE stream and spring monitoring teams.
- Conduct macroinvertebrate samplings at additional long-term monitoring sites – ideally, include Wheless, Stillhouse Hollow, Spicewood, and Tributary 3 at Great Hills Golf Course.
- Conduct annual pebble counts – at a minimum, include Bull Creek above Tributary 7, Tributary 6, Tributary 5, and Wheless tracts.
- Conduct mark-recapture surveys to estimate total population, gather life history characteristics in the wild, and determine responses to springflow cessation, other potential stressors/threats, and habitat enhancement efforts.
- Conduct dye-tracing to delineate the groundwater source areas and flowpaths of caves and springs inhabited by JPS.
- Conduct phylogenetic studies to resolve the taxonomy of the Buttercup Creek cave salamanders and additional population genetic studies to examine whether JPS populations are interconnected by regular dispersers or whether they are demographically isolated.
- Investigate the cause(s) of spinal curvature and other deformities in JPS at Stillhouse Hollow and Barrow Tributary. Possible research could include maintaining JPS from these sites and another site over successive generations in water from Stillhouse Hollow and nitrate toxicity studies.

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