Jollyville Plateau Salamander Interim Report, 2013, for the Balcones Canyonlands Preserve

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Introduction

This document is a summary of research activity for the Jollyville Plateau Salamander (*Eurycea tonkawae*). The City of Austin conducted salamander surveys on the following Balcones Canyonland Preserve (BCP) tracts: Hanks, Franklin, Lanier, managed by the City of Austin Wildlands Division; Stillhouse Hollow and Barrow Preserve tracts, jointly managed by the City of Austin Parks and Recreation Department and Wildands; Sam Hamilton, and Concordia, managed by Travis County. Here, we provide a brief synopsis of each monitoring or research project and summarize the data collected.

We continued long-term population monitoring on a quarterly basis at our standard "count" sites identified above, and all salamanders captured at each site were photographed for the purposes of mark-recapture so that population demographic analyses could be conducted in the future. A summary of the total number of salamanders counted at each site for each quarter are included in this report. Analysis of survival and size distributions (using photographs and mark-recapture data) have not been completed and are not included in this report.

This year we did not conduct 3-day "robust-design" mark-recapture surveys at Lanier, Ribelin and Wheless as we have done in years past due to limited staff availability and priorities. Occupancy monitoring is now a higher priority since it incorporates a better sampling design and allows us to cover a broader territory. We completed occupancy surveys on Bull Creek Tributary 7 and Bull Creek mainstem/Tributary 8 in April and May 2013 and include a brief summary of our findings in this report.

Additionally, we also completed a short-term analysis of salamander movement at Lanier from January thru April 2013. The goal of this project was to determine whether *E. tonkawae* travel throughout stream habitat or if they are solely concentrated near springs. A summary of our findings is included here.

In addition to population surveys, U.S. Fish and Wildlife Service (USFWS) Staff including Pete Diaz in collaboration with the U.S. Geological Survey and City of Austin Watershed Protection Department staff collected salamanders from Lanier Spring (as well as many other central Texas *Eurycea* localities) and placed a semi-permeable membrane passive water sampler device (SPMD) in May for pollutant screening including heavy metals, polycyclic aromatic hydrocarbons and a suite of pesticides. This was the first part of a two year sampling; samples will also be collected in 2014. Chemical analysis data are not yet available from the SPMD project.

We also collected water-borne stress hormones from salamanders at Lanier, Franklin/Pit Spring, Barrow and Tributary 4 of Bull Creek for the second year in a row. Preliminary analyses are provided.

Additional details about these projects including background and methods are available in the project Quality Assurance Project Plans (QAPP) (listed below) and referenced reports therein.

Long-term Population Monitoring

We conducted count surveys using a drive survey technique at 13 sites in 2013. Salamanders were searched for by flipping rocks and other cover object, captured, and photographed on a standardized grid background. Individuals observed, but not captured were recorded as one of three size classes, ≤1", 1-2" and ≥2". Surveys were primarily conducted on a quarterly basis (every three months). Total counts (includes all size classes) are reported from 12 sites. Baker Spring was infrequently surveyed due to lack of spring flow.

Based on rainfall and hydrologic status of each site during our quarterly sampling, sites appeared to be as dry or drier in the first 3 quarters of 2013 as compared to 2012, although conditions are much improved compared to 2011 (Figure 1). Winter and spring rains in 2013 did not result in scouring of the habitat or deposition of excessive flood debris and appeared to have been beneficial for some *E. tonkawae* populations based on higher-than-average counts during this period (Figure 2). Alternatively, these conditions may prompt migration from subterranean habitat and result in higher salamander abundance at the surface. However, these rains did not provide consistent flow at Tributary 5 or Upper Ribelin during this time, resulting in missed or incomplete surveys.

Drought conditions persisted into the summer of 2013, resulting in most of the non-urbanized sites going dry at some point during the year. Flow was very low in the third quarter at all sites, and even Pit Spring (also known as Franklin) went dry shortly after our survey. This followed with record rainfall in October, postponing surveys so high flow could recede and resulting in spring flow at all sites. Not all fourth quarter surveys have been completed. Recent field visits to some sites revealed heavy scouring and sediment/rock deposition due to the October rains. This effect is particularly pronounced in urban areas.

Despite dry conditions during the summer of 2013, total salamander counts were high on average, particularly when compared to the very dry year of 2011 (Figure 2). We observed more salamanders than have been seen in several years at some sites with high levels of development upstream (Stillhouse, Tributary 5, Tributary 6, Tanglewood), although total counts are well below their historical highs (with the exception of Tributary 6). Counts remain in the low single digits at Spicewood and Balcones District Park springs although habitat associated with Balcones District Park Spring is very small in area.

Figure 1. Total rainfall by quarter in the Bull Creek watershed measured from three FEWS gauges. With the exception of record rainfalls in October, 2013 was a very dry year, although not as dry as 2011.

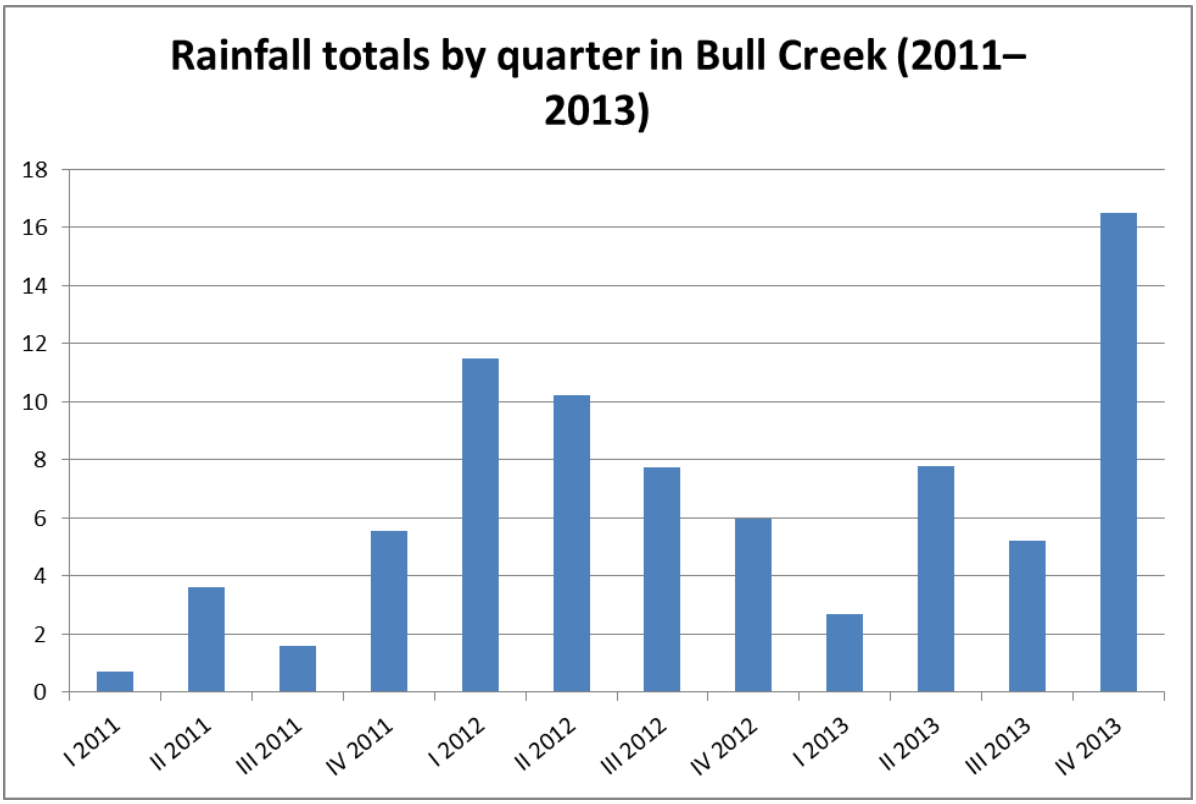
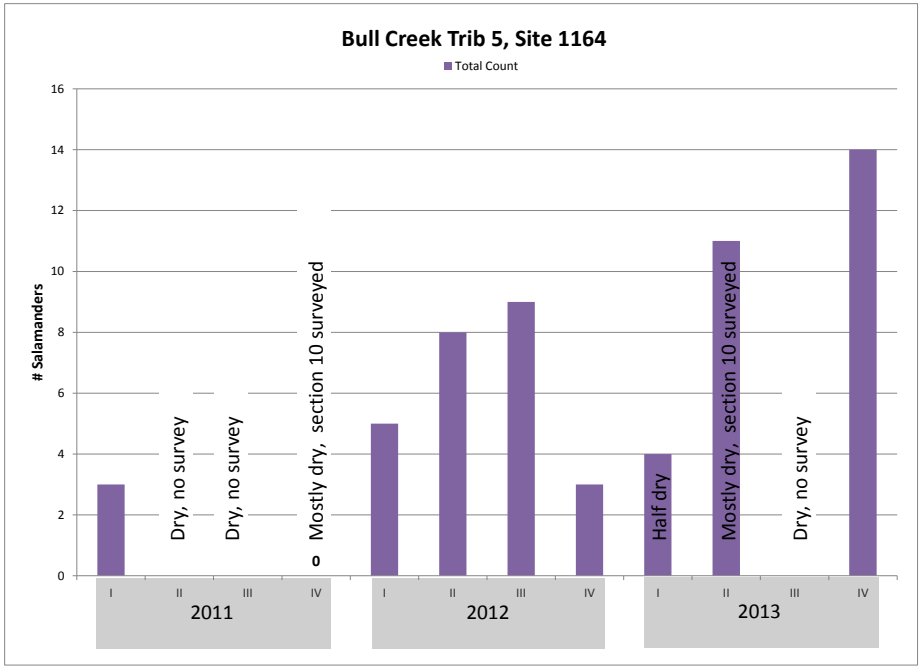
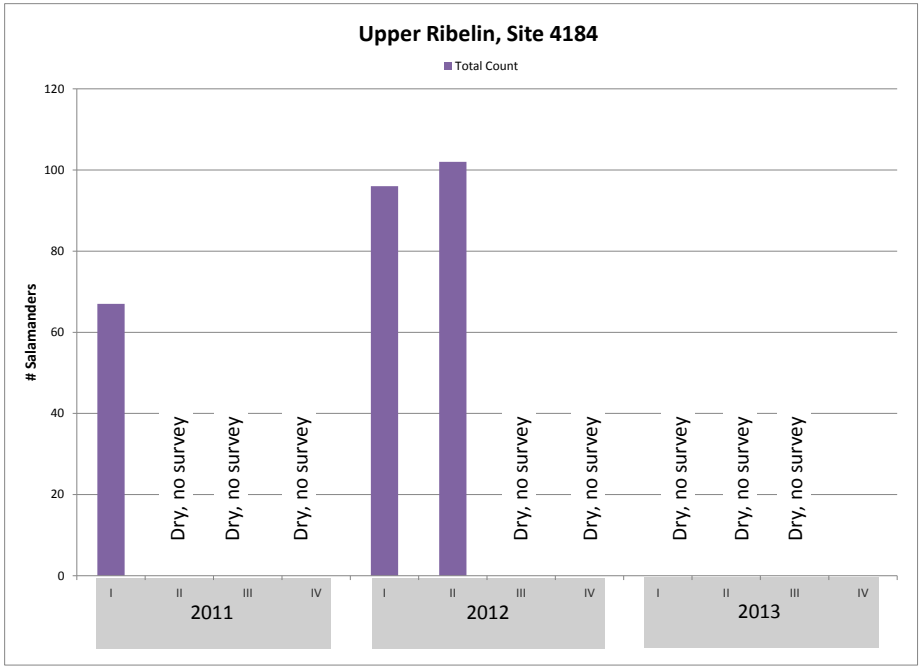
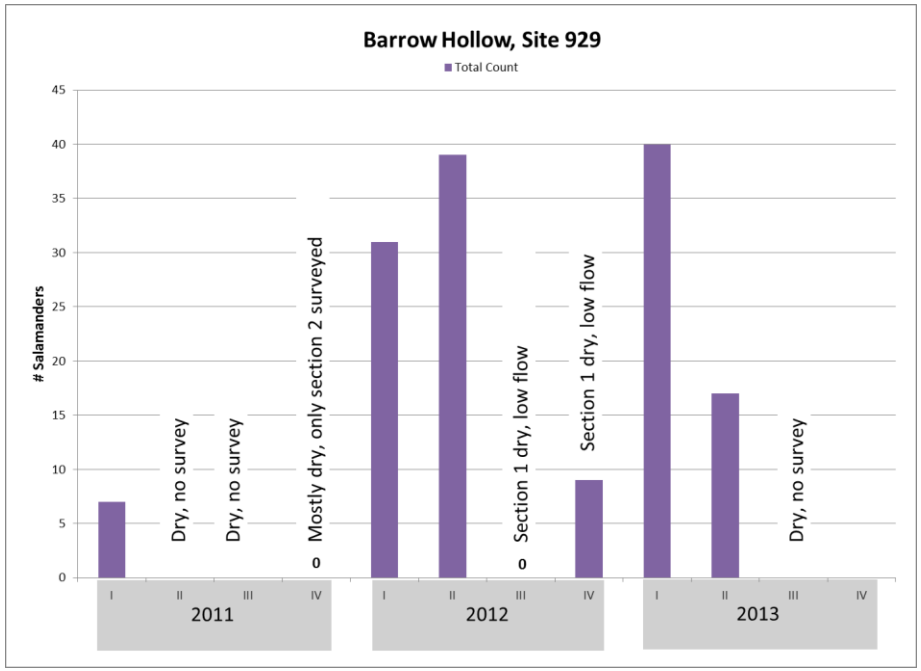


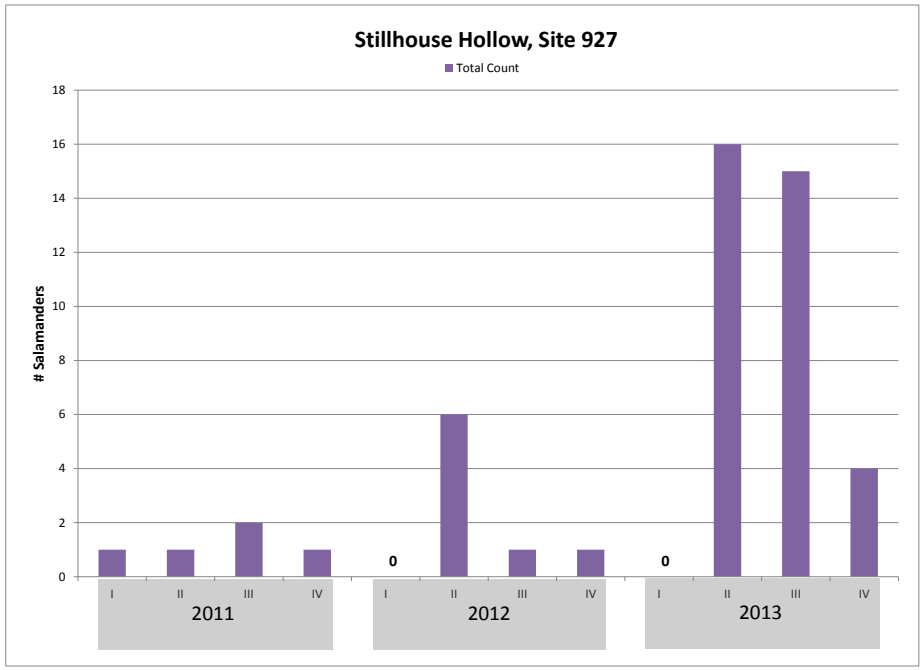
Figure 2: Quarterly Count Summary

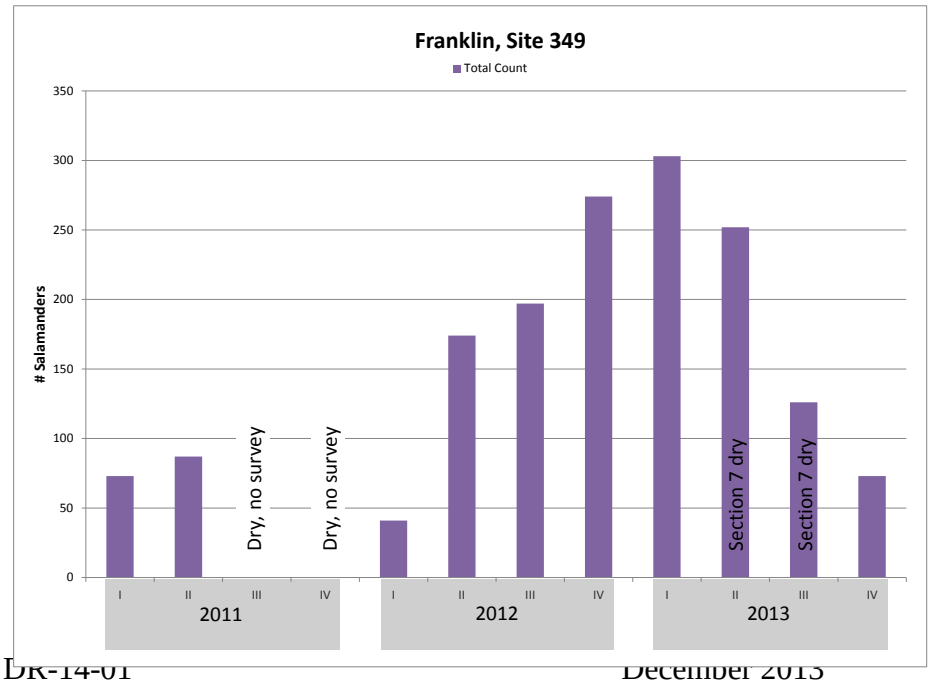
Below are the quarterly results of count surveys conducted at 12 sites between 2011 and 2013. Total salamander counts include the total of all size classes: ≤1 inch, 1-2 inches, and ≥2 inches. Each graph has the same *x*-axis, but different *y*-axes. Missing data are noted on each graph as "no survey" which were due to dry conditions. Zeroes indicate a survey which resulted in no salamander observations. Each quarter is three months long, starting in January, for quarter I. Quarter IV in 2013 is blank in all plots where data have yet to be collected.

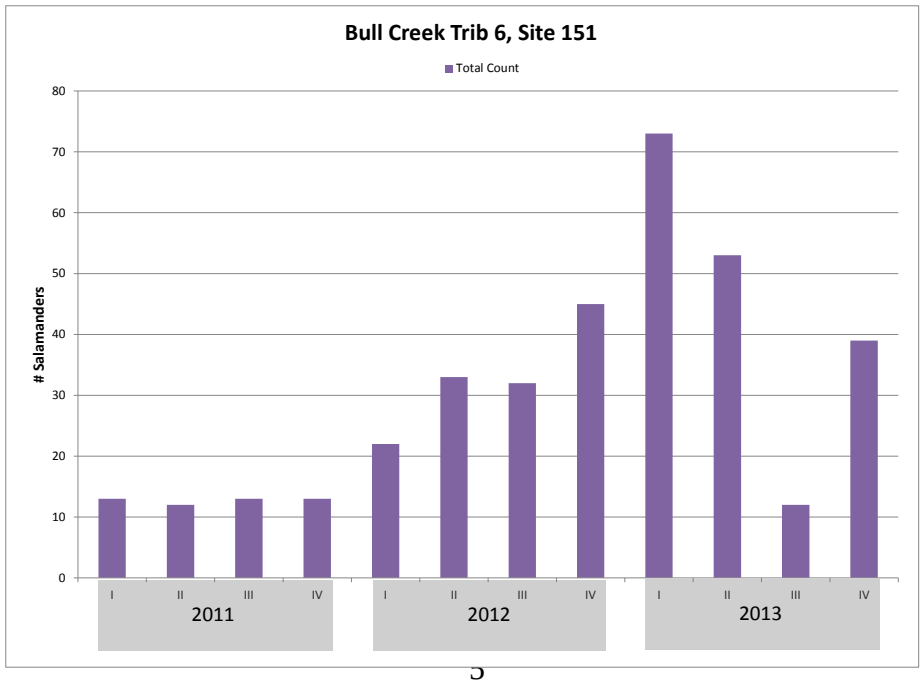


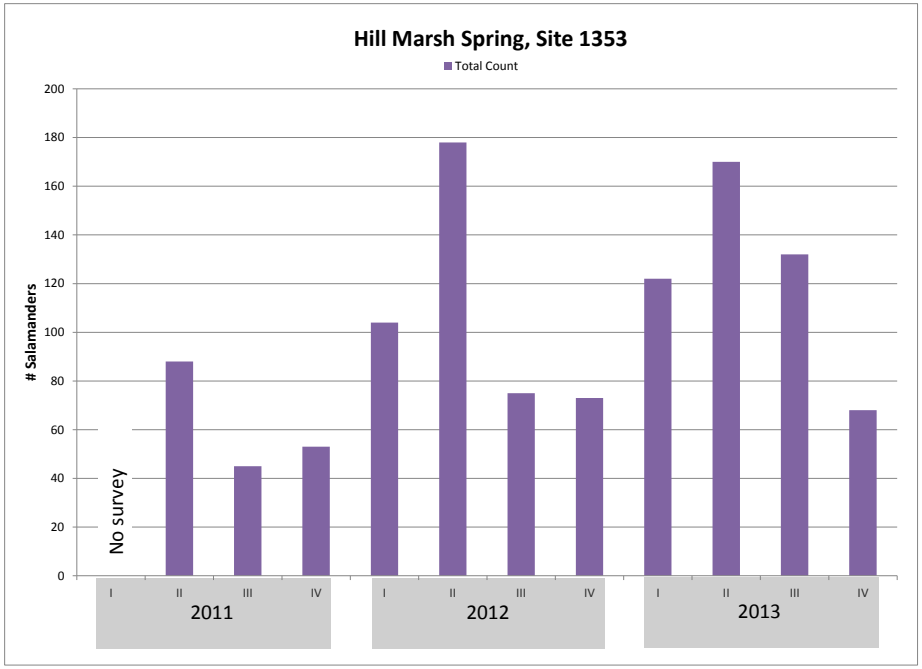


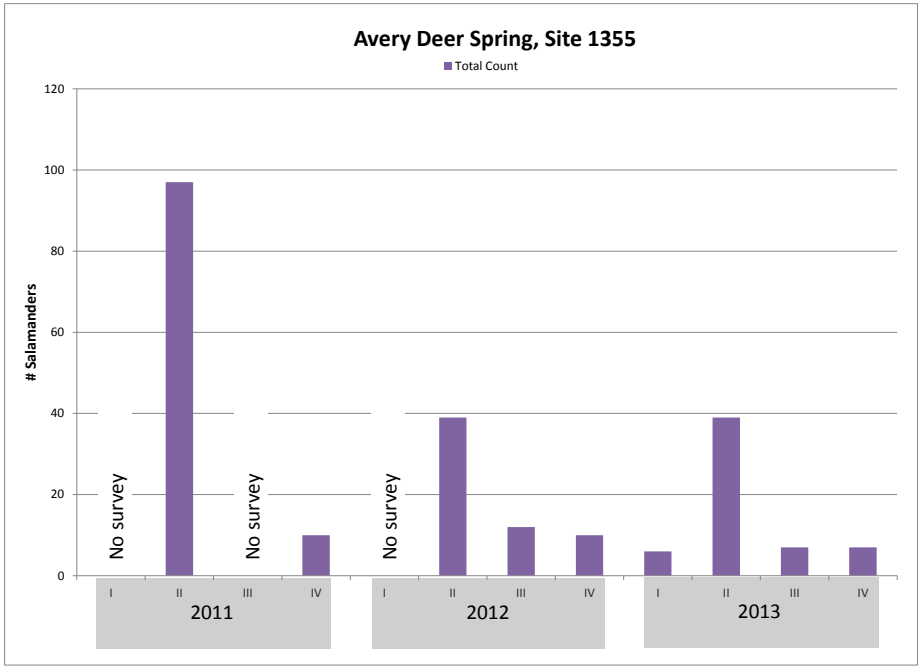

Barrow Hollow, Site 929
Total Count
Salamanders
45
40
35
30
25
20
15
10
5
0
Dry, no survey
Dry, no survey
Mostly dry, only section 2 surveyed
0
Section 1 dry, low flow
0
Section 1 dry, low flow
Dry, no survey
I II III IV
2011
I II III IV
2012
I II III IV
2013

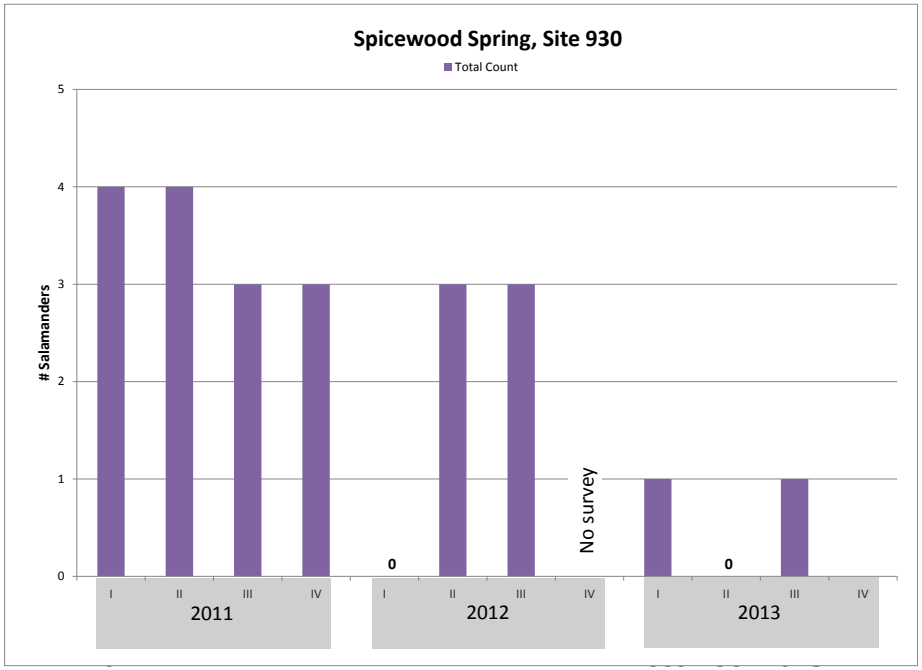

Stillhouse Hollow, Site 927
Total Count
Salamanders
18
16
14
12
10
8
6
4
2
0
0
0
I II III IV
2011
I II III IV
2012
I II III IV
2013

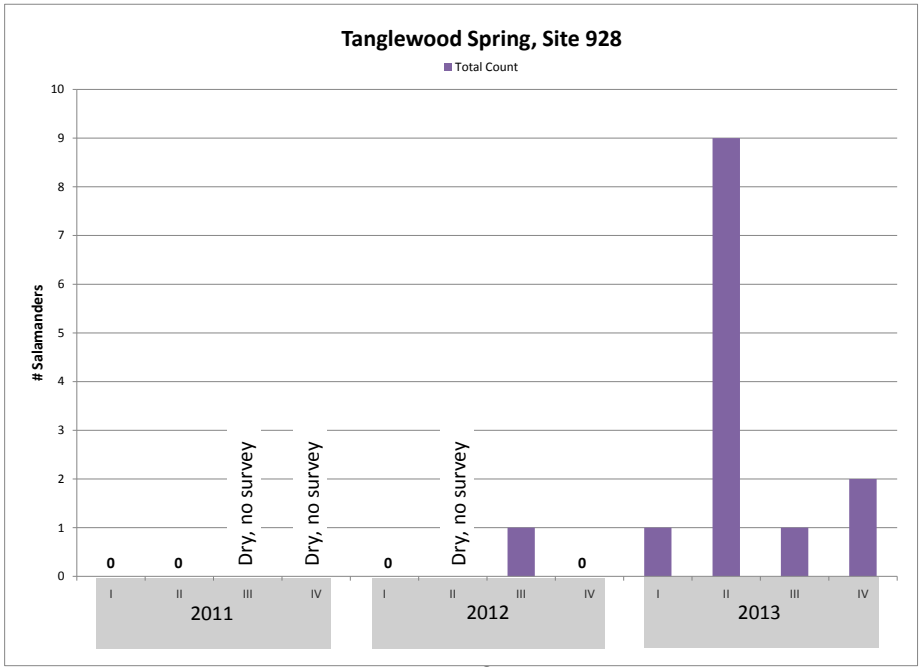

Franklin, Site 349
Total Count
Salamanders
350
300
250
200
150
100
50
0
Dry, no survey
Dry, no survey
Section 7 dry
Section 7 dry
I II III IV
2011
I II III IV
2012
I II III IV
2013

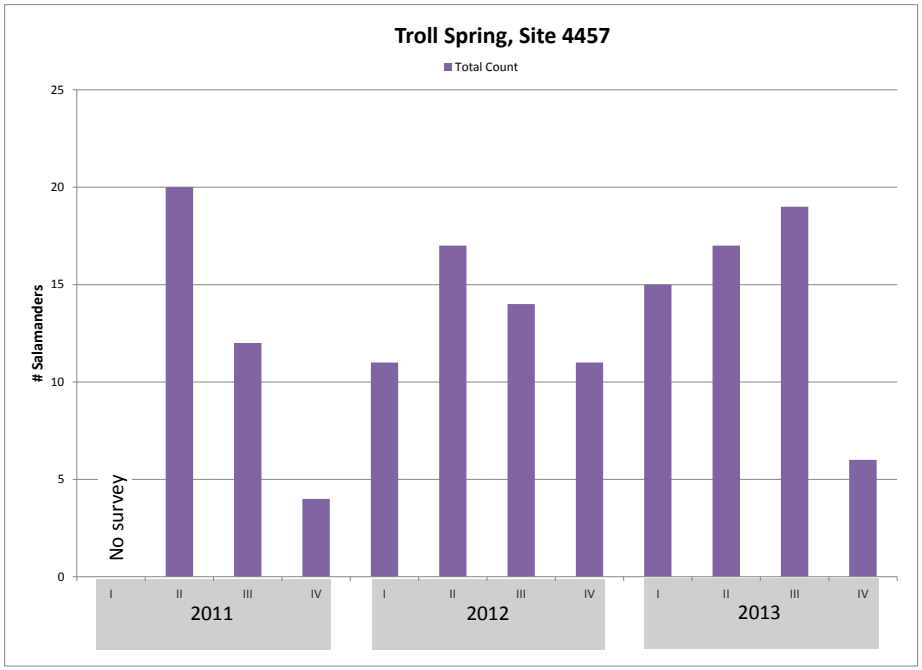

Bull Creek Trib 6, Site 151
Total Count
Salamanders
80
70
60
50
40
30
20
10
0
I II III IV
2011
I II III IV
2012
I II III IV
2013

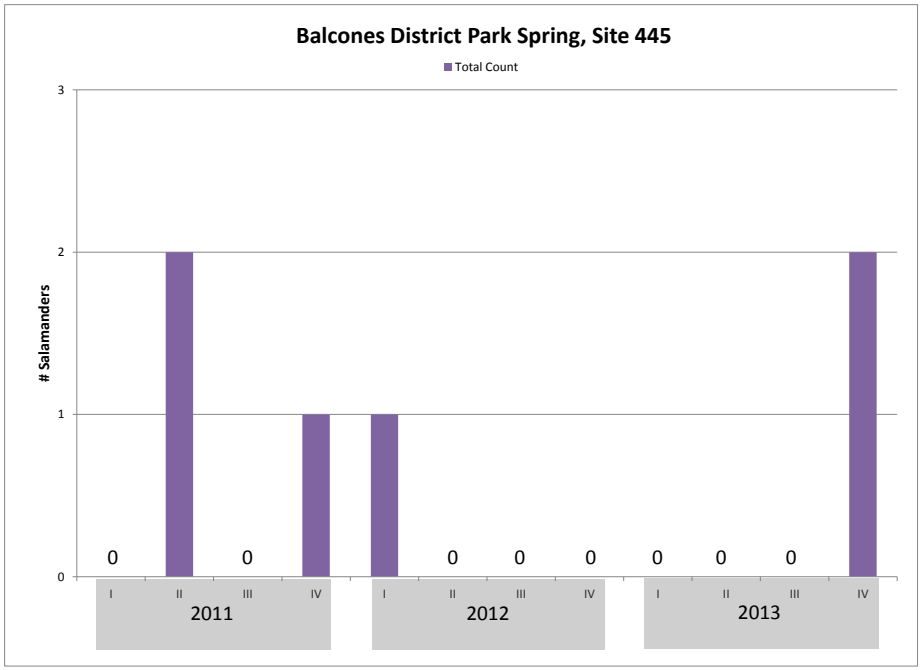

Hill Marsh Spring, Site 1353
Total Count
Salamanders
200
180
160
140
120
100
80
60
40
20
0
No survey
I II III IV
2011
I II III IV
2012
I II III IV
2013


Avery Deer Spring, Site 1355
Total Count
Salamanders
120
100
80
60
40
20
0
No survey
No survey
No survey
I II III IV
2011
I II III IV
2012
I II III IV
2013


Spicewood Spring, Site 930
Total Count
Salamanders
5
4
3
2
1
0
0
No survey
0
I II III IV
2011
I II III IV
2012
I II III IV
2013


Tanglewood Spring, Site 928
Total Count
Salamanders
10
9
8
7
6
5
4
3
2
1
0
0
0
Dry, no survey
Dry, no survey
0
Dry, no survey
0
I II III IV
2011
I II III IV
2012
I II III IV
2013


Troll Spring, Site 4457
Total Count
Salamanders
25
20
15
10
5
0
No survey
I
II
III
IV
2011
2012
2013


Balcones District Park Spring, Site 445
Total Count
Salamanders
3
2
1
0
0
0
0
0
0
0
0
0
I
II
III
IV
2011
2012
2013

Lanier Movement Study (pilot study)

When critical surface habitat was proposed for designation for four central Texas *Eurycea* species by USFWS in 2012 it was limited to spring orifices and 50 m downstream within a stream. This number came from work on *E. naufragia*, which included surveys at two springs (Pierce 2011). Dr. Pierce noted that they never observed salamanders farther than 50 m from the spring orifice (although this was likely due to unsuitable habitat beyond that point; Ben Pierce, pers. comm.). USFWS used this observation as the basis for their proposal critical surface habitat. We believed the proposed surface habitat designation to be a drastic underrepresentation of the potential extent that *E. tonkawae* (and other species) can be found from spring orifice, especially since it was based on an observation more likely reflective of the extent of available habitat at a particular spring rather than species-specific requirements or abilities. However, we only had anecdotal data suggesting that *E. tonakwae* can be found farther than 50 m from springs.

Furthermore, USFWS cited Lucas et al. (2009) as the basis for stating that "populations of salamanders are genetically isolated from one another and neither aquifers nor streams serve as dispersal corridors." While this is true at a larger scale and may help explain current phylogenetic patterns and distributions of species, it is inaccurate to apply to a smaller scale, particularly when referring to within-stream movements of salamanders. As above, we had only anecdoal observations and correlational data to support this claim.

To clarify these issues it was apparent we needed to directly document movement of *E. tonkawae* within the stream channel over a larger area than previously studied. Lanier Spring was the ideal site to do a pilot study. It includes a large population of *E. tonkawae* at a spring emerging from the alluvium and flowing into the main channel of Bull Creek. Additionally, water flows from upstream of the spring run within the channel which is not always the case for Bull Creek headwater springs. For example, the downstream Pit Spring does not commonly have overland upstream flow. The stream channel in this area has loose gravel and cobble substrate and shallow riffles, presumably ideal habitat for salamanders and devoid of any obvious physical dispersal barriers such as deep pools or impoundments. Thus, if salamanders did not move, or were not found far from the spring, it would likely be due to spring-specific habitat requirements and high site fidelity rather than physical barriers or lack of cover. Our goal was to determine whether salamanders disperse within the stream channel where there are no obvious springs, how far and how frequently they move, and whether they really are concentrated at the main spring outlet (although we acknowledge that other portions of the stream may have inconspicuous springs within the channel).

In addition to having suitable habitat to determine movement, Lanier also has a large population of salamanders that were part of a mark-recapture experiment since 2007. Since all individuals were marked and released at the spring site, it would be easy to determine whether those individuals are also found throughout the study area, indicating movement and use of habitat outside the immediate vicinity of the spring.

Our study design included nine 10 m sections, spaced 15 m apart, including the original Lanier Spring site within the channel (site 5) as well as four upstream sites (6–9) and four downstream sites (1–4). Cover was searched exhaustively (taking around 30 minutes with 3 people) in each section and all salamanders were captured and photographed (with the exception of some very small juveniles). Length measurements were taken from photographs and individual visible implant elastomer marks were recorded as well as gravid status.

Surveys were conducted every two weeks between January and April 2013 for a total of eight bi-weekly surveys. We include a summary of the data collected for this study in Tables 1 & 2 and Figures 3–5.

While this was only a pilot study and confined to a single site, our results reveal previously undocumented characteristics of *E. tonkawae* movement and occupancy patterns. First, while most individuals were observed closest to Lanier Spring (which is consistent with the long-standing observation that central Texas *Eurycea* are generally clustered around springs (e.g., Sweet 1982; Bowles et al. 2006), we did observe salamanders in all sections including juveniles [~15 mm total length] up to 80 m downstream of the spring (Figure 4). This suggests that obvious, discrete spring orifices within or adjacent to stream channels (in this case, Lanier Spring) are not necessarily the sole habitat of *E. tonkawae*. This is also evidenced by the multitude of observations and recorded localities of *E. tonkawae* throughout tributaries of Bull Creek (City of Austin, unpublished data). Second, we have thus far documented one individual migrating at least 60 m within a two week period of time and others even farther (up to 80 m) over longer time-periods (as supported by VIE-tagged recaptures). Third, our preliminary results demonstrate that *E. tonkawae* will move both upstream and downstream from Lanier Spring (Figure 5).

Pierce (2011) documented smaller movement distances for *E. naufragia* (maximum of 28 m) at Twin Springs Preserve and Swinbank Spring, and lower movement rates compared to our study, however his study sites were much shorter than ours (28 m and 24 m, respectively) and salamanders were only marked during a single occasion. We hypothesize that differences in movement of salamanders at Lanier Spring compared to Swinbank and Twin Springs are likely due to differences in habitat. Suitable habitat (which we define as flowing water over loose gravel and cobble substrate) is largely unavailable downstream of the study sites at Twin Springs and Swinbank Spring (Ben Pierce, personal communication), and this is in stark contrast to the vast extent of available habitat upstream and downstream of Lanier Spring.

Based on these results and observations of *E. tonkawae* throughout their range, we suspect that movement will be unfettered and occupancy will be extensive where surface habitat is contiguous, cover availability is high and access to subterranean refugia is present (e.g., through interstitial rock spaces). This type of habitat is most common within the incised canyons of the Jollyville Plateau (especially in relatively pristine streams such as the main stem of Bull Creek within the Balcones Canyonlands Preserve) where alluvial deposits within the stream channels provide extensive stretches of habitat.

Table 1. Summary of recaptures from January through April, 2013 at Lanier Spring.

Unique Individuals	187
Total Recaptured at Least Once	81
% Recaptured at least Once	43.3%

Figure 3. Histogram of body length data by survey at Lanier Spring. Survey data is ordered chronologically from top to bottom (Dates 1–8). Total abundance (the sum of frequency in each graph) appears to increase as winter transitions to spring. Reproduction and recruitment is also evident, particularly in survey 8 (April), which had the highest juvenile abundance. This is consistent with previously observed seasonal patterns in reproduction.

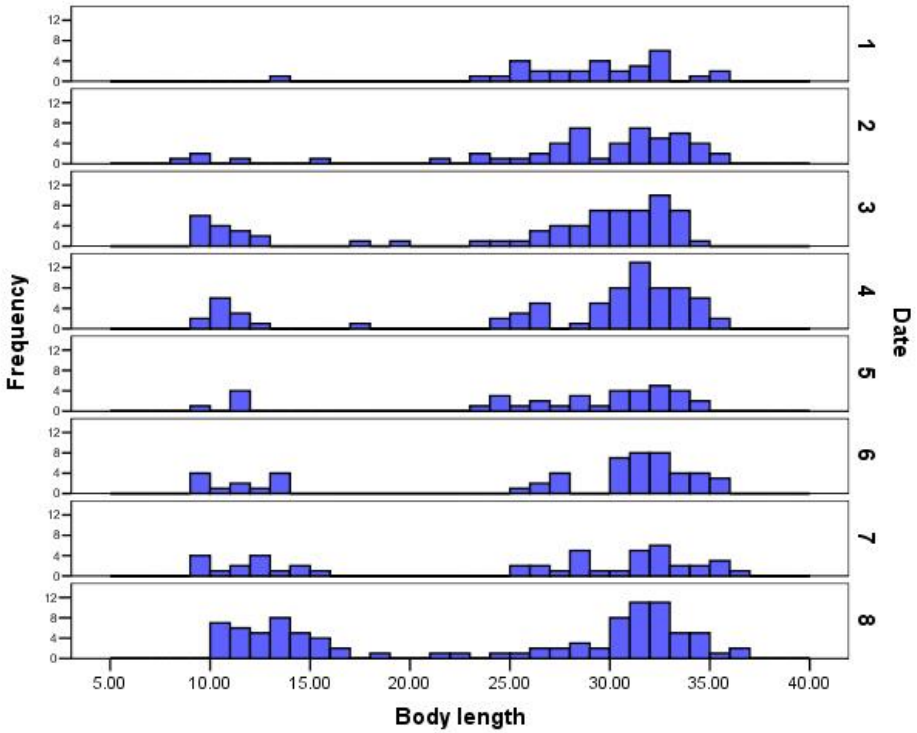


Figure 4. Histogram of body length data by section at Lanier Spring. Survey data is ordered upstream to downstream, from top to bottom (section 5 is at Lanier spring). Total abundance (the sum of frequency in each graph) is highest at the spring and just downstream (section 4), and is also higher downstream vs. upstream. This is likely due to the upstream habitat, particularly sites 8 and 9, being frequently dry but possibly less spring influence (indicated by lower water temperatures).

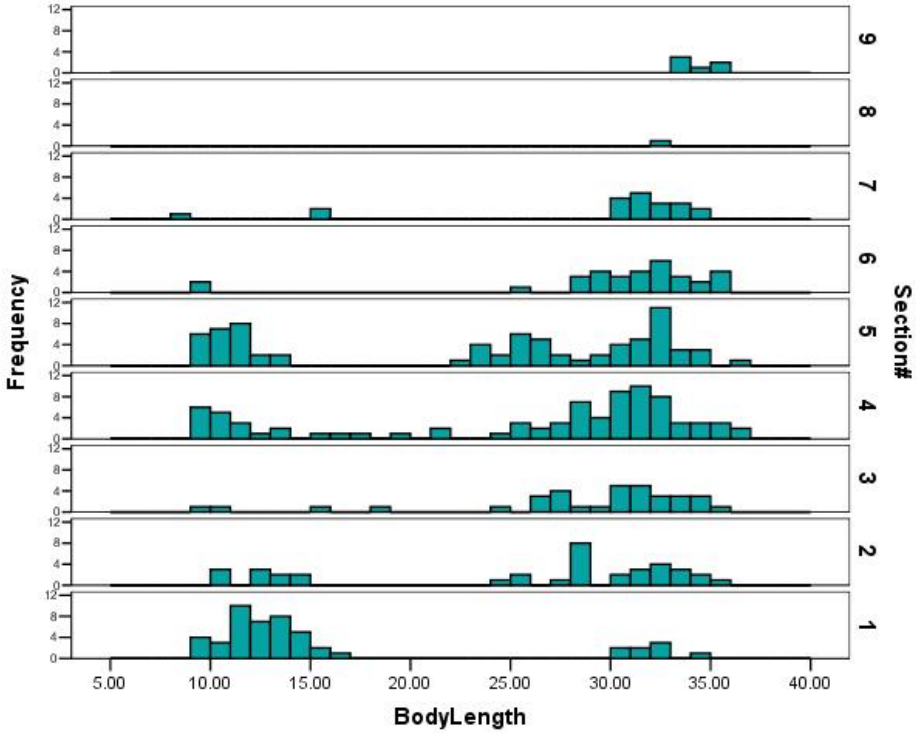
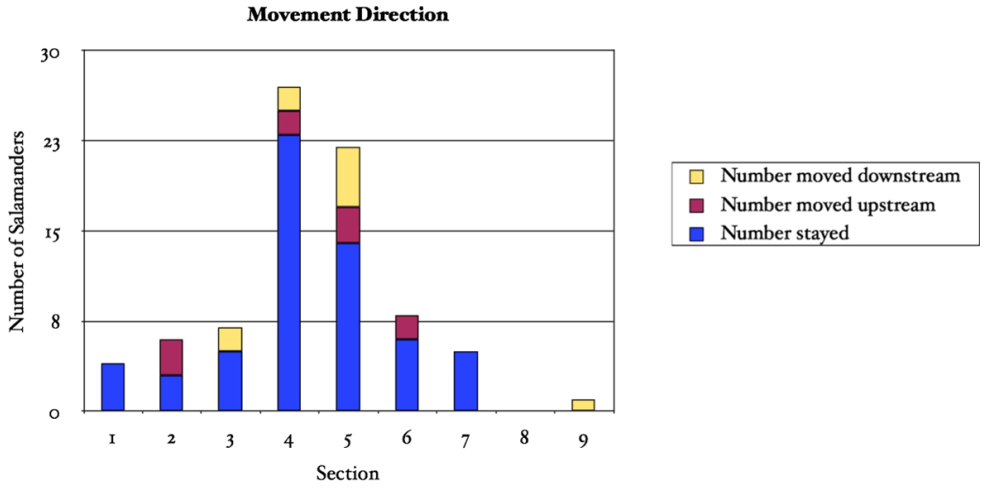


Table 2. Summary of movement by date at Lanier Spring. Percent movement was calculated based on the number moved over the number recaptured (since we can only document movement if a salamander was captured previously) among all sections. These data do not include movement of individuals marked prior to this study.

Original Date	Number recaptured	Number Moved	% moved
1/14/2013	15	3	20.00%
1/28/2013	18	5	27.78%
2/11/2013	21	5	23.81%
2/25/2013	11	3	27.27%
3/11/2013	3	1	33.33%
3/25/2013	8	3	37.50%
4/8/2013	5	2	40.00%

Figure 5. Number of salamanders recaptured and their location relative to their previous position at Lanier Spring from January to April 2013. Most individuals were recaptured at in their original location. There did not appear to be any strong patterns of movement in terms of direction or proportion moved relative to their original location, although sample size was small.



Occupancy Study (year 1)

Site Selection

In the spring of 2013 (19 March– 03 May) we conducted occupancy monitoring on four tributaries of Bull Creek including Barrow Hollow, Tributary 7 (Concordia), Tributary 4 (Spicewood Valley Park and Tanglewood Spring) and Bull Creek mainstem/Tributary 8 (Franklin, Lanier, and Ribelin/Sam Hamilton East tracts). Each reach is within either undisturbed (Mainstem/Tributary 8, Tributary 7) or disturbed (Tributary 4, Barrow) catchments with disturbance defined as having greater than 10% impervious cover. Sites were defined by 10 m sections of linear stream and were selected using a systematic sampling method as follows: starting at a random position (0-100 steps) from the downstream end of each tributary (at the nearest major confluence), we set out to delimit 20 to 30 sites within each tributary, at a minimum distance of 70 m apart (measured as 90 steps). The Mainstem/Tributary 8 reach was almost twice the length of the other tributaries, so sampling intervals were doubled (180 steps) to keep site numbers relatively consistent between tributaries. Each site was flagged, numbered and GPS coordinates were recorded. Light brush removal along each stretch facilitated site access.

The survey design included repeated sampling (k = 3) at each site to account for imperfect detection. Sites were surveyed by City of Austin Watershed Protection Department staff (Nate Bendik and Blake Sissel) for 5 minutes during each visit. The choice of a timed survey was used to ensure that a similar amount of effort was expended to search each site, regardless of variation in site area and total available cover (partly due to differences in wetted width). Salamander presence (and approximate size to the nearest 25 mm) or absence was noted for each visit. Additionally, we recorded several environmental variables. During the first visit we recorded stream depth and velocity in three equally spaced locations at the center of flow within the creek (i.e., the primary path of flow or center of creek if flow was homogeneous) as well as wetted width (bank to bank, perpendicular to the flow direction). At every site visit we also recorded water quality parameters using a Hydrolab minisonde (pH, temperature, dissolved oxygen, specific conductance) and visually estimated the percent area consisting of rock cover (gravel, cobble and boulders), algae or plant cover, and leaf litter or woody debris cover. Cover estimates were not mutually exclusive, such that an area with algae and leaf cover could be on top of rock cover, and all would be included for their respective estimates (thus, they do not need to sum to 100%). Finally, we also noted the presence or absence of fish, crayfish, and tadpoles. Fish species or type (e.g., centrachid, minnow, catfish) was also recorded, if known. Finally, since not all sites had flow during the study period, we recorded hydrologic condition for each site and noted any changes in flow.

A total of 99 sites were chosen among the four reaches. Table 3 provides the breakdown of sites per reach and their hydrologic status during the first survey.

Table 3. Summary of hydrologic status for each reach during occupancy study.

Reach Name	Development Category	No. Sites	No. Wet	No. Dry
Tributary 7	Undisturbed	25	16	9
Mainstem/Tributary 8	Undisturbed	24	11	13
Barrow Hollow	Disturbed	23	17	6
Tributary 4	Disturbed	27	23	4

Many sites were dry despite the relatively wet winter, presumably due to the continued persistence of drought conditions in central Texas. However, the typical hydrologic regimes of these streams are not well documented, so the high proportion of dry sites (particularly in Mainstem/Tributary 8) may be typical. A rainfall event (2–3 inches) in early April resulted in some sites becoming wet when they were initially dry (sites 12, 69, 73, 76 and 77) as well as some sites that were initially wet, dried up, and became wet again (26, 27 & 41). For sites that were initially dry, we did not record stream depth, width or velocity, precluding them from analysis for those covariates. Missing data are easily handled by the statistical models used to estimate occupancy and detection, but there are biological implications to consider where data are missing due to rainfall events that could possibly influence occupancy, since occupancy is assumed to be constant during the sampling period. Thus, we did not include sites that were initially dry in our analysis here, although we did include sites that were wet, then dry, then wet again on the third visit.

Covariate selection and model building

To avoid multicollinearity among predictors we examined correlations among variables expected to co-vary (e.g. temperature and DO) and only included sets that did not co-vary. Additionally, because all surveys were conducted during the same time and temperature during our sampling period gradually became warmer, we also tested for relationships between survey date and temperature.

It was necessary in certain cases to distinguish between period-averaged parameter values and survey-specific values. For example, since detection can vary between surveys, survey-specific values (in our case, water quality parameters) may be useful covariates if they are thought to influence detection. However, because consistent occupancy is a requirement of the model, only single values (e.g., period-averaged values) can be used as covariates on occupancy. We hypothesized that temperature could have an effect on detection, so we chose to use survey-specific temperature (*temp.s*) as a covariate in comparison to models with constant detection, or detection varying among reaches. Temperature could also affect occupancy, in which case we used both period-averaged temperature (*tempavg*) and its standard deviation (*tmp.sd*) as covariates.

To reduce model complexity (and the odds of detecting spurious relationships), we only included parameters in our analysis likely to have a close mechanistic link to salamander presence and/or detection and we did not test for interaction effects. A recent analysis of rock cover availability indicated it was not tightly linked to *E. tonkawae* density (Bendik et al. *In Press*), suggesting that these parameters are unlikely to strongly influence occupancy (at least at the scale we are measuring them). Algae and plant cover are closely linked to nutrient availability, which itself is related to disturbance. Since disturbance is of primary interest and is likely

correlated with algae cover and plant cover was virtually non-existent within these reaches, we also excluded that variable from our analysis. Conductivity is also highly correlated with disturbance, and so was removed in favor of the latter categorical variable. We included stream velocity and depth, but excluded stream width since it is not thought to directly influence salamander presence or absence. Both velocity and depth are more direct measurements of the habitat that are likely to affect salamander ecology. We used the following models for occupancy and detection probability:

$$logit(\psi) = \alpha_1 + \alpha_2 * Disturbance + \alpha_3 * tmp.sd + \alpha_4 * tempavg + \alpha_5 * DO$$

$$+ \alpha_6 * depth + \alpha_7 * velocity \text{ (full model)}$$

with

$$logit(p) = \beta_1$$

(i.e., constant detection among all sites)

or

$$logit(p) = \beta_1 + \beta_2 * temp.s$$

or

$$logit(p) = \beta_1 + \beta_2 * Reach.$$

We tested each occupancy (ψ) model with each parameterization of detection (p). Iterations of the occupancy model tested included full model (as above), water quality only (*temp.sd, tempavg, DO*), and habitat (*depth, velocity*). Furthermore, each model was tested with and without *Disturbance* as a parameter. Finally, we also calculated a "baseline" occupancy rate for each reach separately as well as for all sites combined.

Inference

We used the R package 'unmarked' to construct models of occupancy rates and detection probability and used AIC-based model selection to draw inferences regarding the relative important of covariates on occupancy and detection. Models with a difference of less than 2 AIC were considered equivalent, following common practice and suggestions from Burnham and Anderson (2002). Model specification follows the form (~ p ~ ψ), where 1 indicates intercept only (i.e., parameter is constant across all sites); covariates are specified by name. Table 4 below shows the AIC score and parameterization for each model ("Trib" is an alias for *Reach*). We include the model syntax used for reference in the following table.

Table 4. Results of occupancy data model selection.

Model	AIC
Full (w/ Disturbance)	
~ Trib3 + Trib2 + Trib1 ~ Disturb + tmp.sd + tempavg + do + depth + velocity	142.72
~ 1 ~ Disturb + tmp.sd + tempavg + do + depth + velocity	143.41
~ temp.s ~ Disturb + tmp.sd + tempavg + do + depth + velocity	143.61
Full (w/o Disturbance)	
~ 1 ~ tmp.sd + tempavg + do + depth + velocity	147.24
~ temp.s ~ tmp.sd + tempavg + do + depth + velocity	147.26
~ Trib3 + Trib2 + Trib1 ~ tmp.sd + tempavg + do + depth + velocity	147.73
Water quality only (w/ Disturbance)	
~ Trib3 + Trib2 + Trib1 ~ Disturb + tmp.sd + tempavg + do	150.27
~ 1 ~ Disturb + tmp.sd + tempavg + do	159.33
~ temp.s ~ Disturb + tmp.sd + tempavg + do	160.30
Water quality only (w/o Disturbance)	
~ Trib3 + Trib2 + Trib1 ~ tmp.sd + tempavg + do	159.10
~ 1 ~ tmp.sd + tempavg + do	160.79
~ temp.s ~ tmp.sd + tempavg + do	161.18
Habitat only (w/ Distrubance)	
~ Trib3 + Trib2 + Trib1 ~ Disturb + depth + velocity	162.26
~ temp.s ~ Disturb + depth + velocity	167.56
~ 1 ~ Disturb + depth + velocity	168.01
Habitat only (w/o Distrubance)	
~ temp.s ~ depth + velocity	170.24
~ Trib3 + Trib2 + Trib1 ~ depth + velocity	170.88
~ 1 ~ depth + velocity	171.01

The most complex models were favored by AIC, and models that included disturbance as a factor were unequivocally superior to those that did not. Furthermore, neither temperature nor reach covariates substantially improved models of detection in comparison to a constant detection model ($\Delta AIC < 2$) except among some reduced-parameter model sets (which were not optimal themselves). Examining the p-values of parameter estimates from the full model (*~ 1 ~ Disturb + tmp.sd + tempavg + do + depth + velocity*) provides some insight into which covariates have more of an impact on the model than others (Table 5).

Table 5. Parameter estimates and significance tests for the model that included all occupancy covariates.

Occupancy	Estimate	SE	z	P(>|z|)
(Intercept)	24.35	13.70	1.78	0.075
Disturb	-2.11	0.99	-2.13	0.033
tmp.sd	-2.24	0.78	-2.89	0.003
tempavg	-0.70	0.69	-1.02	0.310
DO	-0.43	0.29	-1.51	0.131
depth	-10.59	3.42	-3.10	0.002
velocity	-1.44	3.08	-0.47	0.641
	Estimate	SE	z	P(>|z|)
Detection	0.96	0.28	3.43	0.001

For example, velocity, DO, and average temperature do not appear to contribute strongly to prediction of occupancy, although depth, disturbance and variation in temperature do contribute strongly to prediction of occupancy. The importance of temperature variation is particularly interesting, since it may indicate the influence of groundwater at a site as surface water varies in temperature much more than groundwater. In this case, the higher variation in temperature, the less likely the site is to be occupied. This is consistent with the view that *E. tonkawae* are more likely to be found near springs (although spring outlets are not always obvious).

Table 6. Baseline estimates of detection and occupancy (without effect of environmental covariates) for each reach..

Reach	ψ	SE (ψ)	p	SE(p)
Mainstem/Trib 8	0.91	0.10	0.77	0.09
Trib 7	0.36	0.19	0.37	0.19
Trib 4	0.38	0.10	0.75	0.09
Barrow Hollow	0.18	0.09	0.89	0.11

Examination of the above results (Table 6) reveals that occupancy is very high in Mainstem/Tributary 8 in comparison to the other sites, and may be the driving factor behind the significance of the *Disturbance* categorical variable in our models. A post-hoc analysis of the best model from above, substituting *Reach* for *Disturbance*, improves the AIC score (= 138.2), confirming that this is probably the case. However, the importance of temperature variation and water depth did not change according to the parameterization of reach (with, without, or grouped by disturbance), suggesting that these parameters are not confounded by differences among reaches. Thus, there are possibly unmeasured covariates associated with reach that may help further explain differences in occupancy.

The other undisturbed reach, Tributary 7, had more uncertainty associated with its detection and occupancy estimates. Additionally, detection was also much lower in that reach. We are uncertain as to why this might have been then case, although it may have something to do with the predominant soil substrate within that channel making salamander detection more difficult. The lower occupancy of Tributary 7, despite being an undisturbed reach, suggests that habitat suitability may be lower here and thus heterogeneous among reaches. Disturbance may not be the only factor that can result in low salamander occupancy throughout a stream.

Further replication will be required to evaluate the effects of disturbance and habitat suitability on *E. tonkawae* occupancy in streams.

Based on these results and our field observations, we plan to measure additional habitat parameters to help us further understand the determinants of salamander occupancy. For example, the few places we did not observe salamanders within Mainstem/Tributary 8 seemed to be dominated by new travertine deposits. While anecdotal, this could be related both to interstitial space availability (spaces cemented shut by travertine development) as well as prey availability. As fallen leaves are coated with travertine, they are no longer able to be colonized by bacteria, thereby removing a food source for grazers, which are prey items for salamanders. Thus, the presence of fresh leaves coated in travertine may be a suitable parameter, or we may devise categorical levels based on the amount of travertine coated leaves or cemented cobble within a site. Similarly, other hard-packed soils or substrates within the creek may negatively affect salamander presence, and so we intend to investigate ways to measure interstitial space availability as well (e.g., by using a soil compaction gauge).

Additionally, we are pleased to note that, with the exception of Tributary 7, detection rates were very high. These results suggest that it would be more optimal to increase the number of sites surveyed and to decrease the number of surveys per site (k) where detection is high (>0.6) and occupancy is low (<0.4) (see MacKenzie and Royle 2005 for an assessment of survey effort allocation).

We plan to increase our sampling to include as many streams as is feasible, climate permitting. Furthermore, we will reduce our repeated sampling in disturbed streams (where occupancy is expected to be low) to k = 2 (for these four streams, this results in a 17% reduction in time that can be allocated to other survey efforts).

Stress Hormone Study (year 2)

In collaboration with Caitlin Gabor's lab at Texas State University, we conducted a second round of stress hormone (coritcosterone, aka CORT) sampling at two disturbed (Barrow Hollow and Tributary 4) and two undisturbed (Franklin and Lanier) sites in May 2013. Initial results from Barrow Hollow showed many negative CORT values and are not displayed. These may be due to a positively biased measurement error with the background control (stream water CORT concentration is subtracted from all samples), although it could also indicate low (near zero) true values of CORT in the Barrow population. This would be a large change compared to the previous year, where Barrow had significantly higher CORT than the two undisturbed populations.

We also performed agitation tests on a small number of individuals to determine whether they were able to mount a CORT response. Inability to mount a CORT response could be due to chronically stressful conditions. We did not perform an ACTH challenge test as initially proposed in the QAPP. Presented in Figure 6 are the raw CORT values corrected for salamander size (tail width). We presented our preliminary results at the Society for Integrative and Comparative Biology conference in January, 2014, Austin, Texas. We also plan to include two additional sites and three sampling periods in 2014.

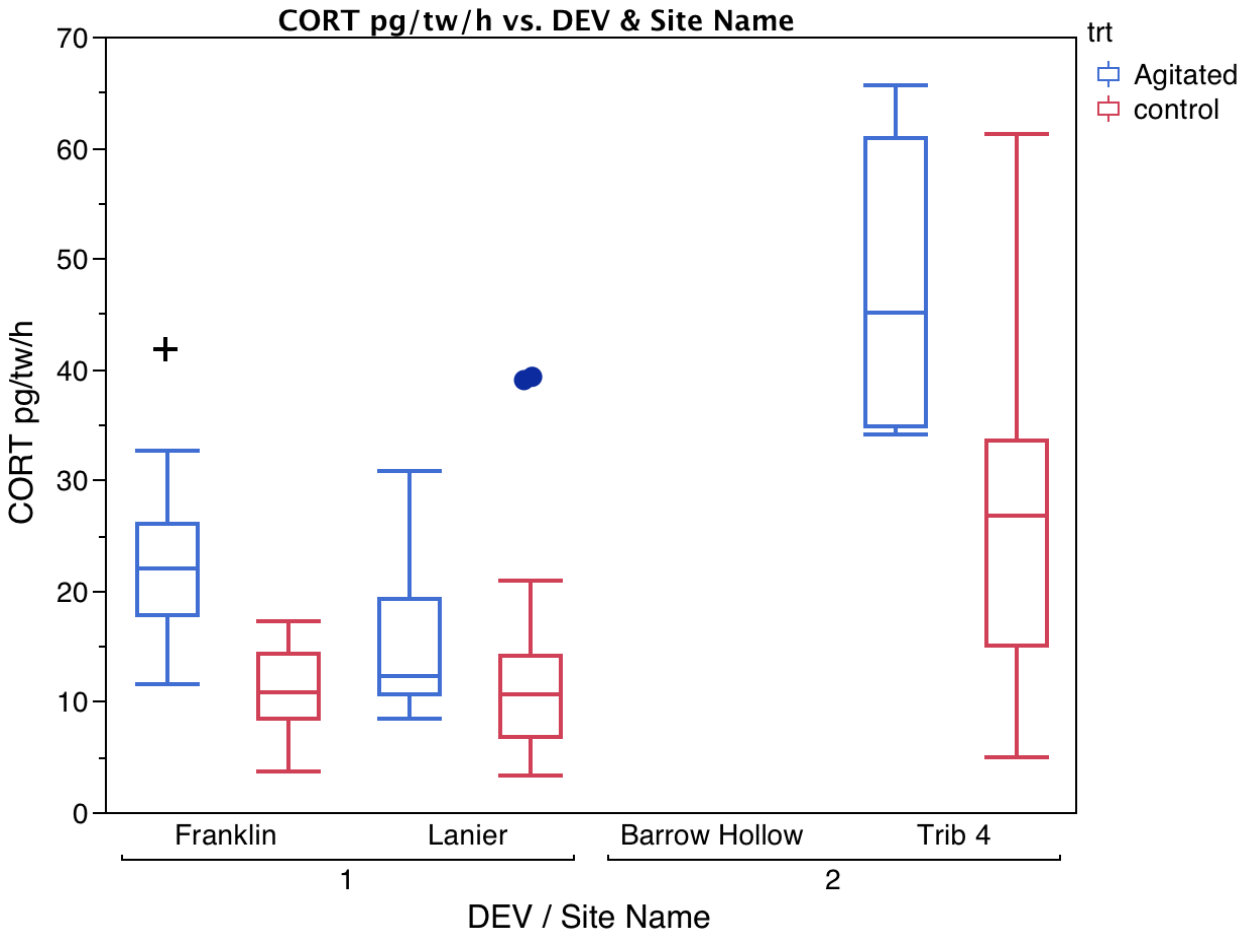


Figure 6. CORT concentrations of agitated and non-agitated salamanders at each study site. Sample sizes for each site are given as Site (non-agitated, agitated): Franklin (16, 12); Lanier (21, 8); Tributary 4 (20, 5).

Toxicant and Water Quality Screening

This project is part of a collaborative effort between the USFWS, USGS and COA to examine the toxicological profile of central Texas eurycid salamanders and their habitat. Results are not available yet from this study. Ten individuals were collected from Lanier Spring, five from Barrow Hollow, nine from Trib 4, four from Troll Spring, and 11 from Trib 6. All individuals were weighed (except those from Lanier) and their body length and tail width was measured from photographs. Figure 7 below shows the relationship between tail width and length, which is a better predictor of mass in comparison to body length or total length.

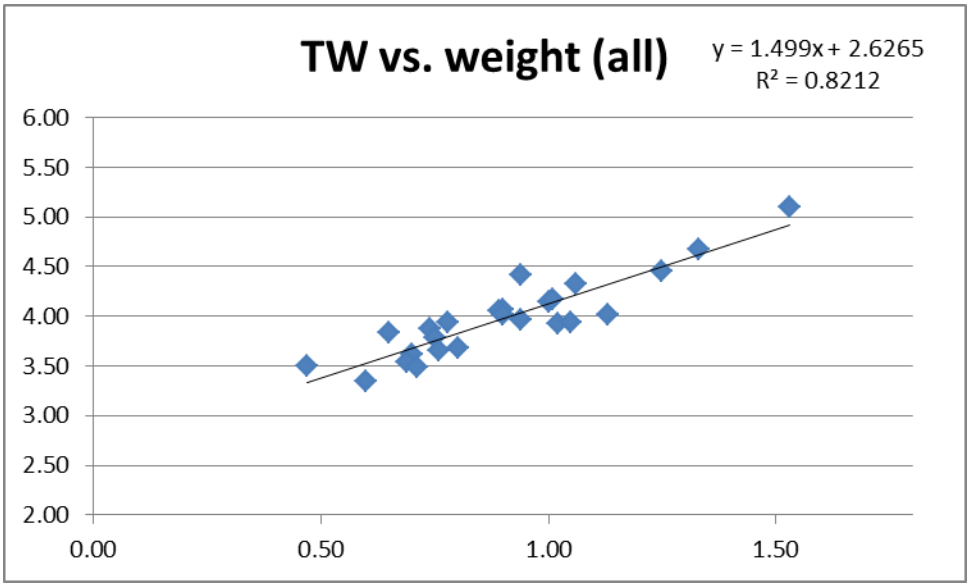


Figure 7. Tail width (tw) measured at the base of the tail (mm) vs. mass (g) for all salamanders collected for toxicant screening.

List of Water Resource Evaluation Quality Assurance Project Plans Associated with this Report

Project 118: Jollyville Water Quality and Salamander Assessment

Project 545: Jollyville Salamander Occupancy Study

Project 547: Assessing Jollyville Plateau Salamander Stress in Urban and Rural streams

Literature Cited

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Collaborators and/or Field Assistants for 2013:

Population studies. **City of Austin**: Blake Sissel, Kira McEntire, Matt Westbrook, Emily Yeoman, Mark Sanders, Laurie Dries, Liza Colucci, Donelle Robinson. **Travis County**: Renee Fields.

CORT study. **Texas State University**: Caitlin Gabor, Drew Davis, Kristina Zabierek.

Toxicity study. **U.S. Fish and Wildlife Service**: Pete Diaz and Eric Orsak. **USGS**: David Alvarez.