Summary of Jollyville Plateau Salamander Data (1997 – 2005) 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 nine years of data shows that salamander counts are declining at three of nine long-term monitoring sites. Salamanders with deformities and disease have been documented at two other sites. The declining counts and health problems appear to be related to water quality and habitat degradation due to urban development. The Jollyville Plateau salamander appears 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 in1997 and 1998 was designed to collect baseline information about these salamanders. The results of that study are documented in the Jollyville Plateau Water Quality and Salamander Assessment (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 efforts to include sites in other watersheds. The original study included monthly surveys. Currently, an effort is made to survey seven of the original sites quarterly. Two of the long-term sites (Barrow, Tanglewood) are surveyed less frequently. Table 1 lists the original and new sites, and Figure 1 presents a map of the sites. In 2005, City staff drafted a Quality Assurance Project Plan that summarizes the current JPS monitoring program.

Bull Creek Watershed – 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.

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 presented in a separate document to the Austin Water Utility's BCP staff.

Brushy Creek – JPS has been found in springs on Avery Ranch. Due to staff limitations, City biologists are unable to survey these sites on a regular basis.

Cypress Creek Watershed – Two springs occur on the Travis County Audubon Society's Baker Sanctuary. Since City biologists have only found salamanders at the larger of these two springs (Baker Spring), quarterly monitoring is now conducted only at this site. The other spring is surveyed occasionally as time is available.

Several springs occur on Travis County-owned lands. JPS has been found at MacDonald Well and springs in SAS Canyon. Salamanders have also been found in Krestchmarr Salamander Cave, which is owned by SAS Institute, though they have given access to Travis County. In 2004, City of Austin and Travis County biologists delineated a survey area for MacDonald Well. Due to time limitations and low or no flow, these sites were not surveyed in 2005. Travis County and City of Austin staff are working to establish routine monitoring for these sites.

Lake Creek Watershed – Only one 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, this site has not been surveyed by City of Austin biologists.

Long Hollow Watershed – This is the only watershed within the known JPS range that has the entire headwaters within a preserve. 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 a "control" site. This is also one of the more ephemeral spring sites and is typically one of the first of the JPS springs to go dry.

Shoal Creek Watershed – Spicewood Spring is the only known site with JPS in this watershed and is one of the original monitoring sites.

Walnut Creek Watershed – Only one small spring pool with JPS is known from this watershed and is a new monitoring site.

West Bull Creek Watershed – Only one known spring with JPS is found in this watershed and is a new monitoring site. West Bull is a large tributary joining with Bull Creek near the confluence with Lake Austin.

Figure 1. Jollyville Plateau Salamander Study Sites

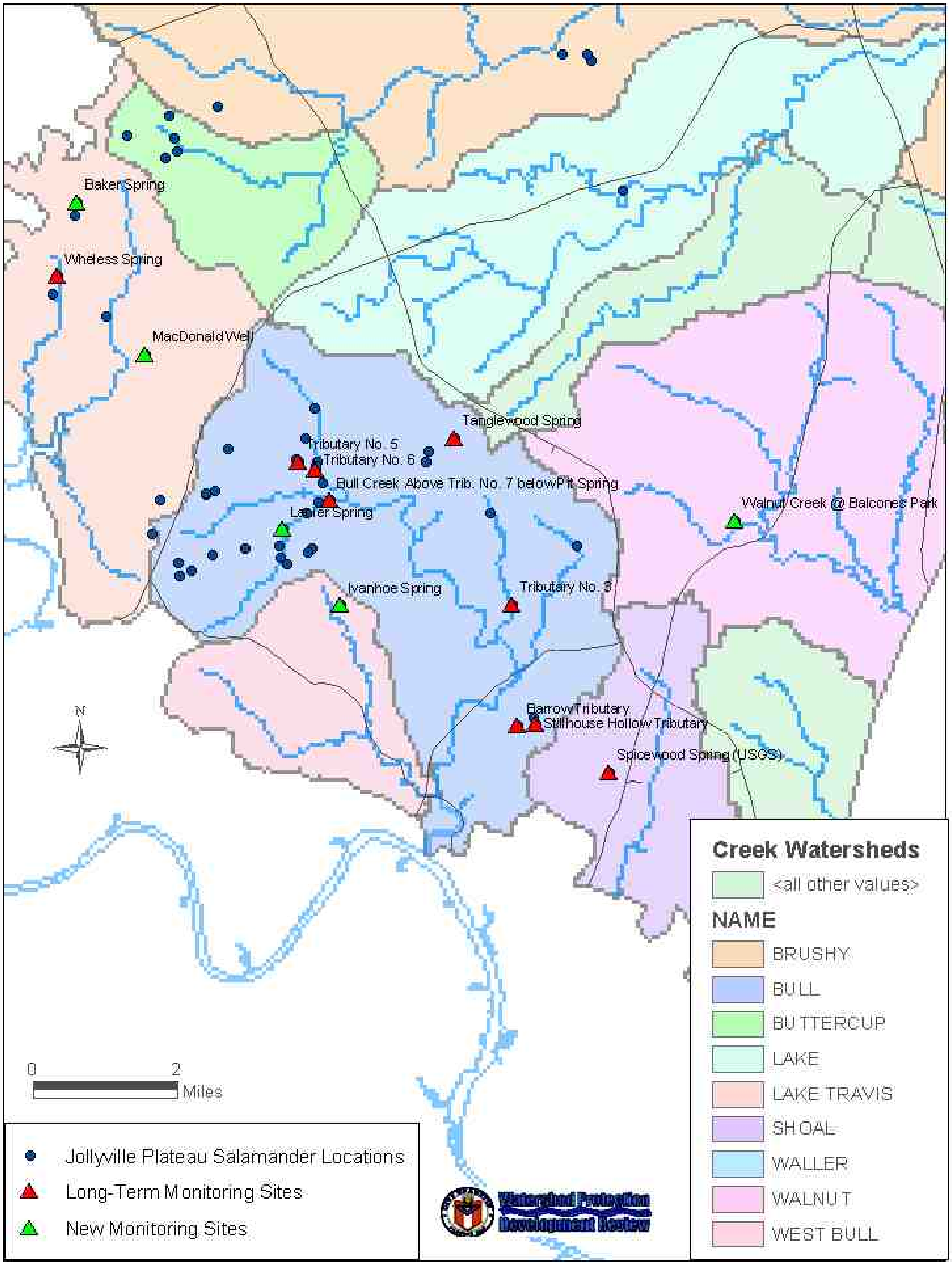

Table 1. Jollyville Plateau Salamander Study Sites

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/Quantiy Sampling?**
Bull Creek Watershed							
25	Barrow Preserve Spring	929	Barrow Preserve Tributary Below Barrow Spring	Urban - old development	City of Austin preserve	Annually to quarterly	Nutrients chemistry flow
24	Stillhouse Hollow Spring	927	Stillhouse Hollow Below Stillhouse Hollow Spring	Urban - old development	Private	Quarterly	Nutrients chemistry flow
31	Tanglewood Spring	928	Tanglewood Tributary Below Tanglewood Spring	Urban - recent development	Private	Annually	Flow
		926	Tributary 3 @ Great Hills Golf Course	Urban - recent development	Private	Quarterly	Flow
		151	Tributary 6 @ Bull Creek (EG)	Urban - recent and continuing development	City of Austin's Hanks tract in the BCP	Quarterly	Nutrients 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 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 chemistry benthic macroinvertebrates flow
	Lanier Spring			Rural	City of Austin's Lanier tract BCP	Quarterly	No
	Cistern Spring			Rural	City of Austin's Franklin BCP	Quarterly	No
Cypress Creek Watershed							
	Baker Spring		Tributary below Baker Spring	Rural	Travis Aubudon Sanctuary BCP	Quarterly	Flow
	MacDonald Well		Tributary below MacDonald Well	Rural	Travis County tract in the BCP		No
	SAS Canyon Spring		Tributary below SAS Canyon Spring	Some existing and ongoing development	Travis County tract in the BCP		No
	Kretschmarr Salamander Cave			Some existing and ongoing development	Private		No
Long Hollow Watershed							
1044	Long Hollow Creek Spring @ Wheless	1045	Long Hollow Creek @ Wheless Tract	Rural	LCRA Wheless tract in the BCP	Quarterly	Flow
Shoal Creek Watershed							
582	Spicewood Spring (USGS)	930	Spicewood Tributary Below Spicewood Spring	Urban – old development	Private	Quarterly to Monthly	Flow
Walnut Creek Watershed							
445	Balcones District Park Spring			Urban – old development	City of Austin park	Quarterly	No
West Bull Creek Watershed							
1072	Ivanhoe Spring			Rural	City of Austin's Ivanhoe tract	Quarterly	No

Jollyville Plateau Salamander Counts over Time, Long-term Monitoring Sites

Figures 2-10 show salamander counts and flow measurements for the nine long-term monitoring sites, from January of 1997 through the fall of 2005. Regression analysis with total count as the dependent variable and date as the independent variable, found significant trends for three sites (Table 2, figures 4, 8, 9). Salamander counts have decreased significantly at Tributaries 5 and 6 in the Bull Creek watershed and at the Spicewood site in the Shoal Creek watershed. Recent and ongoing development is affecting Tributaries 5 and 6, and old development has affected the Shoal Creek site. JPS counts along Tributary 3 also appear to be declining, but additional data are needed to determine whether the trend is real and significant. Counts at the Tanglewood site may be declining, but with infrequent surveys and low initial counts, detecting a significant trend is unlikely.

Table 2. Sites with Statistically Significant Changes in Total Salamander Counts over Time

Site	Pr>F	R^2	Direction	Average 1997 Count	Average 2005 Count
Tributary 6 @ Bull Creek (EG)	0.0014	0.1626	Decreasing	30	8
Tributary 5 Below Hanks Tract Property Line	0.0001	0.3337	Decreasing	42	2
Spicewood Springs and Tributary	0.0066	0.2598	Decreasing	12	2

Jollyville Plateau Salamander Count Data, New Monitoring Sites

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. Based on the preliminary data, the JPS population at Lanier Spring appears to be increasing. This site was heavily impacted by feral hogs until BCP staff installed a perimeter fence in late February/early March 2005. Salamander counts increased from none during a very cursory survey in January 2005 to 50 in July 2005.

Table 3. Salamander Counts at New Monitoring Sites, 2004-2005

Site	Date	Salamander Count (<1"/ 1-2" / >2" / Total)	Flow	Comments
Bull Creek Watershed				
Lanier Spring	1/4/04	0 / 0 / 0 / 0		Very cursory survey; significant damage from feral hogs
	3/16/05	15 / 3 / 0 / 18		Prior to this survey, BCP staff installed a fence around the spring to exclude hogs
	7/1/05	23 / 21 / 6 / 50		
	9/23/05	3 / 23 / 4 / 30		Flow has diminished since the July survey
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	0 / 0 / 1 / 1		
	4/29/05	0 / 1 / 0 / 1		
	10/21/05	0 / 0 / 2 / 2		
Cypress Creek Watershed				
Baker Spring	4/14/04	0 / 6 / 27 / 33	0.07	
	8/24/04	0 / 0 / 1 / 1	--	Too shallow to measure flow
	5/20/05	0 / 3 / 4 / 7	0.02	
	8/26/05	0 / 0 / 2 / 2	0.001	
	Fall 05			Dry
Krestchmarr Salamander Cave	10/1/04	0 / 0 / 4 / 4		
MacDonald Well	8/10/04	2*/ 16 / 7 / 25*		*no flow, only small spring pools; includes 1 dead juvenile found
	Fall 05			Dry
Walnut Creek Watershed				
Balcones District Park Spring	1/13/04	0 / 0 / 3 / 3		Photographed salamander
	4/8/04	1 / 1 / 1* / 3		*Recaptured salamander from 1/13/04
	7/20/04	0 / 0 / 1 / 1		Unable to catch salamander
	10/25/04	0 / 0 / 0 / 0		
	3/16/05	0 / 0 / 1 / 1*		*Recaptured salamander from 1/13/04 and 4/08/04
	7/1/05	0 / 0 / 1 / 1		Unable to catch salamander
	9/23/05	0 / 0 / 1 / 1		Unable to catch salamander
West Bull Creek Watershed				
Ivanhoe Spring	1/13/04	0 / 0 / 0 / 0		Very little flow
	7/20/04	5 / 10 / 2 / 17		
	10/25/04	0 / 2 / 1 / 3		
	3/16/05	15 / 1 / 2 / 18		
	7/1/05	6 / 3 / 1 / 10		
	9/23/05	0 / 4 / 0 / 4		

Jollyville Plateau Salamander Counts and Searches at Other Salamander Sites

The following sites include those that are not monitored on a regular basis due to staff limitations.

Bull Creek Watershed

- City biologists found 2 adult salamanders, including one gravid female, at Moss Gully Spring (City of Austin's proposed WTP4 site) on January 4, 2005, 2 adults on April 4, 2005, and 3 salamanders (1 small juvenile, 2 adults) on May 10, 2005.

- BCP staff found a new JPS site on the proposed WTP4 tract, main branch of Bull creek just below the 4-Points property. On subsequent visits the creek was dry.

- A small spring in the City of Austin's Floral Park was surveyed April 8, 2004 (1 large juvenile, 1 adult) and July 24, 2004 (1 small juvenile, 1 large juvenile, 3 adults).

- A new JPS site has been documented at the City of Austin's Spicewood Valley Park. On May 7, 2004 and March 16, 2005, 12 and 14 salamanders were found, respectively, during cursory surveys of a spring and in the main tributary on this property.

- On May 6, 2005, City and Travis County biologists surveyed springs on the Travis County-owned portion of the Ribelin tract in the BCP. A shelter cave and all of the tributaries were surveyed. One adult salamander was found in a spring on a branch of the tributary that flows into the Lanier property. Five salamanders were found during a quick search along the Lanier/Ribelin boundary.

- On July 11, 2005, a very cursory survey on the Lanier tract along the tributary that flows from 3M through the Ribelin tract indicates a robust JPS population currently exists here. More extensive surveys should be conducted to determine the distribution and abundance of JPS along this tributary.

- City staff have conducted periodic surveys of Powerline Spring on the proposed WTP4 tract but have not found salamanders at this site. Another spring (Plunge Pool) on the mainstem of Bull Creek on the proposed WTP4 has been surveyed briefly on a couple of occasions (November 2004, July 2005), but no salamanders have been found. Buzzard Spring on the Franklin tract was briefly surveyed on July 1, 2005, but no salamanders were found. Additional search efforts at these sites are warranted as staff and time allow.

- City staff surveyed Fern Gulley Spring (at the head of Tributary 5) and over 60 feet of springrun below the spring on December 15, 2004. Surveyors found 2 large juveniles. The site had large amounts of sediment.

Brushy Creek Watershed

- City biologists surveyed three springs on Avery Ranch on December 15, 2004 and found 4 salamanders (2 large juveniles, 2 adults) at Hill Marsh Spring. City geologists found 3 salamanders (1 small juvenile, 2 adults) at Hill Marsh Spring on March 23, 2005.

Cypress Creek Watershed

- The second spring (Audubon Spring) on the Audubon tract was surveyed April 14, 2004 and August 24, 2004. No salamanders were found.

- City and Travis County biologists searched several small springs on Travis County-owned lands in the Cypress Creek macrosite of the BCP during the summer 2005. No new JPS sites were found.

Mark-Recapture

City biologists have developed a technique to identify individual salamanders by photographing the unique patterns of melanophores (dark brown pigments) and iridophores (iridescent pigments) on the dorsal surface of the salamander body, particularly the head. Photographs may also be used to examine deformities and other physiological anomalies that may not be obvious in the field, due to the small size of the salamanders. Due to staff and time constraints, mark-recapture has been limited to the smallest JPS populations (Balcones District Park Spring, Spicewood Spring) and the sites where deformities have been observed (Stillhouse Hollow, Barrow Tributary).

At Balcones District Park Spring, 0 to 3 individuals have been observed during surveys since January 2004. The same individual that was initially photographed on January 13, 2004 was subsequently recaptured twice (April 8, 2004; March 16, 2005) over a period of 14 months. On April 8, 2004, this salamander had lost a portion of its tail, which had completely regenerated by March 16, 2005. Biologists were unable to capture the salamander during recent surveys to determine if it continues to be the same or a different individual.

At Spicewood Spring, the same individual initially photographed on August 19, 2005 was recaptured October 28, 2005. The salamander grew from 48 mm total length to 52 mm over the two-month period. Currently, less than three salamanders are typically found at Spicewood Spring.

The mark-recapture data indicate that these JPS populations are very small. Should funding become available at some point in the future, City biologists have developed a monitoring plan to implement mark-recapture on a larger scale using a combination of photography and visible implant elastomers. Mark-recapture would provide data to estimate total population size, movements within and possibly between sites, growth rates, and life history characteristics, as well as responses to threats (e.g., drought) and habitat restoration efforts.

Zara Environmental has conducted mark-recapture of JPS in Testudo Tube Cave using visible implant elastomers. This study will be presented to City of Austin BCP staff in a separate report.

Health Issues in Stillhouse and Barrow Salamanders

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. Barrow has not been surveyed as often, but at least one salamander with a curved spine was documented from this site in May 2005. Spinal curvature has not been observed at any other site. Frequency at Stillhouse Hollow from 1995 to 2005 has ranged from 0 to 50 percent, with an average of about 5 percent. However, not all deformities may be noticed or reported. City biologists recently began photographing all of the salamanders observed at Stillhouse and Barrow. Biologists can then 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, have been observed upon close inspection of photographs of individuals from Stillhouse Hollow and recently Barrow Tributary.

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 obvious signs of recovering from this condition.

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. 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 water quality problem that has been documented at Stillhouse Hollow and Barrow is high nitrate levels, which average about 6.5 mg/L, have exceeded 10 mg/L (Figure 18), and are higher than any other JPS spring site. Nitrate levels in undeveloped Edwards Aquifer springs are typically close to 1 mg/L.

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 (≤15-day) exposures to elevated nitrate levels (Hecnar 1995, Marco et al. 1999, Schuytema and Nebeker 1999).

The chytrid fungus has recently been confirmed in salamanders from Stillhouse Hollow. This pathogen has been linked to the decline and extirpation of frog and toad populations worldwide. Information about the chytrid fungus and recommended methods of disinfection are presented in "Disinfection Protocols to Prevent the Spread of the Chytrid Fungus In Salamander and Other Amphibian Populations in the Austin, Texas Area" (Appendix A). Microscopic examination of an individual sent to the USGS National Wildlife Health Center in 2000 first revealed the presence of the fungus on the tips of the salamander's toes. In March 2005, presence of the fungus on another individual from Stillhouse Hollow was confirmed using chytrid PCR. Tadpoles, which are abundant at most springsites in the Edwards Aquifer, have not been found at Stillhouse Hollow since the late 1980s (David Hillis, University of Texas, pers. comm., 2005; City of Austin data). The chytrid fungus may have contributed to the absence of tadpoles from this site. Tadpoles are still found at Barrow.

Environmental stressors at Stillhouse Hollow (in particular, high nitrate levels and possibly high ion concentrations and/or other factors) may have weakened the salamanders' immunity to chytridiomycosis. Some salamanders exhibit characteristic signs of infection – emaciation, lethargy, pale and/or discolored skin. To date, no tests for the presence of the chytrid fungus in salamanders or anurans have been conducted for the Barrow Tributary. However, due to its proximity to Stillhouse, Barrow is also treated as an infected site.

Table 4. Frequency of Spinal Curvature in Salamanders at Stillhouse Hollow, 1995-2005*

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 Jollyville Plateau Salamander Study			
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
End of Jollyville Plateau Salamander Study			
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
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

Survey Date	Curved Spine (#/Size)	Total # Salamanders	Incidence Rate (%)
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
Average Frequency			**5%**

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

Water Quality Measurements and Habitat Assessments

Water quality samples are taken from five JPS monitoring sites (Table 1). Parameters at Stillhouse Hollow and Barrow include temperature, dissolved oxygen, conductivity, pH, nitrate/nitrite as N (NO3), and 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.

For the JPS monitoring sites, a general habitat assessment is conducted during each survey. Visual estimates are made of embeddedness, plants, leaf litter, and substrate composition. Numbers of fish and crayfish are counted, and benthic macroinvertebrates and amphibian species observed are recorded. A

Habitat Quality Index form is filled out for each site on an annual basis and includes scores for sediment deposition and embeddedness, among other factors.

Water quality parameters were investigated to see if any of them have changed over time at the long-term monitoring sites. The water quality parameters were conductivity, nitrate, orthophosphorus, total suspended solids, chloride, sulfate, and sodium. Three parameters -- conductivity, nitrate, and sodium -- have shown a significant increase at Bull Creek Tributary 6 and/or Tributary 5, which have also had significant declines in salamander counts. Figures 11-18 show the changes in these parameters over time, and Table 5 presents the parameters with statistically significant trends. Statistical analyses of the habitat parameters considered in the original Jollyville Plateau salamander study (bank condition, embeddedness, sediment deposition, bank vegetative protection, channel flow status, and frequency of riffles) are not included here due to the subjectivity of the current methods used (visual estimates). However, these are discussed in more qualitative terms in the sections below.

Table 5. Water Quality Parameters with Statistically Significant Increases over Time

Site	**Parameter**	**Pr>F**	**R^2**	**Direction**	**Average 1997 Value**	**Average 2005 Value**
Tributary 6 @ Bull Creek (EG)	Conductivity	0.0036	0.09	Increasing	912 uS/cm	923 uS/cm
	Nitrate	0.0368	0.04	Increasing	0.44 mg/L	0.91 mg/L
	Sodium	0.0486	0.09	Increasing	38.4 mg/L	47.1 mg/L
Tributary 5 Below Hanks Tract Property Line	Sodium	0.0001	0.43	Increasing	7.1 mg/L	12.5 mg/L

In addition, some parameters appear to be increasing over time that are not yet statistically significant. Chloride and sulfate values appear to be increasing at Tributary 5 above the baseline levels measured at Bull Creek above Tributary 7. When the concentrations of these parameters are compared to those at Bull Creek above Tributary 7, the least developed site, there is overlap for the first several years and then there is complete separation, with the higher concentrations at Tributary 5.

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

<u>Sediment Deposition and Embeddedness</u> – Sediment deposition and embeddedness are the most obvious factors contributing to the decline of salamander counts along Tributary 5. Embeddedness reflects the degree to which rocks (which provide cover for salamanders) are surrounded or covered by fine sediment such as sand, silt, or clay. Increased sedimentation from erosion and flooding 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. Since sediment deposition and embeddedness are estimated visually, both parameters are subject to error and observer bias. More quantitative measurements are warranted to show how well sediment correlates with salamander abundance and distribution. City biologists measured embeddedness and sediment depths during preliminary pebble counts initiated in the fall of 2004. Survey sections with high salamander counts had embeddedness values of less than 15 percent. An average of embeddedness values for all survey sections within each survey area where the pebble counts were conducted is

presented in Figure 11. Embeddedness was highest in Tributary 5, averaging almost 40 percent over the entire survey area (individual sections ranged from 5 to 75%). Figure 12 shows the results of embeddedness scores based on visual estimates, and Figure 13 shows the sediment deposition scores. Tributary 5 has received the lowest scores for both parameters of all the JPS monitoring sites. 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.

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.

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.

Due to staff limitations, the pebble counts in JPS habitat were not repeated in 2005. More general aquatic habitat surveys performed by the City of Austin's water quality team include pebble counts as a quantitative approach to assessing the impacts of sediments. However, variation in methods and locations may mean that these data are not comparable. A consistent approach to pebble counts is under investigation so data may be included in future reporting in this area.

Specific Conductance and Ions – 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 Tributary 6 site, where specific conductance typically averages over 900 uS/cm (figures 14-17). Specific conductance is high at all of the urban sites, with Stillhouse, Barrow, and Spicewood averaging between 900 and 1000 uS/cm. Comparing the salamander count data among the urban sites, the effects of high ion concentrations and specific conductance are not clear. It is possible that elevated ion concentrations could be contributing to the low JPS numbers and declining trends or the higher ion concentrations are indicators of other urban contaminants. In a 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. The Barton Springs/Edwards Aquifer Conservation District recently funded a study by University of Texas at Austin faculty to conduct more in-depth studies of the effects of high conductivity and low dissolved oxygen on *Eurycea* salamanders.

Nutrients – Nitrate is higher at Stillhouse Hollow and Barrow Tributary than any other JPS site, with concentrations at the springs averaging around 6.5 mg/L and maximum concentrations exceeding 10 mg/L (Figure 18). The springs tend to have higher nitrate concentrations than the tributaries. Nitrate concentrations at Spicewood springs and tributary are only slightly lower than those measured in the Stillhouse and Barrow tributaries. Ammonia appears to be higher at Spicewood Springs than at other JPS sites, averaging over 0.15 mg/L and exceeding 0.80 mg/L (Figure 19).

Contaminants – A sampling plan 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. Sediment samples were collected from Bull Creek above Tributary 7, Stillhouse Hollow, Spicewood Tributary, Tributary 6, Tributary 5, and Barrow Tributary. Parameters included metals, total petroleum hydrocarbons, PAHs, organochlorine pesticides, orthophosphate pesticides, herbicides, texture, total organic carbon, and ammonia. The results are presented in Table 6. This and previous sampling efforts have detected elevated PAHs in sediment from the Spicewood tributary, but not from Stillhouse, Barrow, or other sampling sites (City of Austin 2001).

Table 6. Contaminants in Sediments from Six Jollyville Plateau Monitoring Sites

Above threshold effects concentration (TEC)*		<D.L. = below detection limits						
Above probable effects concentration (PEC)*			Sites - 2005 Data					
Parameter Type	**Parameter**	**UNITS**	**Stillhouse**	**Barrow**	**Spicewood**	**Trib 6**	**Above Trib 7**	**Trib 5**
Carbon	Organic carbon	MG/KG	60200	78600	58200	85300	61100	61600
Grain SizeSubstrate	Texture clay <0.002mm	(%) Percent	10	8	14	18	10	16
Grain Size Substrate	Texture gravel	(%) Percent	7.67	16	11	3	33	13
Grain Size Substrate	Texture sand 0.05-2.0	(%) Percent	60.3	42	61	55	35	34
Grain Size Substrate	Texture silt 0.002-0.05	(%) Percent	22	35	14	24	22	37
Metals	Arsenic	MG/KG	1.1	2.2	2.4	3.6	2.6	4.5
Metals	Cadmium	MG/KG	0.16	0.23	0.32	0.20	0.12	0.31
Metals	Chromium	MG/KG	3.4	10.2	16.7	10.2	3.8	9.7
Metals	Copper	MG/KG	5.2	15.4	20.5	4.5	2.0	5.2
Metals	Iron	MG/KG	1410	4510	7700	6550	2220	7000
Metals	Lead	MG/KG	6.4	28.5	34.6	8.3	2.9	7.0
Metals	Mercury	MG/KG		0.033	0.053	0.013		
Metals	Nickel	MG/KG	7.7	13.0	15.1	8.6	6.6	10.0
Metals	Zinc	MG/KG	6.8	45.2	50.4	16.6	4.4	16.5
Nutrients	Ammonia as n	MG/KG	29	90	56	56	34	71
Organochlorines	4_4'-ddt	UG/KG	<D.L.	<D.L.	8.59	<D.L.	<D.L.	<D.L.
Organochlorines	Dieldrin	UG/KG	<D.L.	2.5	<D.L.	<D.L.	<D.L.	<D.L.
Organochlorines	Heptachlor epoxide	UG/KG	2.7	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.
PAHs	Acenaphthene	UG/KG	<D.L.	<D.L.	34	<D.L.	<D.L.	<D.L.
PAHs	Acenaphthylene	UG/KG	<D.L.	<D.L.	31	<D.L.	<D.L.	<D.L.
PAHs	Anthracene	UG/KG	<D.L.	<D.L.	131	<D.L.	<D.L.	<D.L.
PAHs	Benzo(a)anthracene	UG/KG	<D.L.	35	1220	<D.L.	<D.L.	<D.L.
PAHs	Benzo(a)pyrene	UG/KG	<D.L.	59	1640	<D.L.	<D.L.	<D.L.
PAHs	Benzo(b)fluoranthene	UG/KG	<D.L.	114	1490	44	<D.L.	<D.L.
PAHs	Benzo(ghi)perylene	UG/KG	<D.L.	42	707	<D.L.	<D.L.	<D.L.
PAHs	Benzo(k)fluoranthene	UG/KG	<D.L.	<D.L.	1730	<D.L.	<D.L.	<D.L.
PAHs	Chrysene	UG/KG	<D.L.	74.3	2260	<D.L.	<D.L.	<D.L.
PAHs	Dibenz(ah)anthracene	UG/KG	<D.L.	<D.L.	206	<D.L.	<D.L.	<D.L.
PAHs	Fluoranthene	UG/KG	<D.L.	111	3710	18	<D.L.	<D.L.
PAHs	Fluorene (9h-fluorene)	UG/KG	<D.L.	<D.L.	45	<D.L.	<D.L.	<D.L.
PAHs	Indeno(123-cd)pyrene	UG/KG	<D.L.	38	1190	<D.L.	<D.L.	<D.L.
PAHs	Phenanthrene	UG/KG	<D.L.	43	1100	<D.L.	<D.L.	<D.L.
PAHs	Pyrene	UG/KG	<D.L.	80.8	2650	<D.L.	<D.L.	<D.L.
PAHs	Total pahs - COA	UG/KG	<D.L.	597.1	18144	62	<D.L.	<D.L.
Sample Descriptors	Percent moisture	(%) Percent	50	62	58	46		59

*TEC and PEC are consensus-based sediment quality guidelines derived from multiple studies and other guidelines and restricted to freshwater studies on sediment-dwelling organisms (MacDonald et al. 2000).

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 20). This may be due to input from anthropogenic sources such as stormwater outfalls and golf course irrigation.

Most of the benthic macroinvertebrate sampling is conducted at three Bull Creek sites – Bull Creek above Tributary 7, Tributary 5, and Tributary 6. Interestingly, of these three sites, Tributary 6 has had both higher numbers of benthic macroinvertebrates and macroinvertebrate taxa than the other two sites (figures 21 and 22). However, both of these parameters appear to have declined at the Tributary 5 and Tributary 6 sites since 1997, compared to the more rural Bull Creek above Tributary 7 site. The Bull Creek Tributary 3 at Great Hills Golf Course has also had relatively high numbers of macroinvertrebrates and taxa, although macroinvertebrate sampling is no longer conducted at this site. Sample sizes for Tributary 3 and the other JPS sites are too limited to draw many conclusions.

Plauditus virilis (McDunnough) was found only at Bull Creek above Tributary 7. The species of the small Nearctic genus *Plauditus* are highly sensitive to nutrient enrichment (N.A. Wiersema- personal correspondence). *Plauditus* species have proven to be useful indicator species in the Balcones Plateau region of Texas (Wiersema 1999). *Plauditus virilis* has not been observed in the urban canyon tributaries 5 and 6.

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.

Reproductive Cycles and Flow

As discussed in the original Jollyville Plateau salamander study, 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 23 shows the cyclical nature of the percent in the smallest size class. The percents and the counts in each size class from all sampling efforts are combined and plotted by month in Figures 24-32. The percent of salamanders in the smallest size class peaks in May, 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, three months after the smallest size class peaks. The percent in the largest size class is high from October through April, with a peak in December, and low from May through September. Bowles et al. 2006 present similar results, although the size classes were divided into two categories (<1”, >1 inch) instead of three. However, because JPS spends part of its life in the aquifer, Bowles et al. avoid drawing any conclusions regarding seasonal reproduction, survival, or population size.

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 flows measurements have been too sporadic in recent years. The summed flows are plotted by month (Figure 33). 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 median 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 34). The lag is approximately four months. Figure 35 shows the percent of small salamanders together with the flow from four month 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 Springs. The egg was collected by 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.

General Status Assessment

City staff have 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 in karst often flows 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 Stillhouse Hollow Springs in the Bull Creek watershed, and injections near Spicewood Spring in the Shoal Creek watershed (City of Austin, unpubl. data). 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.

JPS is not found at all springs. Almost all of the known locations and sites with highest JPS counts include springs that are located near or in a creek and provide abundant baseflow.

Bull Creek – Sites where JPS is most abundant include Bull Creek above Tributary 7 and Lanier Spring in the BCP. Preliminary habitat assessments were conducted in this area on the City of Austin's BCP properties (Franklin, Lanier, proposed WTP4) on July 5 and July 11, 2005 to assess the probable extent of known and/or likely JPS habitat. Known and/or likely JPS habitat included (from upstream to downstream) the upstream end of proposed WTP#4 just below the 4-Points property, Lanier Spring, and below Pit Spring above Tributary 7 on the Franklin tract. The tributary that flows from 3M through the Ribelin and Lanier tracts also appears to support a large JPS population and should be surveyed more extensively to determine the extent of the JPS abundance and distribution along this stream reach. All of these sites are downstream of properties with existing, ongoing, or future planned development.

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 spring-run. 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 at the head of the spring.

JPS counts along Tributaries 5 and 6 are declining. The most obvious factor affecting Tributary 5 is high sediment levels, and high ion concentrations along Tributary 6. The Tributary 3 at Great Hills Golf

Course site may also be declining, but more data are needed to show whether this is real and statistically significant. The Tanglewood site is not surveyed on a regular basis because of the low salamander counts and staff limitations.

As discussed above, chytridiomycosis has been confirmed in the Stillhouse Hollow population, and deformities have been documented in both the Stillhouse and Barrow populations. Nutrients, particularly nitrates, and ion concentrations are elevated at these sites.

<u>Brushy Creek</u> – JPS is only known from a few sites on Avery Ranch, which is in a rapidly urbanizing area.

<u>Buttercup Creek</u> – 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.

<u>Cypress Creek</u> – 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, although the headwaters of the springs on the Audubon tract appear to be within this preserve. 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 Wharton 1997).

<u>Lake Creek Watershed</u> – Only one spring with JPS is known from this watershed. The spring is located within the SH45 right-of-way, and the watershed lies within a rapidly urbanizing area.

<u>Long Hollow</u> – This is the only creek within the known range of the JPS that appears to have the entire headwaters and most of the creek within an existing preserve (LCRA's Wheless tract). The survey area is near the upper reach of the creek and is one of the first JPS sites to go dry. When flowing, large numbers of JPS are found in the survey area, and JPS has been found along much of the creek.

<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. Salamander counts at this site have declined significantly in recent years.

<u>Walnut Creek Watershed</u> – Only one tiny spring pool with JPS is known from this watershed, which is located within a park that is surrounded by urban development. One to three salamanders have been found at this site since 2004. One of the salamanders observed over a 14-month period was the same individual, suggesting that this is a very small population.

<u>West Bull Creek Watershed</u> – 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

- Salamander counts are declining at 3 of 9 long-term monitoring sites.
- Salamanders with curved spines and other deformities have been observed at 2 of 9 long-term monitoring sites.
- Infection with the chytrid fungus has been confirmed in 1 of the 9 long-term monitoring sites.
- The declining counts and health problems appear to be related to water quality and habitat degradation due to urban development.
- 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, spring source areas are unknown.
- The Jollyville salamander continues to exhibit seasonal reproductive cycles, which may be regulated by rainfall/flow.

Sampling Plan and Research Recommendations

As funding is available, the following recommendations are made for future JPS monitoring:

- Conduct quarterly salamander counts at all of the nine long-term monitoring sites, with possibly more frequent habitat measurements at Tributary 3 and Spicewood Springs to determine if these sites are also experiencing significant decreases in salamander counts.
- Expand survey efforts to include at least one site in each watershed where JPS is known to occur.
- Continue to take flow measurements with each salamander count.
- Collect water quality samples at all of the monitoring sites during JPS survey efforts. Coordinate with Water Resources Evaluation stream and spring monitoring teams. Include parameters that have been identified as degrading at sites where the salamander counts are declining: nitrate, specific conductance, sodium, sulfate, and chloride.
- Conduct macroinvertebrate sampling – at a minimum, include Wheless, Barrow, and Stillhouse Hollow.
- 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 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 population genetic studies to determine 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. Ideally, sample water quality from at least two sites at both Stillhouse Hollow (main spring, side spring, and ideally one additional site below the confluence of main and side spring) and Barrow (main spring and tributary where the long-term monitoring site is). Possible research could include maintaining JPS from these and another site over successive generations in water from Stillhouse Hollow and nitrate toxicity studies.
- Determine the extent to which the chytrid fungus is present in other Jollyville Plateau salamander populations. City biologists are currently investigating the least intrusive measures of obtaining tissue samples for chytrid PCR, including tissue swabs. Any dead salamanders or anurans should be collected for chytrid PCR (Appendix A).

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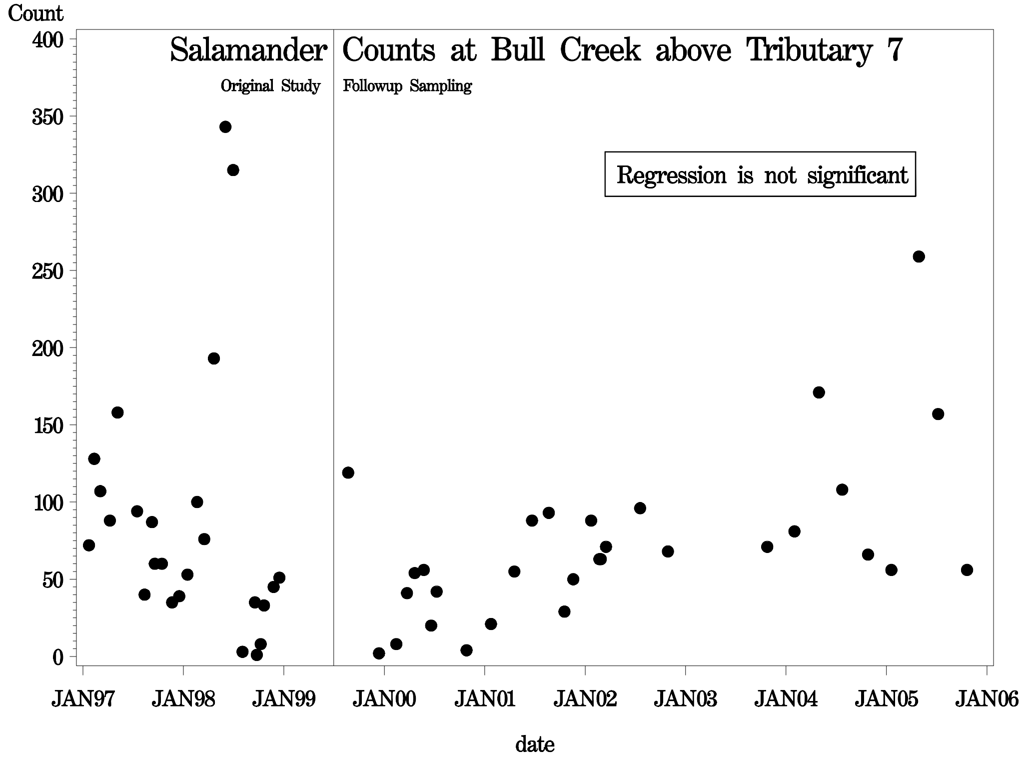
Figure 2. Salamander Counts at Bull Creek above Tributary 7



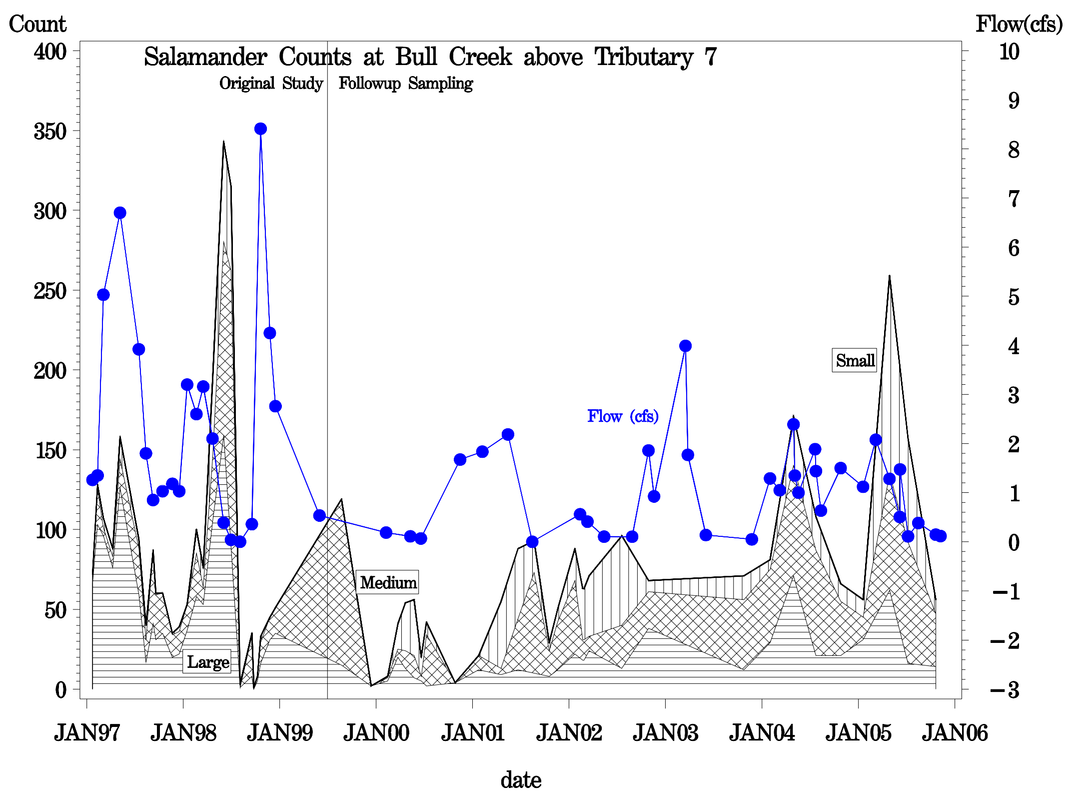
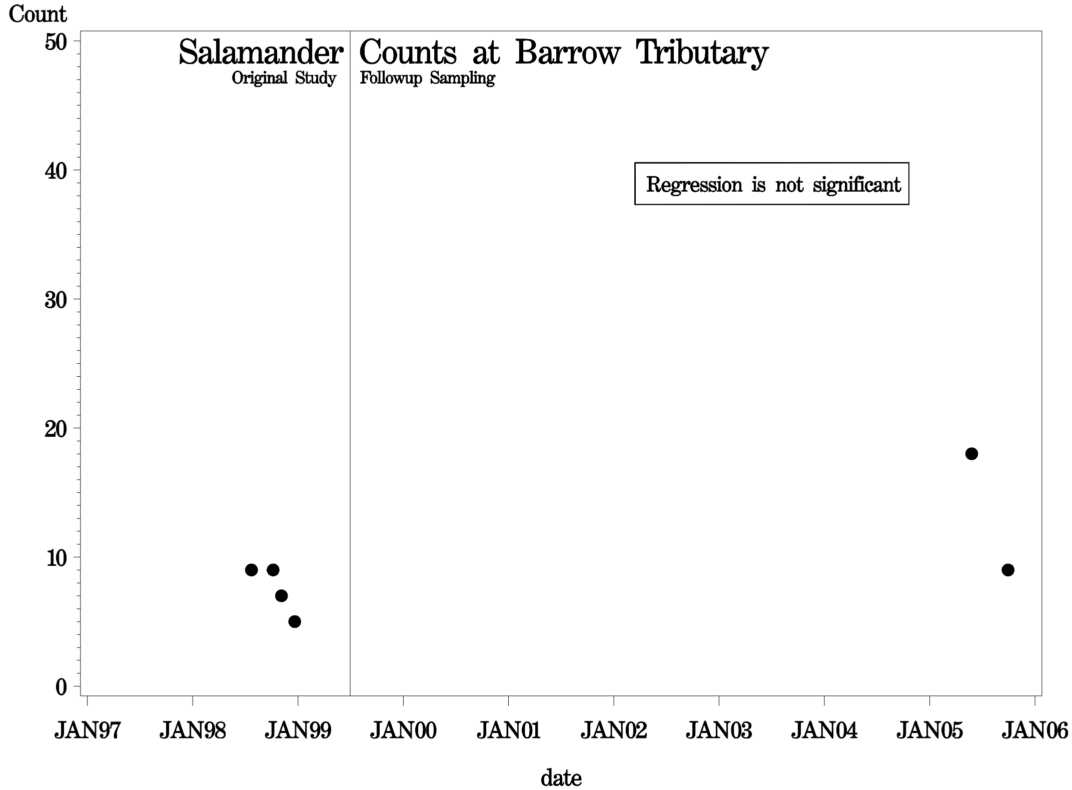


Figure 3. Salamander Counts at Barrow Tributary



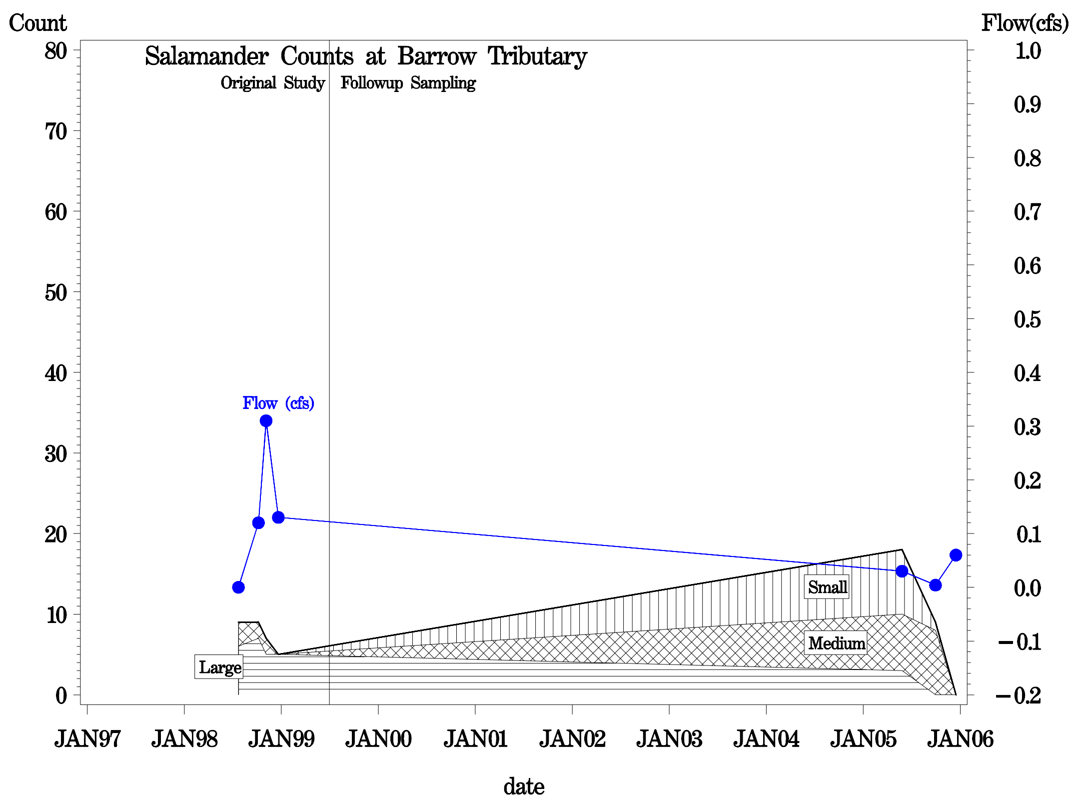
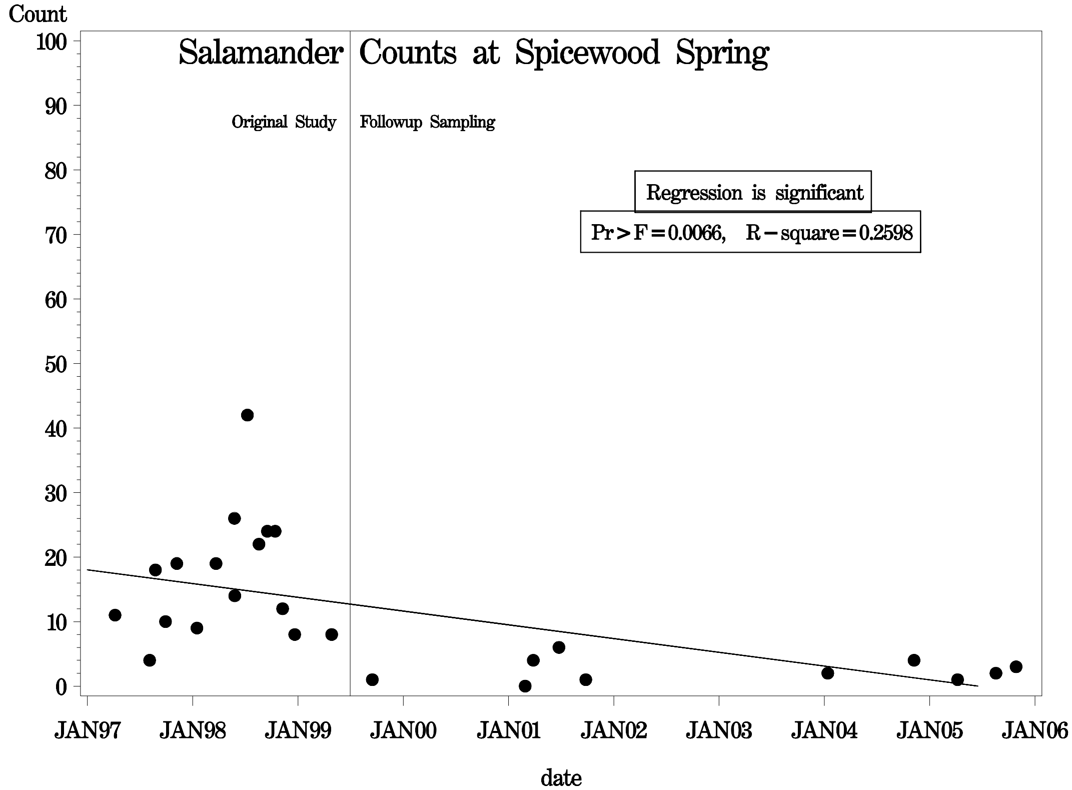


Figure 4. Salamander Counts at Spicewood Spring



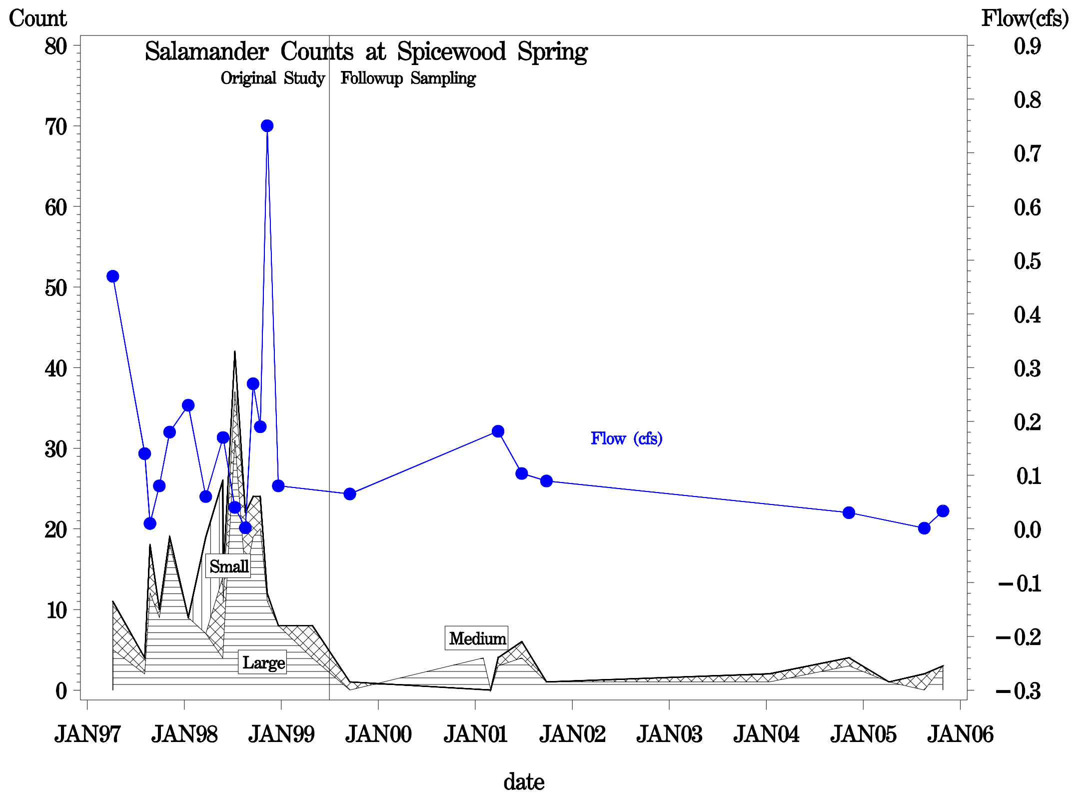
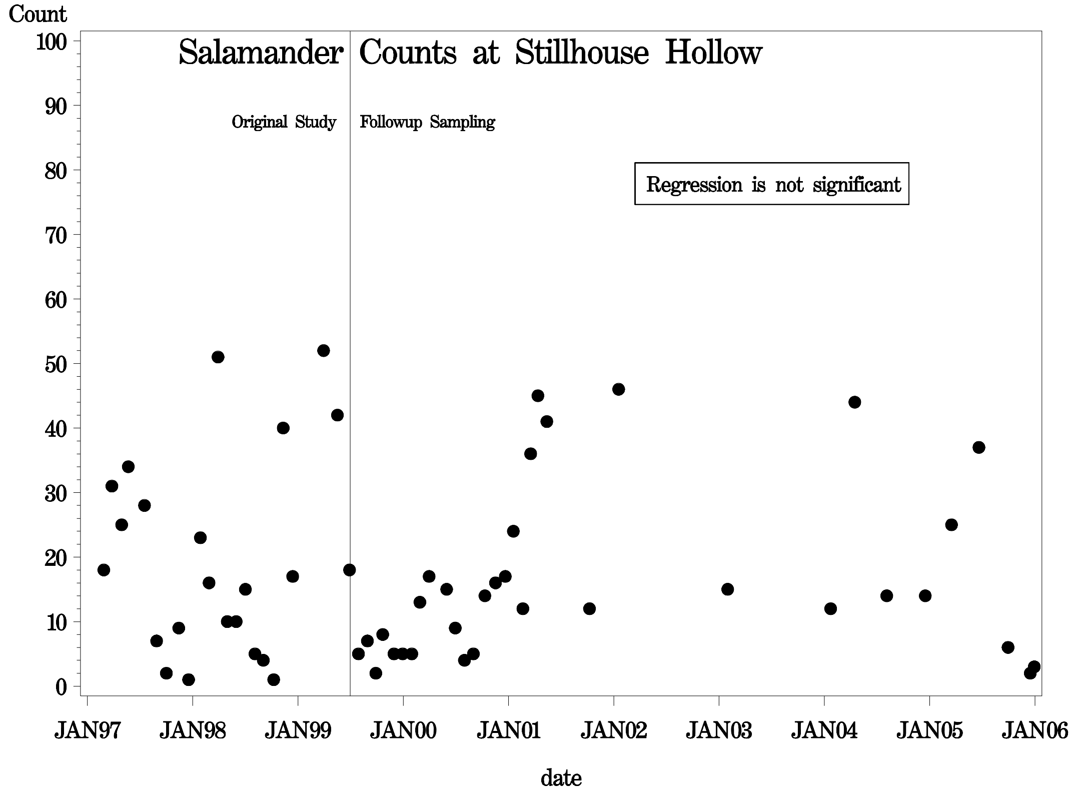


Figure 5. Salamander Counts at Stillhouse Hollow



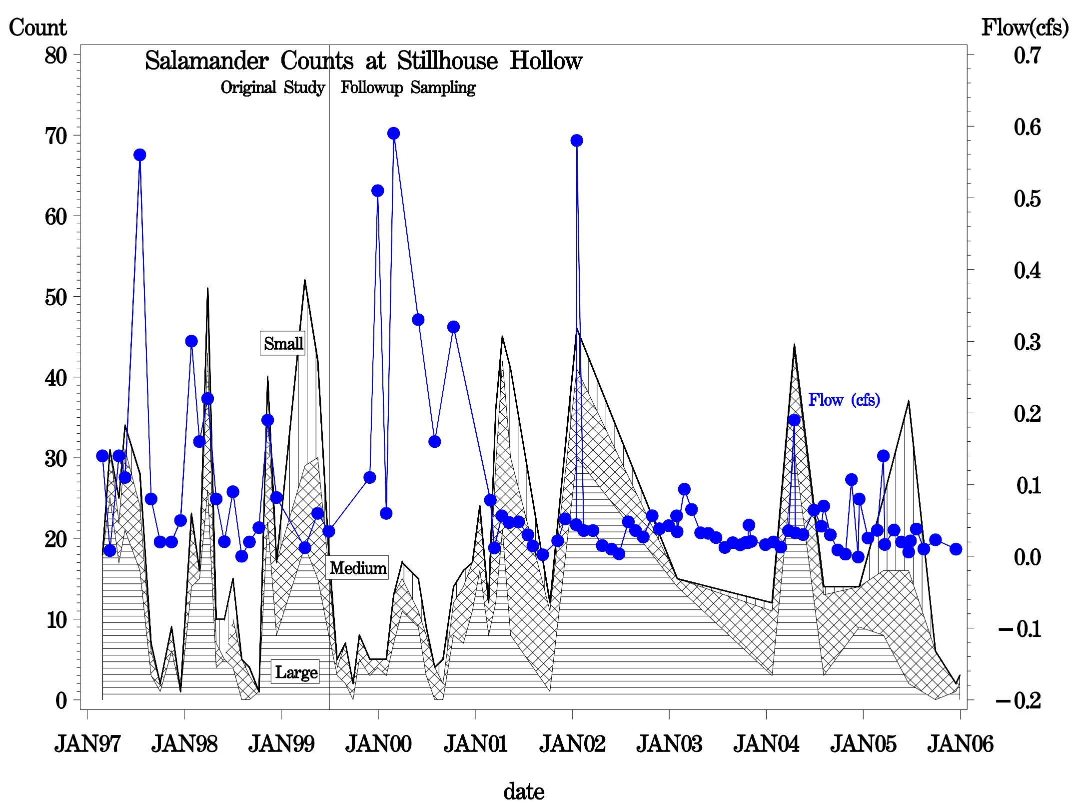
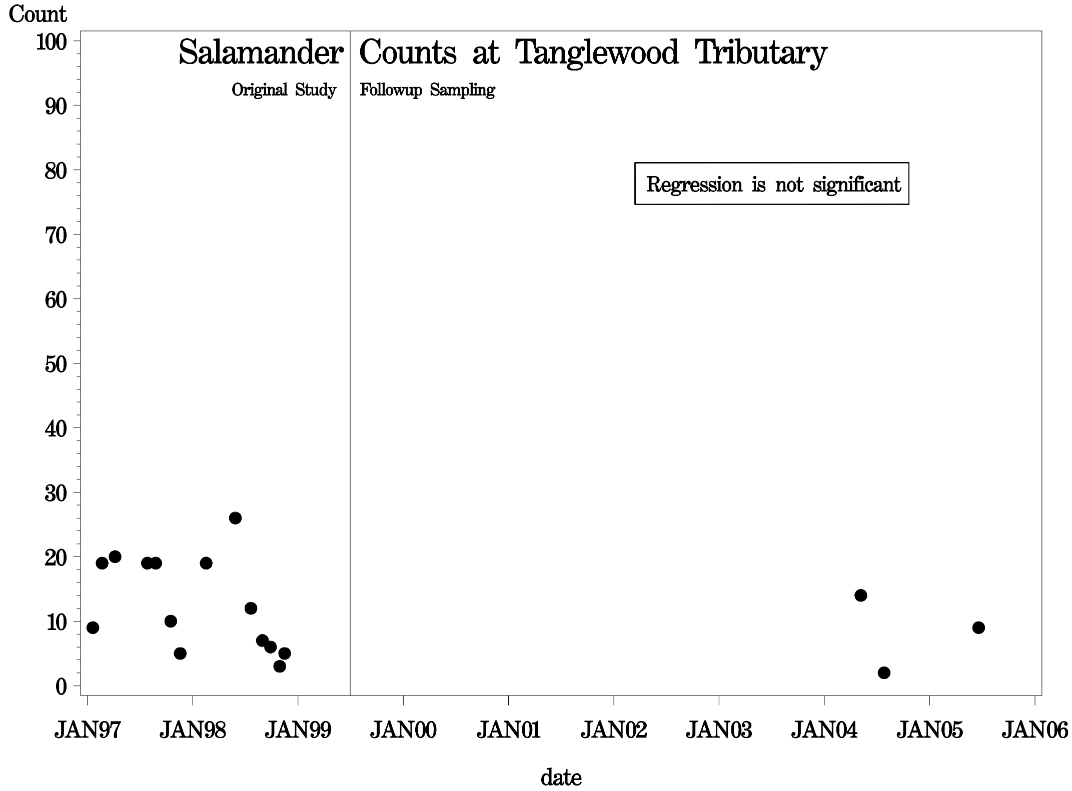


Figure 6. Salamander Counts at Tanglewood Tributary



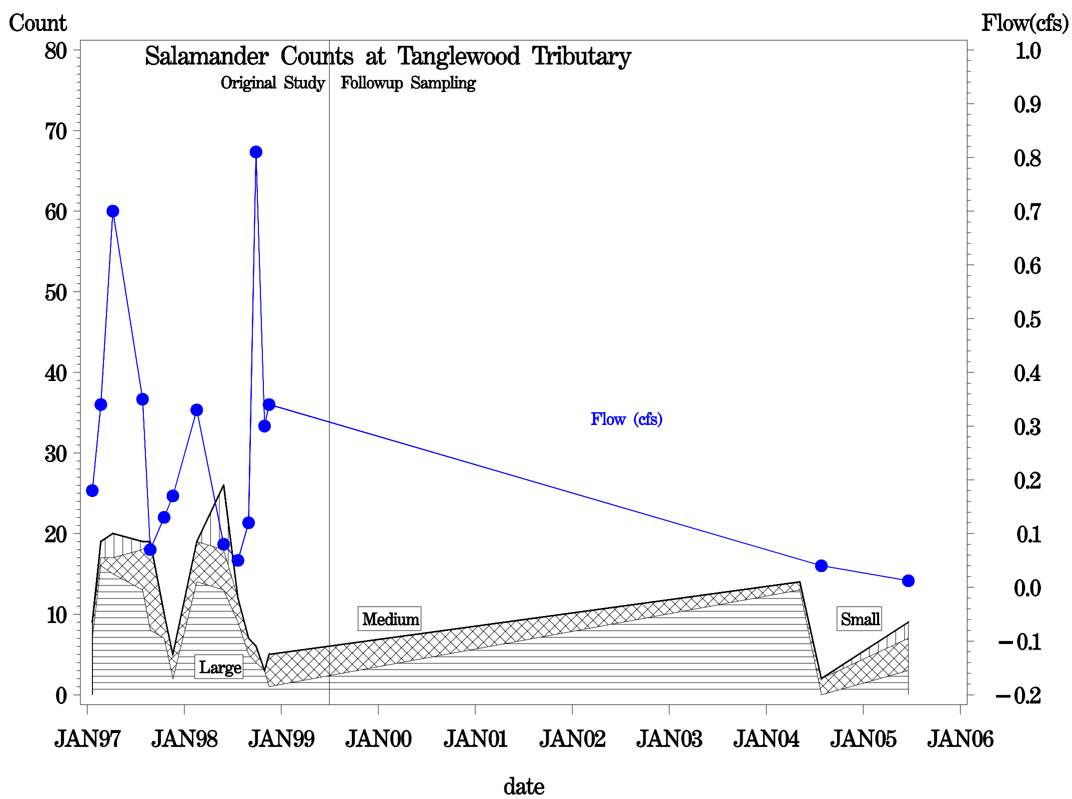
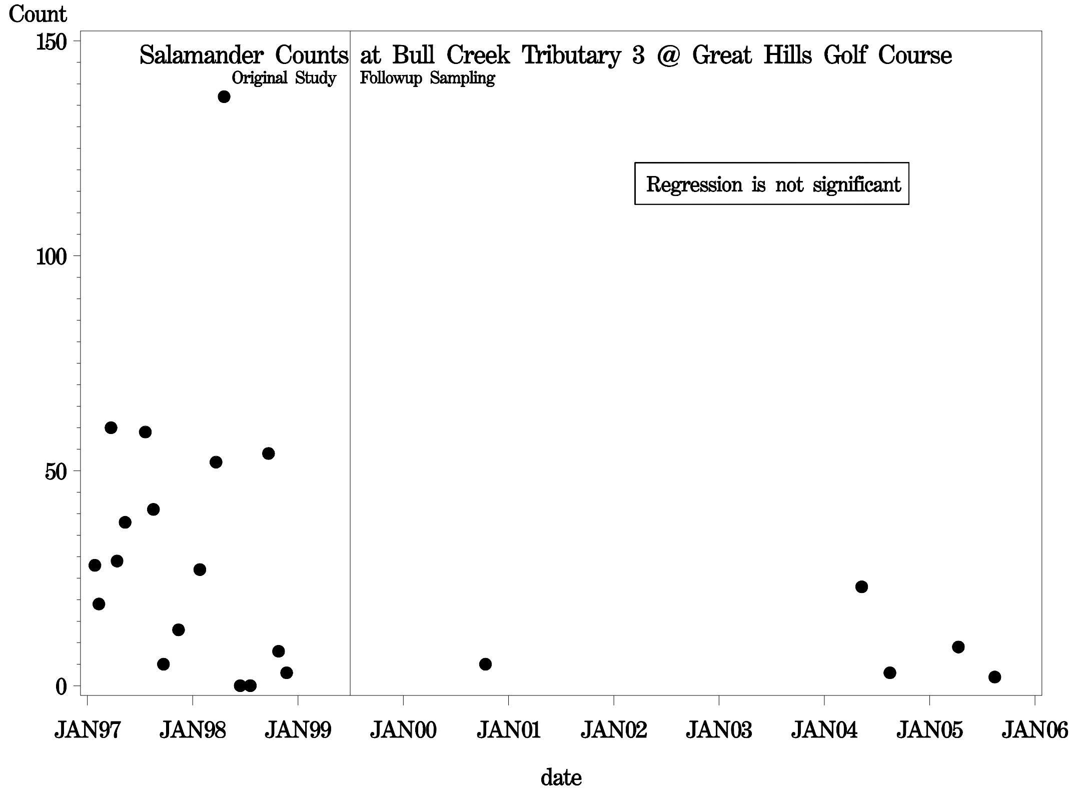


Figure 7. Salamander Counts at Bull Creek Tributary 3 @ Great Hills Golf Course



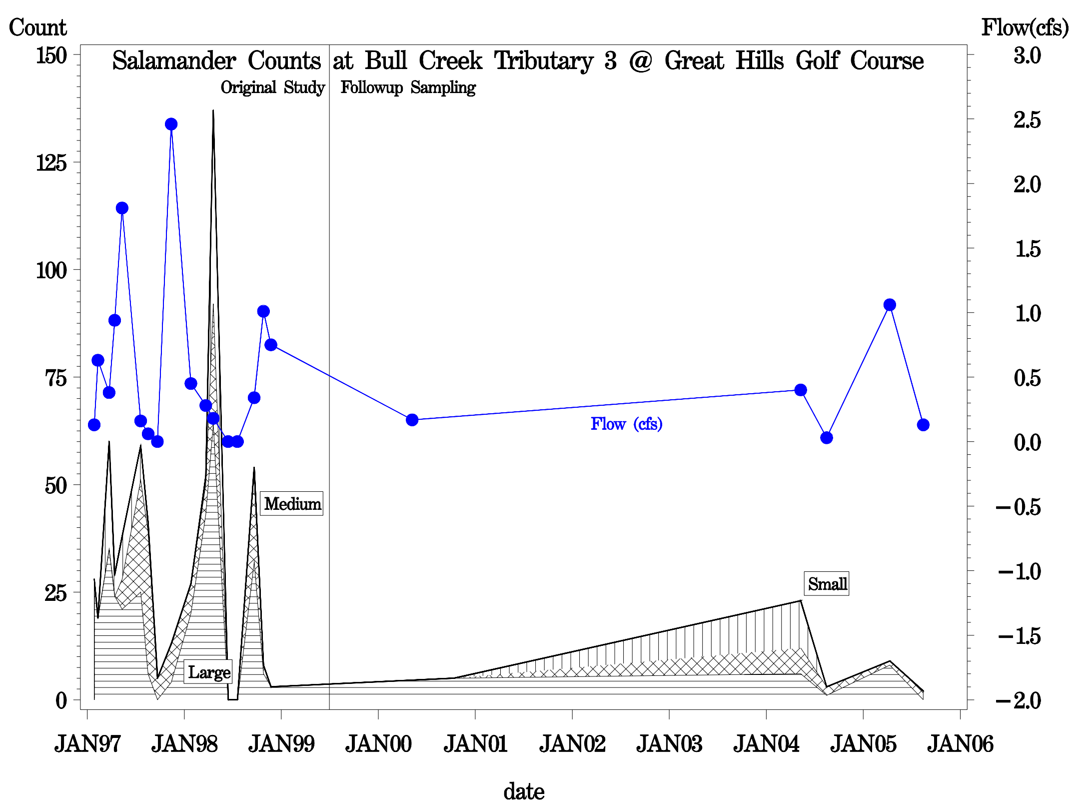


Figure 8. Salamander Counts at Bull Creek Tributary 5

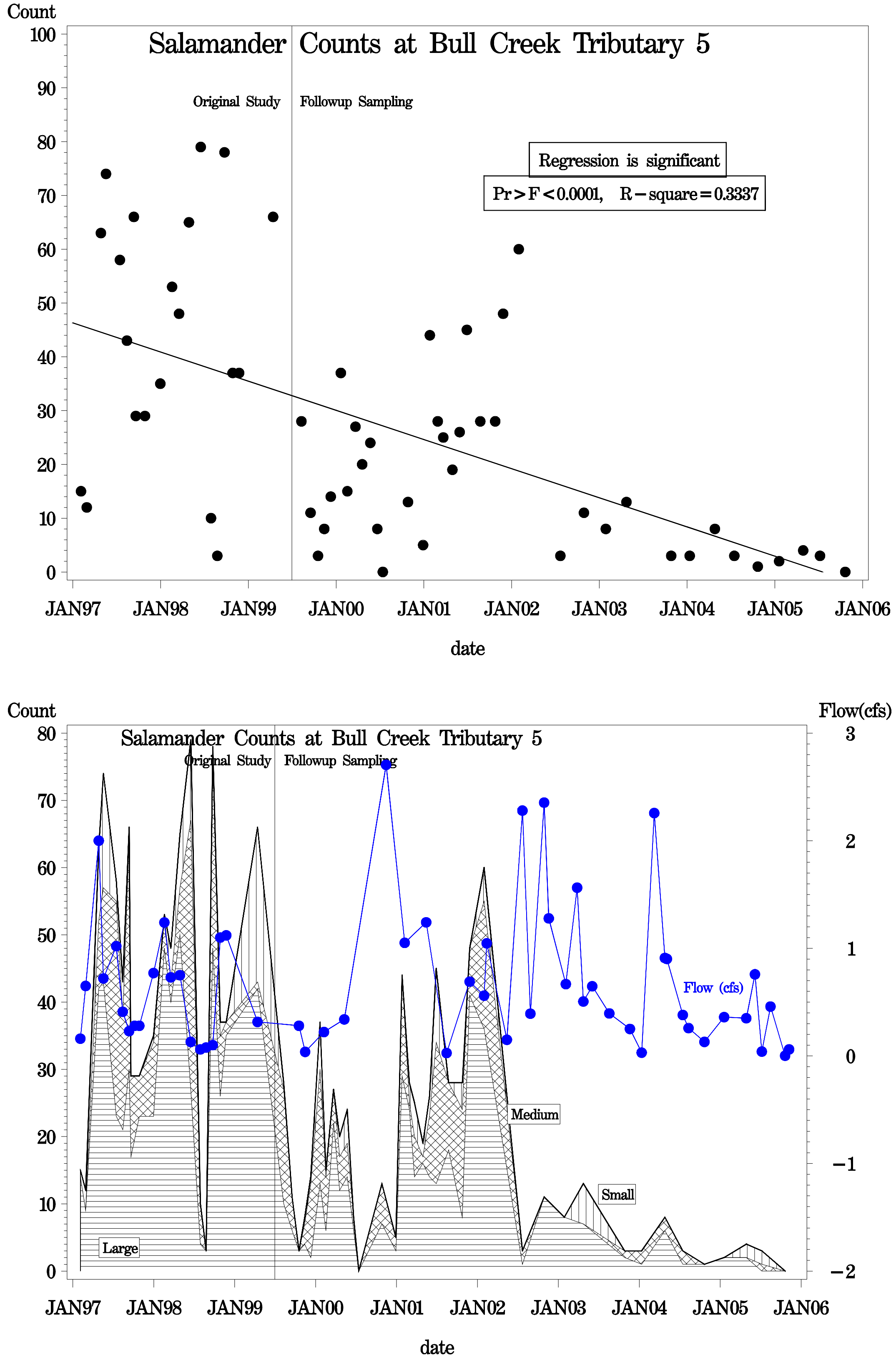
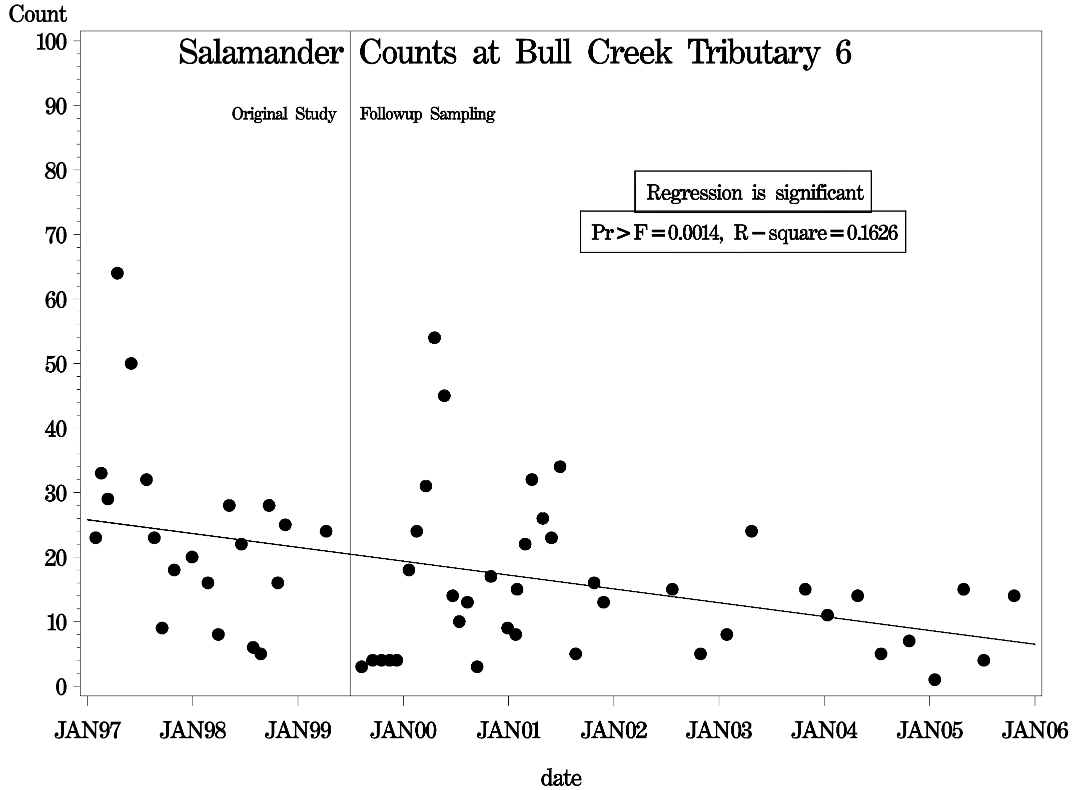


Figure 9. Salamander Counts at Bull Creek Tributary 6



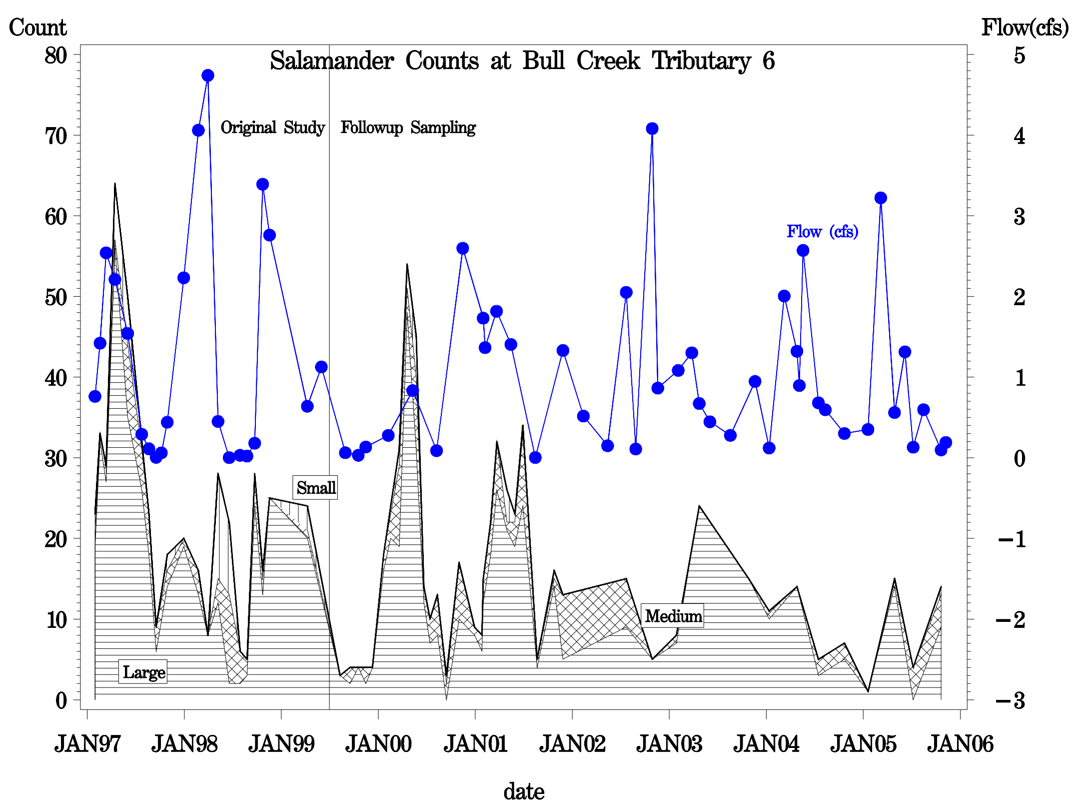
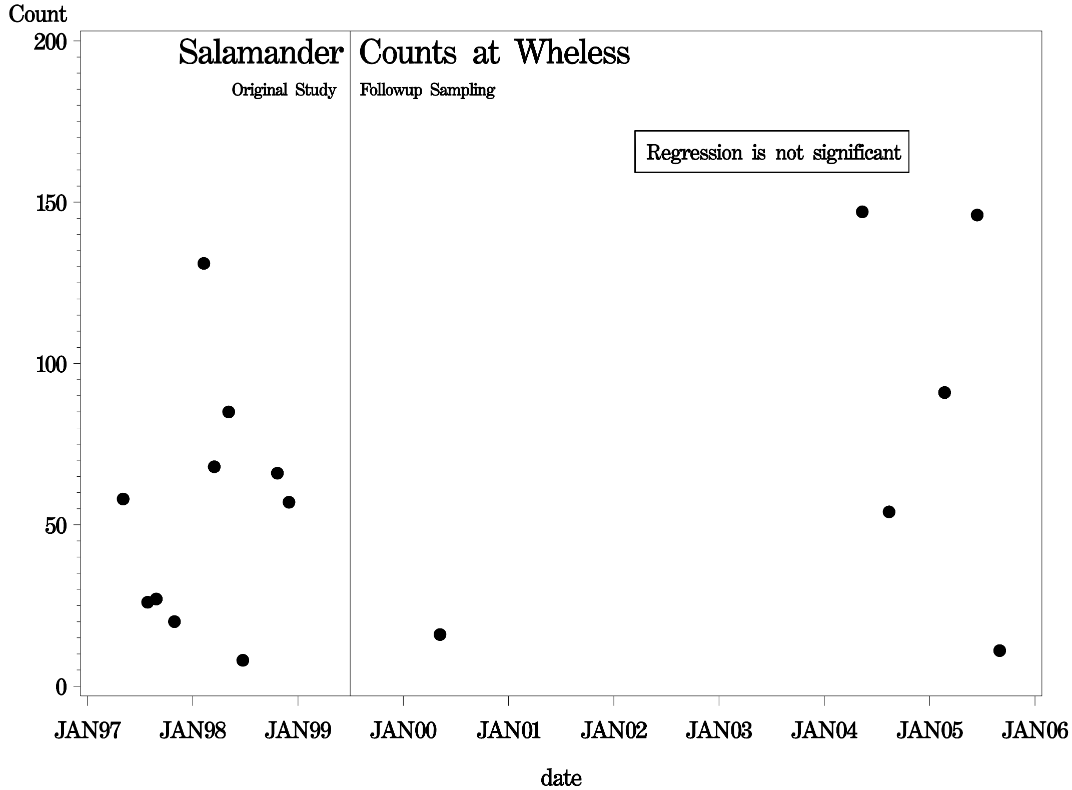


Figure 10. Salamander Counts at Wheless



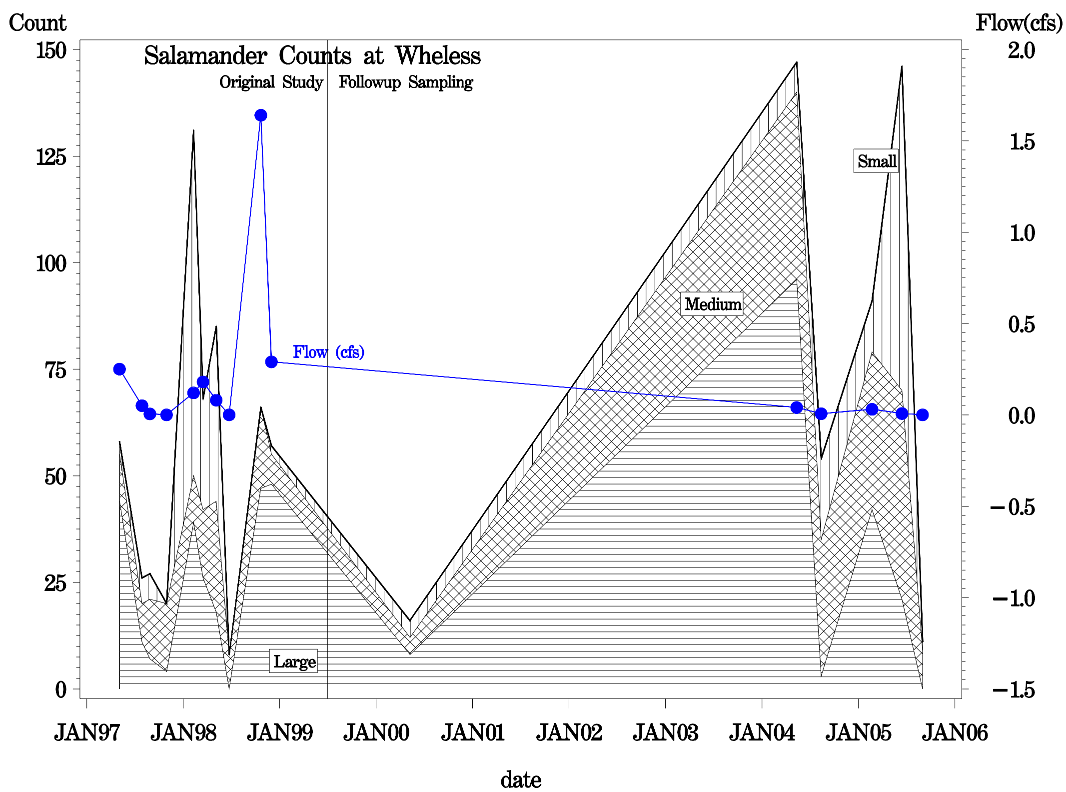


Figure 11. Embeddedness, From Pebble Count Measurements

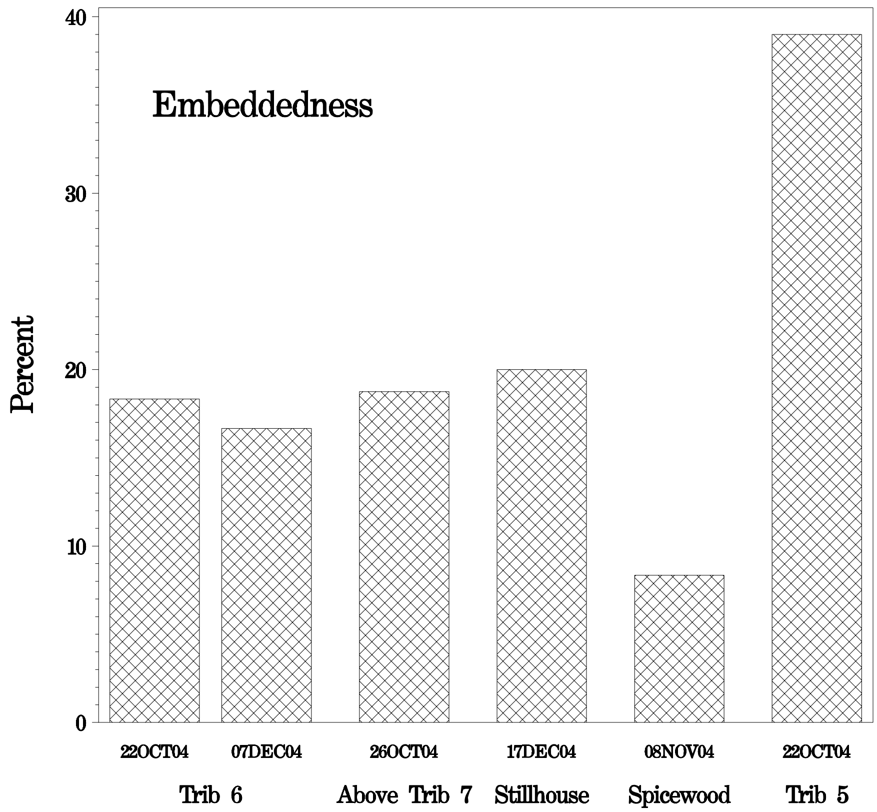
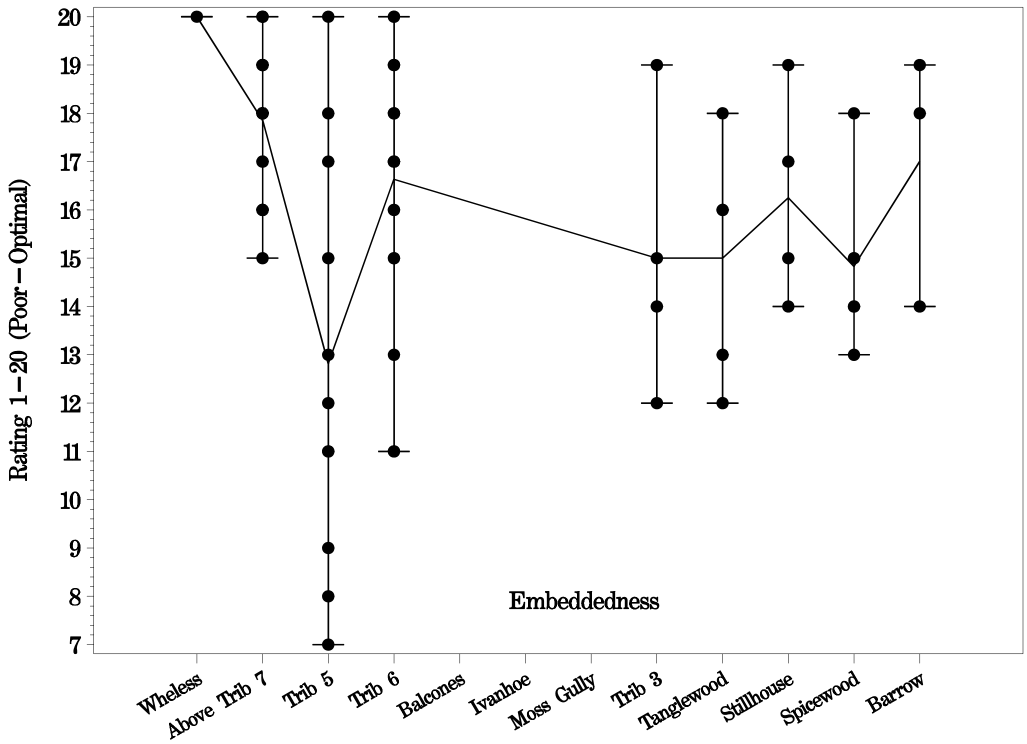


Figure 12. Embeddedness, From Visual Estimates



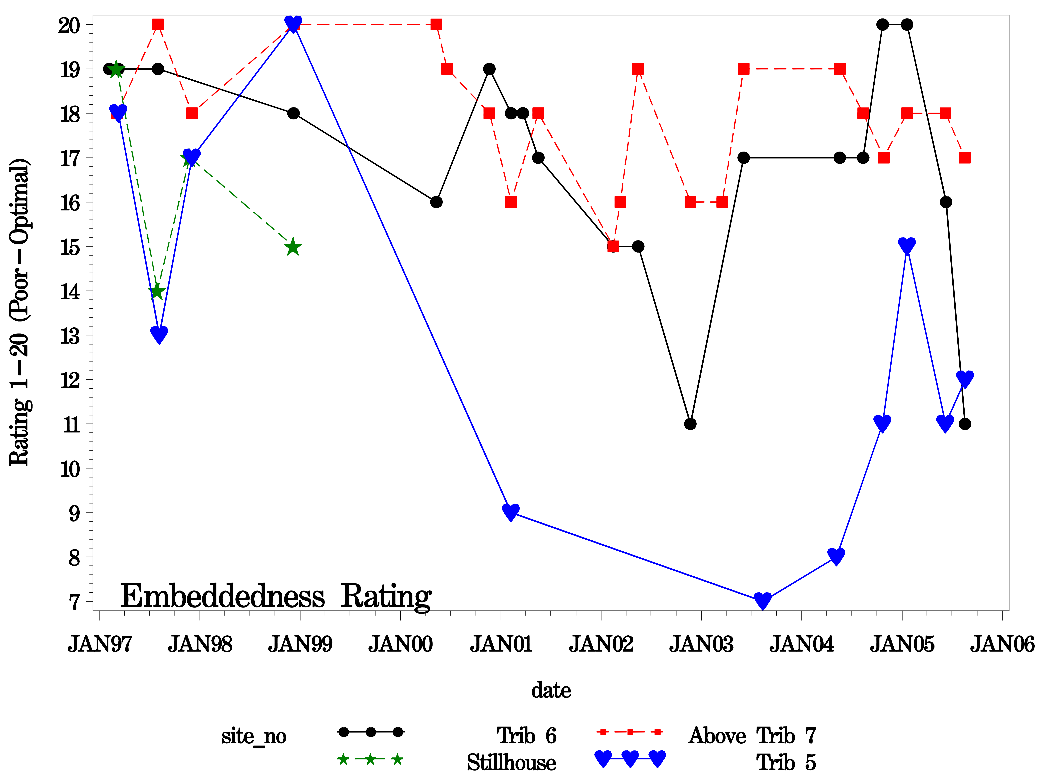
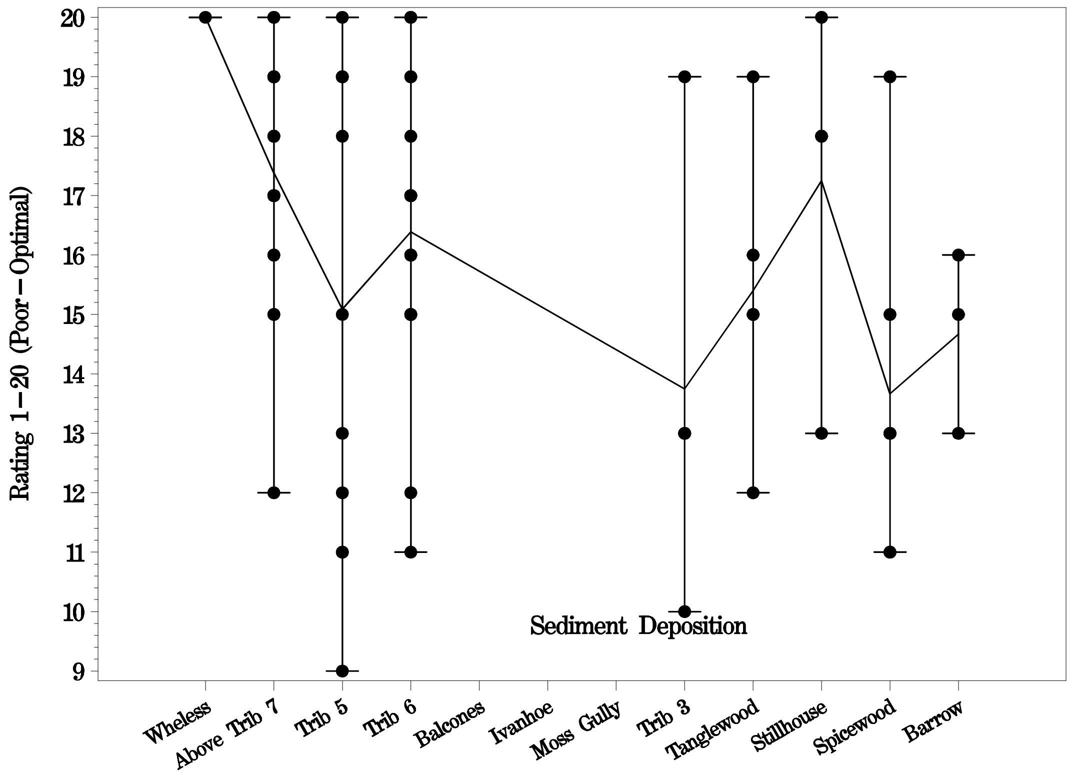


Figure 13. Sediment Deposition, From Visual Estimates



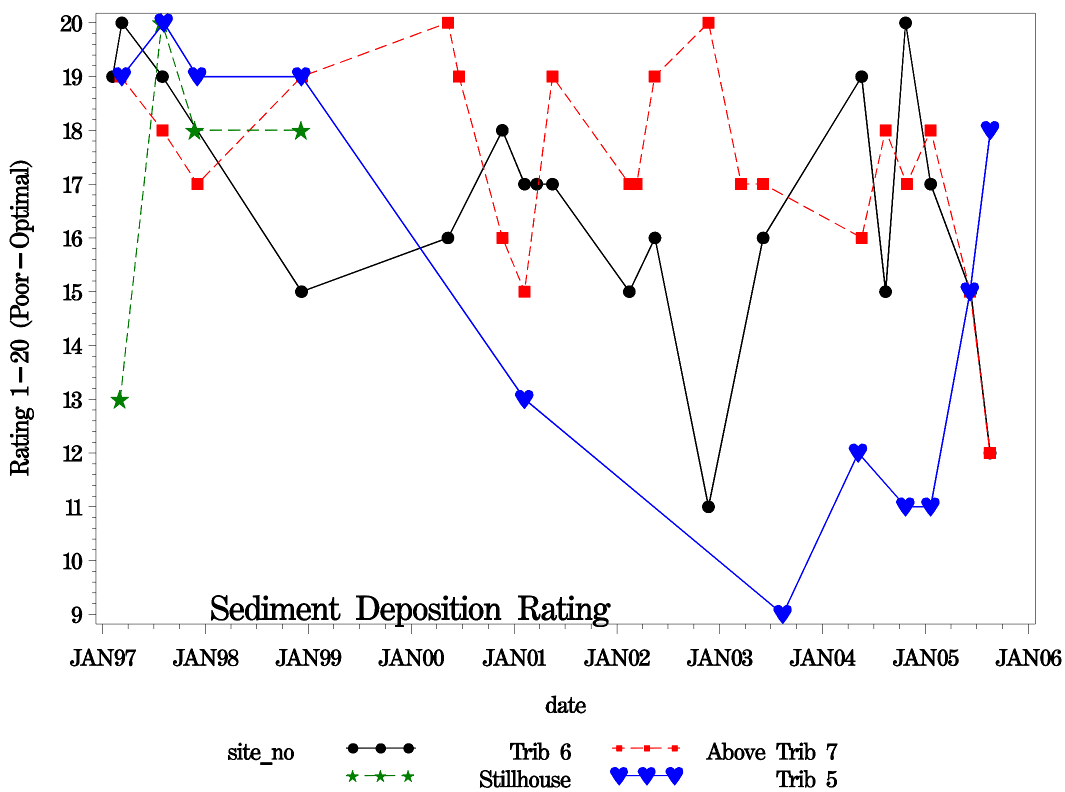
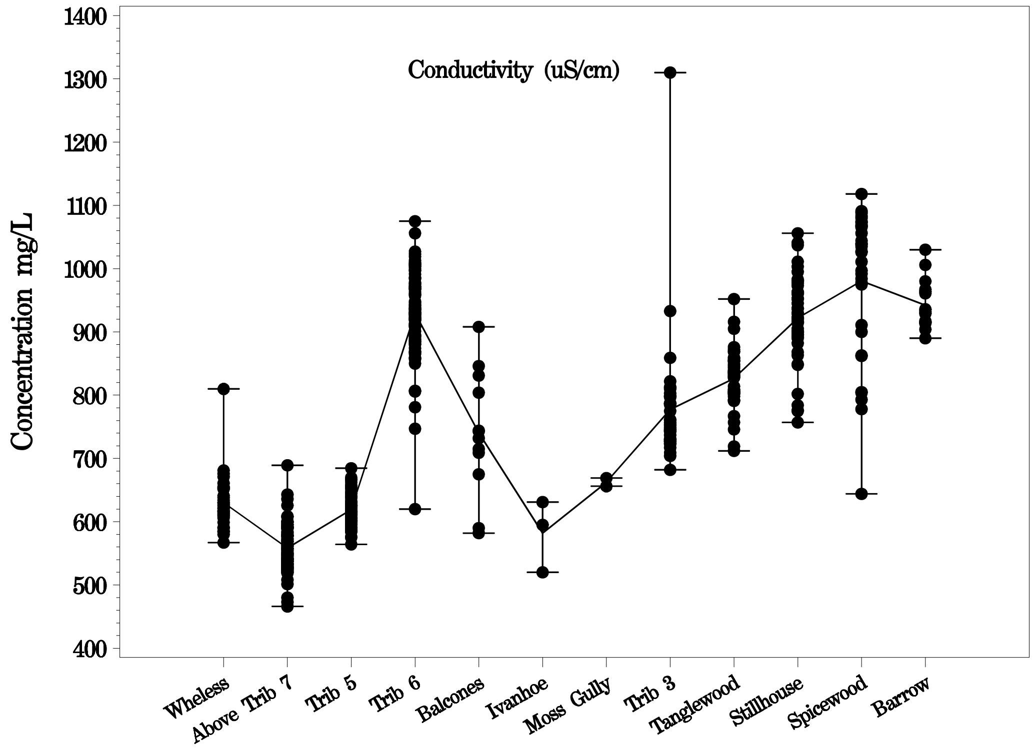


Figure 14. Specific Conductance



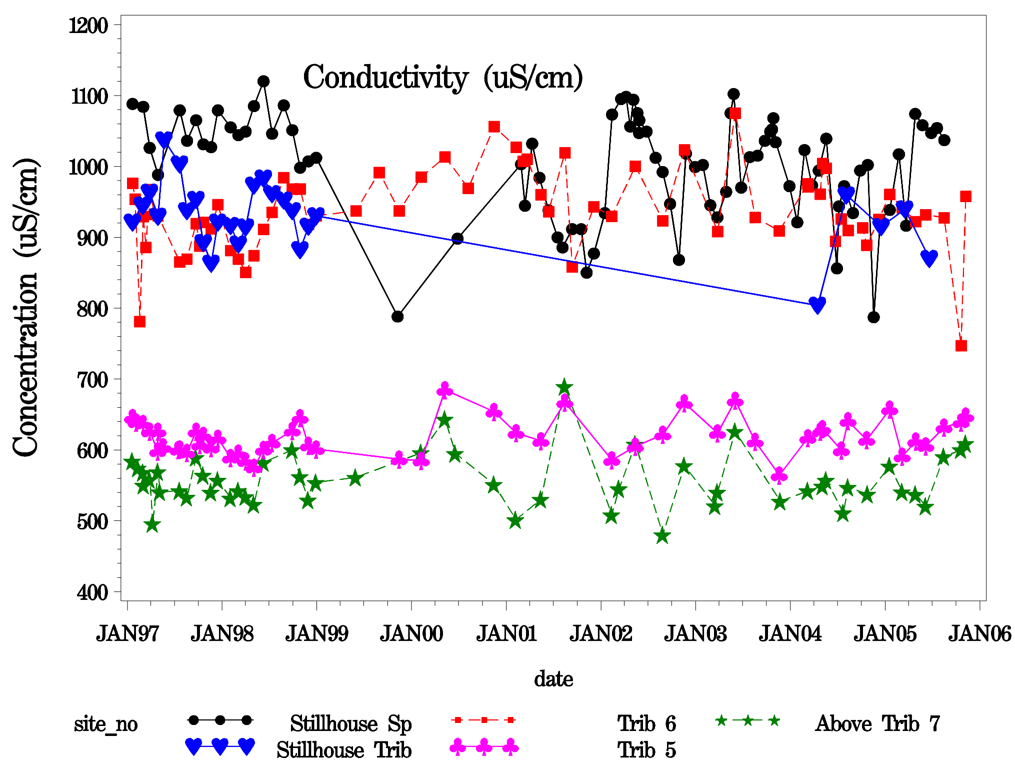
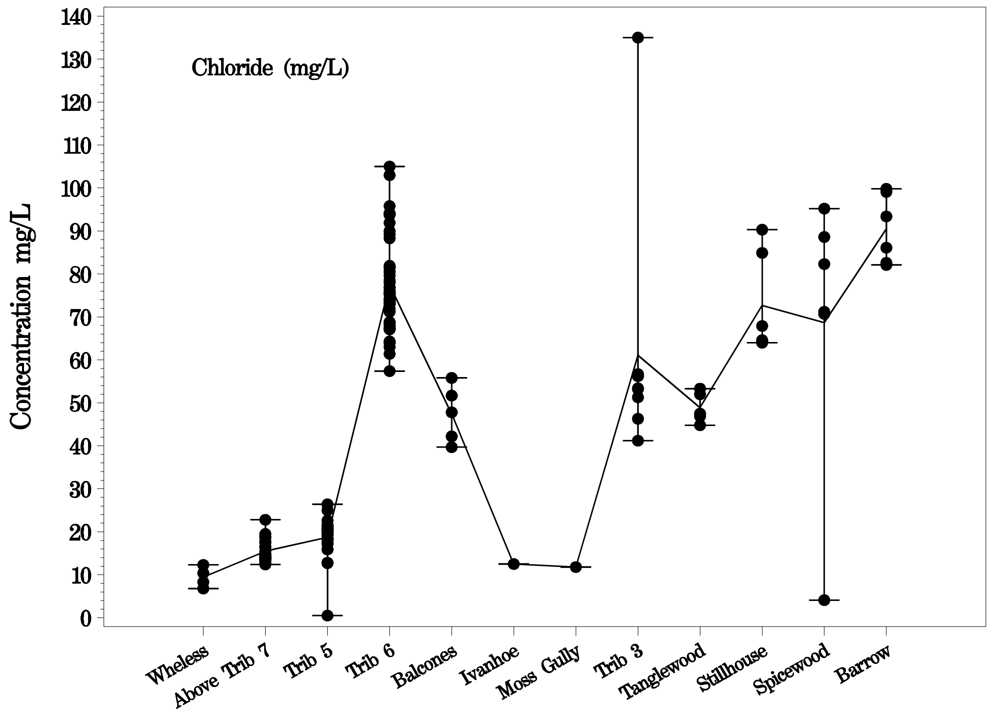


Figure 15. Chloride



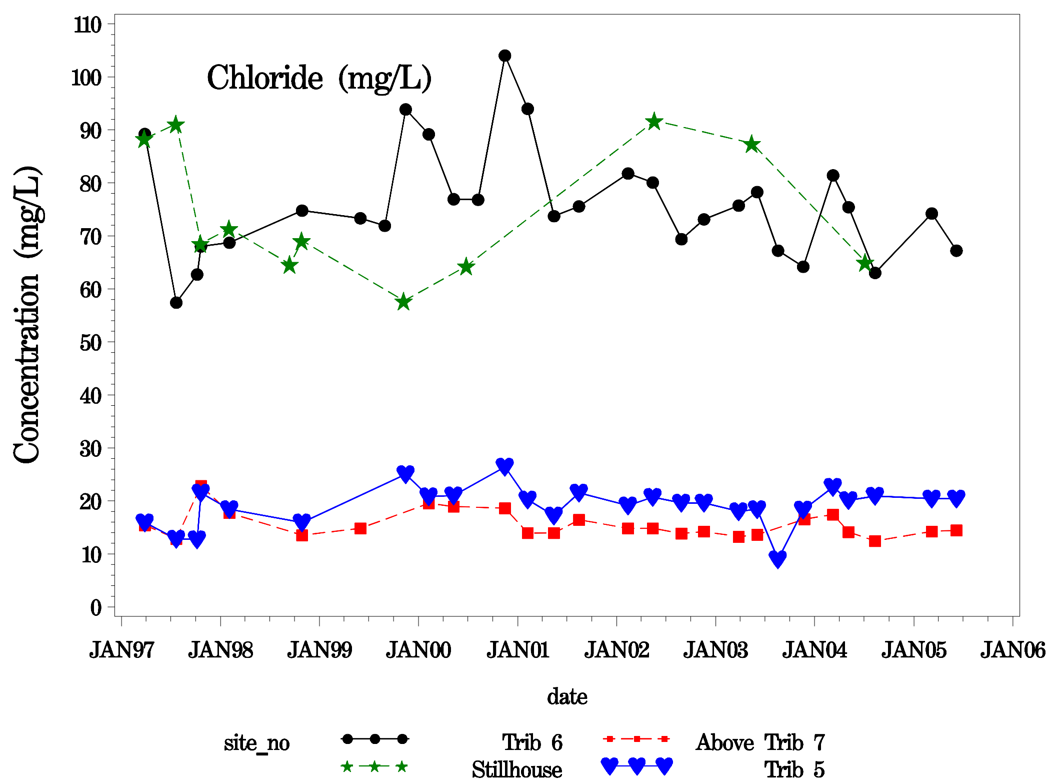
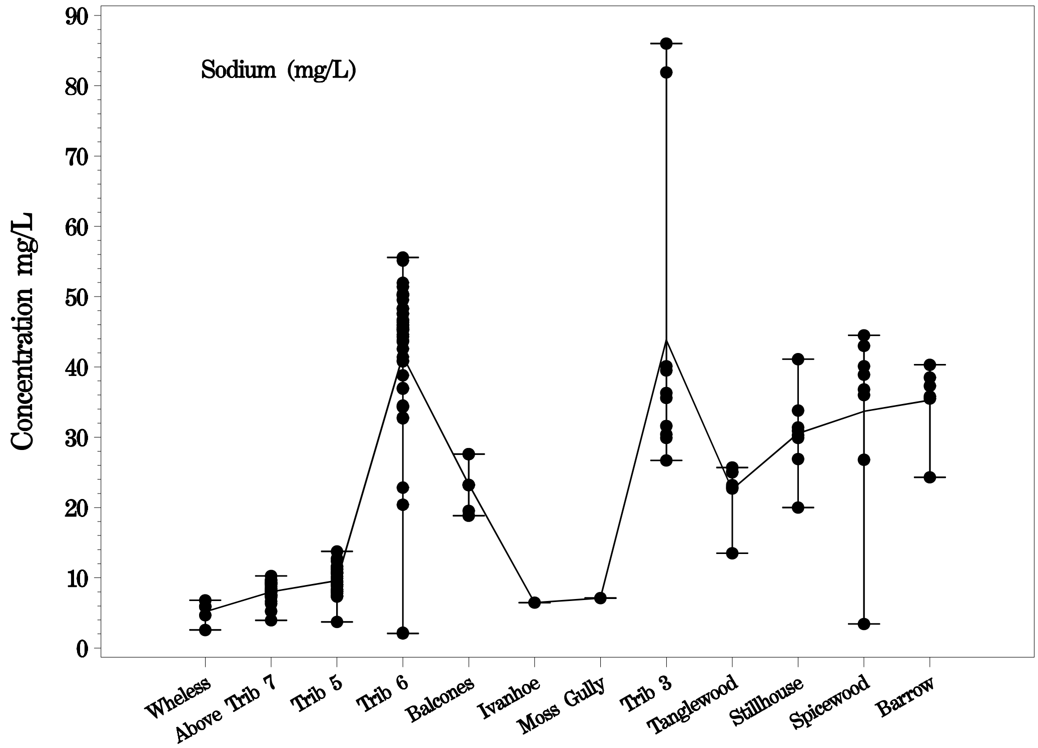


Figure 16. Sodium



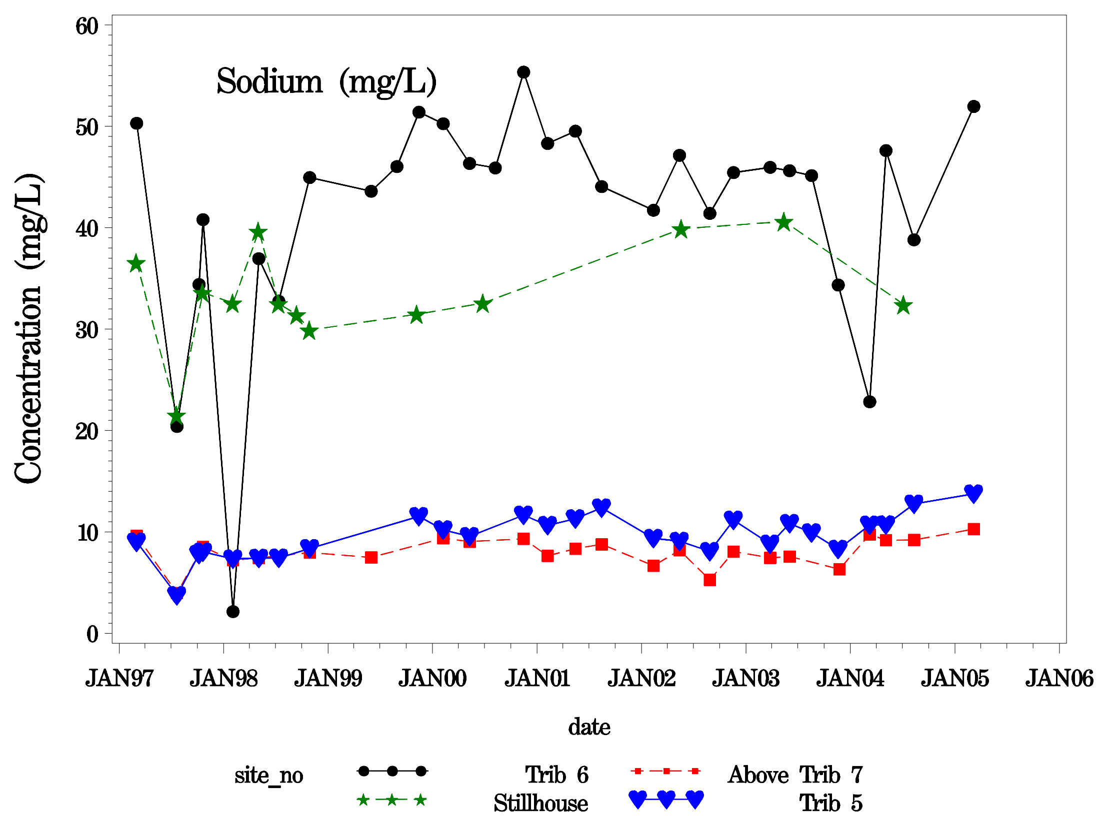
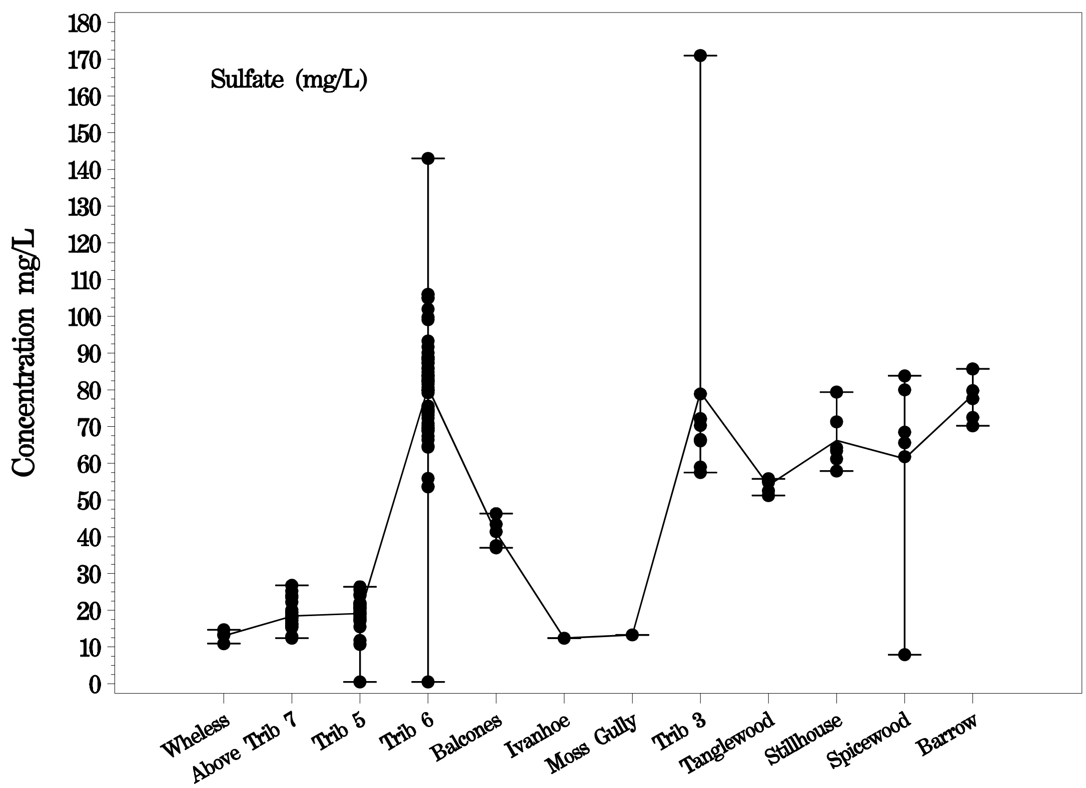


Figure 17. Sulfate



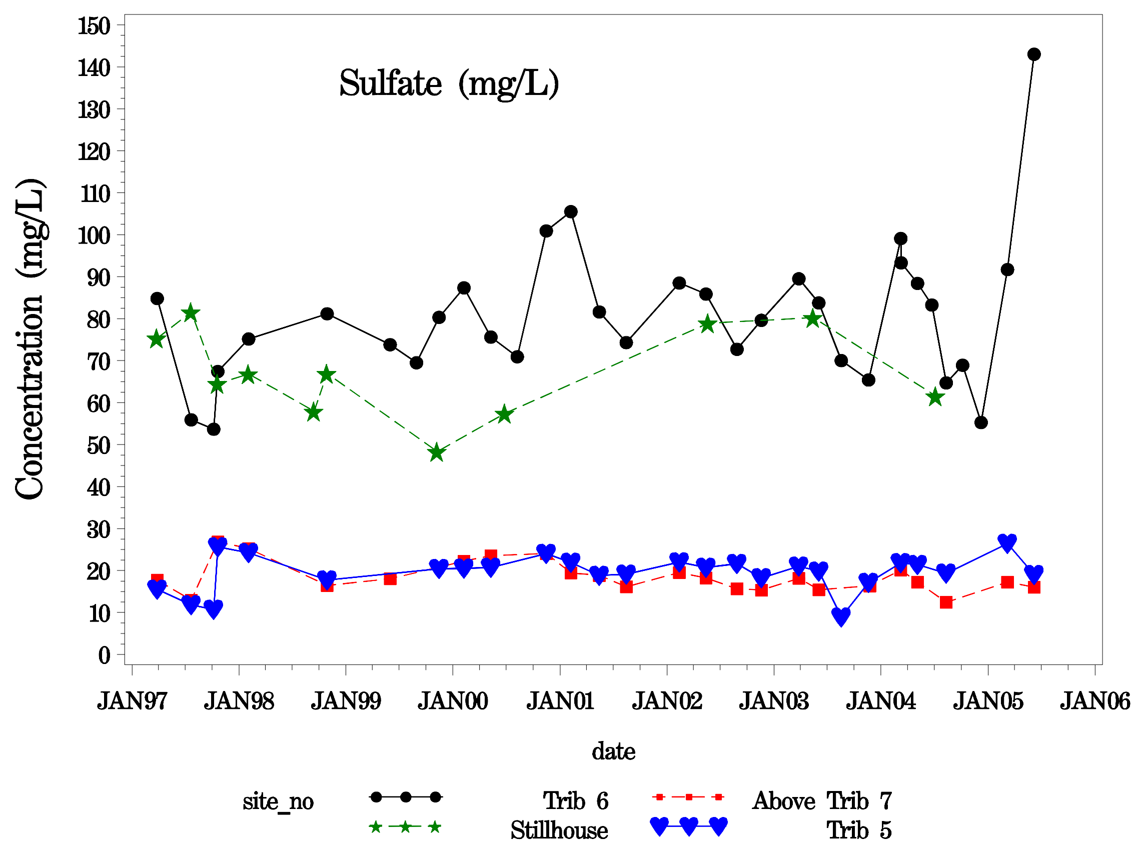
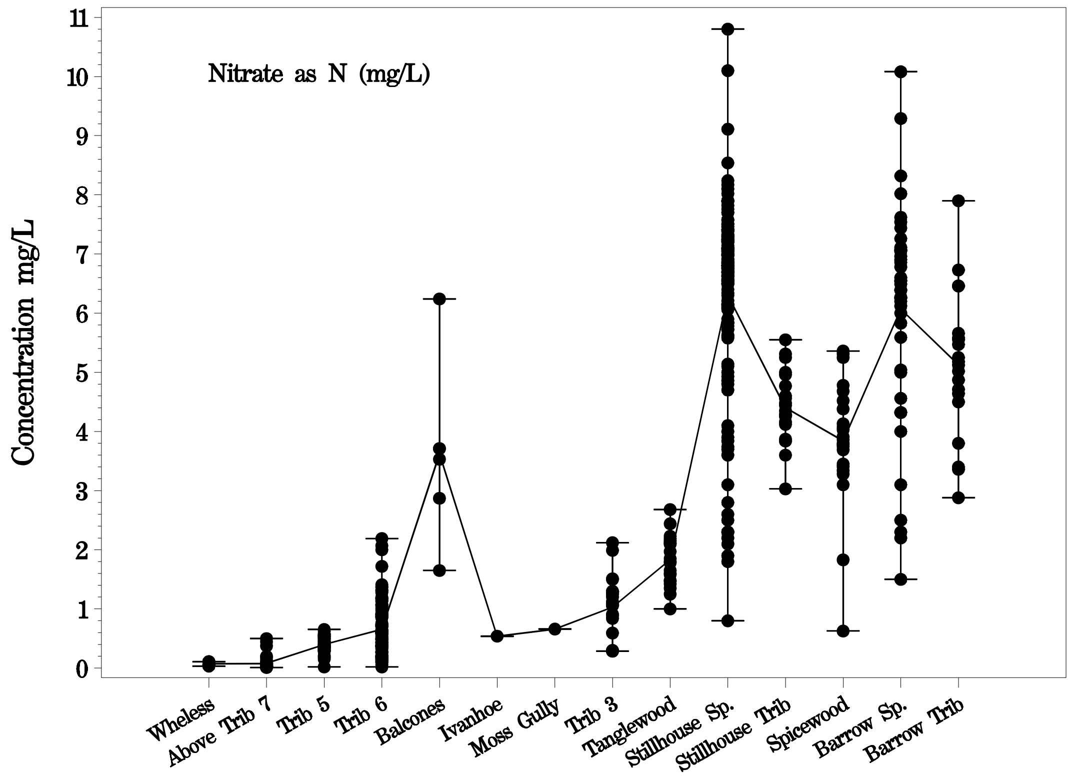


Figure 18. Nitrate



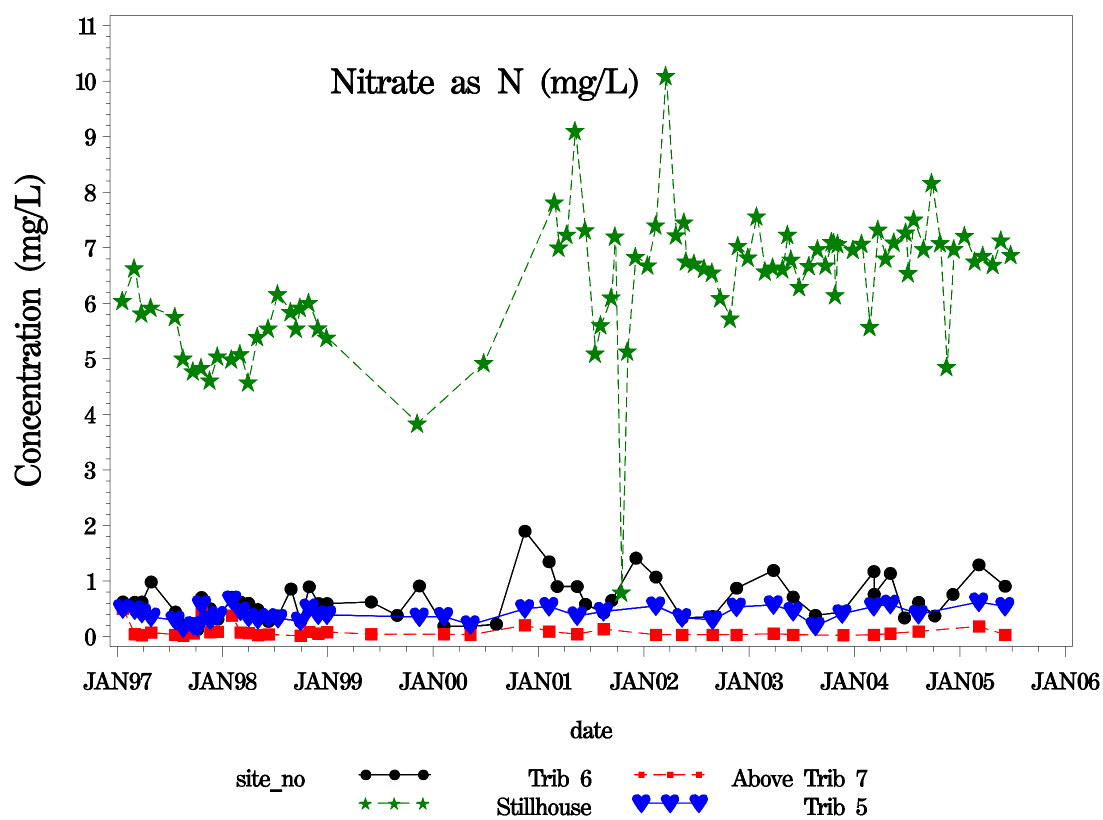


Figure 19. Ammonia

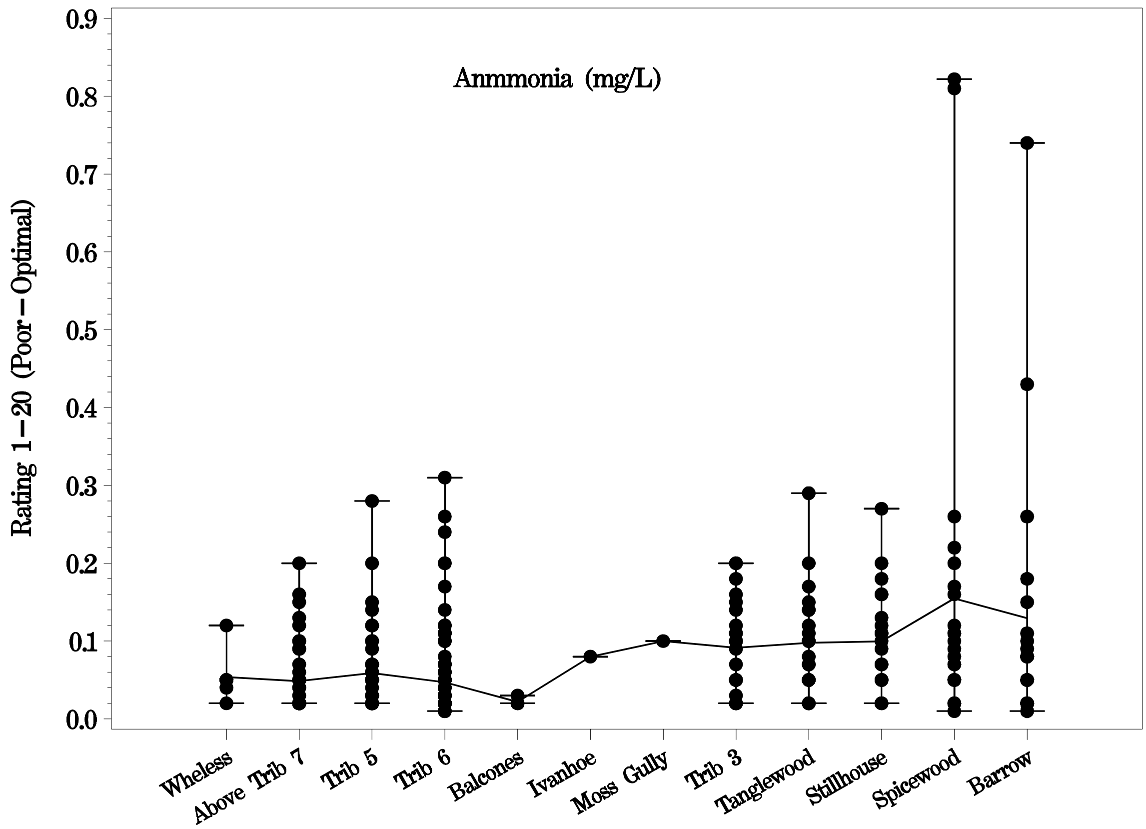
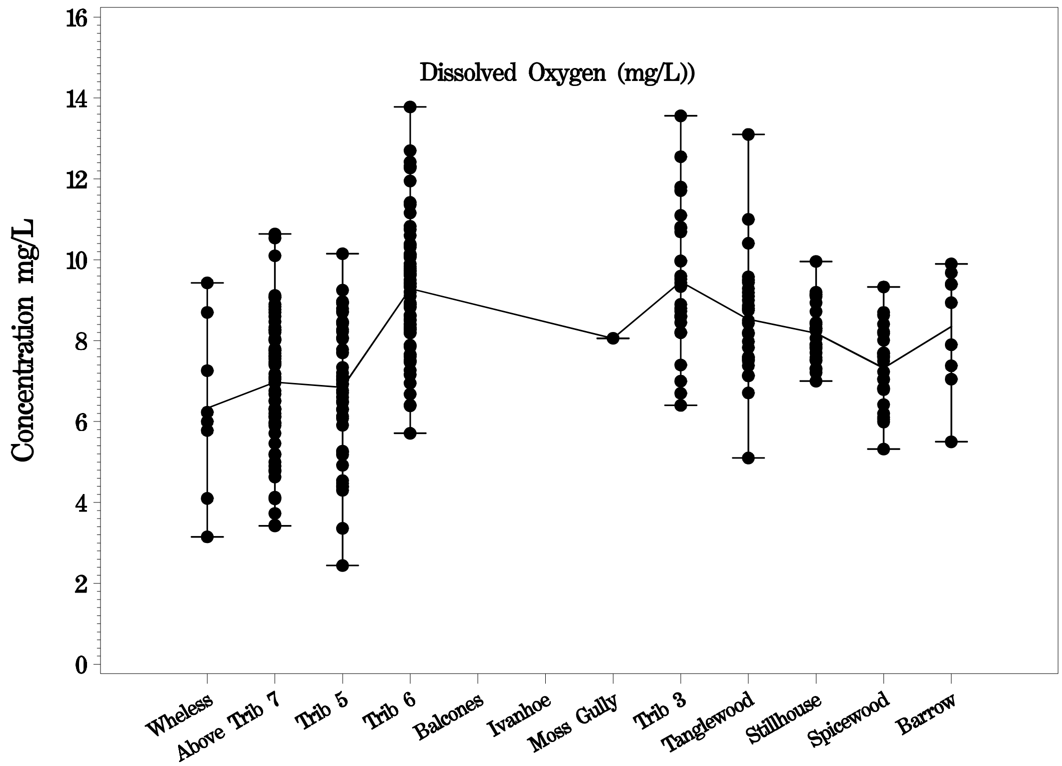


Figure 20. Dissolved Oxygen



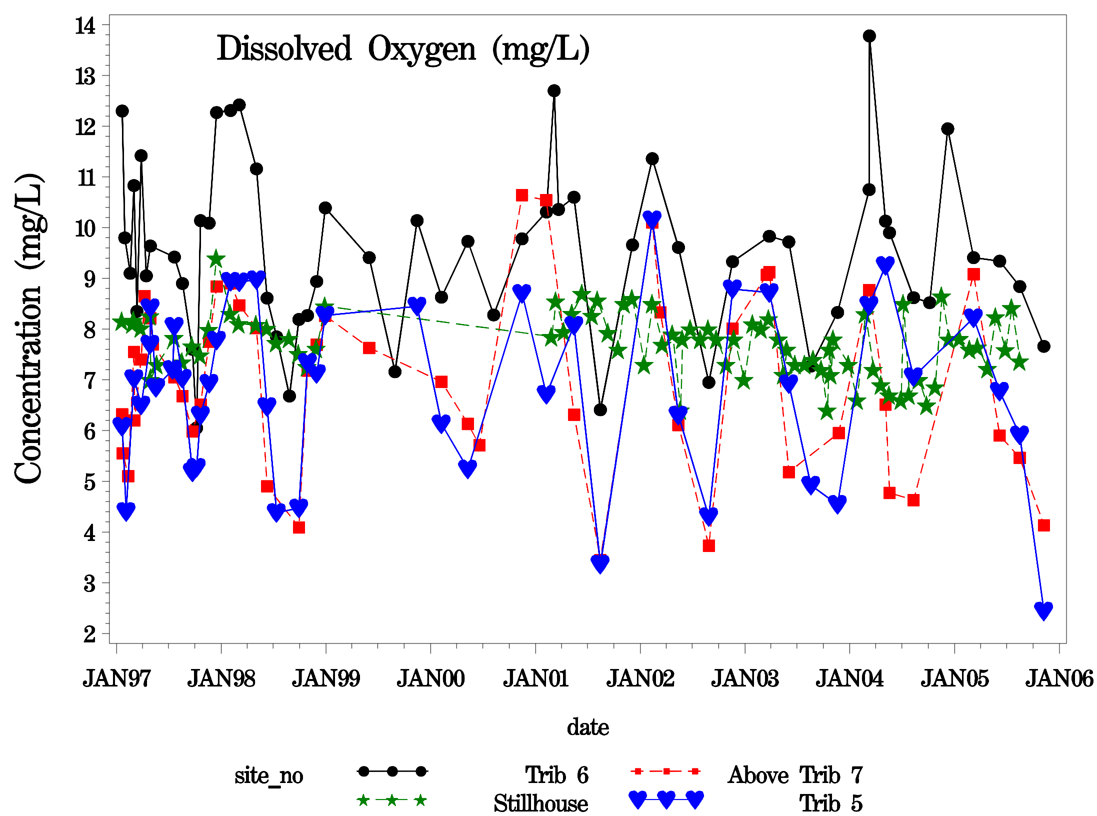
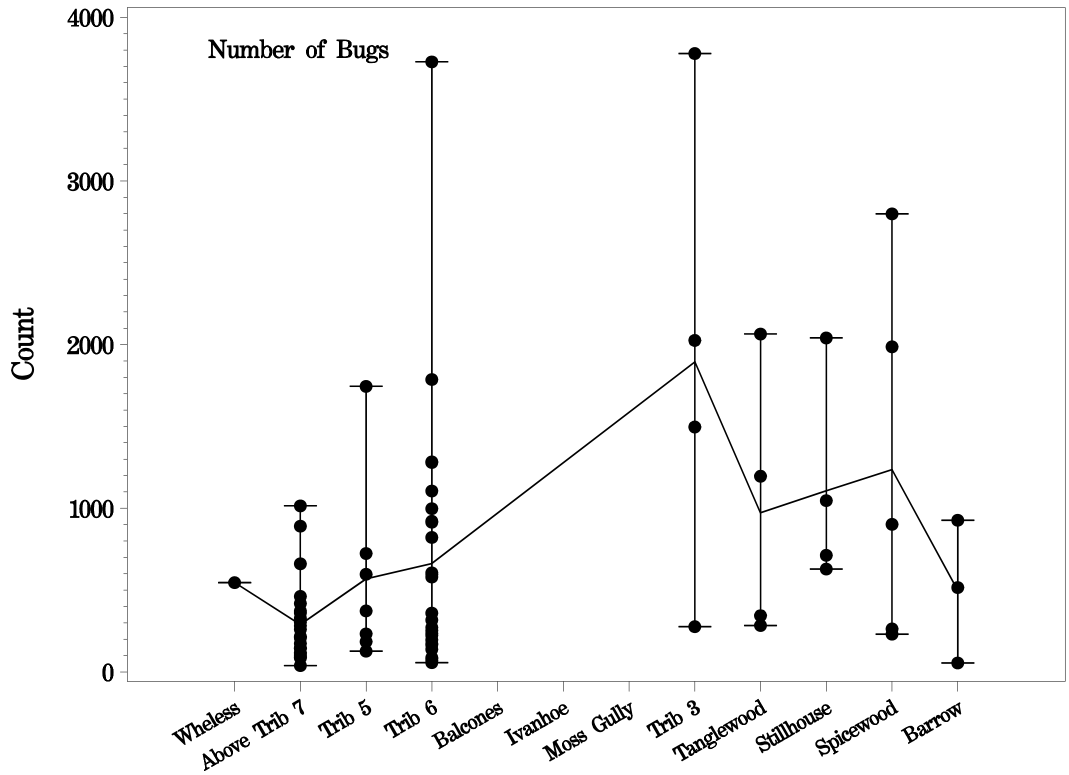


Figure 21. Number of Benthic Macroinvertebrates



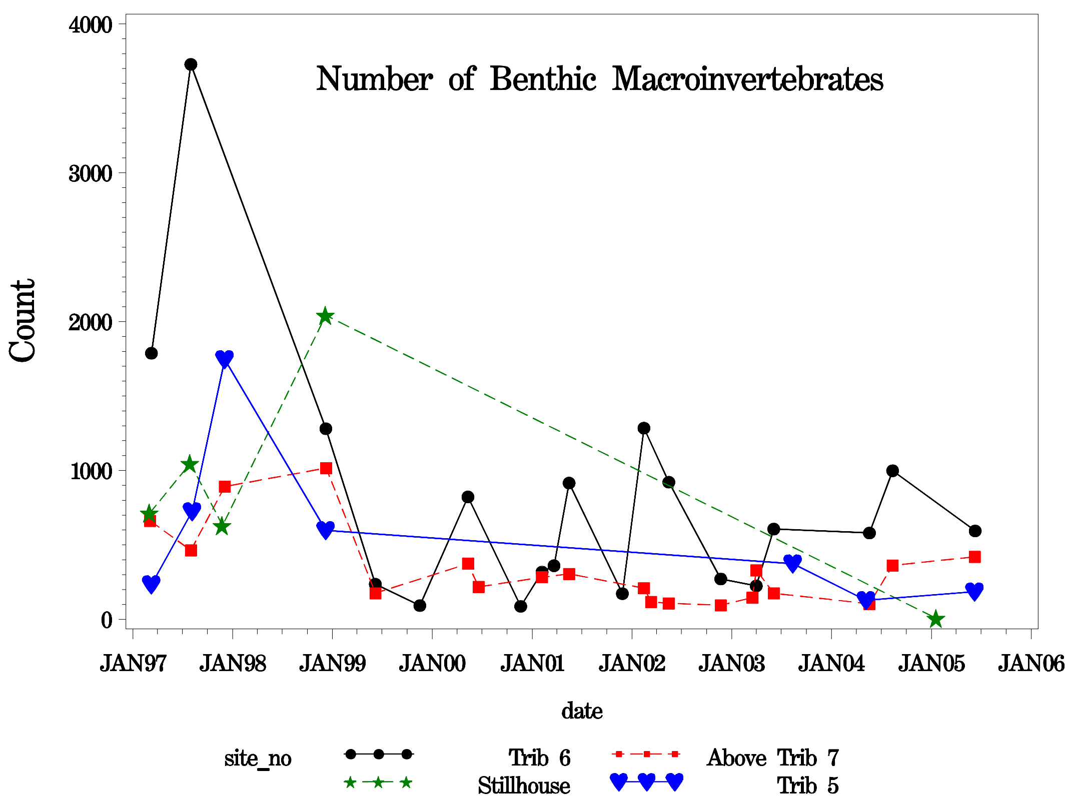
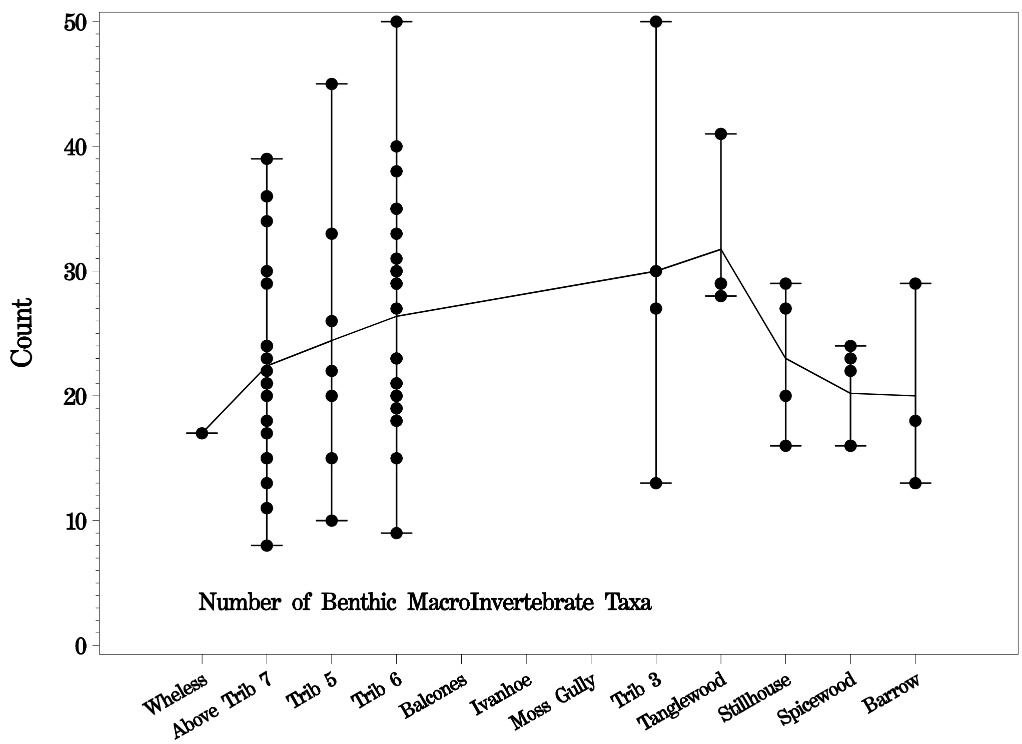


Figure 22. Number of Benthic Macroinvertebrate Taxa



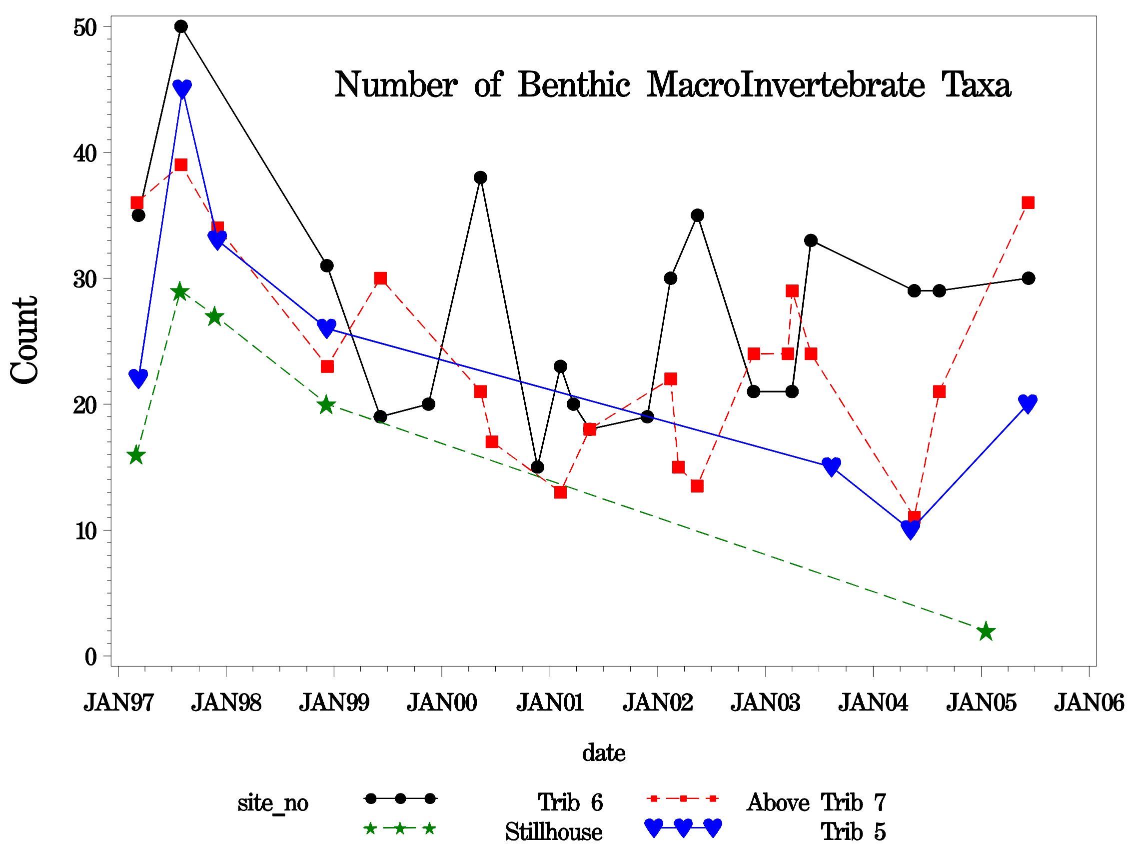


Figure 23. Percent of Salamanders by Size Categories, All Long-term Monitoring Sites

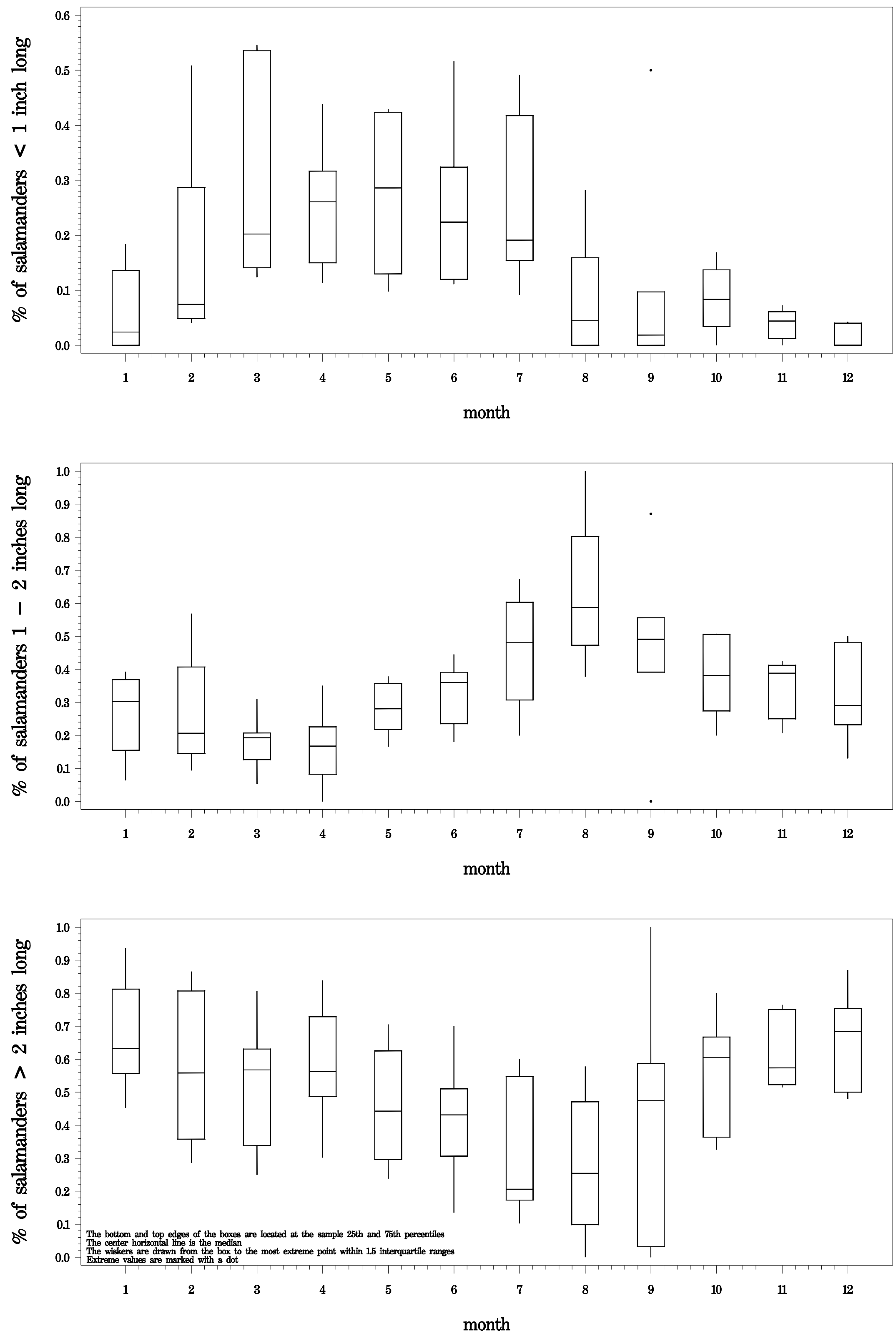


Figure 24. Percent of Salamanders in Size Categories, All Long-term Monitoring Sites

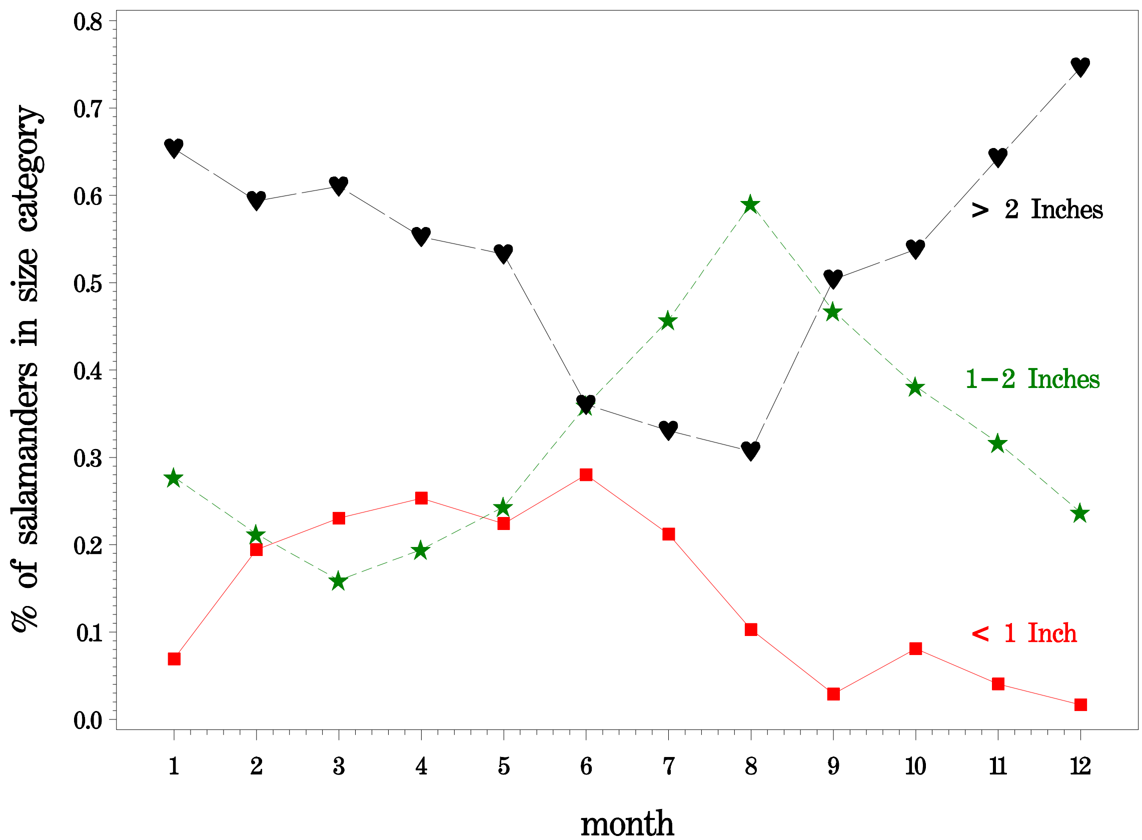


Figure 25. Percent of Salamanders by Size Categories – Spicewood Spring

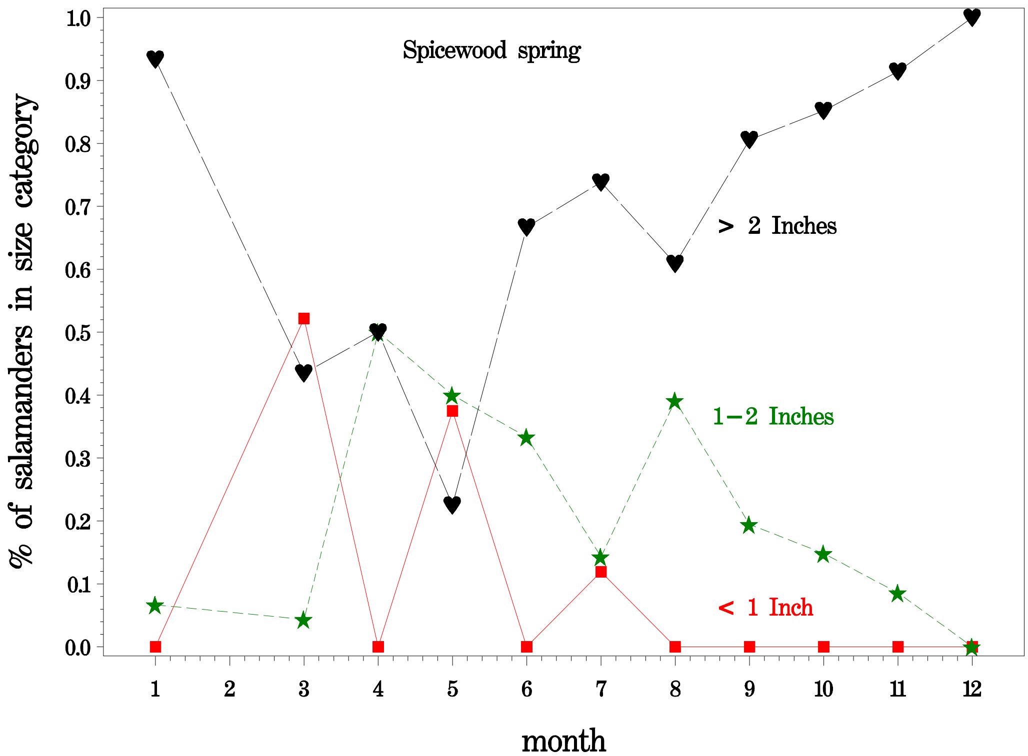


Figure 26. Percent Percent of Salamanders by Size Categories – Stillhouse Hollow Spring

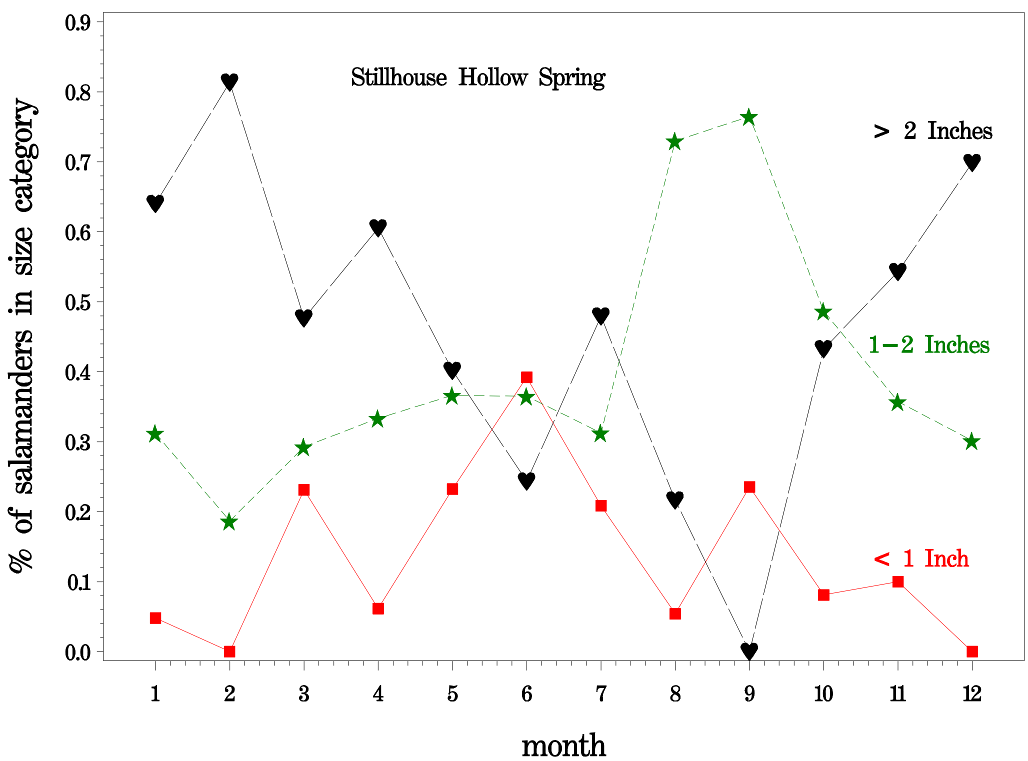


Figure 27. Percent Percent of Salamanders by Size Categories – Tanglewood Spring

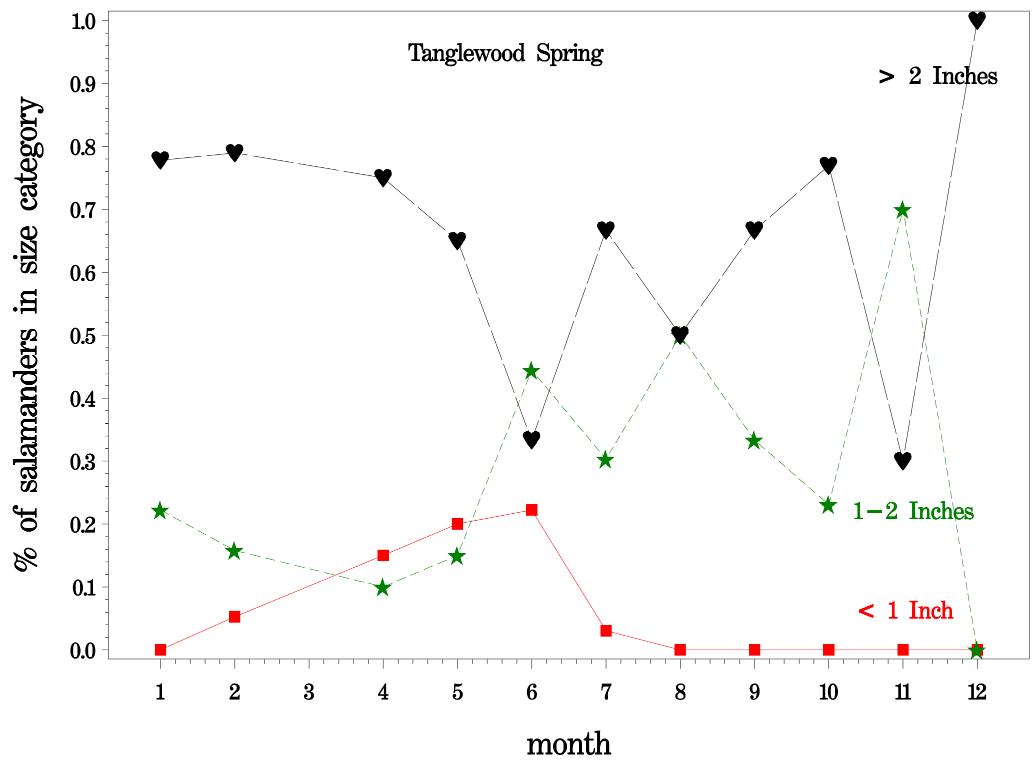


Figure 28. Percent of Salamanders by Size Categories – Tributary 3

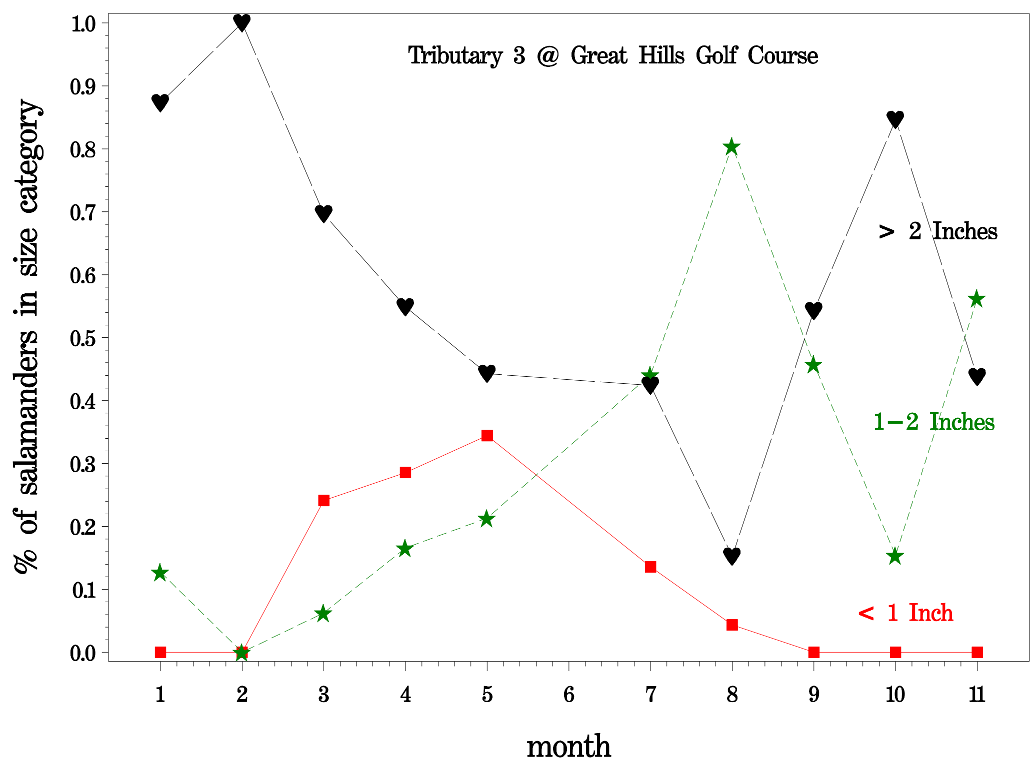


Figure 29. Percent of Salamanders by Size Categories – Tributary 5

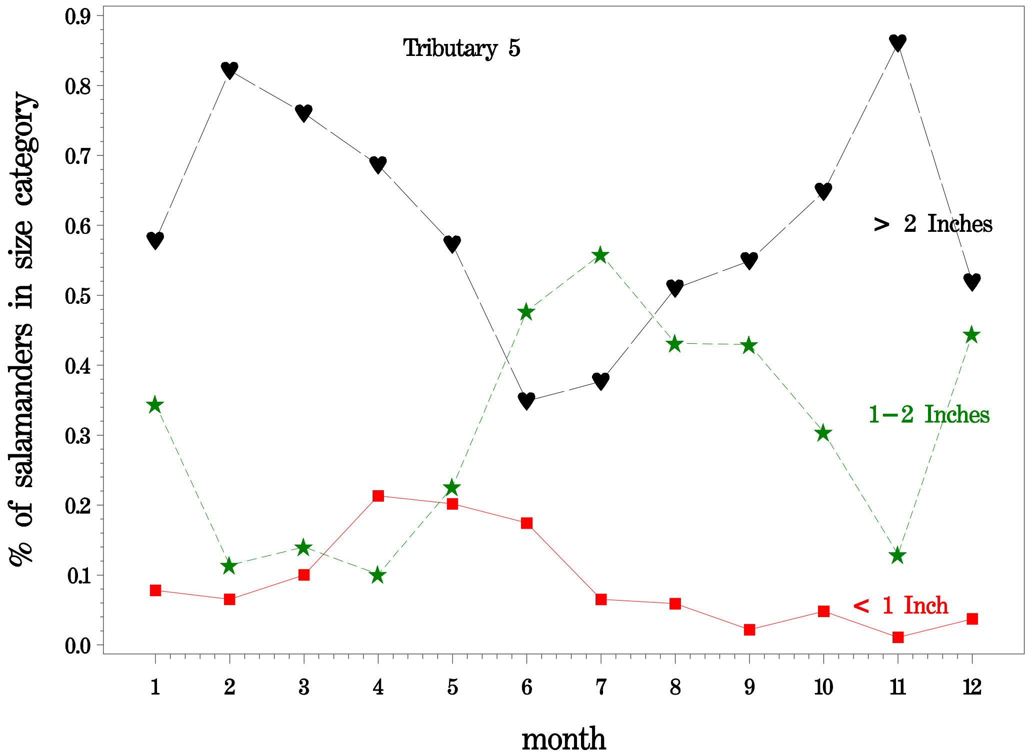


Figure 30. Percent Percent of Salamanders by Size Categories – Tributary 6

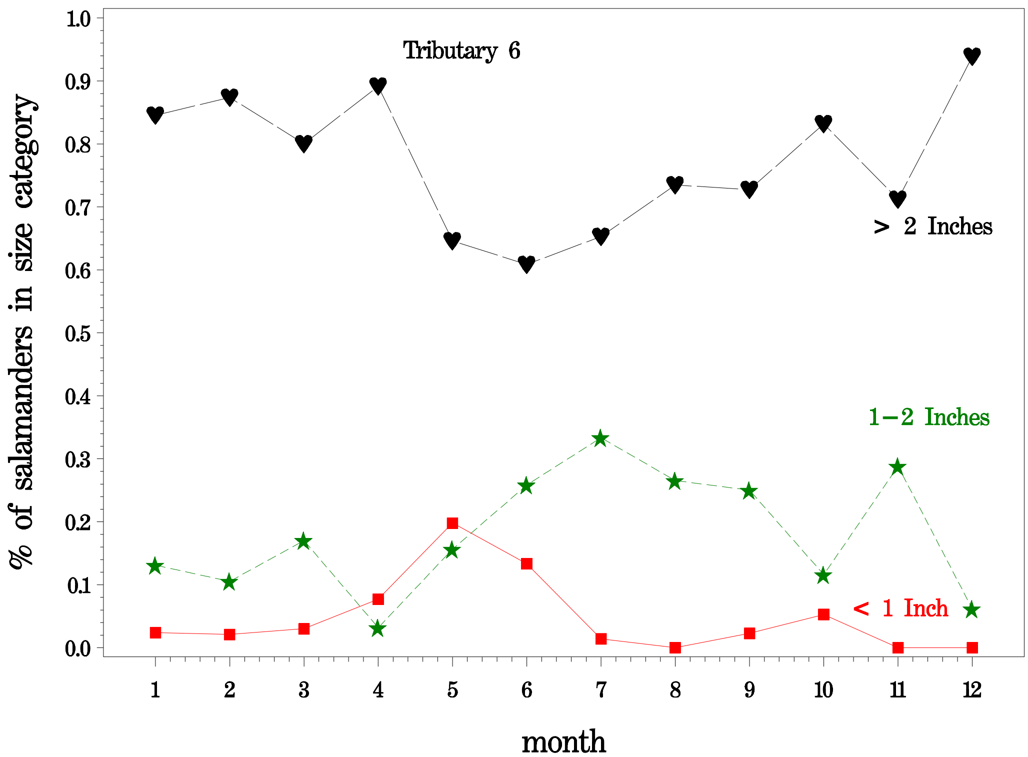


Figure 31. Percent of Salamanders by Size Categories – Bull Creek Above Trib 7

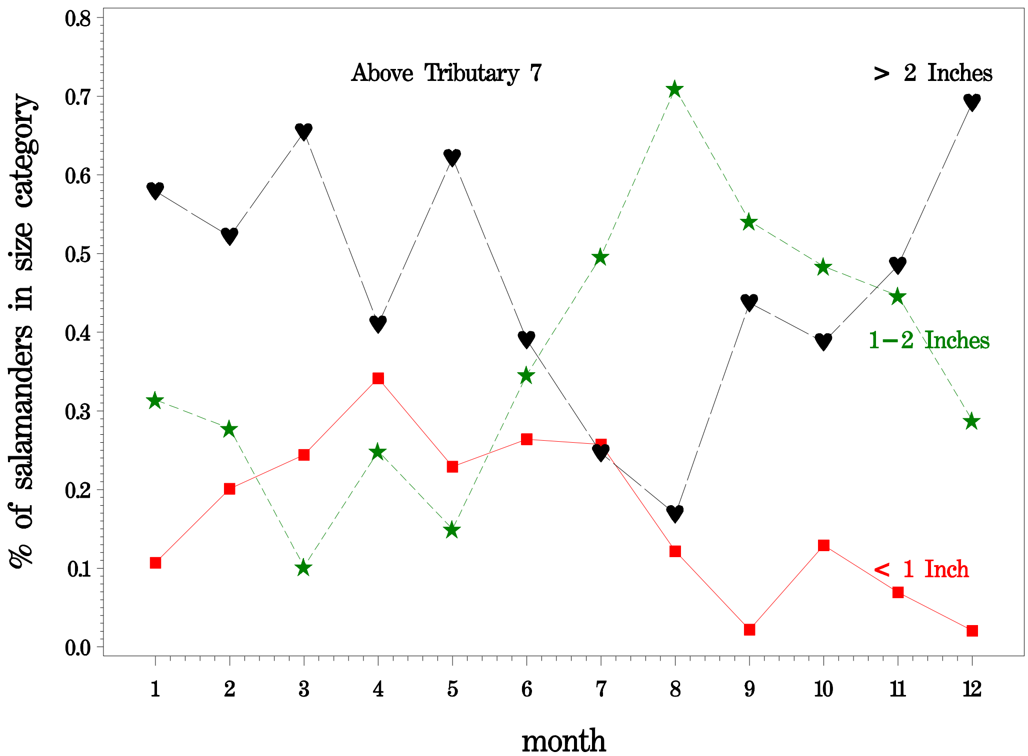


Figure 32. Percent of Salamanders by Size Categories – Wheless

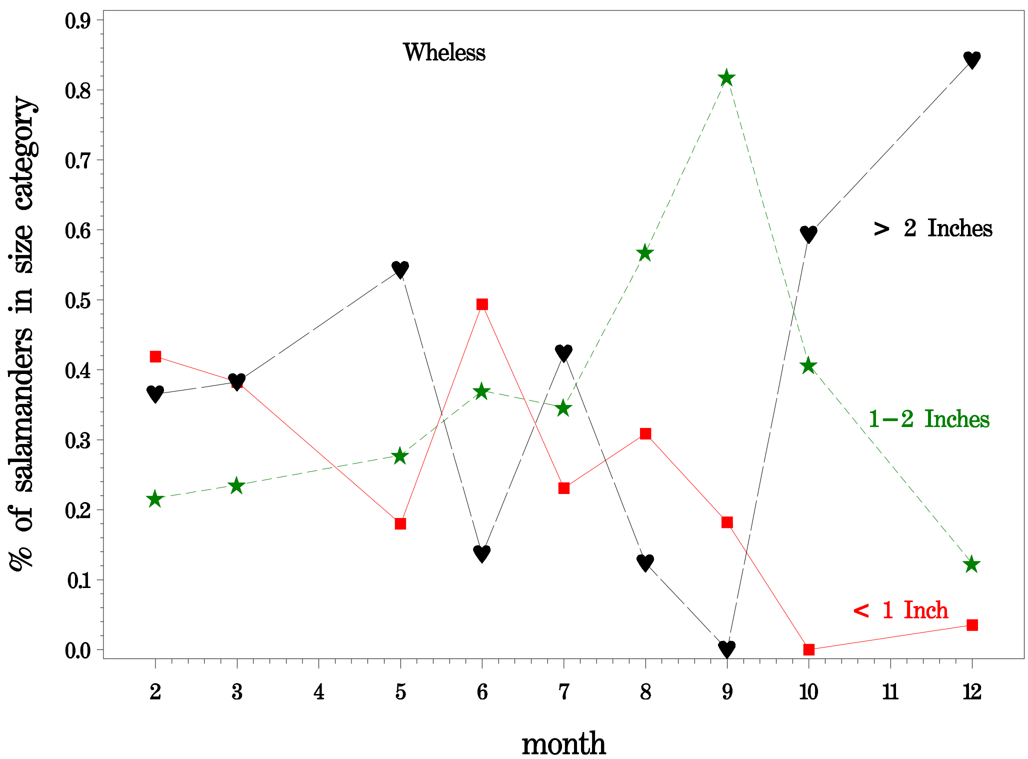


Figure 33. Flow at Bull Creek Tributaries 5, 6, and Above Trib. 7

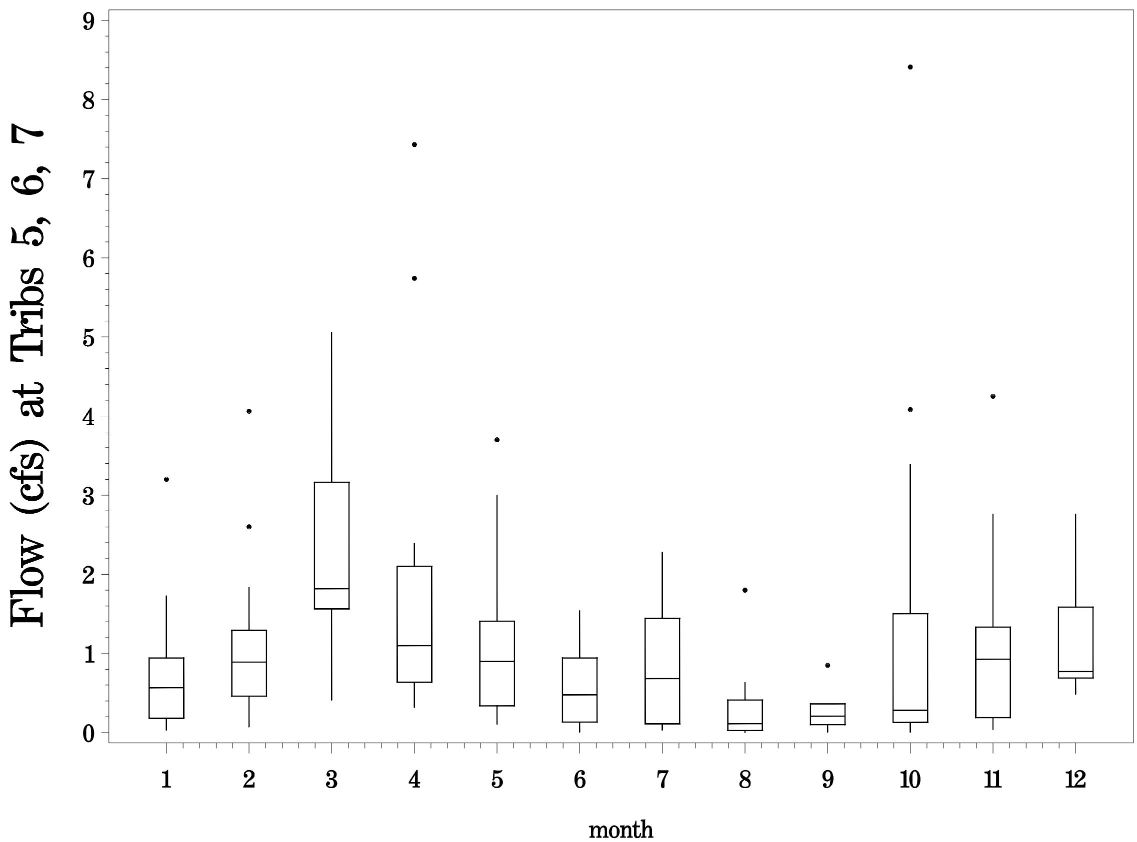


Figure 34. Percent of Salamanders <1 inch Long and Flow at Tribs 5, 6, Above 7

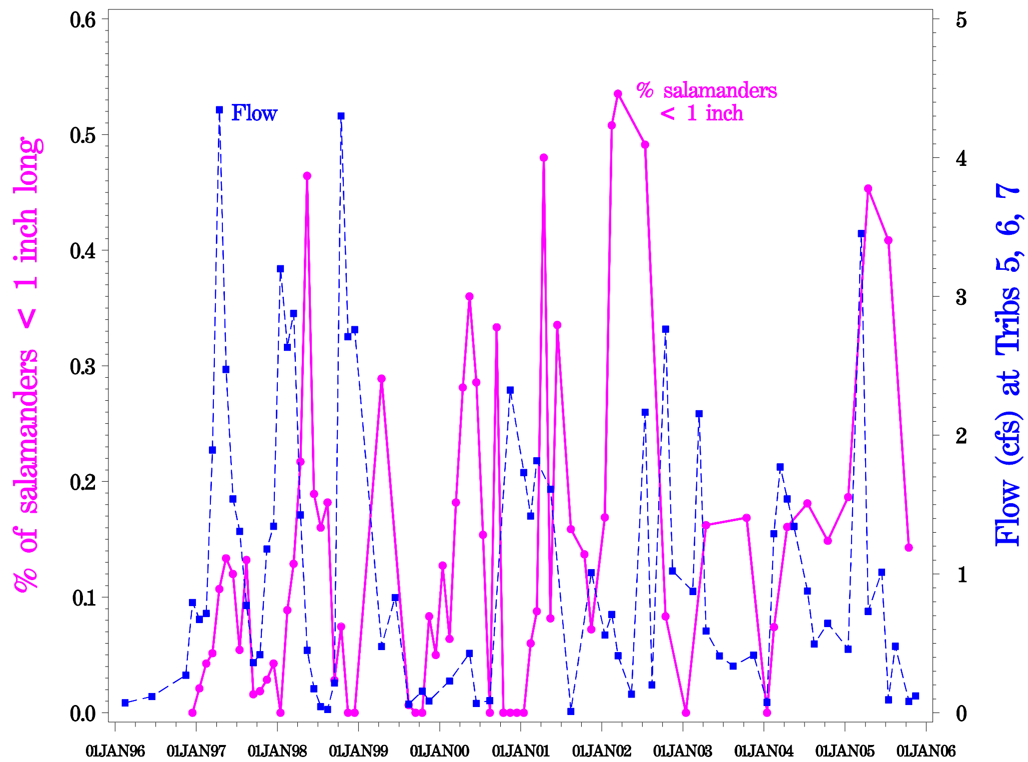
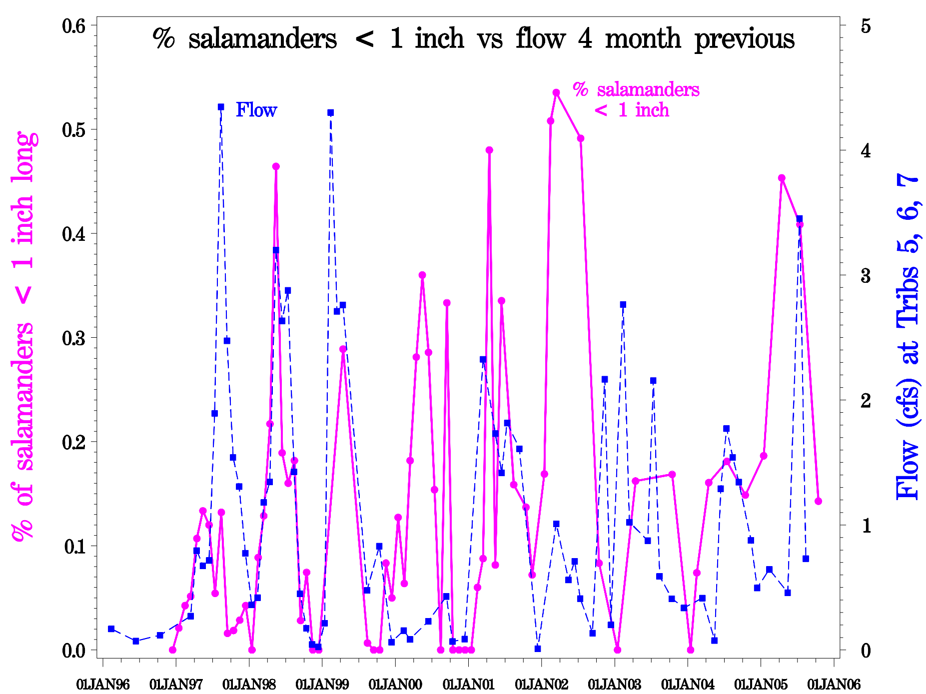


Figure 35. Percent of Salamanders <1 inch Long and Flow 4 Month Previous



Appendix A: Disinfection Protocols to Prevent the Spread of the Chytrid Fungus In Salamander and Other Amphibian Populations in the Austin, Texas Area

Background: The chytrid fungus (*Batrachochytrium dendrobatidis*) is highly pathogenic and has been identified as the cause of rapid declines of an increasing number of wild and captive amphibians in Australia, New Zealand, Europe, Central America, South America, and North America. Evidence suggests the chytrid fungus is an emerging infectious disease that may have originated in Africa and been introduced to other continents through the export and international trade of African clawed frogs (*Xenopus laevis*) during the 20th century. It appears to be spreading rapidly. While it is found primarily in water and moist soil, both aquatic and terrestrial amphibians have been infected. Potential modes of dissemination include movement of water, damp soil, and animals (amphibians, human activities, possibly birds and fish). The fungus can survive without a host and remain infectious for several weeks in sterile aquatic environments.

Chytridiomycosis has been reported mainly in frogs and toads and recently in salamander populations. Infection occurs through waterborne zoospores that invade and colonize keratin skin cells, resulting in a thickening of the skin. Common clinical signs of infected amphibians include lethargy, anorexia, skin discoloration, and sloughing skin. The mechanism by which the fungus results in mortality remains a mystery, but it is believed to interfere with respiration and possibly hydration.

The chytrid is lethal primarily to adult frogs and toads. Infections are limited in amphibian larvae, since they do not have keratin in their body skin. Keratinized skin occurs in the mouthparts of tadpoles and the toes of larval salamanders. However, post-metamorphic amphibians have keratinized skin over their entire body. Salamanders appear to be more resistant than frogs and toads but have also experienced population declines in some regions, and they can transmit the chytrid fungus to more susceptible anuran species.

Some amphibian populations appear to be recovering following initial declines although the disease is still present, suggesting the possibility that some individuals may be more resistant and/or that environmental conditions influence resistance to and the severity of chytridiomycosis.

The greatest risk of transmitting the chytrid fungus occurs when amphibians are placed together in contact in the same container or in containers reused for holding amphibians without disinfection between amphibians.

The Chytrid Fungus in Austin, Texas: To date, the chytrid fungus has been confirmed in Jollyville Plateau salamanders at Stillhouse Hollow in the Bull Creek watershed. Environmental stressors at this site (in particular, high nitrate levels and possibly nutrients) may have weakened the salamanders' immunity to chytridiomycosis. Some salamanders exhibit characteristic signs of infection – emaciation, lethary, pale and/or discolored skin. The Stillhouse population also has a high rate of deformities, including curved spines and missing limbs, toes, and eyes. No tadpoles have been observed at Stillhouse Hollow in the last decade, although they were reported here in the late 1980s. Stillhouse Hollow should be treated as a site contaminated with the chytrid fungus and

possibly other pathogens, and appropriate precautions should be implemented to prevent the spread of this disease to other areas.

The Barrow tributary flows into Stillhouse Hollow; this tributary is not surveyed often for salamanders, but at least one salamander with a curved spine has been documented from this site. Tadpoles are still found at Barrow. To date, no tests for the presence of the chytrid fungus in salamanders or anurans have been conducted for the Barrow tributary. However, due to its proximity to Stillhouse, Barrow should also be treated as a contaminated site.

Methods of disinfection: A study conducted by Johnson et al. 2003 tested the fungicidal effects of several different disinfection treatments, including chemical disinfectants, UV light, heat, and dessication. Only UV light was ineffective at killing the chytrid fungus. The following table is a summary of the study results:

Chemical/product/method	Active ingredient (%)	Exposure time	100% kill
Sodium chloride	**10**	**5 min, 2 min**	**Yes**
	10	1 min, 30 s	No
	5	**5 min**	**Yes**
	5	2 min, 1 min, 30 s	No
	2.5 to 1	5, 2, 1 min, 30 s	No
Household bleach	**4 to 1**	**10, 5, 2 and 1 min, 30 s**	**Yes**
(active ingredient: sodium hypochlorite)	**0.2 to 0.01**	**10 min**	**Yes**
	0.4	5, 2, 1 min and 30 s	No
	0.4	10, 5, 2, 1 min and 30 s	No
Potassium permanganate	**2**	**10 min, 5 min**	**Yes**
	2	2 min, 1 min	No
	1	**10 min**	**Yes**
	1	5, 2, and 1 min	No
	0.1 to 0.001	10, 5, 2, 1 min	No
Formaldehyde solution	**1**	**10 min, 5 min**	**Yes**
	1	2 min, 1 min	No
	0.1	**10 min**	**Yes**
	0.1	5, 2 and 1 min	No
	0.01 to 0.001	10, 5, 2, 1 min	No
Path-X agricultural disinfectant	**1×10^{-2} to 1×10^{-3}**	**5, 2 and 1 min, 30 s**	**Yes**
(active ingredient: DDAC)	**1×10^{-4}**	**5 min, 2 min**	**Yes**
	1×10^{-4}	1 min, 30 s	No
	1×10^{-5} to 1×10^{-6}	5, 2, and 1 min, 30 s	No
Quaternary ammonium compound 128	**Full strength to 1×10^{-3}**	**5, 2, and 1 min, 30 s**	**Yes**
(active ingredient: DDAC)	1×10^{-4} to 1×10^{-6}	5, 2, and 1 min, 30 s	No
Dithane	1×10^{-2} to 1×10^{-6}	5, 2, and 1 min, 30 s	No
Virkon	**1 mg/ml**	**5 min, 20 s**	**Yes**
Ethanol	**70%**	**5 min, 20 s**	**Yes**
Benzalkonium chloride	**1 mg/ml**	**5 min, 20 s**	**Yes**
UV light	1000 mW/m^2	21, 12, 2 and 1h 30, 15, 10, 5 and 1 min	No
Dessication		**6, 5, 4 and 3 h**	**Yes**
		2 h 40 min to 1 h	No
Heat	**100°C (212 °F)**	**1 min**	**Yes**
	60°C (140 °F)	**5 min**	**Yes**
	47°C (116.6 °F)	**30 min**	**Yes**
	37°C (98.6 °F)	**4 h**	**Yes**
	32°C (89.6 °F)	**96 h**	**Yes**
	26°C (78.8 °F)		No
	23°C (73.4 °F)		No

Based on the Johnson et al. study, the following table summarizes recommended methods for disinfecting equipment. Concentrations and exposure times for some disinfectants (bleach) may be increased to kill other potential pathogens such as Ranaviruses. Using a combination of the following methods should also be considered.

Purpose	**Disinfectant**	**Concentration (%)**	**Minimum time**
Disinfecting surgical equipment and other instruments	Ethanol	70%	1 min
	Vircon	1 mg/ml	1 min
	Benzalkonium	1 mg/ml	1 min
Disinfecting equipment and containers	Sodium hypochlorite (bleach)	>1% (I would recommend 5-10%, which will also kill viruses)	1 min (again, I would recommend 10-15 minute minimum)
	DDAC	0.1%	0.5 min
	Complete drying		3 hours
	Hot wash	60^{o}C (140 ^{o}F)	5 min
		47^{o}C (116.6 ^{o}F)	30 min
		37^{o}C (98.6 ^{o}F)	4 h
Disinfecting footwear	Sodium hypochlorite (bleach)	1% (I recommend 5-10%)	1 min (I recommend >10-15 minutes)
	DDAC	0.1%	0.5 min
	Complete drying		3 hours
Disinfecting cloth (bags, clothes)	Hot wash	60^{o}C (140 ^{o}F)	5 min
		47^{o}C (116.6 ^{o}F)	30 min
		37^{o}C (98.6 ^{o}F)	4 h

<u>General Hygiene Protocols</u>:

1. A good reference is the Declining Amphibian Task Force's Fieldwork Code of Practice: http://www.fws.gov/ventura/es/protocols/dafta.pdf

2. Always move from uninfected or least infected areas to infected areas. When working in a stream or watershed, always begin sampling at the highest elevation (or furthest up-stream site) and move in a downstream direction. By moving upstream, personnel could be carrying infectious agents against the flow of water, and could carry agents to headwaters that were free of the agent.

3. Adjacent streams in the same major catchment are likely to have similar agents present, but the possibility of introducing new agents is greatly increased by moving between major catchments or over much longer distances.

4. Note the presence/absence of tadpoles and frogs. If you see a dead frog, preserve it in 70% ethanol and call 974-2204 (Lisa O'Donnell). If possible, wash and disinfect between frog-less sites and sites still having frogs.

<u>Disinfection Protocols During Sampling at Barrow and Stillhouse Hollow</u>:

1. If multiple sites are to be sampled in one day, sample Barrow and Stillhouse at the very end.

2. If both Barrow and Stillhouse Hollow are to be sampled in the same day, sample Barrow first and then Stillhouse Hollow.

3. Dedicate a separate pairs of boots (rubber boots or waders are ideal, since they are easiest to clean and protect clothing from water) and dip nets for these sites. Clean and store them separately at the end of each field day.

4. If Barrow and Stillhouse are surveyed on the same day, use different sets of nets and containers to avoid indirect contact between salamanders from the two sites.

5. Any salamanders that exhibit unusual morphology or behavior may be collected for further observation or pathology. If salamanders are collected from these sites, ensure they are separated from salamanders from different sites to avoid contact between them (e.g. via handling, reuse of containers) or with other captive animals.

6. Adult toads and frogs should only be handled wearing a fresh set of gloves, which should be disposed of in a sealed plastic bag after each individual.

<u>Disinfection Protocols Following Sampling at Barrow and Stillhouse Hollow</u>:

1. Remove mud, snails, algae, and other debris from footwear (preferably rubber boots because they are easiest to clean), clothing, and equipment -- nets, containers, etc.

2. At the vehicle (well away from the springruns), spray or immerse boots in >5% bleach. A spray bottle can be used or the bleach and water mixed onsite in a large container (e.g., ice chest). Without rinsing, allow to air dry completely. Once dry, rinse with tap water to remove any residual bleach and air dry again before reusing.

3. Ideally, place all nets, containers, rulers, pens/pencils, tape measure, and other field equipment in >5% bleach while onsite and allow them to soak until you get back to the office/lab. Without rinsing, allow to air dry completely. Once dry, rinse with tap water to remove residual bleach and air dry again before reusing. Alternatively, bag all equipment and disinfect back at the lab.

4. Wipe all electronic equipment (camera, flow meter, pH/con meter) with an alcohol pad or sponge dipped in bleach solution. Allow to air dry completely.

5. For the Hydrolab, ideally bleach and replace the DO membrane, or wipe the outside of the instrument with alcohol or mild bleach and soak probes in tap water.

6. Use an alcohol-based disinfectant such as Purell for hands.

7. Wash all clothing in hot water and dry on high heat.

8. Used cleaning materials (liquids, etc.) should be disposed of safely and if necessary taken back to the lab for proper disposal. Used disposable gloves should be retained for safe disposal in sealed bags.

References

The following website is an extensive bibliography on the chytrid fungus with direct links to the articles: http://www.jcu.edu.au/school/phtm/PHTM/frogs/chart.htm

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