Estimation of Jollyville Plateau Salamander (*Eurycea tonkawae*) Populations Using Surface Counts and Mark-Recapture

Lisa O'Donnell[1], Andy Gluesenkamp[2], Chris Herrington[3], Martin Schlaepfer[4], Martha Turner[3], and Nathan Bendik[3]

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[1]City of Austin, Austin Water Utility, Wildland Conservation Division, Balcones Canyonlands Preserve, Austin, TX
[2]Texas Memorial Museum, University of Texas, Austin, TX and 700 Billie Brooks Drive, Driftwood, TX
[3]City of Austin, Watershed Protection and Development Review Department, Environmental Resource Management Division, Water Resources Evaluation Section, Austin, TX
[4]State University of New York – College of Environmental Science and Forestry, Syracuse, NY

Abstract

The Jollyville Plateau salamander (Eurycea tonkawae) is a perennibranchiate salamander found only in springs, spring runs, and subterranean streams in the Northern Edwards Aquifer northwest of Austin, Texas. This species is currently a candidate for listing under the Endangered Species Act due to threats from urban development throughout its limited range and corresponding negative trends in surface counts at several long-term monitoring sites. During 2007, the City of Austin initiated mark-recapture surveys of E. tonkawae populations at three spring sites and has compared the results with surface count surveys, which have been conducted as part of a long-term monitoring program since 1997. The mark-recapture study was conducted monthly over an 8-month period using Pollock's robust design. The original purpose of the mark-recapture surveys was to assess the potential impacts of a proposed water treatment plant (WTP4), which was subsequently relocated to an alternate site. While documenting the effects of WTP4 is no longer necessary, this study provided a unique opportunity to compare the utility of mark-recapture and surface counts. Mark-recapture surveys are considerably more labor-intensive, yet provide critical information that cannot be obtained solely from surface counts, including detection probabilities, total population size, vital rate estimates (emigration/immigration, persistence), and surface movement. During this study, detection probabilities varied from month-to-month, but the mean probabilities were similar across all three sites. These preliminary results indicate that, under ideal habitat conditions (i.e., consistent spring flow, suitable cover, and few predators), surface counts should represent a consistent fraction of the total population and thus provide a reliable index of the total population size. Continuing mark-recapture at a subset of the monitoring sites is recommended to better understand how populations respond under less than ideal conditions, monitor variability in detection probabilities over a longer period of time, adjust surface count data as needed, and gather other data that cannot be obtained from surface counts.

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Introduction

The Jollyville Plateau salamander (*Eurycea tonkawae*, hereafter abbreviated as JPS) is a rare aquatic salamander found only in springs, spring runs, and subterranean streams in the Northern Edwards Aquifer northwest of Austin, Texas (figures 1 and 2). Its range includes nine creek watersheds: Brushy, Bull, Buttercup, Cypress, Lake, Long Hollow, Shoal, Walnut, and West Bull (Figure 3). Spring pools, spring runs, and riffles dominated by spring flows provide the ideal surface habitat. Surface populations of JPS are typically found under loose rock substrates that are free of sediment and may also be found in leaf litter and aquatic plants. Anecdotal evidence suggests that egg deposition occurs underground. Because this species remains aquatic throughout its life, it depends on the quality and quantity of groundwater for its survival. It is typically found in clean, clear, flowing water that has a narrow temperature range (average 18-21^{o}C) and mostly neutral pH (average 6.9-7.8) (Davis et al. 2001, Bowles et al. 2006).

Figure 1. Range of *Eurycea tonkawae* within the Edwards Aquifer, Travis and Williamson counties, Texas.

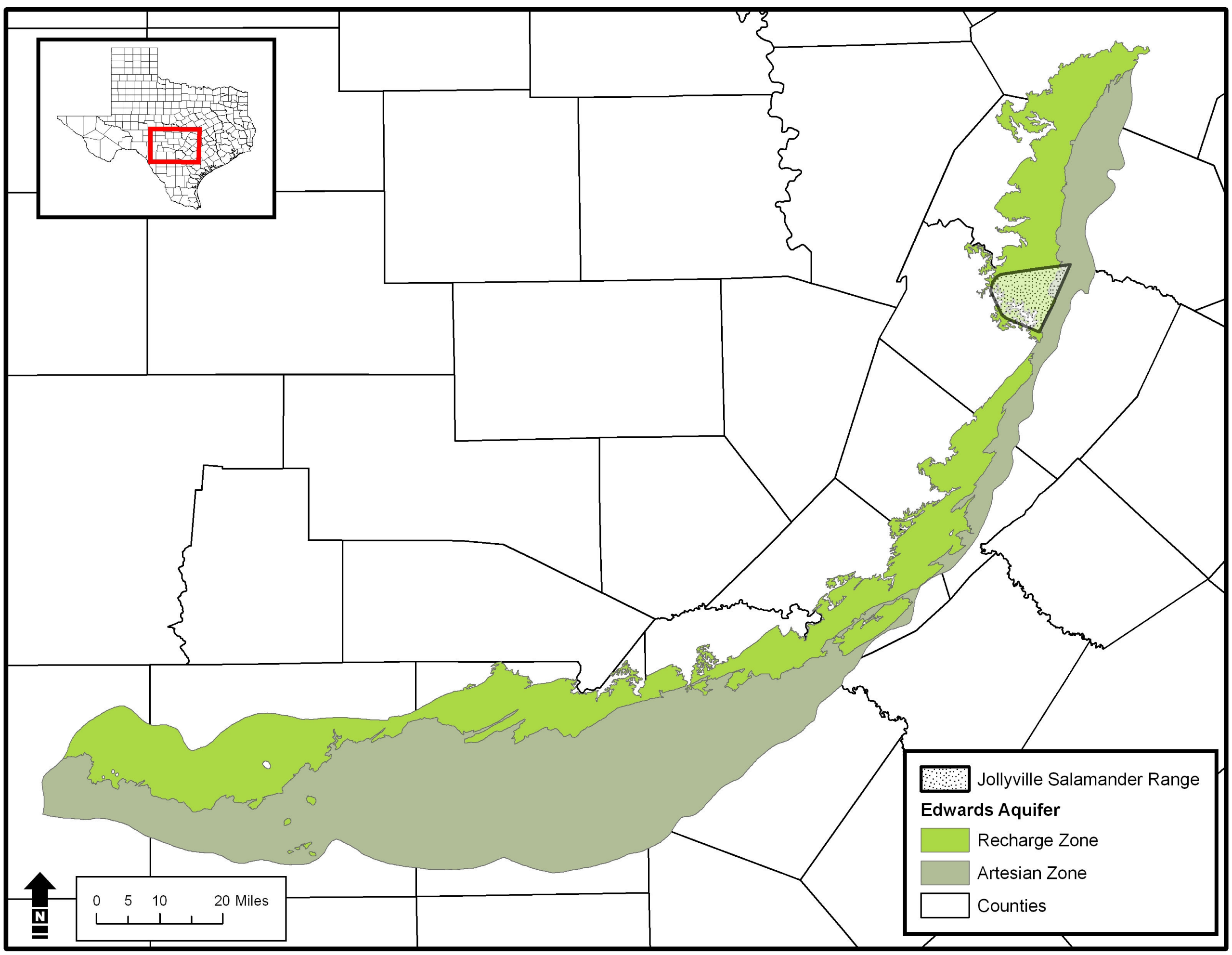


Figure 2. Known *Eurycea tonkawae* locations within the Northern Edwards Aquifer, Travis and Williamson counties, Texas.

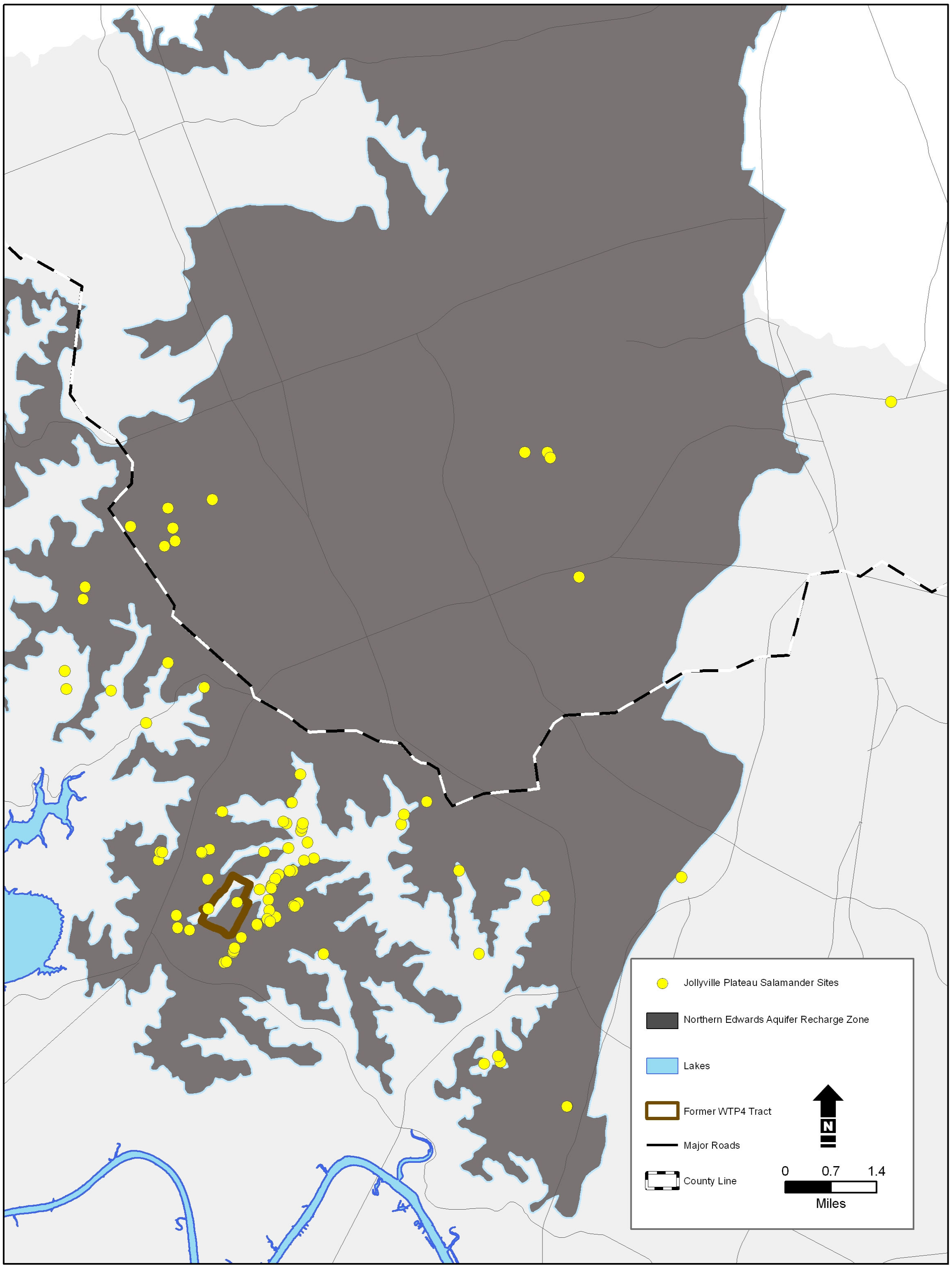
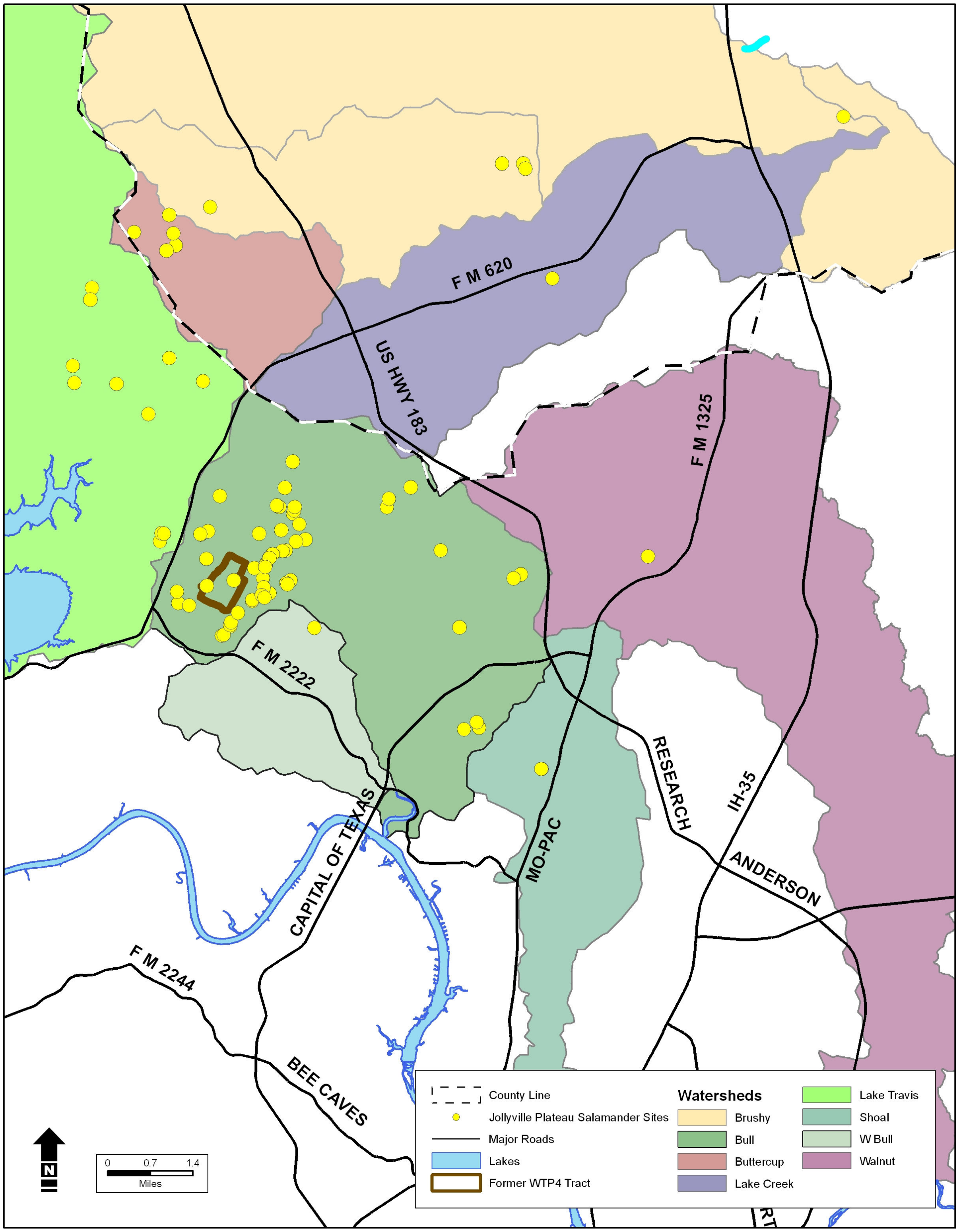


Figure 3. Known *Eurycea tonkawae* locations within creek watersheds, Travis and Williamson Counties, Texas. The Lake Travis watershed includes both Cypress Creek and Long Hollow watersheds.



The JPS is threatened by rapid expansion of urban development throughout its limited range. Significant negative trends in JPS numbers have been documented at four of nine long-term monitoring sites, and JPS with deformities have been found at two sites (O'Donnell et al. 2006). All of these sites occur downstream of areas where the recharge zone and creek headwaters have been developed. In June 2005, the Save Our Springs Alliance petitioned the U.S. Fish and Wildlife Service (USFWS) to add the JPS to the list of threatened or endangered species. On December 13, 2007, the USFWS made a 12-month finding which stated that listing the JPS as endangered or threatened is warranted but precluded due to other listing priorities (USFWS 2007).

The City of Austin initiated an intensive two-year study in 1997 and 1998 to collect baseline information about the JPS (Davis et al. 2001, Bowles et al. 2006). This effort established nine monitoring sites in three watersheds (Bull, Long Hollow, and Shoal) where surface counts were conducted. From 1999 to 2003, City of Austin biologists continued to conduct surface counts at some of these original monitoring sites, but on a less frequent basis. Beginning in 2004, monitoring efforts were expanded to include all nine long-term monitoring sites as well as new sites in other watersheds (Cypress, Walnut, and West Bull). The purpose of these surveys was to assess trends in relative abundance, habitat conditions, and seasonal variation in reproduction (O'Donnell et al. 2005, 2006).

In March 2007, the City of Austin began implementing a revised monitoring plan to determine whether construction of a proposed water treatment plant (WTP4) in upper Bull Creek would have a negative effect on JPS populations downstream of the plant site (City of Austin 2005). The revised monitoring plan included mark-recapture surveys at a subset of sampling sites (double-sampling) to allow for estimation of detection probabilities (i.e., the probability of capturing an animal that is actually present) and thus more accurate estimates of population size. With construction scheduled to begin in October 2007, mark-recapture and surface count surveys were conducted monthly, as staff and resources allowed, to collect as much baseline data possible. Following a City Council directive to re-evaluate the location of WTP4, monitoring was temporarily suspended to evaluate the 8-months of data. In December 2007, the City Council voted to purchase an alternate site to move WTP4 out of the Bull Creek watershed.

While documenting the effects of WTP4 was no longer necessary, the 2007 study offered a unique opportunity to test a different survey method. Mark-recapture is a considerably more labor-intensive method but provides information that cannot be obtained solely from surface counts, including the estimation of detection probabilities, total population size, vital rates (immigration/emigration, persistence), and surface movement. These data can then be used to examine the reliability of using surface counts as indices of total population size. Because surface counts do not include an estimate of detection probability, it is uncertain how widely abundance estimates vary in comparison to the true population size and the extent to which total population size may be underestimated. Combined with population genetics and dye-tracing studies, mark-recapture can also be used to determine whether populations are isolated or interconnected by dispersing individuals (metapopulations). Finally, mark-recapture can offer insight as to how populations respond to changing environmental conditions such as drought, increasing frequency and intensity of storm flows, and habitat degradation from urbanization. This information in turn can be used to evaluate population viability and promote a better understanding of this species.

Materials and Methods

Study Area

The original WTP4 site (now the "former WTP4 site") was situated on 102 acres on upper Bull Creek, between the mainstem and Tributary 8 (figures 4 and 5). Monitoring sites were selected along these two creeks because they support the largest JPS populations in the Bull Creek watershed with potential to be affected by development of the former WTP4 site. Both the mainstem of Bull Creek (hereafter referred to as Bull Creek "Mainstem") and Tributary 8 have some existing development at their headwaters, and construction of additional developments was initiated in October 2007. All of the headwaters in the Bull Creek watershed are developed or slated for future development, so a site in the more remote Long Hollow watershed was selected as a control. The Long Hollow watershed lies along the western edge of the Northern Edwards Aquifer and supports the largest known JPS population. Long Hollow is the only JPS-inhabited watershed that has its headwaters and possibly the recharge area within existing preserves (Shade et al. 2008). This control site was selected to document "natural" population variations in the absence of human influence. Long Hollow differs from Bull Creek in that its flow tends to be more ephemeral.

Between 2005 and 2007, surveys to determine the distribution of JPS and identify potential monitoring sites were conducted along accessible sections of Bull Creek Mainstem, Bull Creek Tributary 8, and Long Hollow. JPS habitat potential was classified as known (based on historic or recent observations), likely (proximity to visible spring openings), potential (shallow flowing water over loose, unembedded rock substrate, presence of ferns and/or aquatic plants indicating regular flow), or unlikely (none of the above, including dry substrates and deep pools with predatory fish). GPS coordinates for each habitat type were recorded. Cursory JPS surveys were conducted in all known, likely, and potential habitat areas and a few unlikely areas. The aerial extent, dates, and results of the distribution surveys are shown in Figure 4.

Priority for selecting monitoring sites for this project included location with respect to the former WTP4 site, relative abundance of JPS, and prior survey history. For Bull Creek Mainstem and Tributary 8, the primary objective was to have monitoring sites upstream and downstream of the potential influence of the former WTP4 site. Sites with large numbers of JPS (i.e., greater than 50 animals) were preferred to increase statistical power (Otis et al. 1978). To provide as much baseline data as possible, existing monitoring sites were included. While one of the original goals was to have a pair of mark-recapture and surface count sites above and below the former WTP4 site and along Long Hollow, the distribution of the JPS populations and/or denial of access to private property precluded this possibility.

Based on the selection priorities, seven monitoring sites were originally established (Figure 5, Table 1). Upper Ribelin was not surveyed during 2007 due to time constraints, and surveys at Lanier Riffle were discontinued after the June 2007 survey due to low numbers of salamanders. A description of each monitoring site, including a map of the survey areas, is provided in Appendix A.

Figure 4. Aerial extent and dates of *Eurycea tonkawae* distribution surveys conducted along the Mainstem and Tributary 8 of Bull Creek and Long Hollow, Travis County, 2005 and 2007.

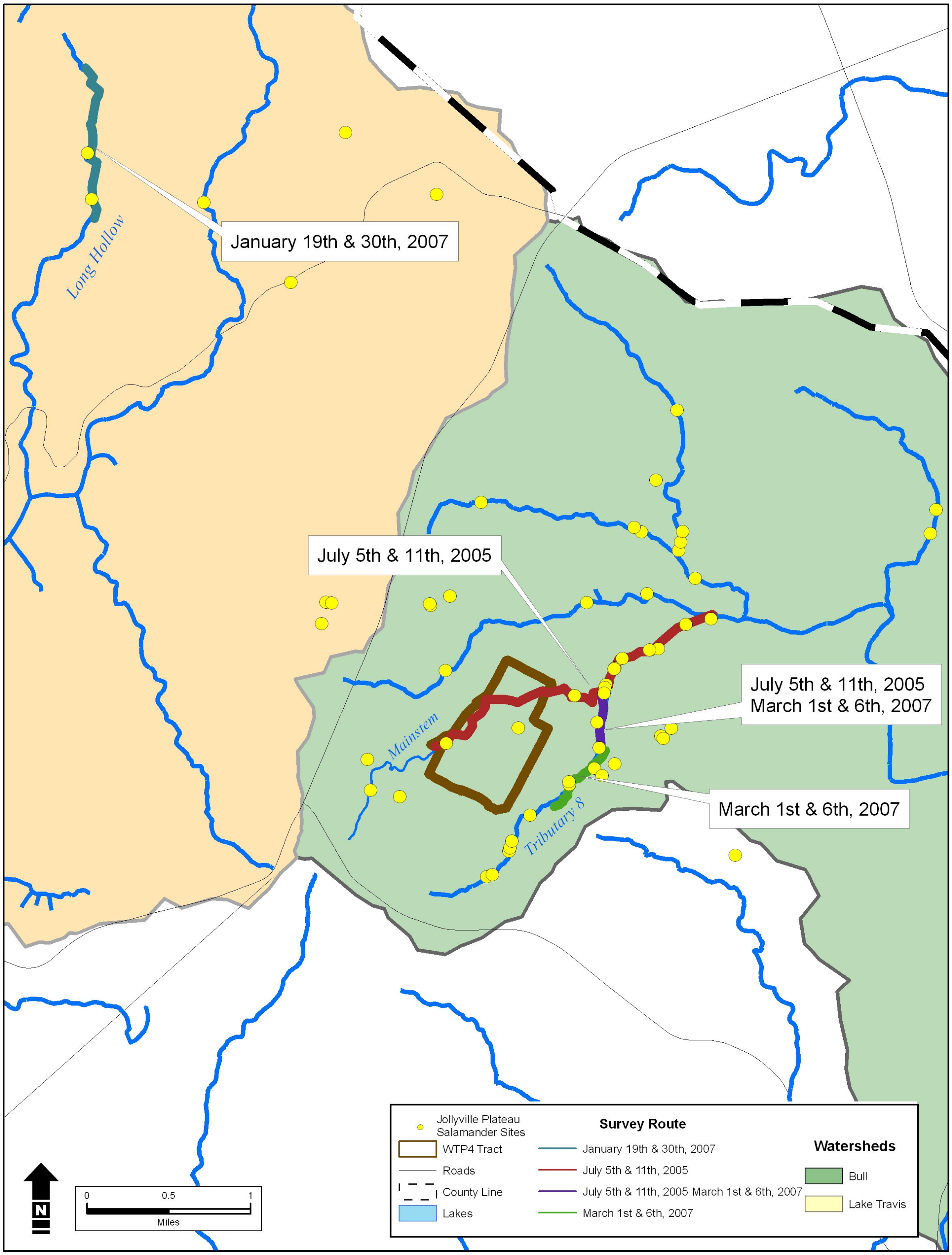


Figure 5. Location of 2007 *Eurycea tonkawae* monitoring sites, Travis County, 2007. Upper Ribelin was not surveyed during 2007 due to time constraints, and surveys at Lanier Riffle were discontinued after the June 2007 survey due to low numbers of salamanders.

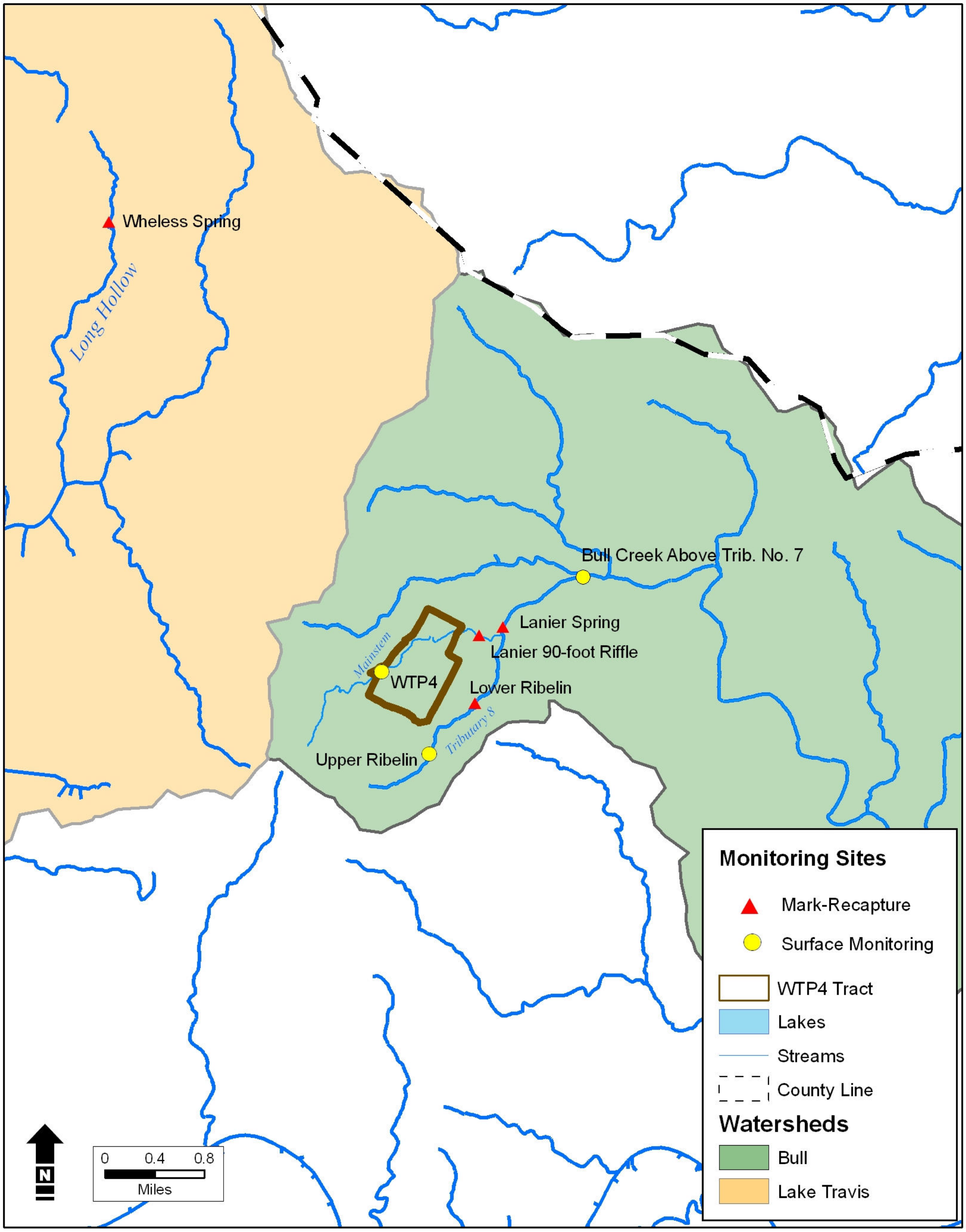


Table 1. Location of 2007 *Eurycea tonkawae* monitoring sites, survey method used, and property ownership. Original long-term monitoring sites (surface counts initiated in 1997) are highlighted. A description of each monitoring site, including a map of the survey areas, is provided in Appendix A.

Monitoring Site	Watershed	Above or Below Former WTP4 Site	Survey Method	Property Ownership
WTP4 at low water crossing	Bull Creek, Mainstem above Tributary 8	"Above"*	Surface count	City of Austin
Lanier 90-foot Riffle**	Bull Creek, Mainstem above Tributary 8	Below	Mark-recapture	City of Austin
Upper Ribelin**	Bull Creek, Tributary 8	Above	Surface count	Private
Lower Ribelin	Bull Creek, Tributary 8	Below	Mark-recapture	Private
Lanier Spring	Bull Creek, Mainstem below Tributary 8	Below	Mark-recapture	City of Austin
Bull Creek above Trib 7	Bull Creek, Mainstem below Tributary 8	Below	Surface count	City of Austin
Wheless Spring	Long Hollow	NA (control)	Mark-recapture	LCRA

*WTP4 at low water crossing is the most upstream site on Bull Creek Mainstem that was accessible but was still technically below the potential influence of the former WTP4 site.

** Upper Ribelin was not surveyed during 2007 due to time constraints, and surveys at Lanier Riffle were discontinued after the June 2007 survey due to low numbers of salamanders.

Field Methods

Two different survey methods were used during this study: surface counts and mark-recapture. Surface counts were conducted at three of the seven monitoring sites, including the long-term monitoring site on Bull Creek (Bull Creek Above Tributary 7). Mark-recapture was conducted at the remaining four sites, including the long-term monitoring site on Long Hollow (Wheless Spring). The survey method used at each monitoring site is shown in Figure 5 and Table 1. With the start date for construction of WTP4 originally projected for October 2007, mark-recapture and surface count surveys were conducted monthly (as staff, resources, and weather allowed) to collect as much pre-construction baseline data as possible.

Surface Counts

The surface count method consisted of surveying a defined area by turning over rocks and searching through leaf litter and aquatic plants. Each JPS observed was recorded based on size class [<25 mm ("small"), 25-50 mm ("medium"), >50 mm ("large") total length (TL)]. Clear acrylic boxes were used to help view the substrate, especially in areas with turbulent water flow. Consistent with previous surveys

(Davis et al. 2001), surface counts were conducted under baseflow conditions [< 0.5 inch (1.75cm) rainfall within the previous 24 hours] to eliminate potential variables introduced by storm flows. Surveys were not time-constrained, but total survey times were recorded for each site.

Mark-Recapture

The mark-recapture method followed Pollock's robust design (Kendall and Hines 1995, Kendall et al. 1997, Kendall and Bjorkland 2001, Bailey et al. 2004a-c, Thompson 2004, Amstrup et al. 2005). This design consists of sampling at two time scales: long-term primary periods (this study, once per month), with each primary period containing a short-term series of consecutive secondary periods (this study, three consecutive days). For secondary periods, multiple sampling events are conducted over a time interval that is short enough to assume the population is demographically closed (no births, no deaths, no immigration, and no emigration). The assumption of closure is relaxed between the primary periods, during which population gains and losses are expected to occur. A combination of open and closed population models allows for estimation of detection probabilities and population size within the survey area and within a primary sampling period. These parameters are then integrated into the estimation of "persistence". This term is usually referred to as survival, but persistence is used here because it was not practically possible to distinguish permanent emigrants from deaths within plots, temporary immigration into plots, and temporary emigration out of plots.

Mark-recapture was conducted from March through October 2007, resulting in 8 primary sampling periods for Lanier Spring and Wheless Spring, each of which contained three secondary periods. The Lower Ribelin site had only 6 primary sampling periods from May to October. At the beginning of each primary period, minnow seines were placed across the width of the stream at the boundaries of each survey area to restrict horizontal (surface) movement into and out of the sampling area within primary sampling periods. The bottom of each seine was buried under sand and rocks down to the bedrock substrate. To qualitatively verify the assumption of closure (i.e., animals did not move in or out of the main survey area within primary sampling periods), searches for marked salamanders were conducted above and below the seines in areas where habitat was available. The seines were removed at the end of each primary period.

JPS were collected using two techniques. The first was to carefully lift rocks and other substrate and collect observed salamanders with small dip nets. The second employed a large net (e.g., 46 x 22 cm) with the bottom edge placed flush on the substrate to avoid any gaps under which salamanders might escape. The water in front of the net was swept by hand to flush salamanders into the net. This latter method worked best in areas with soil substrates, leaf litter, and undercut banks where visibility was low.

All JPS greater than 16 mm snout-vent length (SVL) were collected for mark-recapture. Initial attempts to individually mark or "batch mark" (with a single mark) juveniles shorter than 16mm SVL was discontinued because the animals were too small to safely and reliably apply a mark. Such small juvenile JPS were subsequently counted, but not marked.

Immediately following collection, JPS were placed in fine mesh boxes in the spring runs near the collection sites (Figure 6). Rocks were placed in the boxes to provide cover, which allowed the salamanders to remain in their habitat under natural conditions prior to and after processing. To ensure

JPS were released near their collection site, the mesh boxes were placed in one to three locations within each survey section and flagged to identify their location. Thus, marked animals were released within a few meters of their initial point of capture.

Figure 6. Mesh box used for keeping *Eurycea tonkawae* in their natural habitat before and after processing.

Unmarked JPS greater than 16 mm SVL were anaesthetized in a solution of 0.25g Tricaine S (MS-222)/L of spring water for about 5 minutes. Once an individual salamander was fully anaesthetized, it was placed in a 4-oz write-on Whirl-Pak® with a small amount of anesthesia solution. SVL and TL were measured to the nearest millimeter using a 6-inch (15 cm) ruler. Gravid individuals were noted.

JPS were marked using a combination of elastomer marking (Northwest Marine Technology Inc., Shaw Island, Washington) and photo-identification. For the elastomer marking, sterile 28-gauge insulin syringes were used to inject tiny amounts (2-20 uL) of visible implant elastomer (VIE) just underneath the skin to form a soft colored bead. Each JPS was given three VIE marks using a combination of up to six different colors (blue, red, orange, yellow, white, black) in five locations on the body: dorsal surface of the trunk, immediately posterior of the insertion of the left and right forelimbs; immediately anterior of the insertion of the left and right hindlimbs; and the left side of the tail, approximately 5-15 mm posterior of the vent (Figure 7). All but the white and black colors are fluorescent. The use of six colors in five locations, with a maximum of three marks per individual, allowed for 2,550 unique combinations. Because of the unlikelihood of any movement between the Bull Creek and Long Hollow sites, the same list of color combinations was used so that up to 2,550 JPS could be marked at both of these areas.

Figure 7. Locations of visible implant elastomer (VIE) marks on *Eurycea tonkawae* individual. Selecting from 6 colors, VIE marks were injected in three of the first five locations. The sixth site was discontinued.

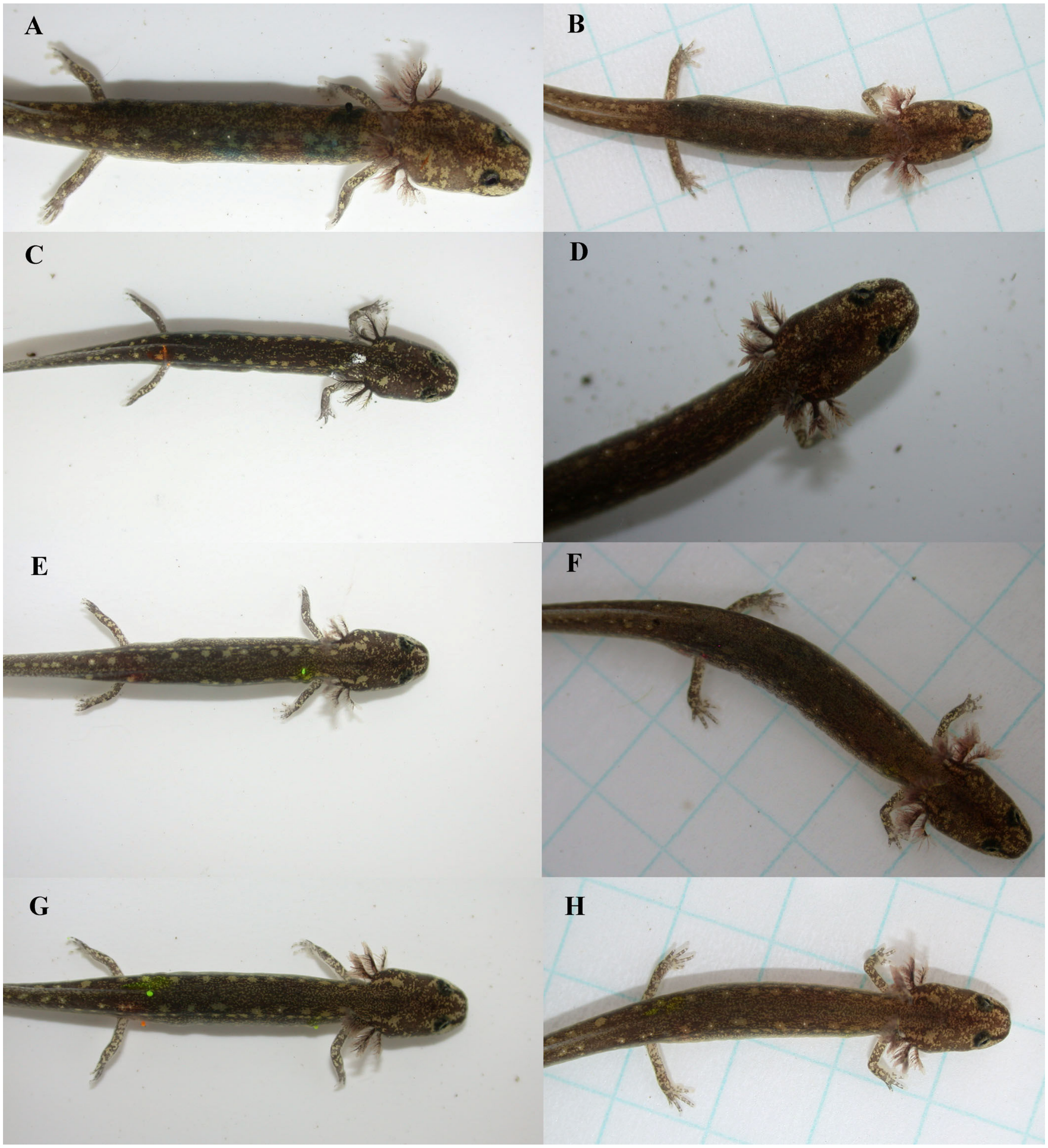
During the first few mark-recapture efforts, tissue samples (~2-3 mm tail tips) for population and phylogenetic work were collected from individual JPS. This included samples from 69 individuals at Lanier Spring, 18 individuals from Lanier Riffle, and 95 individuals from Wheless Spring. Tail tips were collected using iridectomy scissors that had been sterilized in ethanol. Both wild and captive *Eurycea* salamanders appear to recover quickly from this procedure and typically regenerate their tails in a little over a month. Tail tips were stored individually in 2 ml o-ring vials with 95% ethanol and provided to Dr. Paul Chippindale (University of Texas at Arlington) for genetic research.

Following marking with VIE, the anesthesia solution in the Whirl-Pak® was replaced with fresh spring water to begin the recovery process. All data written on the Whirl-Pak® were transcribed to field data sheets. As the last step, each salamander was photographed, placed back in its original mesh box near the collection site, and allowed to fully recover before release.

The primary purpose of the photographs was to document the retention of the VIE marks and the ability to recognize individuals based on unique patterns of melanophores and iridiphores distributed across their dorsal surfaces (for example, see figures 8-10). Melanophores are skin cells containing dark brown pigments, and iridophores are skin cells containing white pigments. Juvenile salamanders tend to have tightly clustered melanophores that give them a darker appearance than adults. However, the distribution of iridophores does not change through ontogeny, and individual-specific patterns created by iridophores, clusters of melanophores, and intervening unpigmented spaces are recognizable throughout ontogeny (Figure 9). Photo-identification using these natural patterns has been used successfully on many different species, including *Eurycea* (Bailey 2004) and other salamander species (Loafman 1991, Heyer et al. 1994, Doody 1995, Smith 2004, Gambel et al. 2007). Photographs have also been used previously to identify JPS individuals in the field and captivity (O'Donnell et al. 2006).

Figure 8. *Eurycea tonkawae* initial capture (March 19, 2007) and recapture (March 31, 2007) from Lanier Spring. VIE color combination is Yellow-Blue-Red, with no marks in positions 4 or 5. The initial capture in the first photo has been anaesthetized in MS-222.

Figure 9. Photographs of individual *Eurycea tonkawae* on the date of first capture and subsequent recapture. VIE marks cannot be seen in all images, but unique pigment patterns are consistent through time, allowing individuals to be accurately identified months after initial capture. Individual images are as follows: Black-Blue-Black on 3-12-2007 (A) and on 7-17-2007 (B), no mark-White-Black-Orange on 5-22-2007 (C) and on 9-17-2007 (D), White-Yellow-no mark-Red on 4-24-2007 (E) and 7-18-2007 (F), no mark-Yellow-Yellow-Orange on 5-21-2007 (G) and on 7-17-2007 (H).



Processing recaptured JPS was similar to that of unmarked animals, except that they were not anaesthetized and were instead placed in Whirl-Paks® with spring water. The color and location of each VIE on the body was identified and double-checked by at least one other person. A UV light was used to illuminate the fluorescent VIE marks, and an optivisor helped to magnify the marks. As time and staff allowed, previous datasheets were searched to verify that the database contained an initial capture with that color combination.

In the majority of cases, VIE marks were correctly identified in the field. The most common problems with VIE included absence of marks, usually due to incomplete injection of elastomer, mark migration during or following injection, and misidentification of VIE color in the field. Misidentification of marks was by far the most common source of VIE problems and was minimized through the use of UV lights, magnification lenses, and independent confirmation of all marks by two or more observers.

The combination of VIE and photography provided verification that recaptures had been correctly identified. If an animal was recaptured but could not be identified with 100% certainty using the VIE method (e.g., VIE had been lost or was not clearly visible), the photo catalogue system was used in combination with biological information such as size and location to confirm the individual's identity. Photographs also allowed for closer examination and documentation of any obvious external health problems.

Habitat Sampling

Habitat quality parameters were recorded in each section of each survey area. Parameters included the number and species of potential predators such as fish, crayfish, and juvenile watersnakes; crayfish numbers according to size (<25 mm, 25-50 mm, > 50 mm); presence of other amphibians; visual assessments of substrate composition, embeddedness, percent cover and type of aquatic plants such as algae, bryophytes, and macrophytes; and percent cover of leaf litter/woody debris. Embeddedness is defined as the degree to which rocks are surrounded or covered by fine sediment and was estimated visually by percent categories. Flow was measured using a Marsh-McBirney Flowmate® meter at the same location at each monitoring site to calculate discharge. Temperature, pH, dissolved oxygen, and specific conductance were measured using a Hach Minisonde®. These water quality measurements were generally taken near the center of each survey section. The only exception was those sections containing a large spring (Lanier Spring, Wheless Spring), in which case the measurement was taken near the spring outlet. Photos were taken of each survey section. Since the focus of this study was on mark-recapture, with the exception of water quantity data, these results are presented and discussed in Appendix B.

Observations of Surface Movement

Surface movement within the Lanier Spring and Wheless Spring study sites was documented by recording the section in which each marked animal was captured. These two mark-recapture sites were divided into different sections based on habitat differences (Appendix A). Lower Ribelin was too short to contain multiple sections, so documenting surface movement within this study area was not possible. In addition, two surveys were conducted beyond the study area at Lanier Spring on May 14 and June 12, 2007. These data and results are discussed in Appendix C.

Animal Care Protocols

Throughout the mark-recapture project, every effort was made to reduce the amount of stress on each salamander. These steps were needed to minimize negative behavioral responses to capture and promote equal recapture probability among individuals, which was critical to the success of the project. Prior to initiating mark-recapture in the field, the potential effect of using the VIE method was researched. VIE has been used in a variety of fish, anurans, and salamanders, including the slimy salamander (*Plethodon albagula*) (Taylor et al. 2006), San Marcos salamander (*E. nana*) (Joe Fries, U.S. Fish and Wildlife Service, *pers. comm.*), the Texas blind salamander (*E. rathbuni*) (Gluesenkamp and Krejca 2007), JPS in Testudo Tube Cave (Gluesenkamp and Krejca *in prep.*), and the City of Austin's captive breeding program (O'Donnell et al. 2006). For the duration of this project, one biologist, Dr. Andrew Gluesenkamp, was responsible for the majority of the VIE marking. After training and initial supervision, only a few other biologists were allowed to mark JPS. With experience, injecting 3 VIE marks took less than one minute per animal. Needles were disinfected with ethanol after each injection. Before and after processing, animals were kept in fine mesh boxes in the spring runs near the collection site to keep them in their natural habitat for the majority of the time. Processing was conducted as quickly as possible, with animals kept shaded and at or close to the spring run temperatures at all times. JPS were placed in sterile Whirl-Paks® that were only used that day. Small UV lights were used to pinpoint the light on each individual VIE mark and avoid shining the light on the salamanders' eyes. Using these protocols, mortality was very rare (<1% of the total number of marked animals). Any dead salamanders that were found were preserved in 95% ethanol and included in collections for genetic research.

The chytrid fungus has been documented throughout the JPS' range (O'Donnell et al. 2006), and stringent measures were followed (O'Donnell et al. 2005) to prevent the spread of this and other pathogens. Separate sets of equipment were dedicated to the Bull Creek and Wheless sites, and all equipment was cleaned, disinfected, and dried completely at the end of each primary sampling period.

Data Management

The City of Austin used both Access (2003) and Oracle databases for the survey data. The Access (2003) database was developed specifically for the mark-recapture data. Mark-recapture data for each monitoring site were entered into structurally identical but separate Access databases. Sample events, observations, individuals, and photo references were stored in normalized Access tables and joined with system-assigned numeric key fields. Entry forms were utilized to enforce table logic on entry. Habitat characterization data and population counts, including counts obtained during mark-recapture surveys, were entered and maintained in a field sample database, which is an existing Oracle database maintained by the City of Austin.

Data QA/QC

Data quality checks were essential to verifying the correct identity of all initial captures and recaptures. The first step was to confirm that field observations were accurate (i.e., VIE combinations were correctly identified) by inspecting each photograph taken in the field and confirming the combination on the salamander. Those individuals that were correctly identified in the field were labeled as "confirmed" in the database. Entries were labeled "unconfirmed" when the following occurred:

1) Few or no VIEs were visible in the photos.
2) Photos were blurry or missing.
3) Individual VIEs were misidentified (e.g., black mistaken for blue).
4) VIE combinations were misidentified.
5) Two different salamanders were given the same combination (duplicates).
6) Individuals did not have recorded marking events (no initial capture).
7) Individuals appeared to have diminishing SVLs, i.e. subsequent captures had smaller SVL values than previous captures. SVL values with a difference of 5mm or more were flagged as problems.

Most of the above problems were solved by (i) trying to positively identify as many VIEs on the individual as possible; (ii) searching through all individuals in the database with those particular VIEs (in the same location on the body); and (iii) using natural pigment patterns to match individuals with their initial captures.

For duplicate combinations, individuals were first matched with their initial captures based on their natural pigment patterns (see example in Figure 10). Unique numbers were then assigned to the two sets of individuals to separate them in the database. For diminishing SVLs, if the VIE pattern of the individual in question matched that of its initial capture, its SVL was treated as an error and not used in the analyses.

Data for all individuals with "confirmed" entries (entries for which photos of sufficient quality were available to identify individuals) were used in the data analyses. For "unconfirmed" entries, individuals with missing or poor quality photos were included in the analyses if there was no reason to assume they had been incorrectly identified in the field (i.e, decreasing SVLs). These entries were identified as "unconfirmed" but "good" for use in the data analyses. Any other problems that were irresolvable (no matches found, erroneous SVLs) were labeled "unconfirmed" and "bad," and were excluded from the analyses.

Because so few small juvenile JPS (<16mm SVL) were marked with "batch" marks, which was discontinued after the second month of mark-recapture surveys, these individuals were not included in the mark-recapture data analyses.

Results of Photographic Data QA/QC

Comparison of photographs of individuals collected on different dates was the most critical part of the data QA/QC process and resolved most of the problems due to misidentification in the field. In nearly all cases, if appropriate images existed for at least two dates, it was possible to identify individuals based on comparison of pigment pattern alone. This provided confirmation that 84-97% of the individual recaptures were correctly identified (Table 2). Photos taken on June 12 and 13 at the Lanier Riffle and Lower Ribelin sites were lost due to camera malfunction and thus were not available to confirm the identity of the JPS recaptures for those days. This lowered the percent confirmed to 84% and 90% for these sites, respectively. However, assuming identifications in the field were correct if there were no decreasing SVLs or other problems, 98-100% of all individuals were correctly identified. Unconfirmed individuals with no or poor quality photographs and invalid SVL (0-1.8% of observations) were assumed to be incorrect identifications; these were included in total counts of JPS but were not used in the mark-recapture data analyses.

Table 2. Results of photographic data QA/QC. Includes total number of *Eurycea tonkawae* observations confirmed using photographs and unconfirmed (photos missing or poor quality). Total numbers include all unique captures within the primary study area and in adjacent sections used to check the horizontal closure assumption. Unconfirmed observations were assumed to be "good" and used in the data analyses provided there were no accompanying erroneous data such as decreasing snout-vent lengths. Unconfirmed observations were assumed to be "bad" if there were irresolvable problems (no matches found, erroneous snout-vent lengths).

Site	Lanier	Lanier Riffle	Ribelin	Wheless
Total Unique Captures	1018	32	606	2638
Number Confirmed	987	27	546	2533
% Confirmed	97	84*	90*	96
Number Unconfirmed	31	5	60	105
% Unconfirmed	3	16	10	4
Number "Good"	1016	32	595	2634
% Good	99.8	100.0	98.2	99.8
Number "Bad"	2	0	11	4
% Bad	0.2	0.0	1.8	0.2

*Photos taken on June 12 and 13 at the Lanier Riffle and Lower Ribelin sites were lost due to camera malfunction and thus were not available to confirm the identity of the recaptures for those days.

Figure 10. Photos of two *Eurycea tonkawae* that were accidentally marked with the same VIE combination (Red-Blue-Orange) at Lanier Spring on March 19, 2007 (photos 1 and 2). Photos 1A and 2A show both salamanders under anesthesia immediately after marking. Both JPS were recaptured on June 11, 2007 (photos 1B and 2B) and other dates. VIE marks and natural pigment patterns were used to tell these individuals apart. An example of the unique natural pigment patterns are indicated on each photo.

Missing Data/Incomplete Surveys

Incomplete mark-recapture surveys occurred at Lanier Spring on March 13 and Wheless Spring on March 11 and 12 and on August 20 (Appendix D). The first Lanier Spring survey on March 13 had to be terminated due to weather; 18 JPS were marked, and 14 had to be released before processing. Because the full primary period had to be postponed until March 19-21, the March 13 data were omitted from the mark-recapture analyses for Lanier Spring. Upper Ribelin was not surveyed during 2007 due to time constraints, and surveys at Lanier Riffle were discontinued after the June 2007 survey due to low numbers of salamanders, thus these sites were not included in the data analyses. Surface counts were not conducted in May or August due to flooding conditions.

Data Analyses

Different statistical tools were used to analyze the mark-recapture data. Within primary sampling periods, the program CAPTURE (Otis el al 1978, White et al. 1982) was used to quantify potential behavioral or group responses to capture and marking and verify assumptions of closure (Appendix E). An additional test for closure within primary periods was performed using the program CLOSETEST (Stanley and Burnham 1999, Stanley and Richards 2005). MARK version 4.3 was used to identify models of how detection and persistence probabilities vary in space and time that best fit the data. Finally, RDSURVIV (Kendall et al. 1997, Lindberg et al. 2001) was used to evaluate the goodness-of-fit of a set of pre-defined models for qualitative comparison to the best selected models from MARK.

For the models run in MARK, capture history is a function of various biological factors, including an animal's probability of initial detection (p), probability of recapture (c), persistence (S, the probability that an animal found within a sampling site would be found within the same site during the next primary sampling period), and movement patterns into and out of each site (emigration (γ') and immigration (γ"), respectively). Parameter and model notations are listed in tables 3 and 4.

Table 3. MARK parameter notations.

Model Notation	Brief Description
S(s)	persistence variable across months
S(.)	persistence constant across months
p(s) c(s)	capture and recapture probabilities variable across months
p(s) = c(s)	capture and recapture probabilities variable across months, but equal to one another within any given month
p(.) c(.)	capture and recapture probabilities constant across months
p(.) = c(.)	capture and recapture probabilities, equal, and constant across months
markov	Markovian movement. γ' and γ", the probability of an animal re-entering or leaving a plot is conditional upon where it was found in the previous primary period. May vary across months, or may be constant (in which case it is labeled markov cte)
random	Random movement. γ' = γ"; the probability of an animal re-entering or leaving a plot is not conditional upon where it was found previously. May vary across months, or may be constant (in which case it is labeled random cte)
no mov	No movement into or out of plot. γ' = γ" = 0

Table 4. MARK model notations.

Parameter Notation	Brief Description
S	Persistence
p	Probability of capture
c	Probability of recapture
γ'	The probability of being off the study area, unavailable for capture during primary trapping session (i) given that the animal was not present on the study area during primary trapping session (i − 1),and survives to trapping session (i). Thus, (γ' – 1) is the probability of a temporary emigrant returning to the sample area. Sometimes called "immigration".
γ''	The probability of being off the study area, unavailable for capture during the primary trapping session (i) given that the animal was present during primary trapping session (i − 1), and survives to trapping session (i), i.e., probability of temporarily emigrating from sample area
N-hat	Estimated number of individuals available for capture within a defined area within a given primary period. N-hat = n/p, where n is the total number of individuals captured
N_{super}	Estimated number of individuals available (e.g., surface) and unavailable (e.g., subsurface) for capture within a defined area within a given primary period. Requires temporary migration to be random ($\gamma' = \gamma''$). N_{super} = N-hat/(1- γ)

*cte = constant time and error

MARK was used to test a small number of *a priori* questions:

1) Is persistence variable across months, or constant?
2) Are p and c variable across months, or constant?
3) Are p and c equal or unequal?
4) Is movement in and out of sample plots between primary sampling periods variable across months or constant? Furthermore, are such movements random or Markovian, (whereby the probability of an animal entering or leaving a plot is conditioned upon where it was in the previous primary sampling period)?

The most parameterized model (i.e., the model with the largest number of estimated parameters) possible was evaluated first, and then more simplified models were evaluated. The primary interest was in the values of p and c, and then S, and finally the gammas (γ' and γ''). Thus the procedure began in a reverse order, by first testing for evidence of temporary emigration/immigration, then S, then p and c, to yield the "best first-pass model". This best first-pass model was then checked by re-testing the emigration/immigration and S components.

At each stage, the model with the lower Akaike's Information Criteria (AICc) score was chosen if the difference between models was greater than 2. For models with similar AICc (where the difference between them less than 2), the model with fewer parameters was chosen. The parameter values of the best model were then reported. Models that yielded illogical parameter estimates or parameter estimates with confidence intervals potentially indicating non-convergence were excluded.

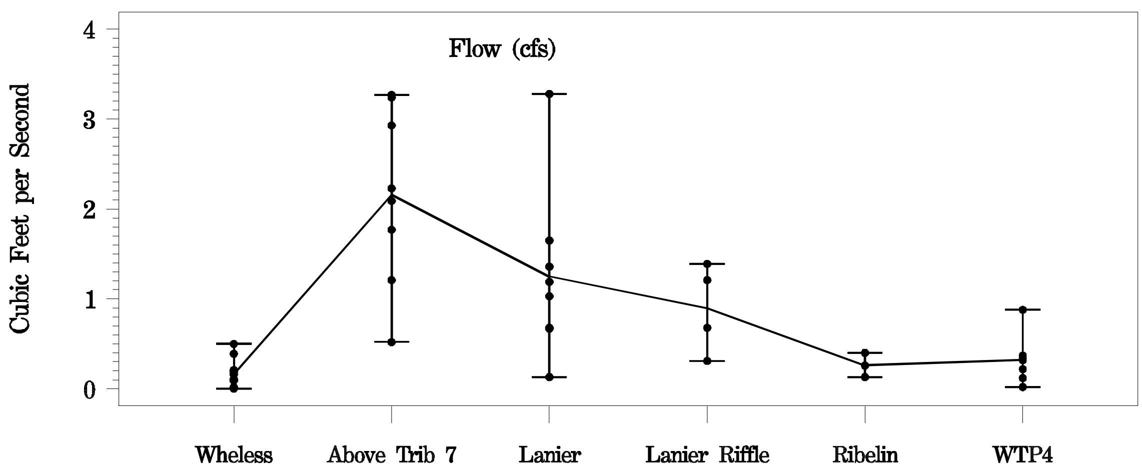
Results and Discussion

Note: Since the primary focus of this report is on the mark-recapture and surface count data, water quality, general habitat and natural history observations for 2007 are provided in Appendix B.

Water Quantity

This study was unique due to the significant amount of rainfall that occurred throughout the 8-month period (March-October 2007), with rain occurring almost every month. Except for the main spring pool at the Wheless Spring site, all of the sites flowed continuously. Wheless Spring was not flowing on the first two days of the first primary period (March 10 and 11), but was flowing on March 12 following rainfall during the previous night. Except for a tiny pool near the head of the spring, the Wheless spring pool was dry again during the October 15-17 surveys. In contrast, flows from the spring pool at Lanier Spring remained fairly consistent (0.03-0.06 cfs) throughout the study. Highest and most variable flows were measured in the creek channel at the most downstream sites, Lanier Spring and Bull Creek Above Tributary 7 (Figure 11).

Figure 11. Water flows (cfs) at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.



Two rain events occurred during the May and September primary periods that altered flow conditions during the Bull Creek mark-recapture surveys. Rains during the morning of May 16 caused the creek to rise and flow over the seine nets at all three mark-recapture sites. Because of the high flows, the few JPS that were found were clinging to tree roots and other vegetation along the banks. Rains during the morning of September 11 resulted in the seine nets being pulled up from the bottom at the Lanier Spring and Lower Ribelin sites.

Spring flow undoubtedly has a major influence on salamander numbers, reproduction, and distribution. For example, flow and dissolved oxygen are critical for development of the eggs and exchange of gases across the gills and skin of amphibians (Boutilier et al. 1992, Duellman and Trueb 1994, Seymour 1999). Previous analyses have found that the pattern of flow and percent of small juvenile JPS is similar but offset by a lag of approximately four months (O'Donnell et al. 2006). Although no direct relationship has been detected beyond presence/absence of spring flows and JPS, the variability in JPS numbers appears to be tied to surface flows. For example, when a spring starts flowing after a dry period, salamanders are presumably re-surfacing from the subsurface and concentrated near the spring outlet. With sustained flows, this initial peak in numbers is followed by a decrease as individuals disperse away from the springs. As flows begin to subside, individuals begin moving back toward the springs, resulting in another peak in numbers, followed by a decrease as flows dissipate. This pattern of JPS numbers in response to surface flow and other habitat conditions was observed at all of the spring sites (Figures 12-16). Numbers also declined following flooding events at the downstream sites, particularly Bull Creek Above Tributary 7. The most upstream sites, WTP4 and Wheless Spring, are more susceptible to spring flow cessation.

Figure 12. Rainfall, flow, and *Eurycea tonkawae* surface count data for WTP4, 2007. No surveys were conducted in May or August 2007 due to flooding rains. Small = individuals <25 mm total length (TL); Medium = individuals 25-50 mm TL; Large = individuals >50 mm TL.

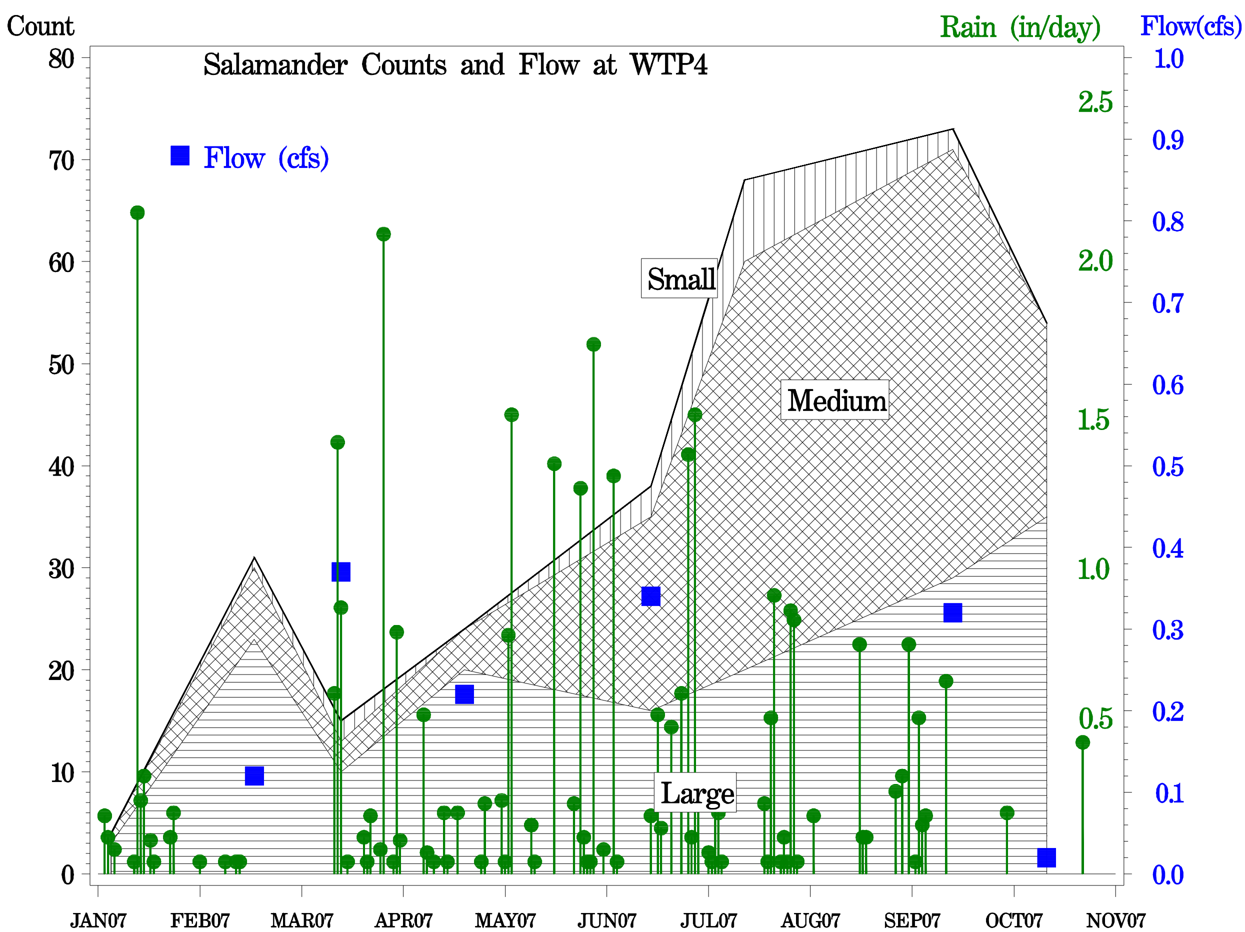


Figure 13. Rainfall, flow, and *Eurycea tonkawae* surface count data for Bull Creek Above Tributary 7, 2007. No surveys were conducted in May or August 2007 due to flooding rains. Small = individuals <25 mm total length (TL); Medium = individuals 25-50 mm TL; Large = individuals >50 mm TL.

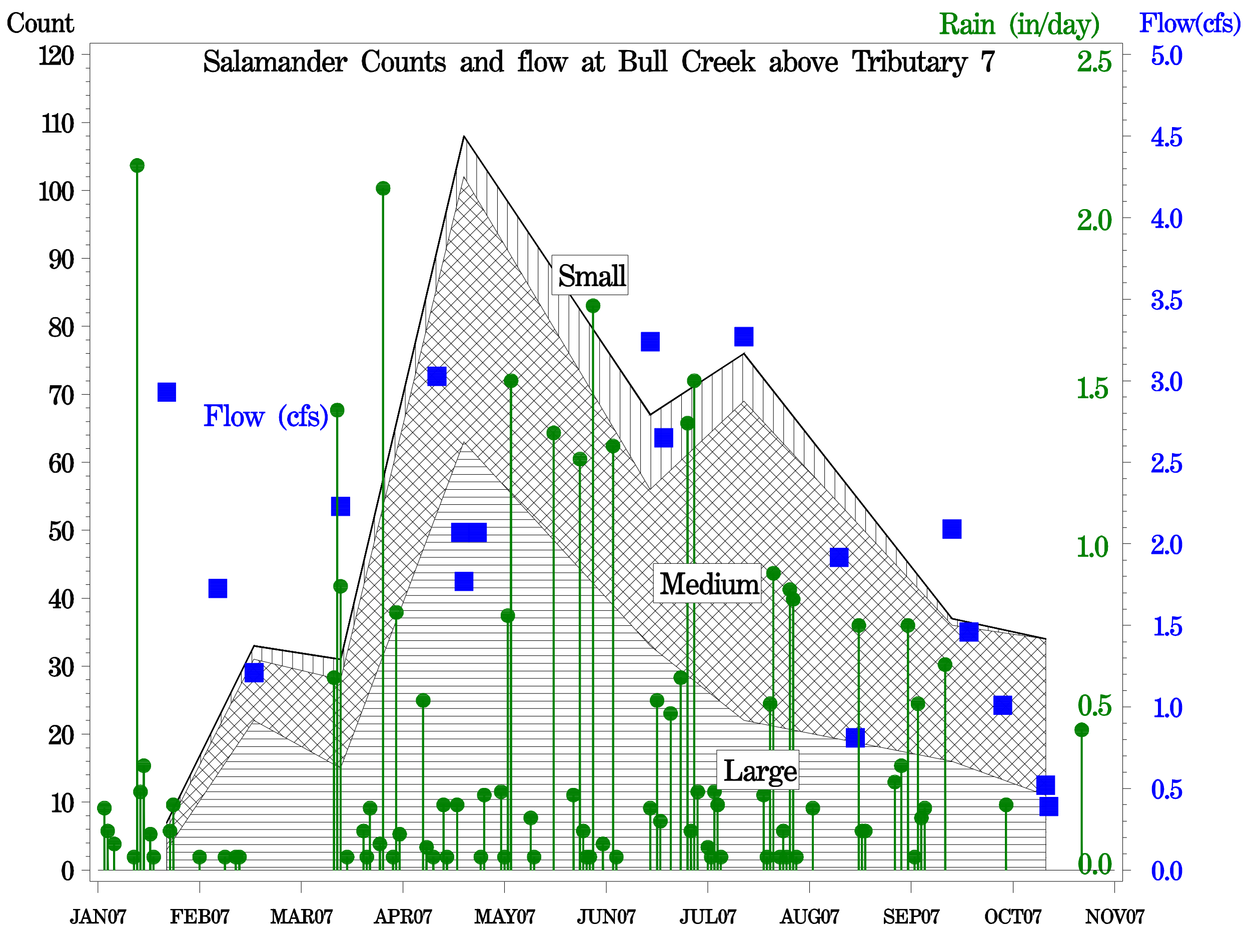


Figure 14. Rainfall, flow, and *Eurycea tonkawae* counts from the first day of each primary session of mark-recapture at Lanier Spring, March-October 2007. All data prior to March 2007 were from surface count surveys. Small = individuals <25 mm total length (TL); Medium = individuals 25-50 mm TL; Large = individuals >50 mm TL.

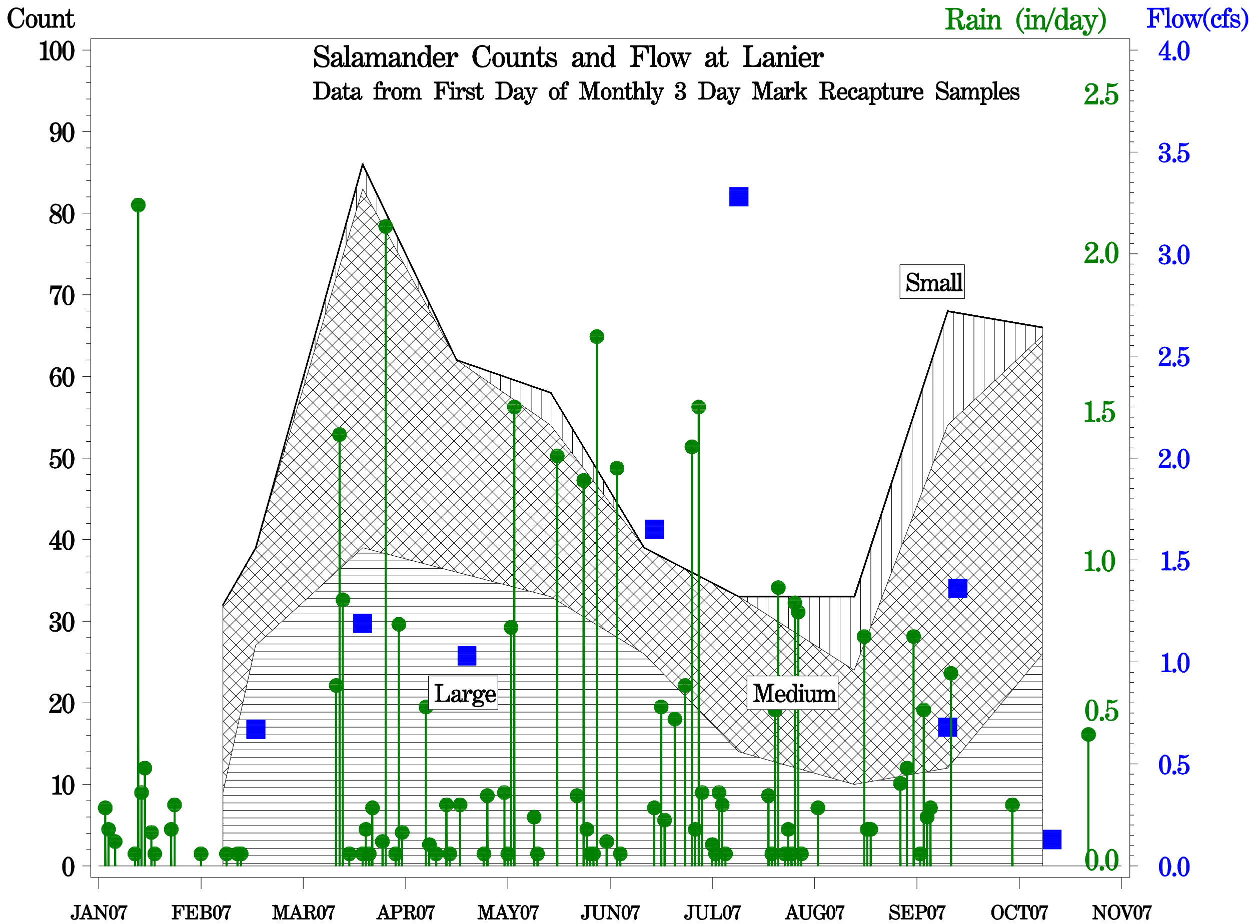


Figure 15. Rainfall, flow, and *Eurycea tonkawae* counts from the first day of mark-recapture at Lower Ribelin, May-October 2007. Small = individuals <25 mm total length (TL); Medium = individuals 25-50 mm TL; Large = individuals >50 mm TL.

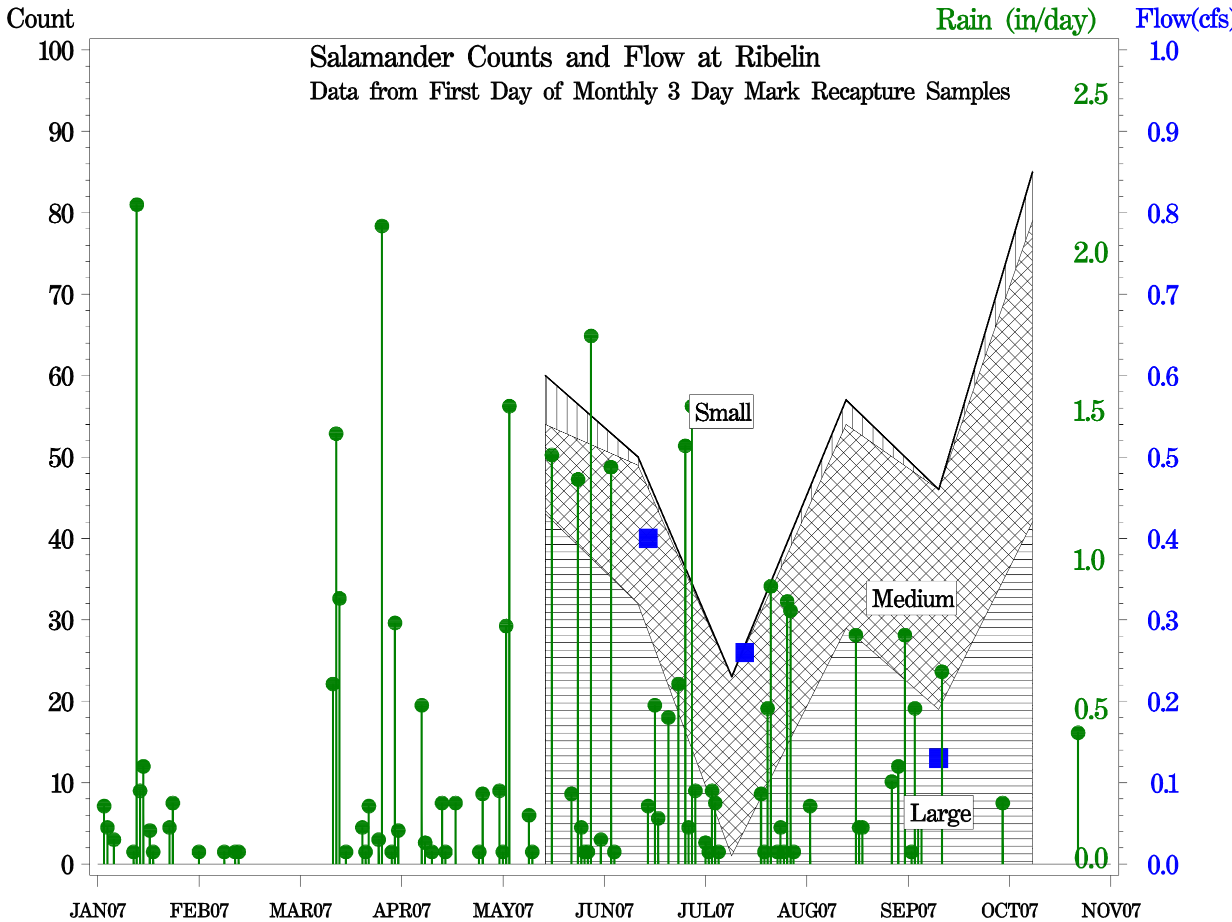
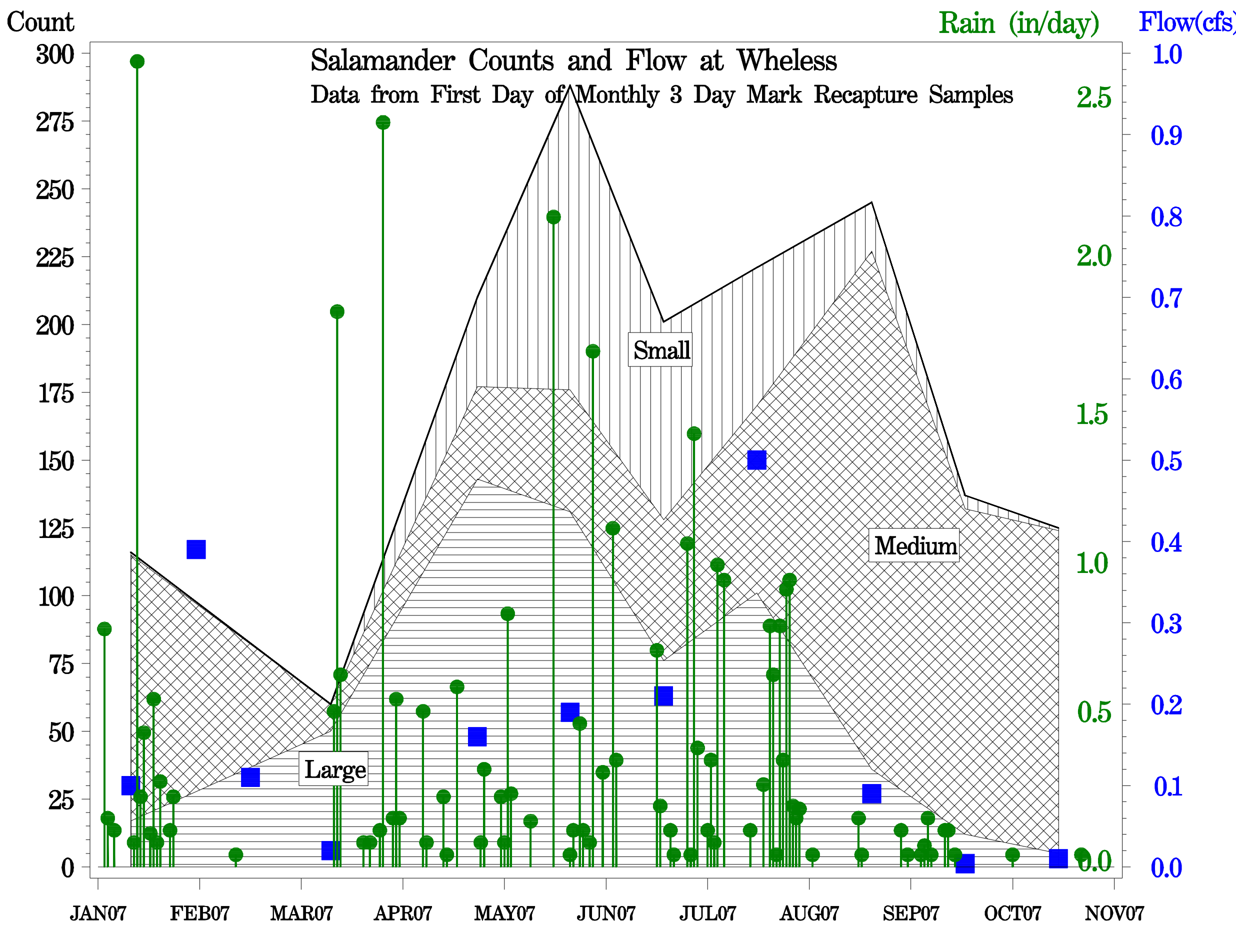


Figure 16. Rainfall, flow, and *Eurycea tonkawae* counts from the first day of mark-recapture at Wheless Spring, March-October 2007. All data prior to March 2007 were from surface count surveys. Small = individuals <25 mm total length (TL); Medium = individuals 25-50 mm TL; Large = individuals >50 mm TL.



Surface Counts and Data from First Day of Mark-Recapture Surveys

For the purpose of comparing surface count and mark-recapture data, the total number of JPS observed on the first day of each primary period of the mark-recapture surveys, including the total collected for mark-recapture and small juveniles, was used as the surface count equivalent. Surface count surveys were intended to provide an additional control to evaluate whether mark-recapture had a negative effect on the study populations (O'Donnell et al. 2005). For example, if sites monitored using mark-recapture sampling had steadily declining salamander numbers while sites monitored using surface counts were stable or increasing, this could indicate a negative impact of mark-recapture sampling on population numbers. However, this pattern was not observed, and each site appeared to respond to its own unique set of variables (figures 17-22). Thus, no negative effects of mark-recapture could be discerned based on a comparison of the mark-recapture and surface count data.

Figure 17. *Eurycea tonkawae* surface counts (Bull Creek Above Tributary 7, WTP4) and counts from the first day of mark-recapture sampling (Lanier Spring, Lower Ribelin, Wheless Spring), 2007. All data prior to March 2007 were from surface count surveys.

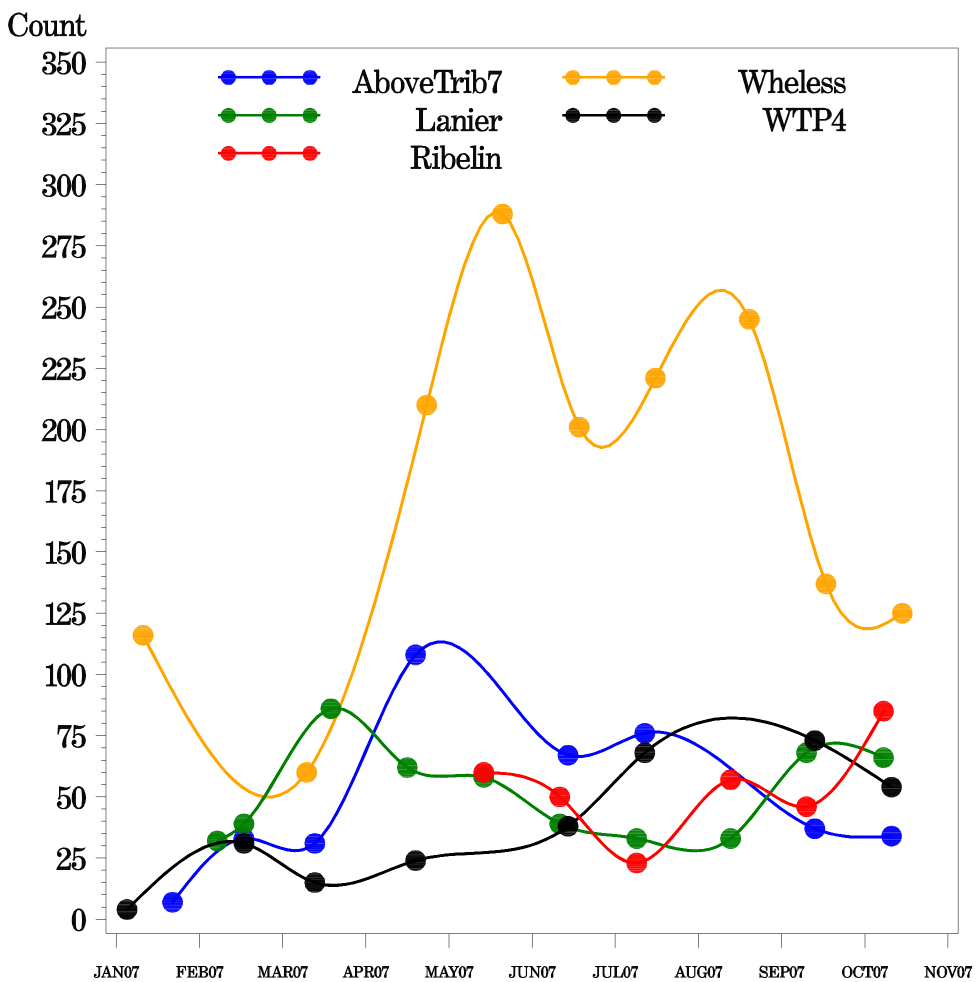


Figure 18. *Eurycea tonkawae* surface count data, 2007. No surveys were conducted in May or August 2007 due to flooding rains.

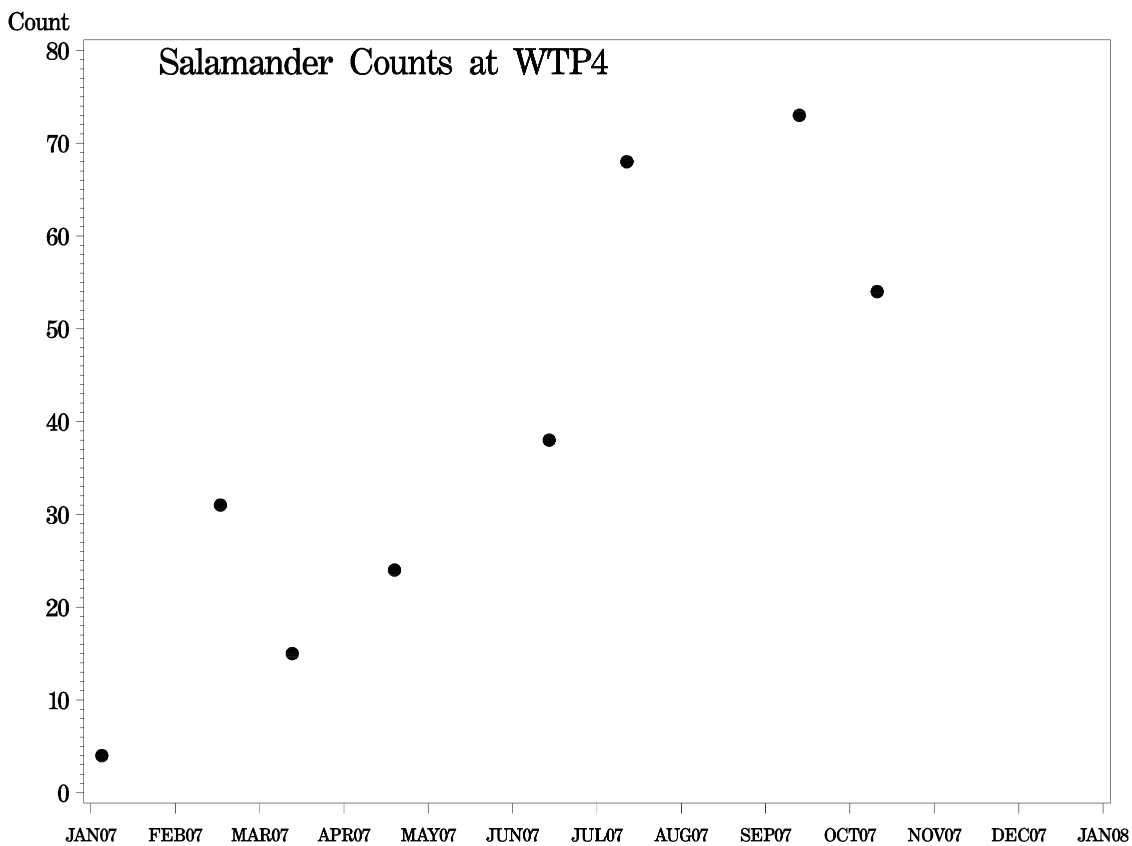


Figure 19. *Eurycea tonkawae* surface count data for Bull Creek Above Tributary 7, 2007. No surveys were conducted in May or August 2007 due to flooding rains.

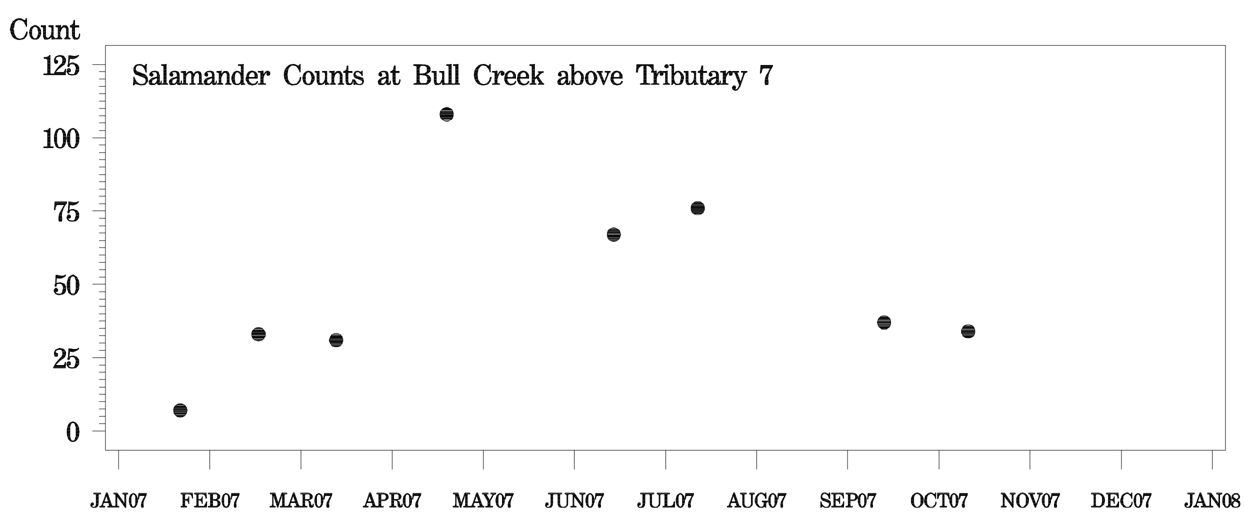


Figure 20. *Eurycea tonkawae* counts from the first day of mark-recapture at Lanier Spring, March-October 2007. Data prior to March 2007 were from surface count surveys.

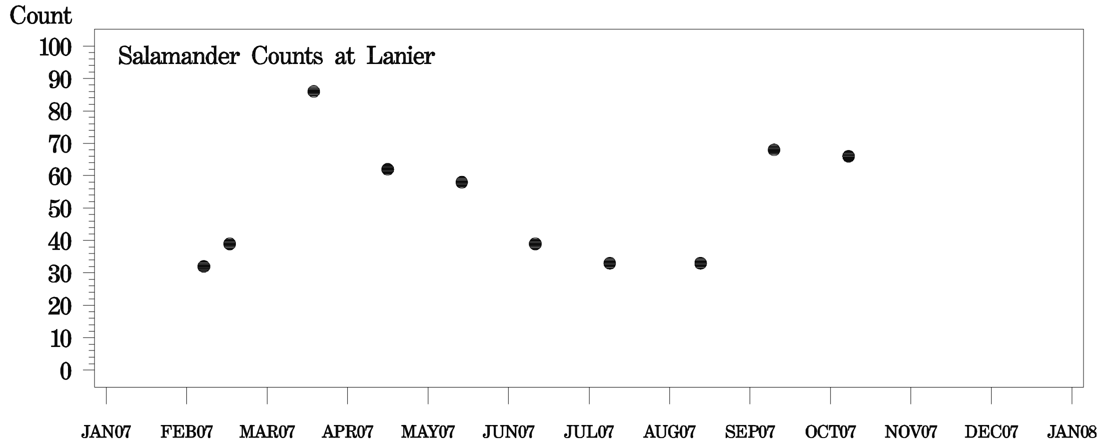


Figure 21. *Eurycea tonkawae* counts from the first day of mark-recapture at Lower Ribelin, May-October 2007.

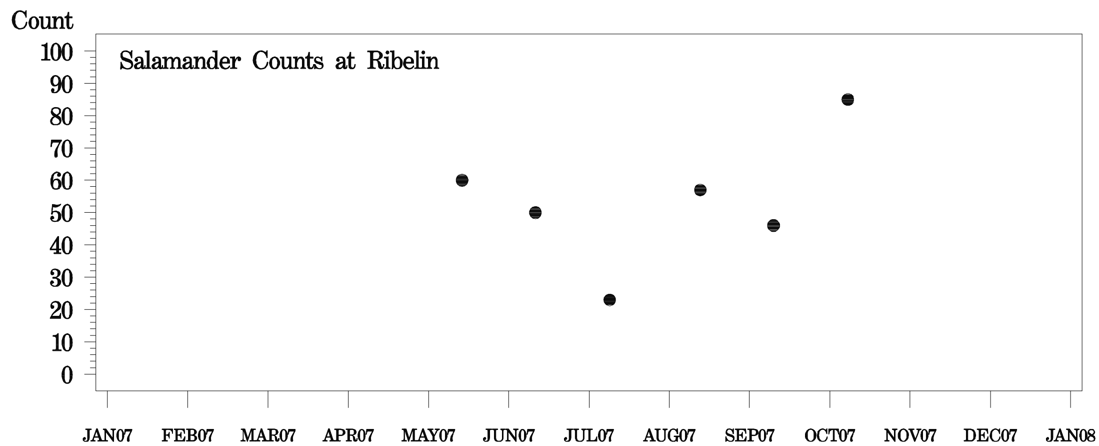
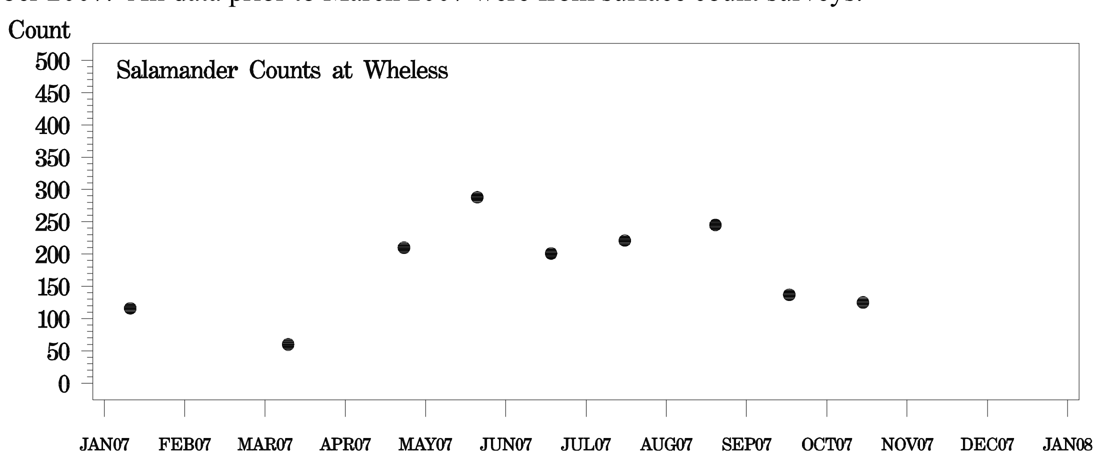


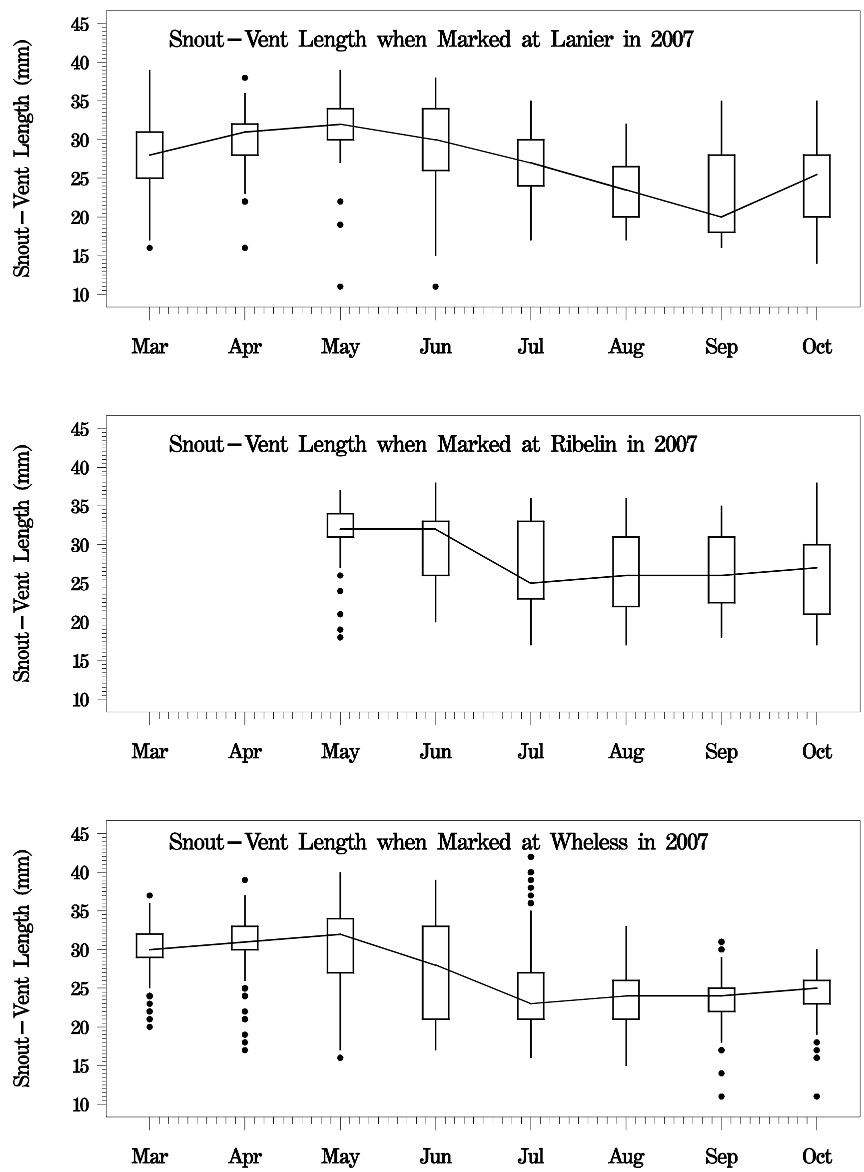
Figure 22. *Eurycea tonkawae* counts from the first day of mark-recapture at Wheless Spring, March-October 2007. All data prior to March 2007 were from surface count surveys.



Reproduction and Recruitment

The increase in JPS numbers during the latter part of the study appears to have been influenced by reproduction and recruitment at most of the monitoring sites. This was most pronounced at the Wheless Spring site, which had the largest numbers of small juveniles, with highest counts in May and June (Figure 16; Appendix D). For the three mark-recapture sites, the numbers of small juveniles seen in late spring and early summer was followed by a decrease in average size of initial captures compared to the beginning of the study (Figure 23), which is attributed to the recruitment of small juveniles into the size classes that were large enough to mark (i.e., >25 mm TL).

Figure 23. Box plots of snout-vent lengths for initial captures of *Eurycea tonkawae* at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. The line connecting each plot is drawn through the mean.



Individual Growth

For Lanier Spring, Lower Ribelin, and Wheless Spring, SVLs ranged from 14 mm to 37 mm (Figure 24), and TLs ranged from 24 mm to 72 mm (Figure 25). As expected, growth rates were highest for the smaller size classes and rates decreased with increasing size. Assuming JPS are about 15 mm TL at hatching, and growth rates are about 8-9 mm per month for individuals ranging from 15-20 mm (Figure 25), JPS could be recruited into the large juvenile class (25-50 mm) within one to two months. Individuals in the large juvenile size class had about half the growth rate of small juveniles. For example, an individual with a TL of 30 mm had an average growth of about 5.6 mm per month.

Figure 24. Estimated growth rate in snout-vent length (SVL) per month (30 days) for *Eurycea tonkawae* caught at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. The average monthly growth rate for an individual with a 20mm SVL is 2.2mm. Negative values likely reflect measurement error.

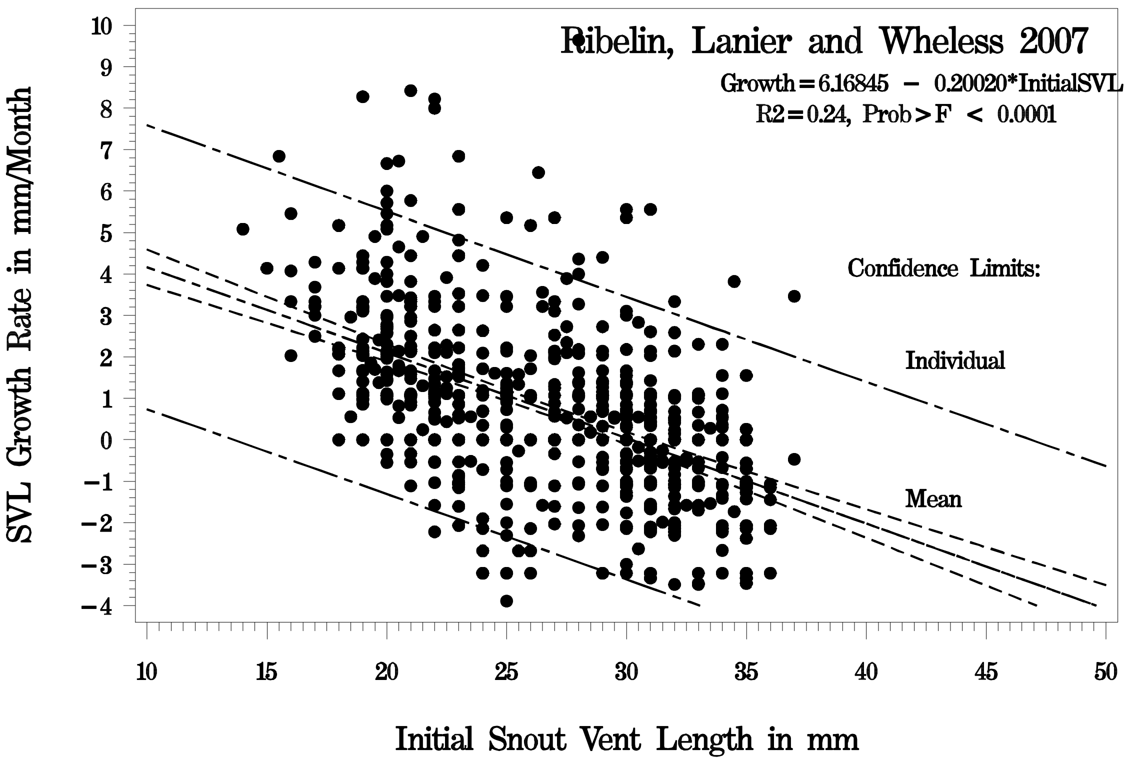
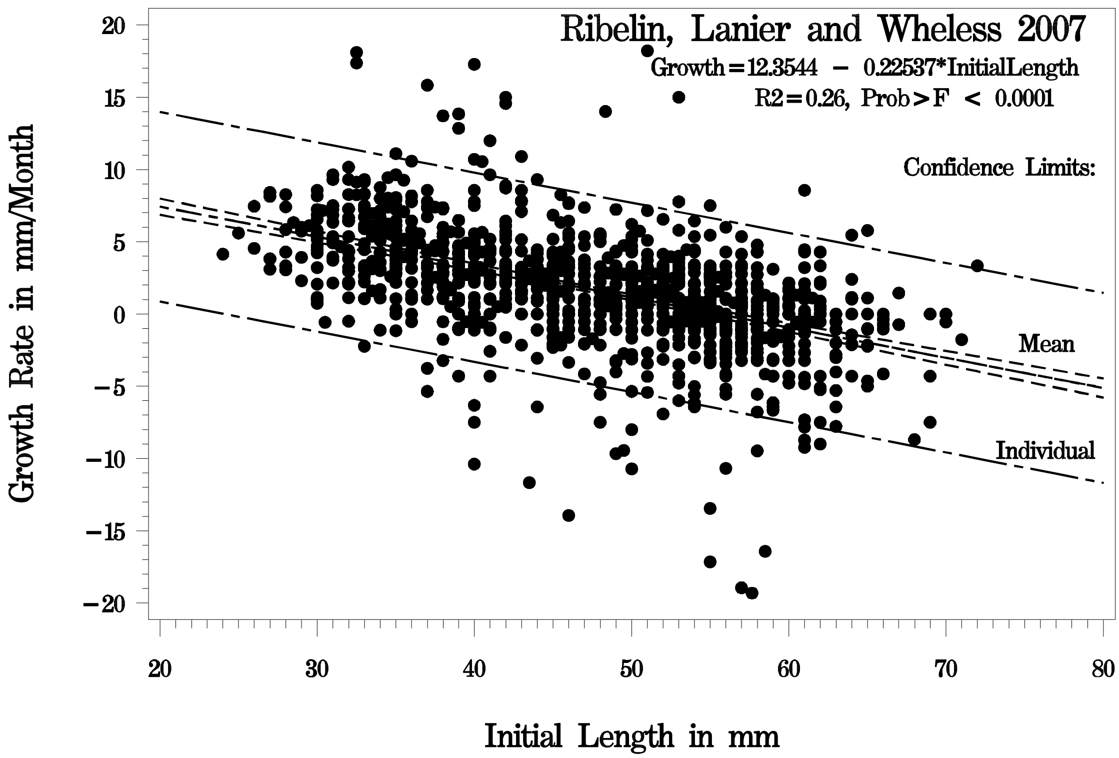


Figure 25. Estimated growth rate in total length (TL) (mm) per month (30 days) for *Eurycea tonkawae* caught at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. Negative values likely reflect measurement error.



Mark-Recapture

Initial Captures and Recaptures

Over the 8-month study period, a total of 2,185 JPS were caught and marked with VIE, including 463 at Lanier Spring, 20 at Lanier Riffle, 281 at Lower Ribelin, and 1,421 at Wheless Spring (Appendix D). In addition, 14 small juveniles (10 at Lanier Spring and 4 at Wheless Spring) were given "batch" marks during the March and April surveys.

Within each primary period, the number of "initial captures" (unmarked animals captured and marked) was generally highest the first day and decreased by about 10-20% on each subsequent day (Figure 26). Conversely, the number of recaptured animals was generally lowest the first day of each primary period, and typically increased on subsequent days (figures 26-29). Following the first month of surveys, recaptures throughout the study period ranged from 35-84% at Lanier Spring, 31-85% at Lower Ribelin, and 19-67% at Wheless Spring (Appendix D).

Rainfall had an obvious influence on the number of initial captures and recaptures on several occasions (figures 27-29). For example, at the Wheless Spring site, the number of initial captures was highest on the third day of the first primary period, March 12, when the main spring pool began flowing again after a major rain event. At the Bull Creek mark-recapture sites, heavy rainfall on the mornings of May 16 and September 11 resulted in lower numbers of both initial captures and recaptures.

Figure 26. Total numbers of *Eurycea tonkawae* initial captures ("mark") and recaptures at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007, across all primary sampling periods, by secondary sampling period.

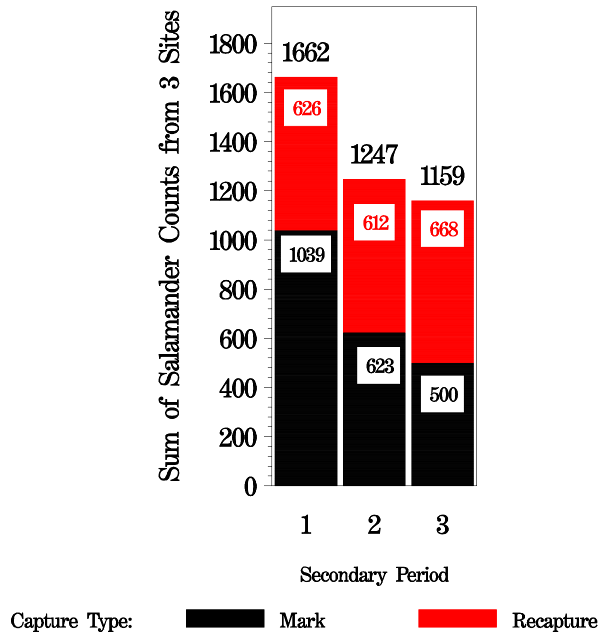


Figure 27. Numbers of *Eurycea tonkawae* initial captures ("mark") and recaptures at Lanier Spring, 2007. Major rain events occurred between the second and third days of the May primary period (May 16) and between the first and second days of the September primary period (September 11). The recaptures in March include individuals recaptured from the March 13 survey, which was discontinued due to weather.

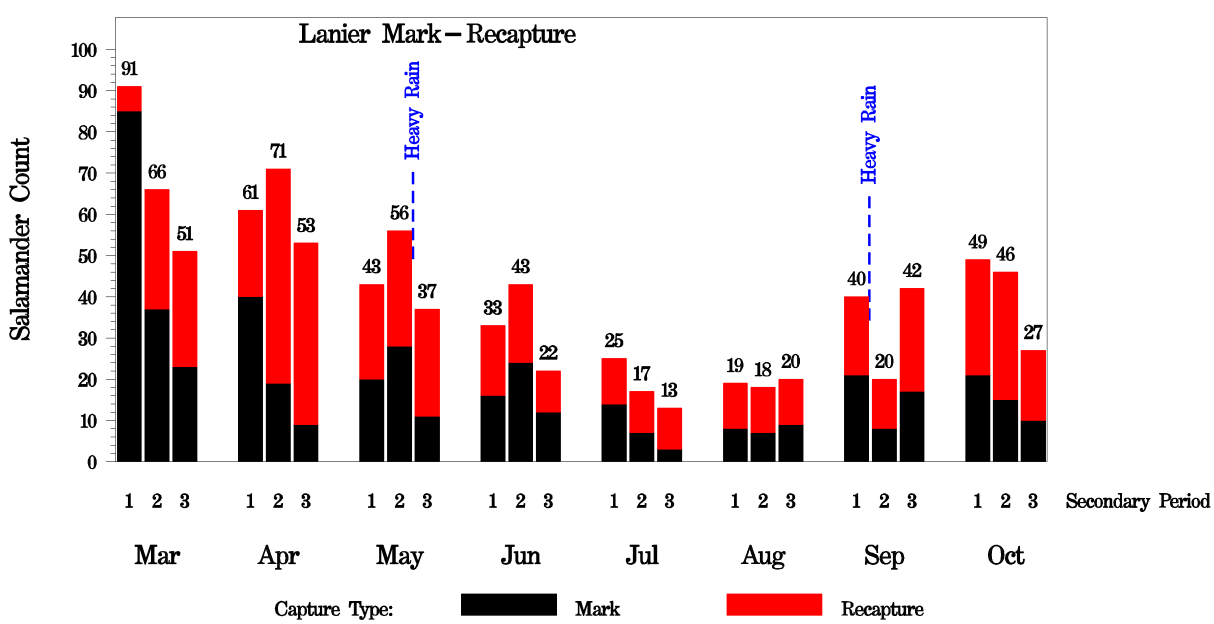


Figure 28. Numbers of *Eurycea tonkawae* initial captures (“mark”) and recaptures at Lower Ribelin, 2007. Major rain events occurred between the second and third days of the May primary period (May 16) and between the first and second days of the September primary period (September 11).

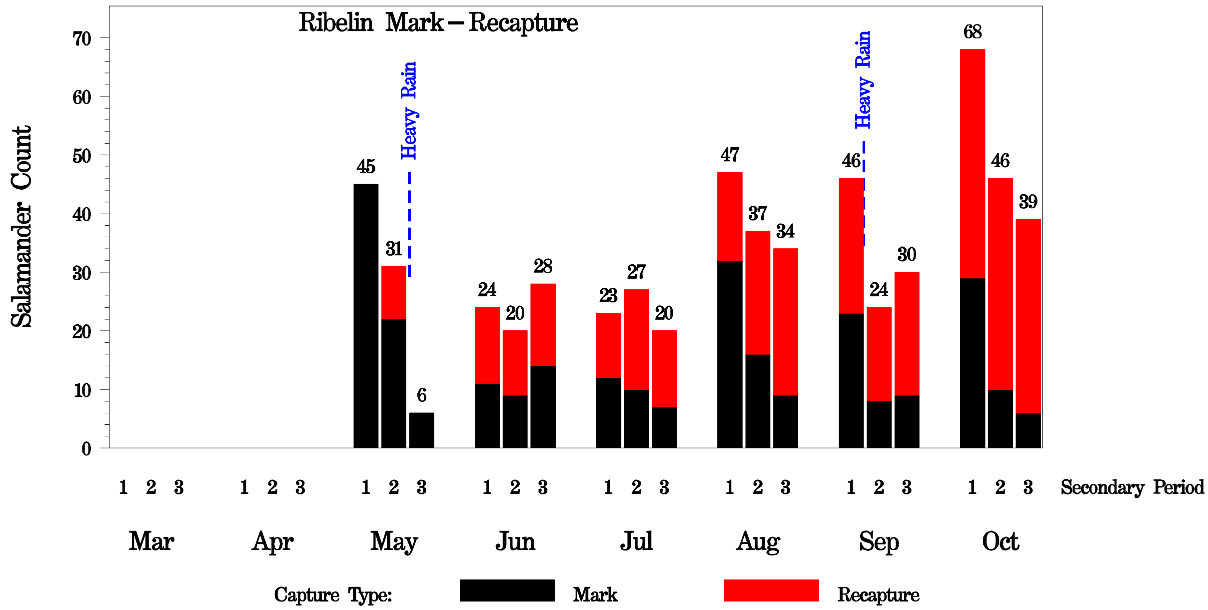
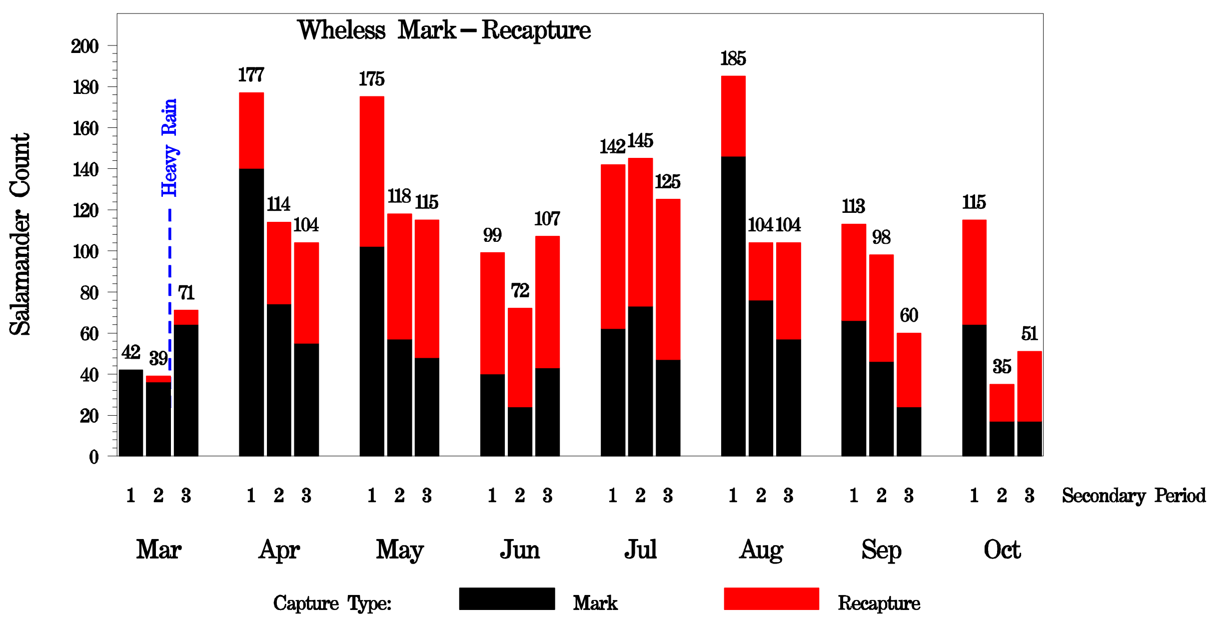


Figure 29. Numbers of *Eurycea tonkawae* initial captures (mark) and recaptures at the Wheless Spring, 2007. A major rain event occurred between the second and third days of the first primary period (March 12). Incomplete surveys were conducted on the second and third days of the March primary period. On the first day of the August primary period (August 20), >29 unmarked and >15 recaptured individuals had to be released before processing.



Frequency of Capture
The number of times individual JPS were caught was consistent across the three mark-recapture sites (Figures 30 and 31). The majority of individuals were caught only one time (42-54%) and in only one primary period (55-63%). The disappearance of JPS following initial capture suggests a "trap-shy" behavioral response (i.e., animals that were not seen again were assumed to have moved beyond the study area or became better at escaping capture). About 10% of the JPS that were caught in more than one primary period had a lapse of at least one month when they were not observed (Table 5). Allowing at least 2 to 3 months between primary periods (i.e., quarterly) could reduce the trap-shy response and increase the number of recaptures. This is consistent with recapture percentages for 2008, which were over 60% for the first day at all three sites following a lapse of 4 to 5 months (Appendix D).

Table 5. Number and percent of *Eurycea tonkawae* observations with at least one month where salamanders were not observed for Lanier Spring, Lower Ribelin, and Wheless Spring, 2007.

# Observations				
# Months between observations w/ at least 1 month missing	**Lanier**	**Wheless**	**Ribelin**	**Total**
2	46	129	44	*219*
3	29	47	5	*81*
4	2	8	4	*14*
5	4	2	1	*7*
6	3	0	0	*3*
7	1	0	0	*1*
Total # observations	*751*	*2130*	*476*	*3357*
% of Total Observations				
# Months between observations w/ at least 1 month missing	**Lanier**	**Wheless**	**Ribelin**	**Total**
2	6.1	6.1	9.2	*6.5*
3	3.9	2.2	1.1	*2.4*
4	0.3	0.4	0.8	*0.4*
5	0.5	0.1	0.2	*0.2*
6	0.4	0.0	0.0	*0.1*
7	0.1	0.0	0.0	*0.0*
Total	*11.3*	*8.7*	*11.3*	*9.7*

Figure 30. Frequency distribution of total *Eurycea tonkawae* captures throughout the 8-month study at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007.

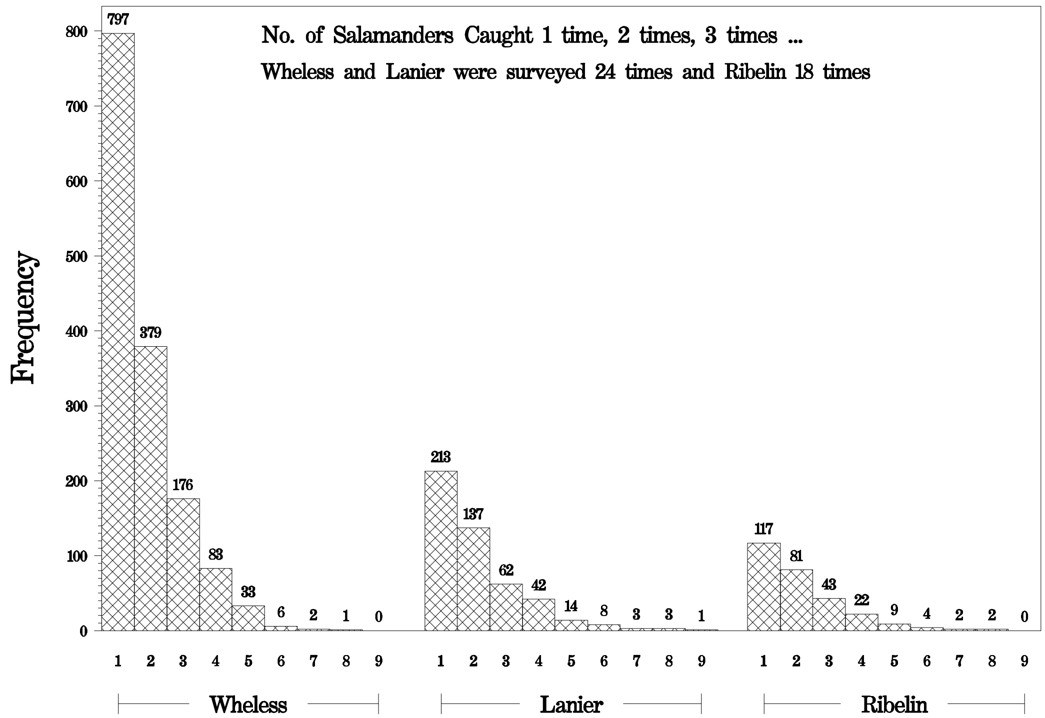
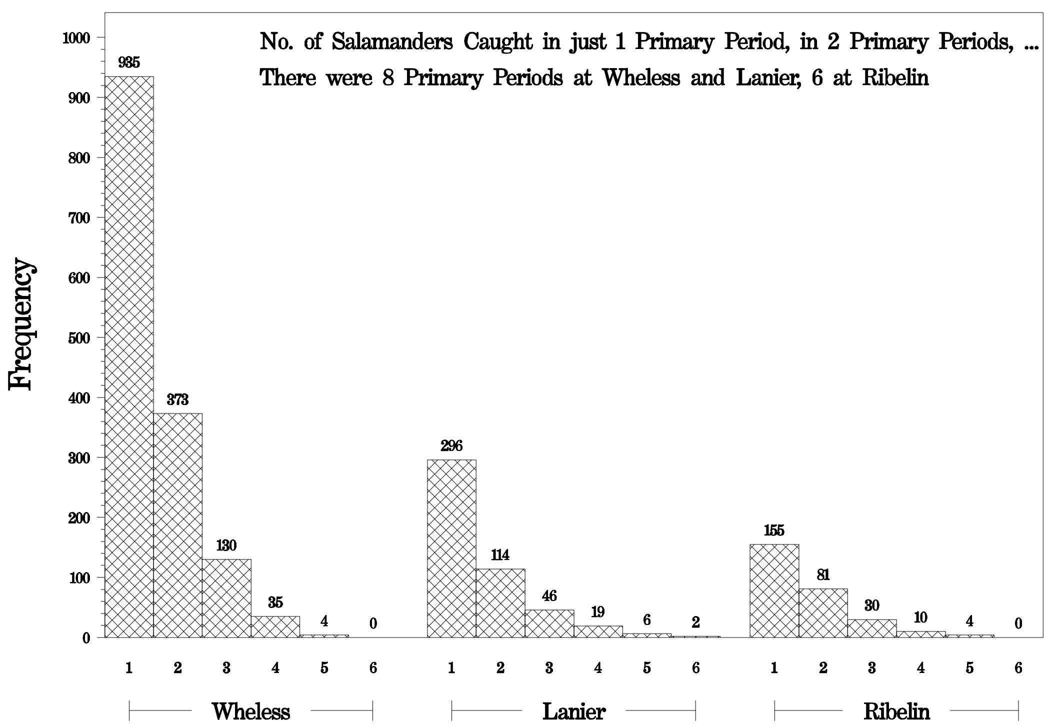


Figure 31. Frequency distribution of number of primary sampling periods in which *Eurycea tonkawae* were captured, at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007.



Assumption of Closure Within Primary Periods **-** *Field Evidence*
Over the 8 primary periods for all three study sites, there were only 15 confirmed violations of closure due to horizontal (surface) movement where marked individuals were captured both inside and outside the net-enclosed study area within the same primary period. This represents less than one percent of the total unique captures (15/2342). Documented closure violations included 4 marked individuals at Lanier Spring and 11 marked individuals at Wheless Spring. No closure violations were documented at the Lower Ribelin site. The low number of salamanders that were found both inside and outside of the nets within the same primary period indicates reasonably good horizontal closure. Since closure violations due to vertical (surface to subsurface) movement could not be controlled or directly quantified, statistical analyses of closure within primary periods were conducted and are presented in Appendix E.

Model Selection - MARK
The best MARK models for Lanier Spring, Wheless Spring, and Lower Ribelin sites can be seen in Appendix F. Initial analysis in CAPTURE suggested that capture (p*)* and recapture (*c)* parameters vary both within and between every primary sampling period (Appendix E). Parameters in such models, however, frequently did not converge (i.e., some parameters were not defined). As a result, more reduced models were selected by choosing models in which p and c were set to a constant, average value within primary sampling periods. To further facilitate convergence of estimated parameters, MARK was run using the Huggins method (1989, 1991).The best models from each location were subject to a goodness of fit test using RDSURVIV.

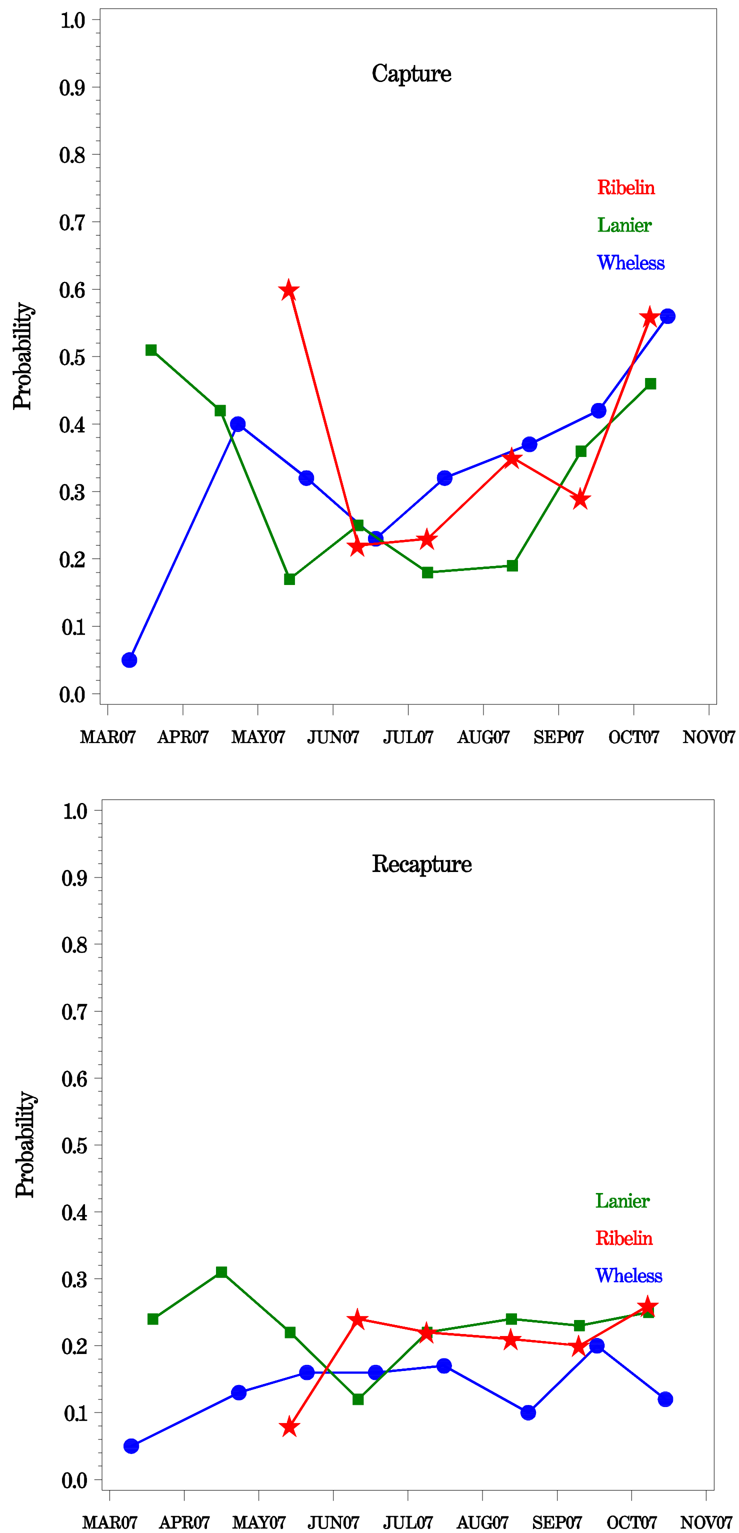
Detection (Capture and Recapture) Probabilities
The capture history at every site supported models where capture (p) and recapture (c) probabilities were distinct from one another (i.e. $p \neq c$) and varied between primary sampling periods. Capture probabilities (p) during the first primary period in March varied between 0.05 and 0.56 (mean = 0.33) at Wheless Spring, 0.17 and 0.51 (mean = 0.32) at Lanier Spring, and 0.22 and 0.60 (mean = 0.38) at Ribelin Spring. Except for the first primary period at Wheless, capture probabilities were at their lowest during the summer months for all three sites (Figure 32).

The capture probability for the first primary period during March at Wheless Spring was problematic due to the very few individuals from day 1 that were recaptured on either day 2 or 3, and very few individuals from day 2 that were recaptured on day 3. Additionally, some of the identities of the recaptures could not be confirmed through QA/QC due to poor or missing photos. Of the 42 initial captures on day one, only five were recaptured with confirmed identity (two individuals on day 2 and three on day 3), and of the 39 animals captured on day two, only one individual was recaptured with confirmed identity on day 3. Because MARK was not able to estimate p for that period, it was fixed at 0.05 based on the actual number of recaptures. The low p value for the first primary session had no effect on the model selection process and had only very minor effects on the population size estimates for the April-October periods. Excluding the March p value of 0.05, capture probabilities for Wheless ranged from 0.23 to 0.56 (mean = 0.37).

Recapture probabilities (c) were almost always lower than p, varying between 0.05 and 0.2 (mean = 0.14) at Wheless Spring, 0.12 and 0.31 (mean = 0.23) at Lanier Spring, and 0.08 and 0.26 (mean = 0.20) at Lower Ribelin (Figure 32). The lower recapture values likely reflect a behavioral response to being captured, as suggested by CAPTURE analyses (Appendix E). It is possible, for example, that JPS are more likely to retreat into subterranean refuges following capture and handling. Bailey et al. (2004a-c) observed a similar trap-shy response in terrestrial salamander populations. Recapture probabilities were only slightly higher than capture probabilities during one of the eight primary periods at Wheless Spring (June), three of the eight primary periods at Lanier Spring (May, July, August), and two of the six primary periods at Lower Ribelin (June, July). This suggests that p and c values should not be equal in selected models.

Capture (p) and recapture (c) probabilities for each primary period are listed in Table 6, and means for the study period are shown in Figure 34.

Figure 32. Estimated *Eurycea tonkawae* detection (capture and recapture) probabilities by month for Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. The probabilities for the March primary period at Wheless could not be estimated using MARK due to the low number of recaptures and were fixed at 0.05.

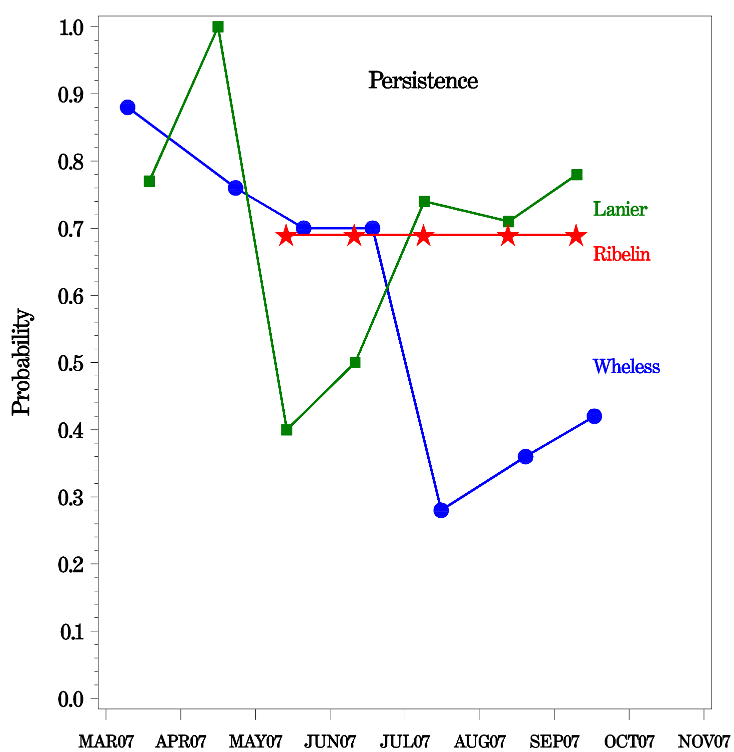


Persistence

Overall, models with persistence estimates that varied from month-to-month performed better than models with constant persistence estimates at Wheless Spring and Lanier Spring. Estimated persistence varied from 0.28 to 0.88 (mean = 0.51) at Wheless Spring and 0.4 to 1.0 (mean = 0.70) at Lanier Spring (Table 6, figures 33 and 34). Persistence was lowest at Wheless Spring in July and at Lanier Spring in May. With the short duration of this study and limited opportunity to survey outside of the study areas, it is not possible to know whether animals that were marked and not seen again died or permanently emigrated from the study area. Thus, in this study, the survival parameter is referred to as persistence. A possible interpretation of the variation in persistence values and low values during summer months is that they reflect emigration and immigration following seasonal water flows. Given the low observed mortality, potential longevity of closely related species (USFWS 2005), presence of few predators in the study areas, and observations of marked JPS beyond the study area, most of the animals that were marked and not seen again were assumed to have emigrated from the study area.

For Lower Ribelin, a model with a constant persistence across primary sampling periods performed best, and monthly persistence was estimated to be 0.69. This means that there was a 69% chance that an animal marked one month would be in the study area and available for capture in the following month. The more discrete nature of the habitat at Lower Ribelin (Appendix A) likely limited the amount of dispersal along the stream bed, and thus, the amount of immigration and emigration that could take place.

Figure 33. Estimated persistence for *Eurycea tonkawae* by month for Lanier Spring, Lower Ribelin, and Wheless Spring, 2007.



Temporary Migration (Emigration and Immigration)

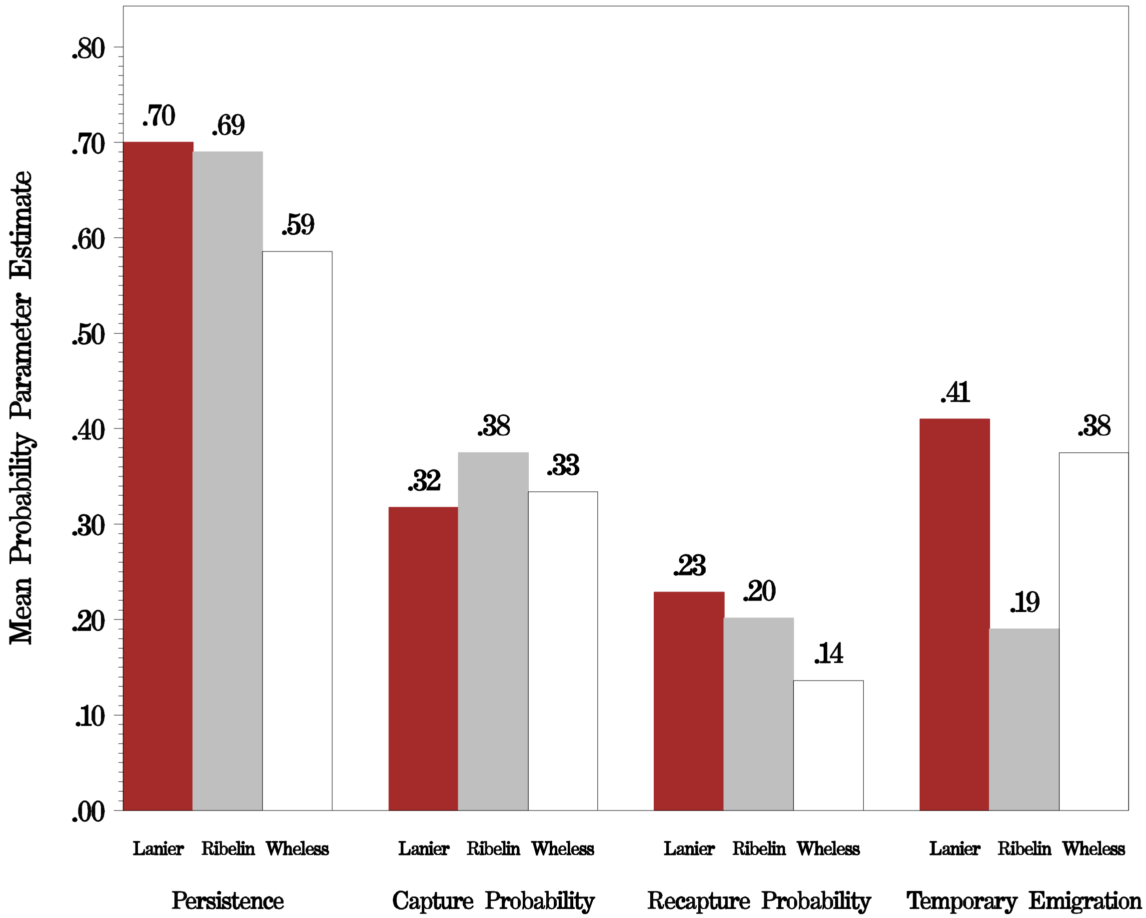
The most parsimonious MARK models suggest that movement into and out of the survey sites between primary sampling periods was random at Wheless Spring, Lanier Spring, and Lower Ribelin. This means that the probability of an animal's movement into or out of a study site was not conditional on where it was located during the previous primary sampling period. Thus, temporary emigration is hereafter referred to as temporary migration to encompass both movement parameters.

Temporary migration estimates were held constant at 0.38, 0.41, and 0.19 for Wheless Spring, Lanier Spring, and Lower Ribelin, respectively (Figure 34, Table 6). These temporary migration values reflect the habitat characteristics of each site. At the Wheless Spring and Lanier Spring sites, suitable surface habitat extends both above and below the survey areas, allowing animals to move horizontally beyond the survey area between primary periods. The temporary migration estimates suggest that approximately 40% of the animals at these two sites marked during one primary sampling period were unavailable for capture during the subsequent primary sampling period. Thus, temporary migration may have been due to upstream, downstream, or vertical (surface to subsurface) movement with respect to the study area. In contrast, the habitat at the Lower Ribelin site is discrete and naturally bounded by stream segments of bedrock with little or no protective cover (Appendix A). This is reflected in the lower temporary migration value, indicating that only a small percentage (19%) of the animals at this site was unavailable for capture during any primary sampling period.

Temporary emigration estimates may actually vary over time considering field observations of salamander abundances before and after drying events, but there is insufficient data at present to estimate a convergent model with time-varying p, unequal p and c, and time-varying emigration/immigration parameters.

Closed-population and open-population models should yield unbiased estimates of the "superpopulation" if the temporary emigration is completely random (Kendall et al. 1997). Completely random temporary emigration implies that, at any given time, the surface population is a random sample of the "superpopulation" (Kendall et al. 1997). Random temporary emigration for all three sites allows for estimation of the superpopulation size, which is discussed below.

Figure 34. Estimated mean persistence, capture probability, recapture probability, and temporary emigration for *Eurycea tonkawae* at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. The mean capture and recapture probability estimates for Wheless Spring include the March primary period, which was problematic and fixed at 0.05 based on the actual number of recaptures.



Surface Population Size

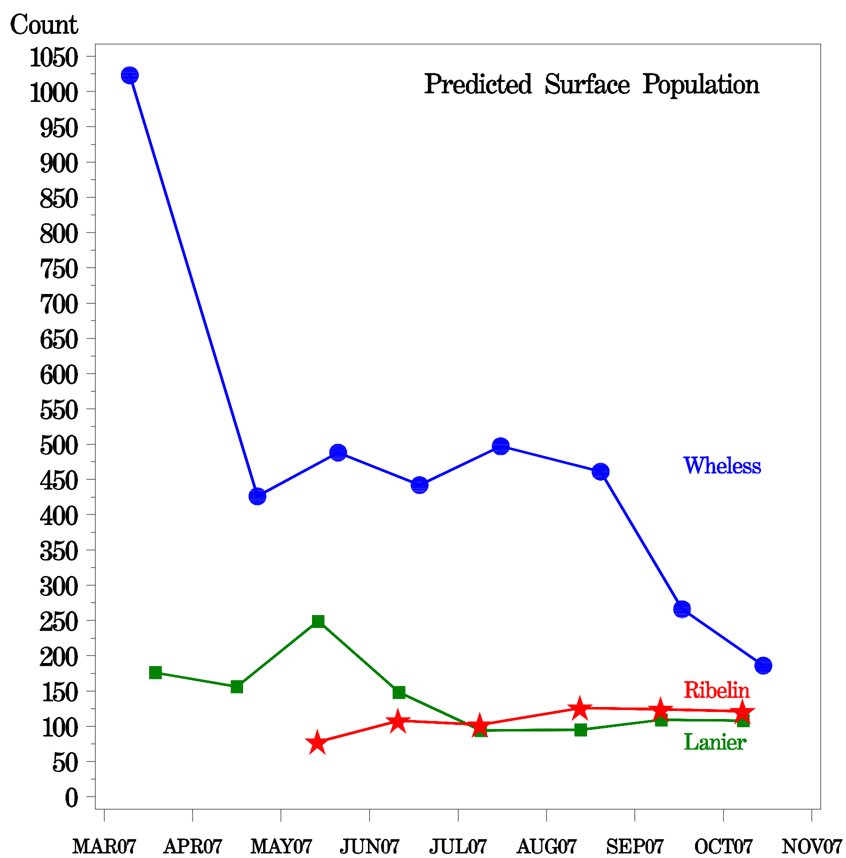
The estimated surface population size for each primary period comprised all JPS that were in a given area available for capture, namely the surface area between the two nets that defined each sampling area. The surface population does not reflect the superpopulation, which consists of all the JPS that could potentially end up in a sampling site, including individuals from the subsurface, during any given sampling period. Estimated surface population sizes within each site varied from month to month and are presented in Figure 35 and Table 6. The means are shown in Figure 37.

Surface population estimates at the Wheless Spring site averaged 474 and varied from 187 (95% confidence intervals (CI): 178-207) during the last month of the study (October) to 1024 (95%CI: 883-1191) during the first month of the study (March). Since MARK was unable to estimate p for the first month, the estimate of 1024 is questionable. Excluding this data point, the mean surface population estimate for the Wheless Spring study site is 396, ranging from 187 in October to 497 (95% CI: 178-591) in July. Looking at the period with the highest and most consistent spring flows (April - August), the estimated surface population size was fairly stable (varied from 426 to 497 (95% CI: 368-593)) and averaged 463.

Surface population estimates at the Lanier Spring site averaged 142 and varied from 94 and 95 (95%CI: 59-197) during July and August to 249 (95% CI: 184-367) in May.

Surface population estimates at the Lower Ribelin site averaged 110 and had the least amount of variation, from 78 (95%CI: 74-91) during the first month of study (May) and 121 to 126 (95%CI: 99-179) from August through October.

Figure 35. Estimated surface population sizes of *Eurycea tonkawae* by month for Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. The estimated surface population for the March primary period at Wheless is questionable, since MARK was unable to estimate the capture probability.



Surface Density Estimates

Approximate wetted areas under bankfull conditions for the primary study areas, which does not include the closure assumption check areas, are 85 m^2 (919 ft^2) for Wheless Spring, 60 m^2 (650 ft^2) for Lanier Spring, and 51 m^2 (555 ft^2) for Lower Ribelin (Appendix A). For JPS that were large enough to mark (large juveniles and adults), this would translate to rough surface density estimates of 5.0 to 5.8 (mean = 5.4) animals/m^2 for Wheless Spring (excludes months of March, September, and October due to drier conditions), 1.6 to 4.2 (mean = 2.4) animals/m^2 for Lanier Spring, and 1.5 to 2.5 (mean = 2.2) animals/m^2 for Lower Ribelin. Because wetted areas were variable and not estimated during each month of this study, these estimates should be interpreted with caution. However, the data suggest that of the three study areas, Wheless Spring supports the highest JPS population density.

Superpopulation Size

The "superpopulation" represents the total population within the surface and subsurface associated with the study area (Bailey et al. 2004a, Kinkead and Otis 2007). It includes both animals available for capture, which is represented by the estimated population size for any given primary period, and those animals unavailable for capture, but that move into or out of the study area at random within any given primary period. The animals that are unavailable for capture are assumed to be located in subterranean refuges.

Superpopulation size estimates can be obtained as follows: N_{super} = N-hat/(1-γ) provided that the temporary migration rates are random (Kendall et al. 1997), which was the case at all three sites. This suggests that estimated populations within each site corresponded to (1-0.38) = 0.63, (1-0.41) = 0.59, and (1-0.19) = 0.81 of the superpopulation for Wheless Spring, Lanier Spring, and Lower Ribelin, respectively. Thus, the superpopulations at these three sites were respectively 1.61, 1.69, and 1.23 times larger than the estimated total surface populations (Table 6). These factors are constant across primary periods because the migration parameter was also constant in the most parsimonious models. Additionally, the calculation for the superpopulation parameter is limited to primary periods where migration could be estimated, which excludes the first and last primary periods.

Superpopulation estimates for Wheless Spring averaged 689 and varied between 426 and 795 (95% CI: 384-949) for the April-September primary periods. At Lanier Spring, superpopulation estimates averaged 240 and varied between 159 and 422 (95% CI: 100-622). At Lower Ribelin, superpopulation estimates averaged 142 and varied between 126 and 156 (95% CI: 94-221). The site with the least potential for surface movement out of the study area (Lower Ribelin) had the least variation in population estimates and the narrowest confidence intervals. Means of superpopulation estimates are presented in Figure 36.

The mean superpopulation estimates represent about half of the total number of individuals that were marked at each site over the 8-month study period (Lower Ribelin = 281, Lanier Spring = 463, Wheless Spring = 1,421). Given the low observed mortality, these data suggest dispersal of individuals beyond the study area.

Figure 36. Estimated superpopulation sizes of *Eurycea tonkawae* by month for Lanier Spring, Lower Ribelin, and Wheless Spring, 2007. Spring flows at Wheless Spring had begun to subside in September, resulting in lower population estimates.

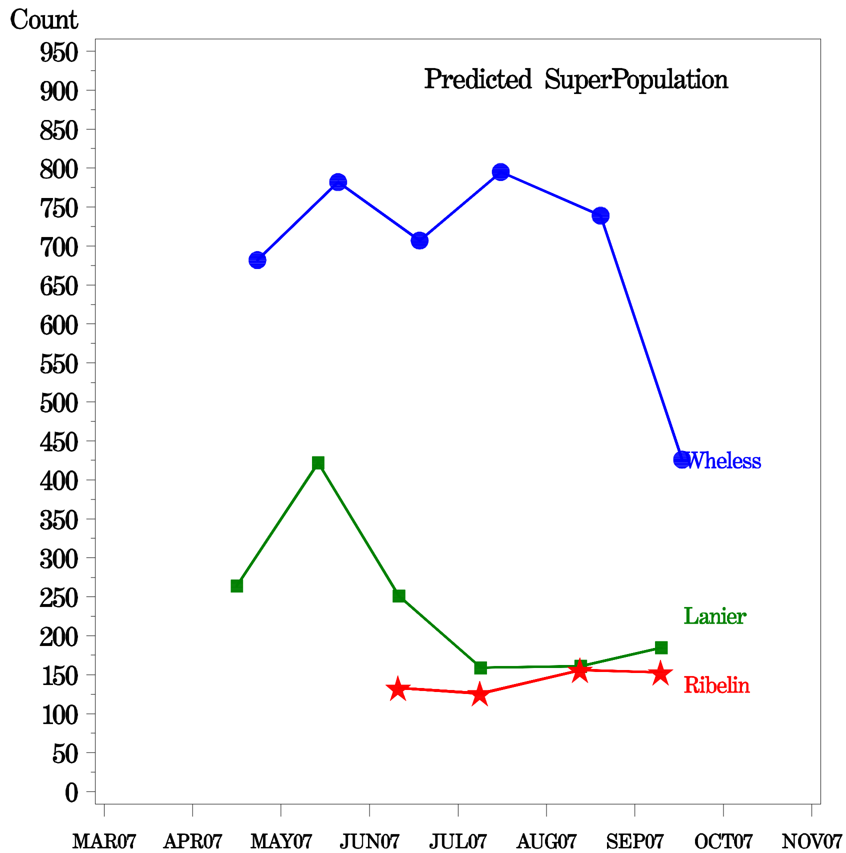


Figure 37. Mean first day count, primary period (3-day) unique captures, estimated total surface population, and estimated superpopulation for *Eurycea tonkawae* at Lanier Spring, Lower Ribelin, and Wheless Spring, 2007.

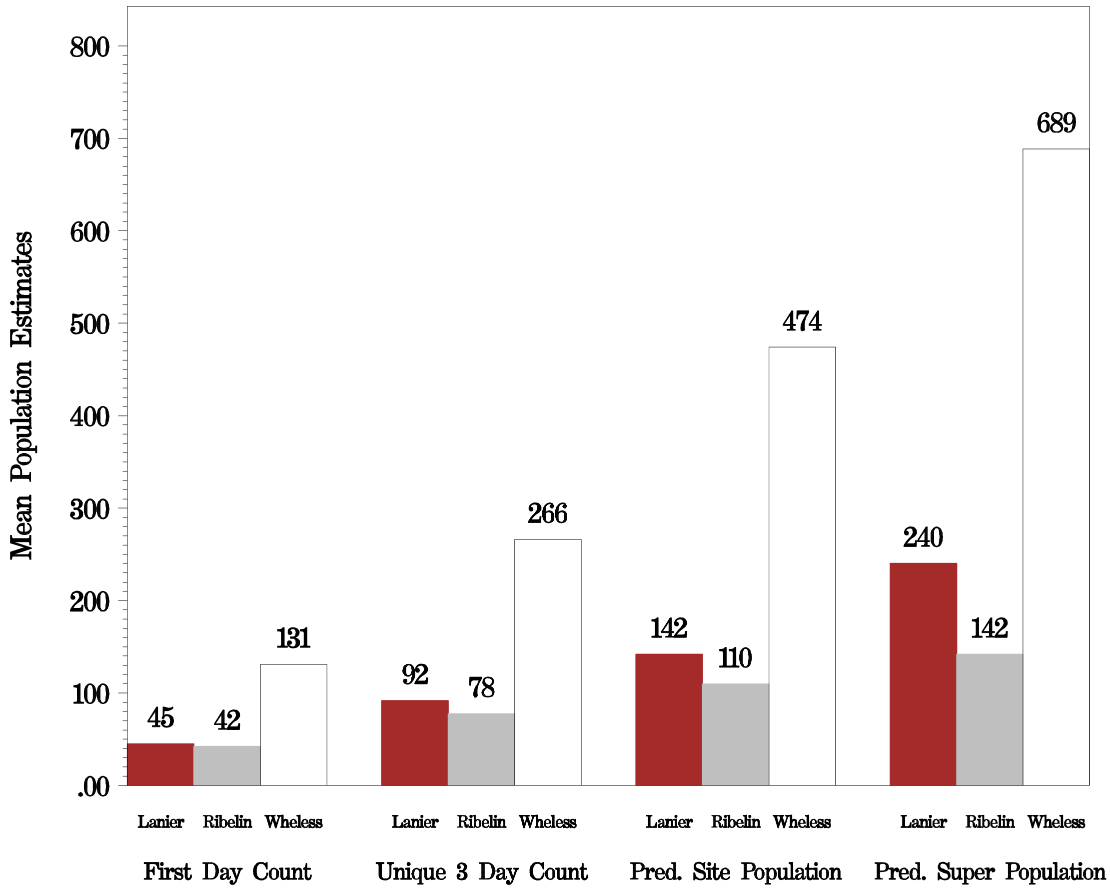


Table 6. Population parameters for *Eurycea tonkawae* mark-recapture datasets from Wheless Spring, Lanier Spring, and Lower Ribelin, 2007.

Site	Date	Period	S	γ	p	c	first day captures	total unique captures	Surface Pop (N)	N lower 95 cL	N upper	Super pop	Super pop lower	Super pop upper
Wheless	10-Mar-07	1	0.88	.	0.05*	0.05*	42	146	1024*	883*	1191*	.	.	.
Wheless	23-Apr-07	2	0.76	0.375	0.40	0.13	177	336	426	389	491	682	622	786
Wheless	21-May-07	3	0.70	0.375	0.32	0.16	176	338	489	427	593	782	683	949
Wheless	18-Jun-07	4	0.70	0.375	0.23	0.16	99	238	442	368	559	707	589	894
Wheless	16-Jul-07	5	0.28	0.375	0.32	0.17	142	342	497	439	591	795	702	946
Wheless	20-Aug-07	6	0.36	0.375	0.37	0.10	185	346	462	412	549	739	659	878
Wheless**	17-Sep-07	7	0.42	0.375	0.42	0.20	113	213	266	239	319	426	384	510
Wheless**	15-Oct-07	8	.	.	0.56	0.12	115	171	187	178	207	.	.	.
Lanier	19-Mar-07	1	0.77	.	0.51	0.24	91	155	176	164	203	.	.	.
Lanier	16-Apr-07	2	1.00	0.408	0.42	0.31	61	129	156	139	197	264	236	334
Lanier	14-May-07	3	0.40	0.408	0.17	0.22	43	107	249	184	367	422	312	622
Lanier	11-Jun-07	4	0.50	0.408	0.25	0.12	33	86	148	111	240	251	188	407
Lanier	9-Jul-07	5	0.74	0.408	0.18	0.22	25	42	94	59	197	159	100	334
Lanier	13-Aug-07	6	0.71	0.408	0.19	0.24	19	45	95	64	175	161	108	297
Lanier	10-Sep-07	7	0.78	0.408	0.36	0.23	40	80	109	92	151	185	156	256
Lanier	8-Oct-07	8	.	.	0.46	0.25	49	91	108	97	139	.	.	.
Ribelin	14-May-07	3	0.69	.	0.60	0.08	45	73	78	74	91	.	.	.
Ribelin	11-Jun-07	4	0.69	0.190	0.22	0.24	24	57	108	80	168	133	99	207
Ribelin	9-Jul-07	5	0.69	0.190	0.23	0.22	23	55	102	76	161	126	94	199
Ribelin	13-Aug-07	6	0.69	0.190	0.35	0.21	47	92	127	106	175	156	131	216
Ribelin	10-Sep-07	7	0.69	0.190	0.29	0.20	46	79	124	99	179	153	122	221
Ribelin	8-Oct-07	8	.	.	0.56	0.26	68	111	122	115	139	.	.	.

* p and c were fixed at 0.05 based on actual number of recaptures within first primary period. Of 42 captured on day one, only 5 were recaptured (2 on day 2, and 3 on day 3), and of the 39 animals captured on day two, only 1 was recaptured on day 3. That makes for a p that is lower than 0.05, thus MARK was unable to estimate this value resulting in a questionable surface population estimate for this period.
**Spring flows at Wheless Spring had begun to subside in September, and the main spring pool was dry by October resulting in lower population estimates.

Comparison of Surface Counts, Surface Population Size, and Superpopulation Size

The ratios of the surface counts (i.e., population indices) and estimated population sizes are presented in Table 7. The surface count/surface population ratios ranged from 0.04 to 0.62 (mean = 0.35) for Wheless Spring, 0.17-0.52 (mean = 0.32) for Lanier Spring, and 0.22 to 0.58 (mean = 0.39) for Lower Ribelin. Excluding the problematic estimates for March, the surface count/surface population ratio for Wheless Spring ranged from 0.22 to 0.61 (mean = 0.39). The surface count/superpopulation ratios ranged from 0.14 to 0.27 (mean = 0.22) for Wheless Spring, 0.10 to 0.23 (mean = 0.16) for Lanier Spring, and 0.18 to 0.30 (mean = 0.24) for Lower Ribelin.

While the range of surface count/population ratios varied considerably by time and site, two major trends are apparent. First, despite the differences in habitat conditions among each site, ANOVA results indicate average surface count/population ratios across the study were not significantly different between sites (d.f.=2, P=0.72). A thorough surface count under ideal conditions (i.e., consistent spring flows) at these sites should, on average, result in the capture of approximately 30-40% of the surface population and 15-25% of the super-population. Second, surface count/population size ratios tended to be lower during the summer months of this study (excluding the March Wheless value; unpaired t-test: d.f.=12, P=0.008); however, additional research is needed to better understand why. For example, increased activity due to sustained surface flows, warmer temperatures, and/or higher prey availability may result in lower capture probability. These in turn may be the result of the unusual amount of precipitation received during 2007, a seasonal effect, a combination of both, or other factors.

The consistency of the average ratio of surface count/population size (and capture probabilities) across sites indicates that a long-term series of surface count data for these or similar sites may provide a reliable index of population size at a given site. However, due to the variable range of surface count/population size at any given time, it is important to interpret any short-term trends in surface counts with caution. For example, surface counts done shortly after dry habitat begins flowing again are unlikely to represent an average survey. This is partly evidenced by the disparity between the first surface counts for the initial Wheless survey (March 10, 2007) and the estimated population size (42 first day, 1024 estimated; this was attributed to the low number of recaptures which complicated model selection). Surveys conducted when habitat is shrinking (i.e., during dry spells) would likely result in the opposite effect: an increase in the ratio of surface count to population size. Thus it is important to take recent site conditions into consideration when interpreting surface count data and to rely on long term trends to assess population status.

Table 7. Ratios of *Eurycea tonkawae* surface count indices and population estimates for Wheless Spring, Lanier Spring, and Lower Ribelin, 2007.

Site	Date	Period	p	first day captures (Index)	Surface Pop (N)	Index/ Surface Pop (N)	Super pop	Index/ Super pop
Wheless	10-Mar-07	1	0.05*	42	1024*	0.04*	.	.
Wheless	23-Apr-07	2	0.40	177	426	0.42	682	0.26
Wheless	21-May-07	3	0.32	176	489	0.36	782	0.23
Wheless	18-Jun-07	4	0.23	99	442	0.22	707	0.14
Wheless	16-Jul-07	5	0.32	142	497	0.29	795	0.18
Wheless	20-Aug-07	6	0.37	185	461	0.40	739	0.25
Wheless**	17-Sep-07	7	0.42	113	266	0.42	426	0.27
Wheless**	15-Oct-07	8	0.56	115	187	0.62	.	.
Lanier	19-Mar-07	1	0.51	91	176	0.52	.	.
Lanier	16-Apr-07	2	0.42	61	156	0.39	264	0.23
Lanier	14-May-07	3	0.17	43	249	0.17	422	0.10
Lanier	11-Jun-07	4	0.25	33	148	0.22	251	0.13
Lanier	9-Jul-07	5	0.18	25	94	0.37	159	0.22
Lanier	13-Aug-07	6	0.19	19	95	0.20	161	0.12
Lanier	10-Sep-07	7	0.36	40	109	0.27	185	0.16
Lanier	8-Oct-07	8	0.46	49	108	0.45	.	.
Ribelin	14-May-07	3	0.60	45	78	0.58	.	.
Ribelin	11-Jun-07	4	0.22	24	108	0.22	133	0.18
Ribelin	9-Jul-07	5	0.23	23	102	0.23	126	0.18
Ribelin	13-Aug-07	6	0.35	47	126	0.37	156	0.30
Ribelin	10-Sep-07	7	0.29	46	124	0.37	153	0.30
Ribelin	8-Oct-07	8	0.56	68	121	0.56	.	.

* parameter was fixed at 0.05 based on actual number of recaptures within first primary period. Of 42 captured on day one, only 5 were recaptured (2 on day 2, and 3 on day 3), and of the 39 animals captured on day two, only 1 was recaptured on day 3. That makes for a p that is lower than 0.05, thus MARK had trouble estimating this value resulting in a questions surface population estimate for this period.

**Spring flows at Wheless Spring had begun to subside in September, and the main spring pool was dry by October resulting in lower population estimates.

Conclusions

This pilot study was originally designed to evaluate the effects of building a water treatment plant upstream of several large JPS populations in the Bull Creek watershed. While WTP4 has been moved to an alternate site, this study provided an opportunity to test and compare mark-recapture with the less labor-intensive surface count surveys. Major rain events facilitated data collection by providing nearly continuous spring flows throughout the 8-month study period.

Population parameter estimates were obtained for detection probabilities, persistence, temporary migration, surface population size, and superpopulation size. Despite differences in habitat conditions and month-to-month variability, mean parameter estimates across all three sites were similar for almost all variables. Mean capture probabilities (0.32 to 0.38) indicate that, on average, surface counts represent about 30-40% of the surface population. These results provide a framework from which to interpret previously collected count data for this species, and show that although capture probabilities may vary from one survey to the next, they seem to be fairly consistent on average among different sites.

Total surface population size estimates under bankfull conditions at the three mark-recapture sites (Lower Ribelin, Lanier Spring, Wheless Spring) averaged 110, 142, and 463, respectively, with approximate surface density estimates of 2.2, 2.4, and 5.4 animals/m^2. These results are consistent with previous conclusions based on surface count data that Wheless Spring supports one of the largest and densest JPS populations.

Because JPS can retreat into the subterranean habitat, it was important to know what fraction of the superpopulation is made up of the visible individuals on the surface. Superpopulations were estimated to be 1.23 to 1.69 times larger than the surface populations. This suggests that when conditions at the surface are suitable (i.e., consistent surface flows), the majority of JPS are on the surface, and that there are not large numbers of individuals in the subsurface. Under these ideal conditions, surface counts at the three mark-recapture sites represented approximately 15 to 25% of the superpopulation.

While the overall means were similar among all three sites, there was month-to-month variability in most of the population parameters. Habitat conditions appeared to be the major influence, particularly surface flows and the amount and extent of suitable cover on the surface (loose, unembedded rocks), both of which provided avenues for dispersal in and out of the study area. As expected, the site with limited suitable cover beyond the study area (Lower Ribelin) had the least amount of variation and narrowest confidence intervals. The sites with habitat beyond the study area (Lanier Spring, Wheless Spring), more variable surface flows, and/or multiple portals to the subsurface (Wheless Spring) tended to have more variability in the parameter estimates.

The results of the 2007 study suggest that surface counts should continue to be a useful tool in evaluating the population status of JPS. They provide a cost-effective alternative to the more labor-intensive mark-recapture method and are a less intrusive method of estimating population size. While the mark-recapture study presented here is by no means an exoneration of surface counts as a poor estimator of population size, it does highlight the ability of surface count surveys under ideal habitat conditions (i.e., continuous spring flows) to show a relatively consistent fraction of the total estimated population size on average across different sites over an extended study period. However, populations may respond differently over a longer period of time and under less than ideal conditions, particularly drought and habitat degradation. If possible, mark-recapture should continue at a subset of sampling sites to monitor changes in detection probabilities, adjust surface count data as needed, and gather other data that cannot be obtained from surface counts, such as dispersal and longevity.

Recommendations

- Continue mark-recapture sampling at a subset of the surface count sites (double-sampling) to further validate and adjust estimates obtained from surface counts, determine whether and how the population parameters change over time, and how populations respond under different environmental conditions (such as habitat degradation from urban development, drought, storm flows). Combined with population genetics and dye-tracing studies, mark-recapture can also be used to determine the degree to which populations are isolated or interconnected by dispersing individuals (metapopulations).

- Conduct mark-recapture on a less frequent basis (e.g., quarterly) to reduce the behavioral trap-shy effect, increase detection probabilities, and allow the habitat to fully recover between sampling events.

- To avoid the possibility of introducing or spreading pathogens among JPS sites, adhere to strict protocols for disinfecting all footwear and field equipment (i.e., see Declining Amphibian Task Force Fieldwork Code of Practice and Appendix A in O'Donnell et al. 2005).

- Ensure all assumptions of the study design are met, such as closure to surface movement, no mortality, and equal capture probability within primary periods. To improve the likelihood of equal capture probability among individuals, precautions should be taken to avoid injury and stress to all individuals during collection and handling. Efforts to maximize horizontal closure should be implemented (i.e., placing a barrier along the upstream and downstream boundaries of the study area), and all individuals should be returned to the same sections where they were collected.

- Conduct surveys under baseflow conditions when there is flow throughout the entire survey area. Investigate more fully the influence of surface flows and other potential covariates on population numbers, dispersal, and vital rate parameters.

- Survey areas outside the bounds of the study sites to document when, how far, and how frequently JPS disperse.

- Continue confirmation of recapture identity by matching VIE marks and natural pigment patterns in photographs. This is the most critical QA/QC step and is essential to producing a robust and reliable dataset. This includes photographing each individual to provide positive identification and correct any errors due to misidentification and/or missing VIE marks.

- Investigate the feasibility of using pattern-recognition software and fewer VIE marks.

- Devise a better photographing system that is less time-consuming and more user-friendly, with more consistent high quality photos. The use of a photography chamber would also make photo checks easier and more accurate. The chamber should enable data QA/QC personnel to identify marks that may have migrated to the ventral areas of the body.

- Include a standard measurement device with all photographs – for example, photographing each animal on a grid so that any erroneous measurements made in the field can be easily checked and corrected.

- Check recaptures while in the field (ideally with a computer) and record the date of the initial marking on the field data sheets. Back up all hard copy and electronic data at the end of each field day. Enter data and download photos the day of or soon after each field day.

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Appendix A. Site Descriptions

Each monitoring site selected for this study was distinct in terms of location and size of springs and seeps (allowing for movement between the surface and subsurface), extent and quality of surface habitat, and flow conditions. All sites selected for this study were still classified as rural and thus relatively pristine (figures A-1 and A-2). New urban development activities commenced near the headwaters of both Bull Creek Mainstem and Tributary 8 in October 2007, near the end of the study.

Mark-Recapture Sites (4): Mark-recapture sites included one long-term monitoring site (Wheless) and three new sites (Lanier Riffle, Lanier Spring, Lower Ribelin). Sites were selected based on previously defined sections (Wheless), stretches of suitable rock substrates (Lower Ribelin), and observed concentrations of JPS.

- *Bull Creek Mainstem, Lanier 90-foot Riffle (below former WTP4 site, above confluence with Trib 8)*

This is the only site along Bull Creek Mainstem where JPS were found below the former WTP4 site and above the confluence with Tributary 8. This 90-foot long, narrow riffle is entirely within the creek channel (Figure A-3). At bankfull conditions, it has an approximate wetted area of 60 m^2 (650 ft^2). While no springs are visible, water quality data (pH, temperature) suggest the presence of one or more small seeps along the banks. Gravel and cobble are the predominant substrates. This short stretch of habitat is bounded above and below by deep pools with predatory fish that do not appear suitable for JPS. Since so few JPS were found at the Lanier Riffle site (high count of 8), the mark-recapture surveys were terminated after four months (April – July 2007).

- *Bull Creek Mainstem, Lanier Spring (below former WTP4 site, below confluence with Trib 8)*

The Lanier Spring site lies along Bull Creek Mainstem below the confluence with Tributary 8. It consists of five sections, including a spring pool (Section 1), spring run (Section 2), and section of creek channel (sections 3-5; Figure A-4). The primary study area included sections 1-3, totaling about 31 m (102 ft), with an approximate wetted area of 63 m^2 (680 ft^2) . Sections 4 and 5 were established as check points to verify the assumption that the primary study area was closed to horizontal (surface) movement during the primary periods; both sections are about 5.5 m (18-18.5 ft) long. The Section 1 spring pool flows from the Glen Rose geologic formation and consists of soil with a light cover of cobble and gravel. Water primrose (*Ludwegia* sp.), other aquatic plants, and leaf litter are commonly found in the spring pool. A short spring run (Section 2) flows from Lanier Spring into the mainstem of Bull Creek (sections 3-5). The creek channel at this location is predominantly riffle habitat with sand, gravel, and cobble substrates. Similar habitat continues upstream and downstream of the monitoring site. However, cursory surveys prior to initiating this study indicated JPS densities were highest near the spring pool.

A fence was installed around the spring pool in early 2005 to preclude further damage from wallowing by feral hogs (O'Donnell et al. 2006). Following fence installation, aquatic macrophytes and JPS numbers began to increase in the spring pool.

- *Bull Creek Tributary 8, Lower Ribelin (below former WTP4 site)*

This site was discovered during reconnaissance surveys along Bull Creek Tributary 8 in early 2007. The property is privately owned, and the property manager was very accommodating of this project and other surveys conducted as part of the WTP4 monitoring efforts. The Lower Ribelin site lies entirely within the creek channel (Figure A-5). The upstream end is bounded by a short ledge beneath which groundwater discharges through visible fractures in the bedrock. Below this spring is a short [(approx. 23 m (74 ft)] stretch of cobble, gravel, and boulders. The creekbed above and below the monitoring site is almost entirely bedrock with little loose rock or plant substrates and so provides little habitat for JPS. At bankfull conditions, the approximate wetted area is 51 m^2 (555 ft^2).

- *Wheless Spring and Long Hollow Creek Below Wheless Spring (control site)*

The Wheless Spring site on Long Hollow was established as a long-term monitoring site in May 1997 (Davis et al. 2001). While JPS have been reported along Long Hollow throughout the LCRA Wheless tract (LCRA 1993), only a few were observed beyond the vicinity of Wheless Spring during the distribution surveys in January 2007. No other large, visible springs with high concentrations of JPS were found downstream.

The Wheless Spring site consists of six sections, including a spring pool (Section 1) and five pools or riffles in the creek channel (sections 2-6; Figure A-6). The primary study area included sections 1 and 3-5, totaling about 69 m (225 ft) in length. Under bankfull conditions, the wetted area is approximately 85 m^2 (919 ft^2). Sections 2 and 6 were selected as check points to verify the assumption that the primary study area was closed to horizontal movement during the primary periods; these two outer sections totaled about 36 m (119 ft). The Section 1 spring pool flows intermittently from the Glen Rose geologic formation and consists of soil with a cover of cobble, gravel, and leaf litter. Wheless Spring (Section 1) flows into Long Hollow Creek via a spring run that is overgrown with greenbriar (*Smilax* sp.) and other thick vines and so is largely inaccessible. The creek is predominantly bedrock covered with a mixture of soil, cobble, gravel, and boulders. The spring run enters about midway along Section 3 of the creek channel. Above this is Section 2, which is almost entirely bedrock and thus has very little JPS habitat. Section 2 is bounded on the upstream end by a grassy swale that is not habitat. Section 4 is a riffle with little shade and the highest percent of plant cover. Sections 5 and 6 are both shady bedrock pools covered with cobble, gravel, and leaf litter. Water quality data (pH, temperature) suggest the presence of many small springs and seeps along Long Hollow Creek below Wheless Spring, including the lower end of Section 3 and the upper end of Section 5. The upper end of Section 5 is typically the last area to go dry.

Surface Count Sites (3): Surface counts sites included one long-term monitoring site (Bull Creek Above Tributary 7) and two new sites (WTP4, Upper Ribelin). Sites were selected based on previously defined sections (Bull Creek Above Tributary 7), accessibility, stretches of noticeable groundwater resurgence and disappearance, and observed concentrations of JPS. Upper Ribelin was not surveyed during 2007 due to time constraints.

- *Bull Creek Mainstem, WTP4 ("Above" former WTP4 site)*

Other than the Lanier 90-foot Riffle site, this was the only accessible JPS site found along the mainstem of Bull Creek above the confluence with Tributary 8. Other sites have been reported upstream of the former WTP4 site but are on private property. While technically downstream from potential influence of the former WTP4 construction site, this was the most upstream monitoring site where access was permitted on Bull Creek. The WTP4 monitoring site lies entirely within the creek channel. It is about 85 m (280 ft) long and extends from the fenceline near the southwest corner of the property downstream to a low water crossing (Figure A-7). The site is characterized by a series of pools and riffles over short sections of cobble and gravel interspersed with long stretches of primarily bedrock substrates. The low water crossing and a pool immediately upstream are predominantly soil and leaf litter. Few JPS have been found below the low water crossing, which is primarily bedrock. At bankfull conditions, the approximate wetted area is 220 m^2 (2,360 ft^2).

No springs or seeps are discernable within the WTP4 monitoring site. However, when this site was dry in 2006, a small pool of water remained visible just upstream of the property boundary, indicating a source spring at this location. When the creek is flowing, habitat upstream of the monitoring site appears to be suitable (riffle over gravel and cobble).

The WTP4 site was not mapped into discrete units until the end of the study, so JPS numbers were recorded for the entire monitoring site.

- *Mainstem Bull Creek Above Tributary 7 (below former WTP4 site and confluence with Trib 8)*

Bull Creek Above Tributary 7 was established as a long-term monitoring site in December 2006 (City of Austin 2001). It is located along Bull Creek Mainstem, is entirely within the creek channel, and is the most downstream of the monitoring sites on Bull Creek. Small springs and seeps occur along the banks. The largest spring in this area, Pit Spring, is upstream of the monitoring site. The site originally consisted of seven sections (Figure A-8), three of which were later discontinued (sections 5, 6, and 8). The sections that continue to be monitored include 2-4 and 7, a series of riffles and pools covering a total length of about 40 m (130 ft). Under bankfull conditions, the approximate wetted area is 70 m^2 (750 ft^2). Sections 2-4 are dominated by gravel, cobble, and sand. Section 7 is downstream of sections 2-4 and is predominantly calcified substrate with some loose rock cover.

- *Bull Creek Tributary 8, Upper Ribelin (above former WTP4 site)*

This site was identified during reconnaissance surveys along Bull Creek Tributary 8 in March 2007. It is about 29 m (96 ft) long with an approximate wetted area of 52 m^2 (565 ft^2) at bankfull conditions. It lies entirely within what appears to be a short gaining (groundwater discharge) and losing (groundwater recharge) section of the creek channel. There appears to be a groundwater resurgence at the upstream end of the monitoring site (the creek goes dry more rapidly above and below the monitoring site). The substrate consists primarily of cobble, gravel, and boulders. Sediment was noticeably more visible in this area near the end of the 8-month survey period. There was not enough time to delineate this monitoring site until after the study (Figure A-9), so only one cursory survey in March and two incomplete surveys in September and October were conducted. However, since this is the most upstream and accessible site on Tributary 8 with large numbers of JPS, efforts to monitor the Upper Ribelin site are encouraged.

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Figure A-1. Land use analysis for areas draining to *Eurycea tonkawae* sites in the Bull Creek Watershed.

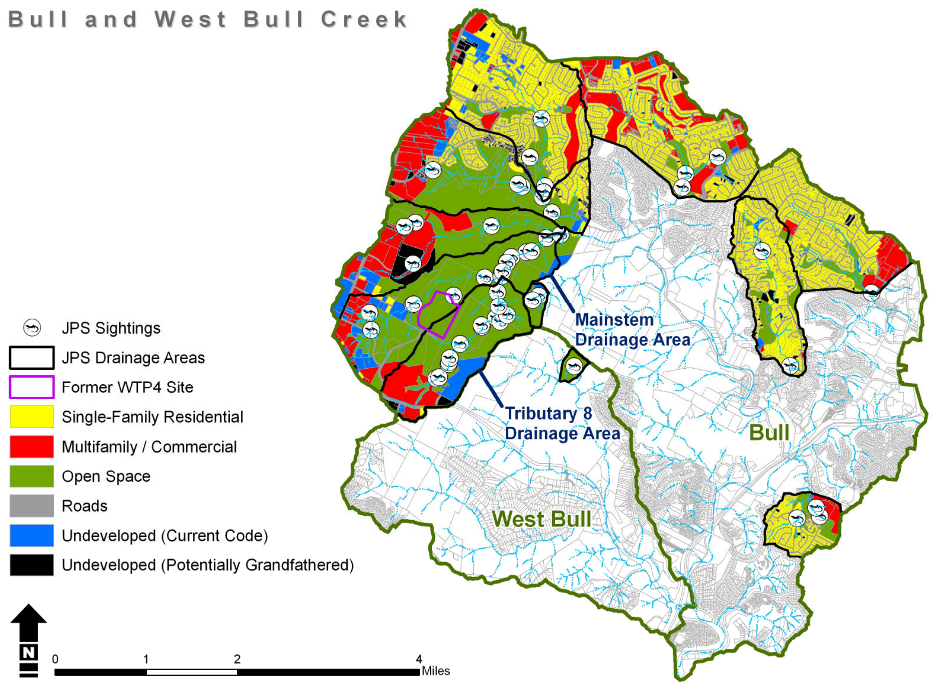


Figure A-2. Land use analysis for areas draining to *Eurycea tonkawae* sites in the Long Hollow (Wheless Spring) Watershed.

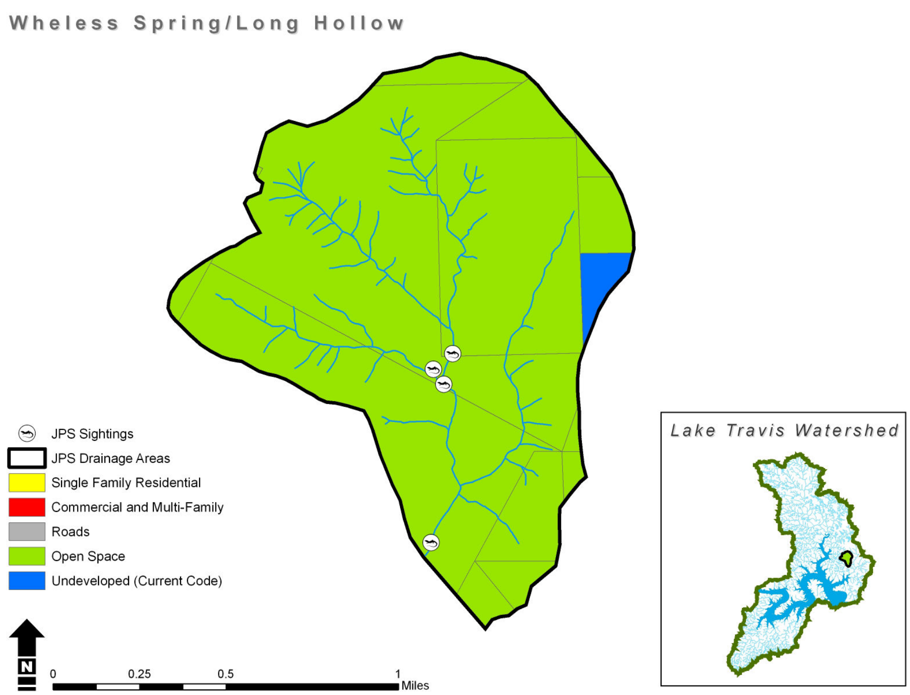


Figure A-3a. Map of the *Eurycea tonkawae* monitoring site on Bull Creek referred to as "Lanier 90-Foot Riffle". Seine nets were placed at the upstream and downstream ends of the site during each primary period. Mark-recapture surveys were terminated after four months (April – July 2007) due to low numbers of *E. tonkawae*.

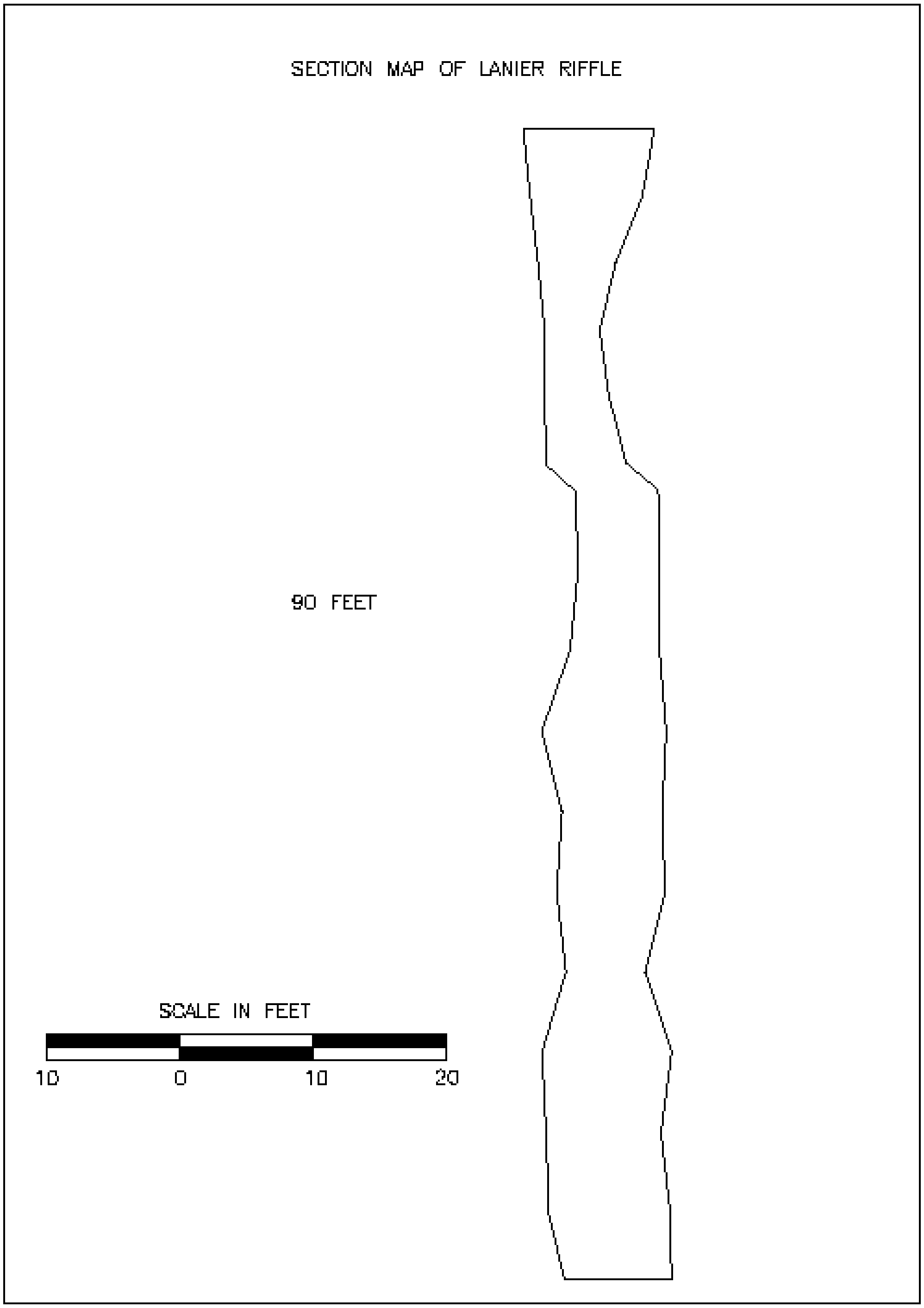


Figure A-3b. Lanier 90-Foot Riffle monitoring site. Photo taken 19 April 2007, looking downstream.

Figure A-4a. Map of the *Eurycea tonkawae* monitoring site on Bull Creek referred to as "Lanier Spring". Primary study area included sections 1-3; seine nets were placed at the upstream and downstream ends of section 3 during each primary period. Surveys were conducted in sections 4 and 5 to check for assumption of closure (no horizontal movement to or from primary study area).

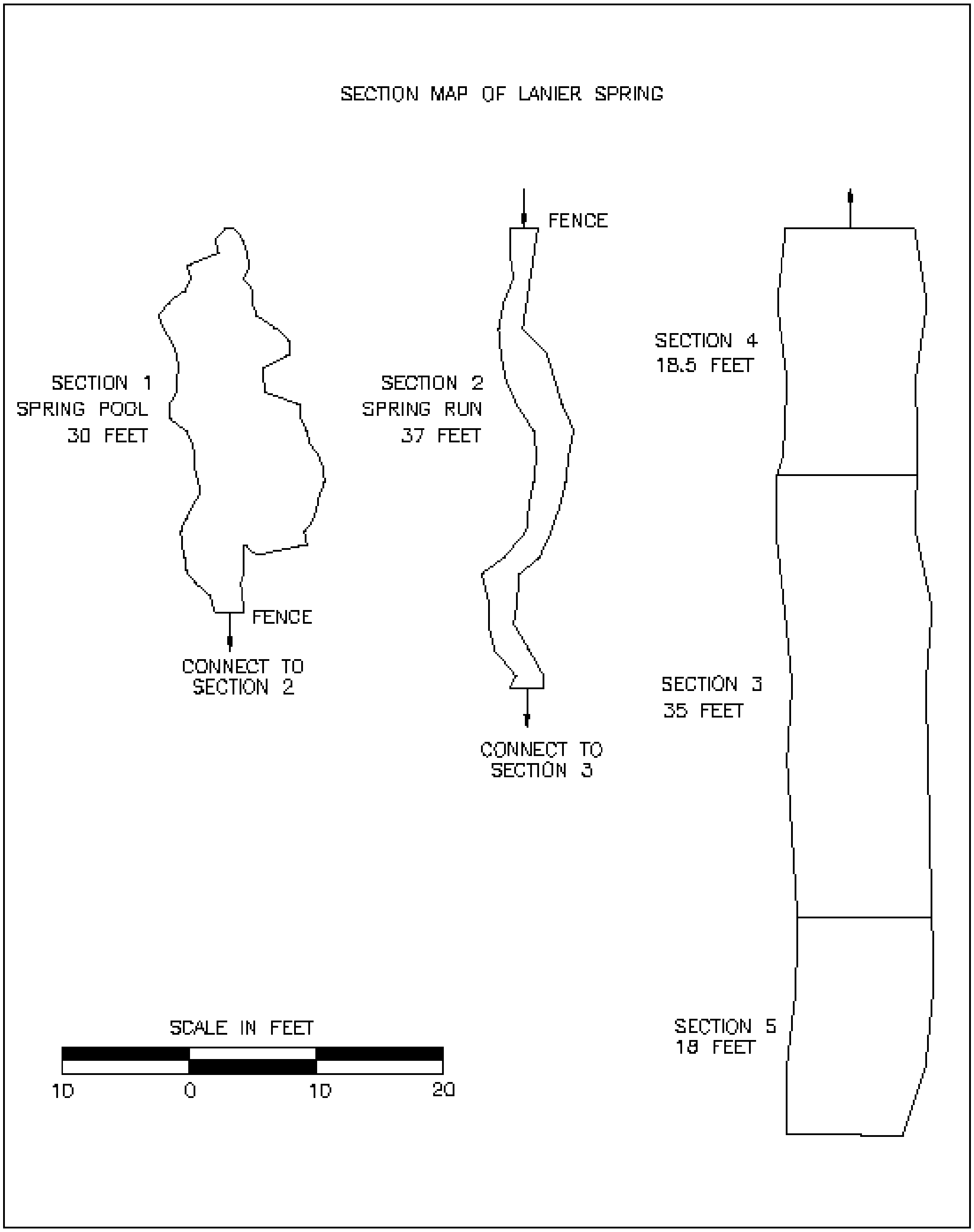


Figure A-4b. Lanier Spring monitoring site: Section 1 Spring Pool, 13 September 2007, and Section 3 (Bull Creek channel) looking upstream to confluence with Tributary 8, 24 May 2007.

Figure A-5a. Map of the *Eurycea tonkawae* monitoring site on Bull Creek referred to as “Lower Ribelin”. Seine nets were placed at the upstream and downstream ends of the site during each primary period. This site was naturally bounded by stream segments of bedrock with little or no protective cover for JPS.

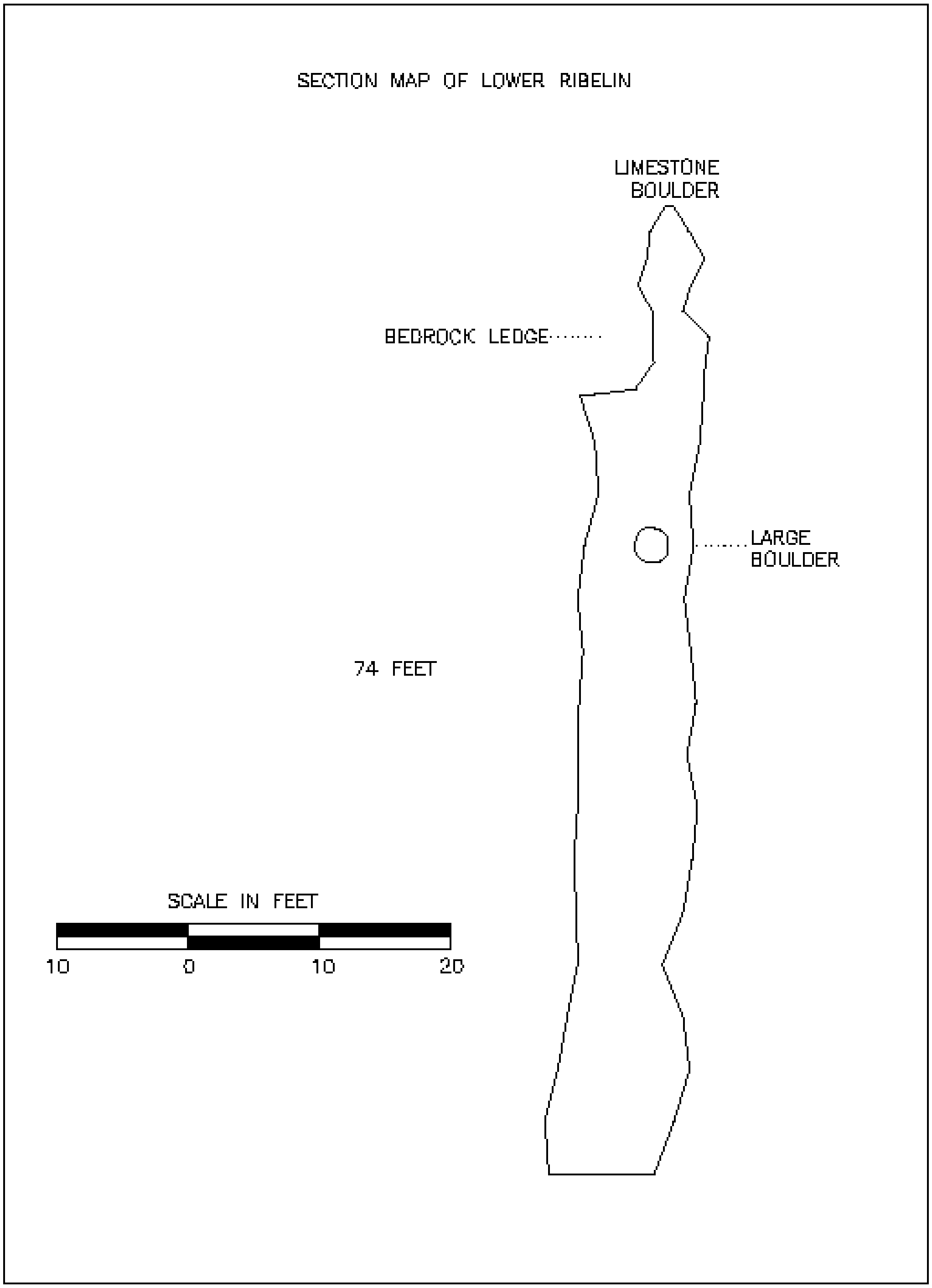


Figure A-5b. Lower Ribelin monitoring site. Photo taken 14 June 2007, looking downstream.

Figure A-6a. Map of *Eurycea tonkawae* monitoring site on Long Hollow Creek referred to as "Wheless Spring". Primary study area included sections 1 and 3-5. Seine nets were placed at the upstream end of section 3 and downstream end of section 5 during each primary period. Surveys were conducted in sections 2 and 6 to check for assumption of closure (no horizontal movement to or from primary study area).

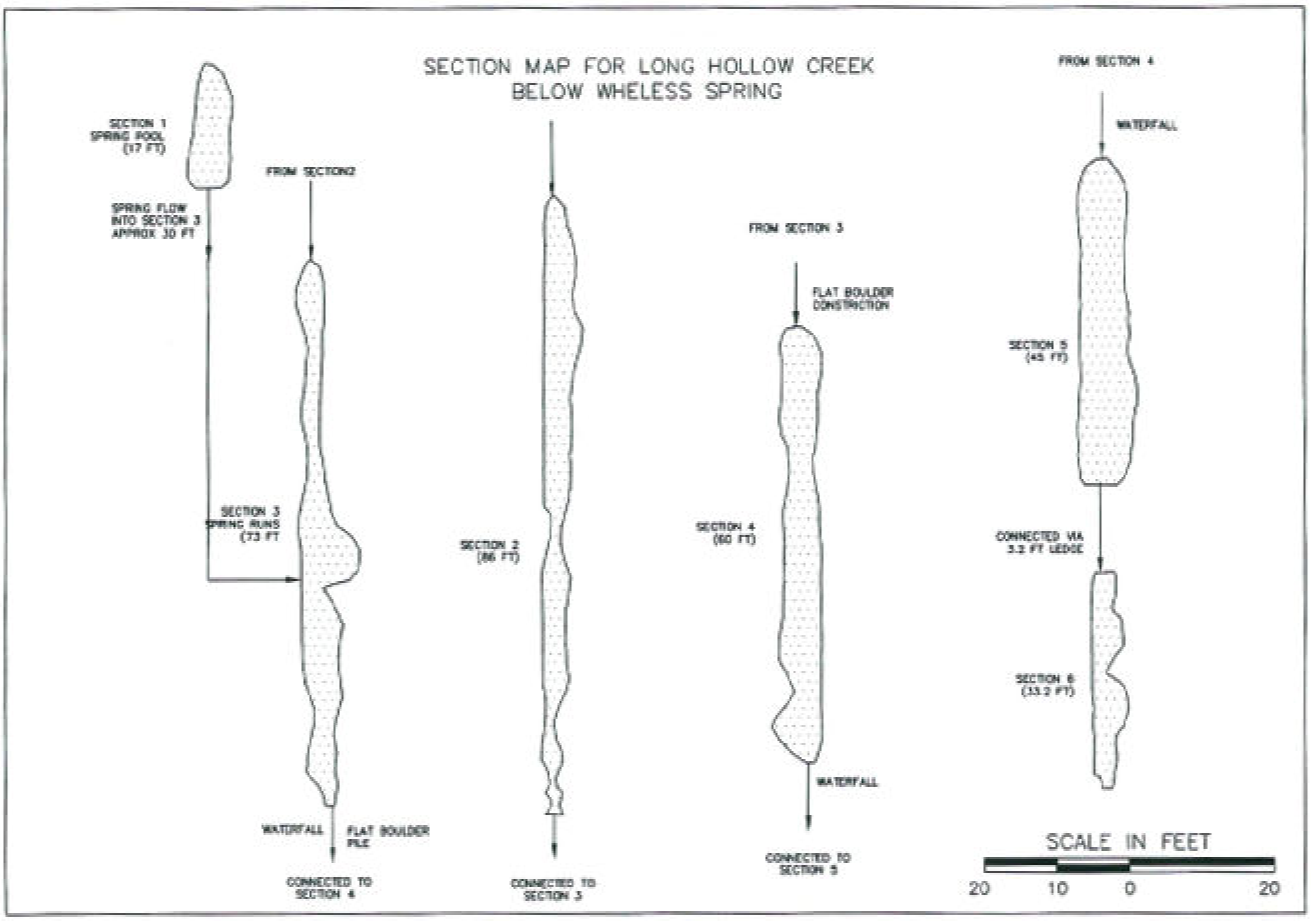


Figure A-6b. Wheless Spring monitoring site: Section 1 Spring Pool and Section 6 (Long Hollow Creek channel) looking upstream, 18 June 2007.

Figure A-7a. Map of the Water Treatment Plant 4 monitoring site.

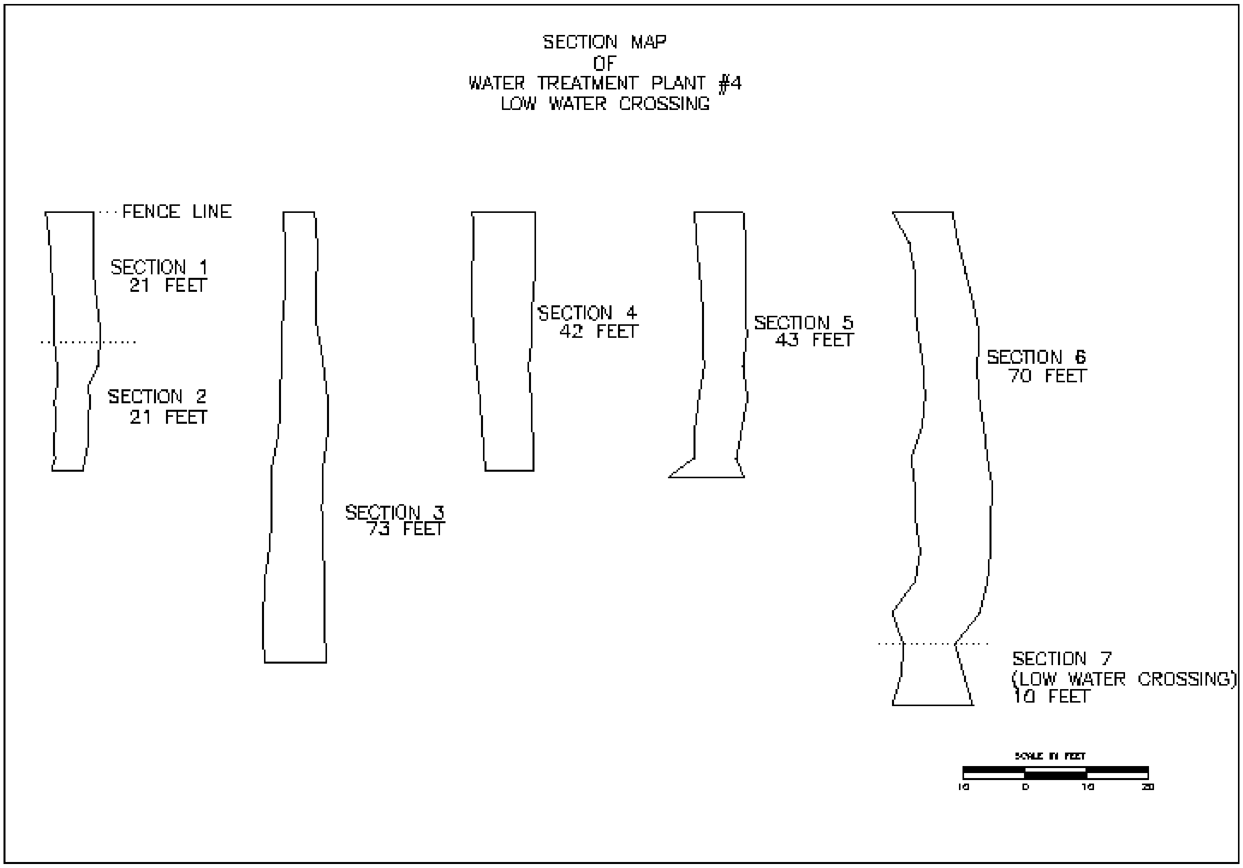


Figure A-7b. Water Treatment Plant 4 monitoring site. Photo taken 19 April, 2007, looking upstream from Section 3.

Figure A-8a. Map of the *Eurycea tonkawae* monitoring site on Bull Creek referred to as "Bull Creek Above Tributary 7". Current survey area includes sections 2, 3, 4, and 7.

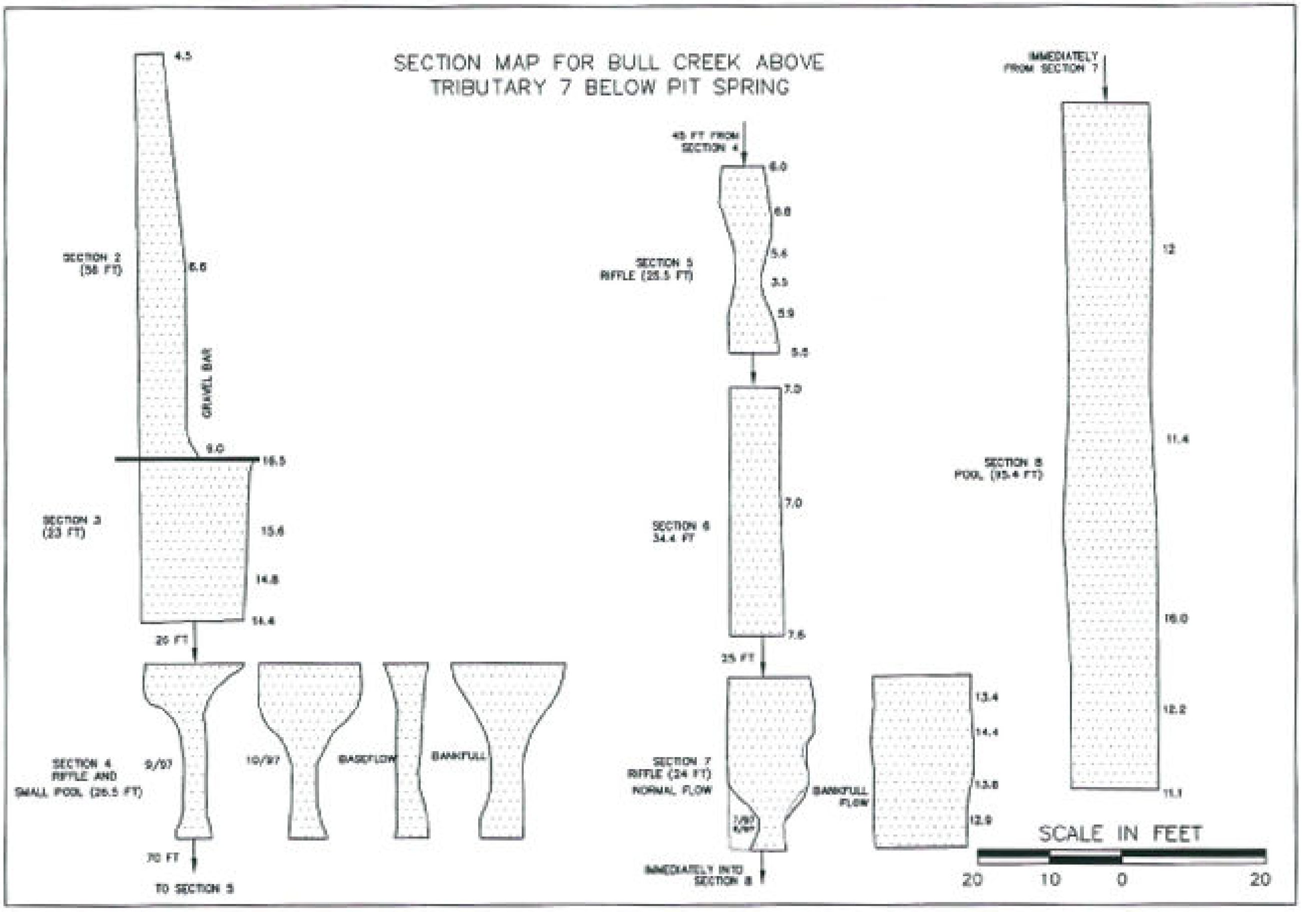
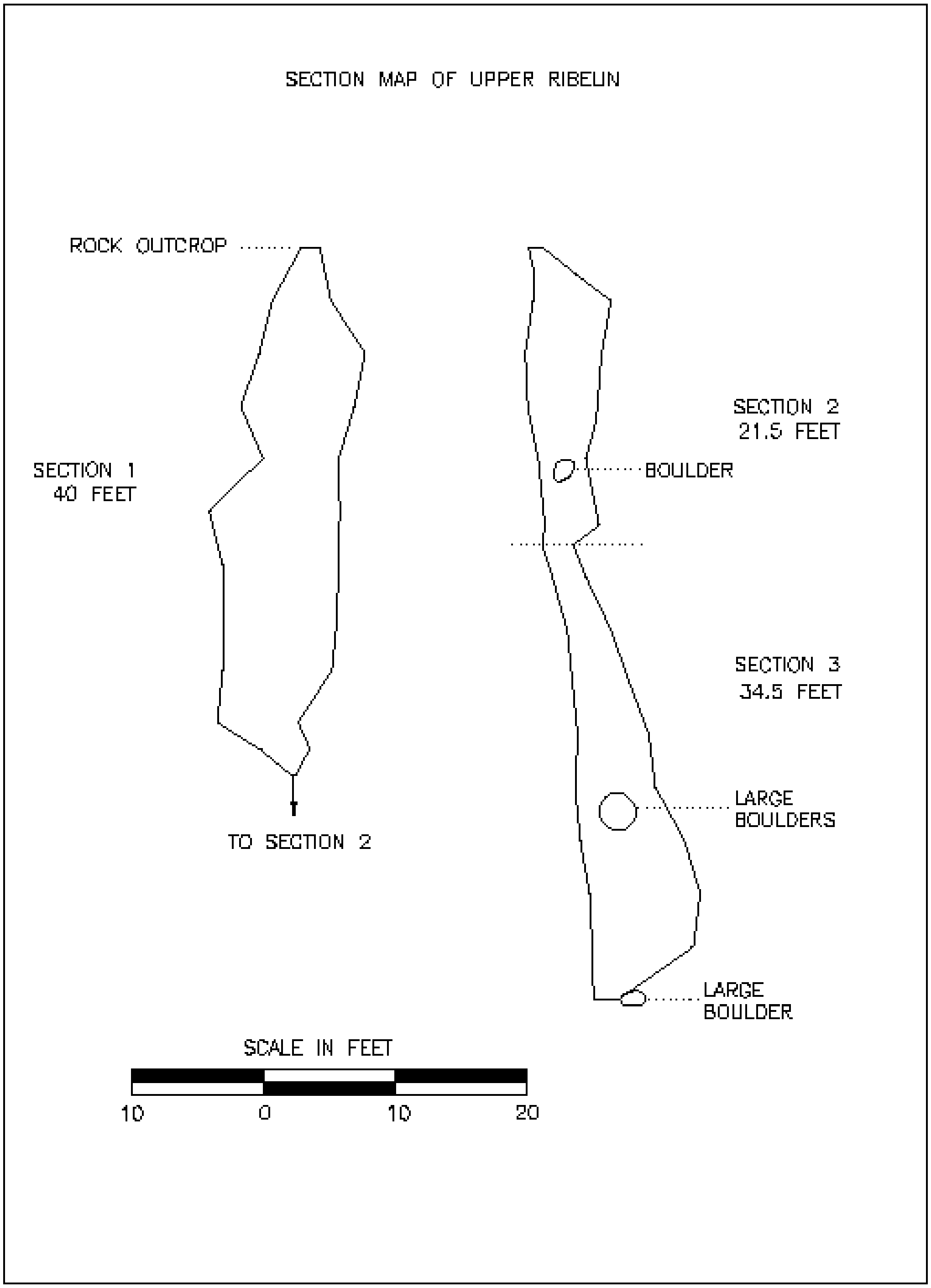


Figure A-8b. Bull Creek Above Tributary 7 monitoring site. Photo taken 19 April 2007, looking downstream from Section 2.

Figure A-9. Map of the *Eurycea tonkawae* monitoring site referred to as "Upper Ribelin".



Appendix B. Water Quality, Habitat, and Natural History Notes, 2007

Water Quality - Because the JPS remains aquatic throughout its life, it is highly dependent upon the quality and quantity of groundwater for its survival. It is typically found in clean, clear, flowing water that has a narrow temperature range (average temperature = 19-20.5^{o}C across each survey site in 2007, Figure B-1) and is mostly neutral (average pH = 7-7.7 across each survey site in 2007, Figure B-2). Carbon dioxide typically makes up about 1 to 2 percent of the total dissolved gases (City of Austin, unpublished data from Jollyville Plateau springs and Barton Springs), producing a slightly acidic pH that quickly dissipates as the groundwater surfaces. This results in a lower pH at spring outlets than in the neighboring creeks. Likewise, water temperatures in creek water tend to be more variable and influenced by air temperatures than spring water. Specific conductance, a measure of the amount of ions in the water, averaged about 540-620 uS/cm across each survey site during this study (Figure B-3). Dissolved oxygen averaged 6-8 mg/L across each survey site (Figure B-4) and was generally lower at the spring outlet than the neighboring creek.

Figure B-1. Water temperature (^{o}C) at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

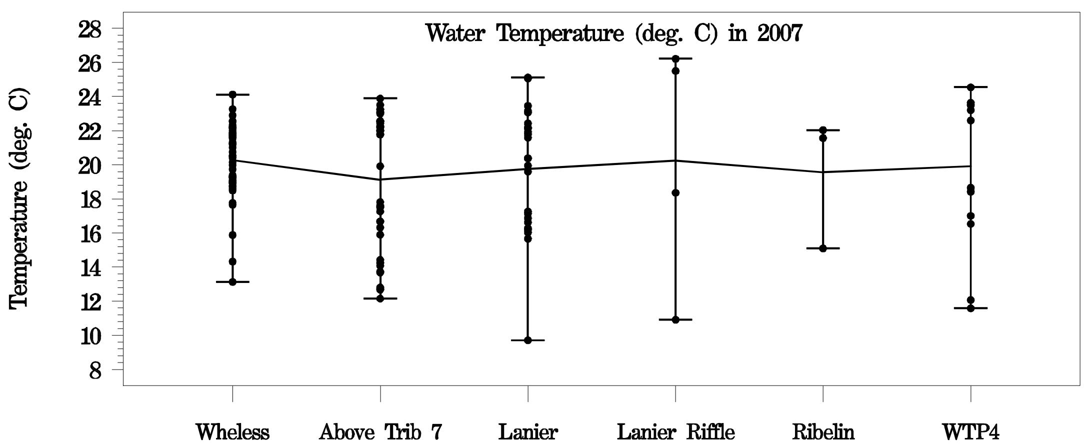


Figure B-2. Water pH at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

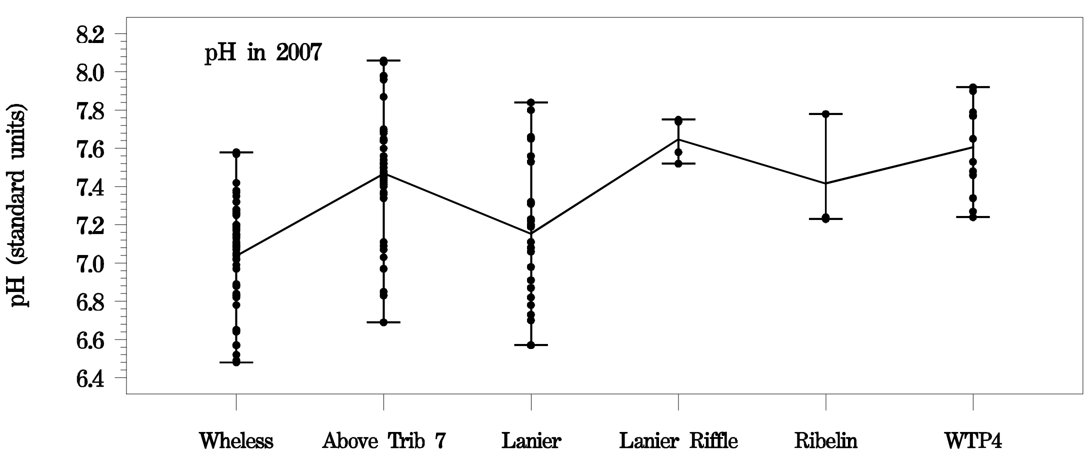


Figure B-3. Specific conductance (uS/cm) at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

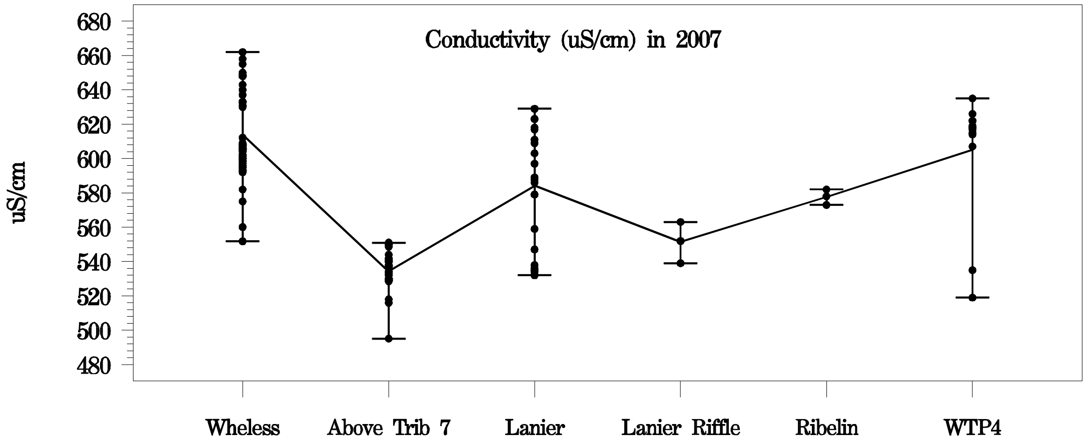
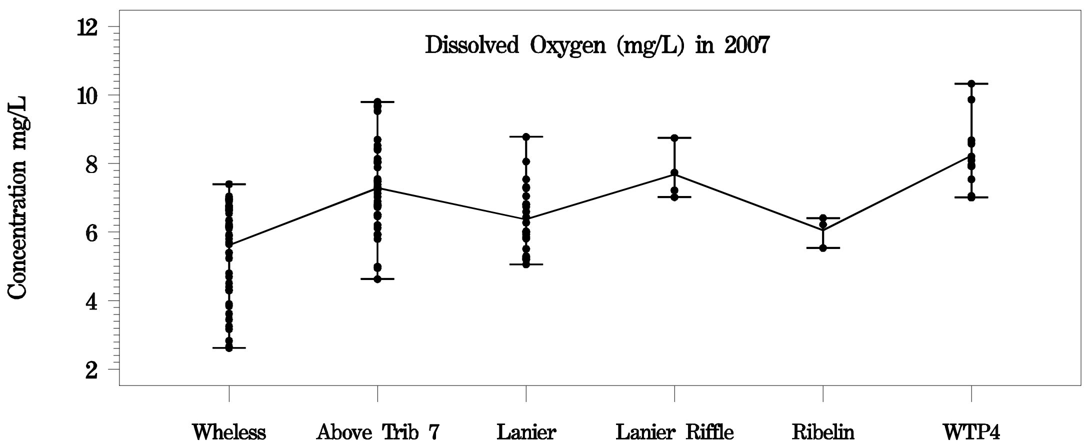


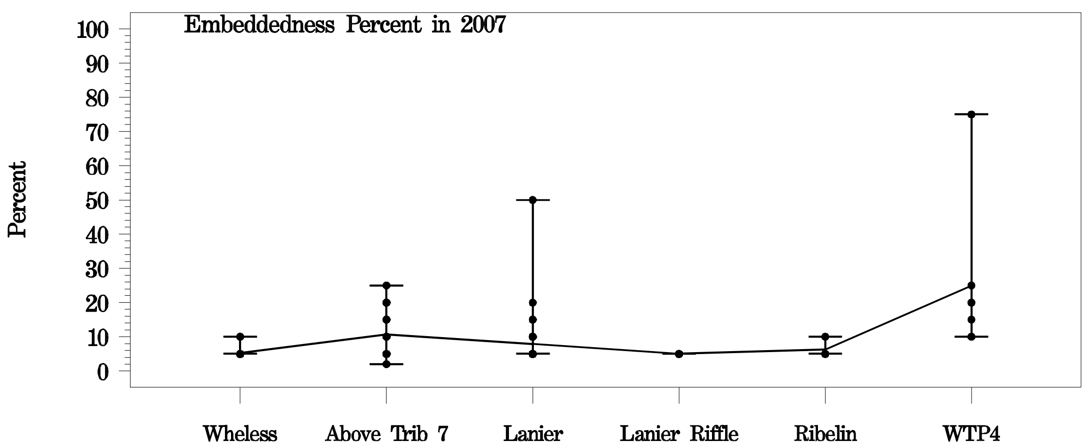
Figure B-4. Dissolved oxygen (mg/L) at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.



***Embeddedness* -** Increased sedimentation from urban development is a major water quality threat because it fills interstitial spaces where the JPS and its prey base, small aquatic invertebrates, live. Increased sedimentation and embeddedness are believed to have contributed to the decline of JPS at a long-term monitoring site on Bull Creek Tributary 5 (O'Donnell et al. 2006). Miller and Hess (2007) found that increased sedimentation contributed to lower abundance of larval southern two-lined salamanders (*Eurycea cirrigera*) in urban and suburban streams in Wake County, North Carolina. They attributed the decline to sediment-filled substrate interstices, which prevented the salamanders from migrating with the water column during dry periods.

All of the JPS sites that were monitored during 2007 as part of this study were still relatively pristine, so embeddedness was typically very low (less than 10%) (Figure B-5). Embeddedness was highest at the WTP4 site (average 25%), which was just downstream of recent construction activity. The embeddedness observed at Lanier Spring was due to sand in the creekbed or native soil substrate in the spring pool and spring run. Embeddedness at Bull Creek Above Tributary 7 was due primarily to sand. Bowles et al. (2006) found that embeddedness due to loose organic detritus and sand did not adversely affect JPS presence, which is consistent with observations during the 2007 study.

Figure B-5. Percent embeddedness at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.



***Predators* –** Potential predators of JPS include predatory fish, large (>2") crayfish (*Procambarus clarkii*), other large invertebrates such as dragonfly (Anisoptera) naiads and giant water bugs (*Lethocerus amercanus*), and possibly small blotched watersnakes (*Nerodia erythrogaster*). The Wheless Spring site tended to be the most predator-free of the monitoring sites. Fish have not been observed at the Wheless site (Figure B-6), and crayfish are rarely observed (Figure B-7). However, large numbers of dragonfly naiads, beetle larvae, and giant water bugs were observed at this site as flows subsided during the August-October surveys. Due to the noticeable increase in giant water bug numbers, the few JPS mortalities documented at the Wheless site during this period were believed to be due to predation by this species. Giant water bug nymphs feed on invertebrates, tadpoles, small frogs, small fish (Robinson 2005) and salamander larvae (Brunkhurst 2004), and were observed preying on tadpoles during the September surveys at Wheless Spring. Intact remains of JPS individuals were also found that appeared to have the body fluids removed. In one case, a dead individual that had previously been marked with VIE was found in this condition with two small red marks on either side of the body, possibly from where it had been held by the front legs of a giant water bug.

While fish were observed at all of the other monitoring sites (Figure B-6), few were considered predatory. Of the known and potential predators, most were found in very low numbers. This included an occasional small (~3-5") green sunfish (*Lepomis cyanella*), which has been observed feeding on JPS (O'Donnell et al. 2006). Small (2-3") yellow bullhead catfish (*Ameiurus natalis*) were seen on rare occasions at the Lanier Spring and Bull Creek Above Tributary 7 sites, and a small largemouth bass (*Micropterus salmoides*) was seen once at Lanier Spring. The most common species were blacktail shiners (*Cyprinella venusta*), which were most frequently seen at the Lower Ribelin site.

Crayfish are opportunistic predators that were common at all but the Wheless Spring site (Figure B-7). JPS were occasionally found with portions of their tails missing, which may have been the result of attempted predation by crayfish and/or other predators.

Juvenile blotched water snakes (*Nerodia erythrogaster transversa*) were seen on several occasions at the Lower Ribelin site and a couple of times at Lanier Spring. This species feeds on frogs, toads, tadpoles, salamanders, and crayfish (Werler and Dixon 2000). While it is a potential predator of JPS, more abundant food is typically available, including tadpoles, crayfish, and small fish.

Cannibalism was documented on a few occasions. Following anesthesia, adult JPS were observed regurgitating small juvenile JPS on two occasions at the Wheless Spring site. Cannibalism by a large adult (approximately 50 mm total length) on a smaller salamander (approximately 25 mm total length) was also observed on one occasion at the Lanier Riffle site.

Figure B-6. Approximate numbers of fish at *Eurycea tonkawae* monitoring sites during 2007 surveys. Many of the fish species, such as minnows, are not predatory. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

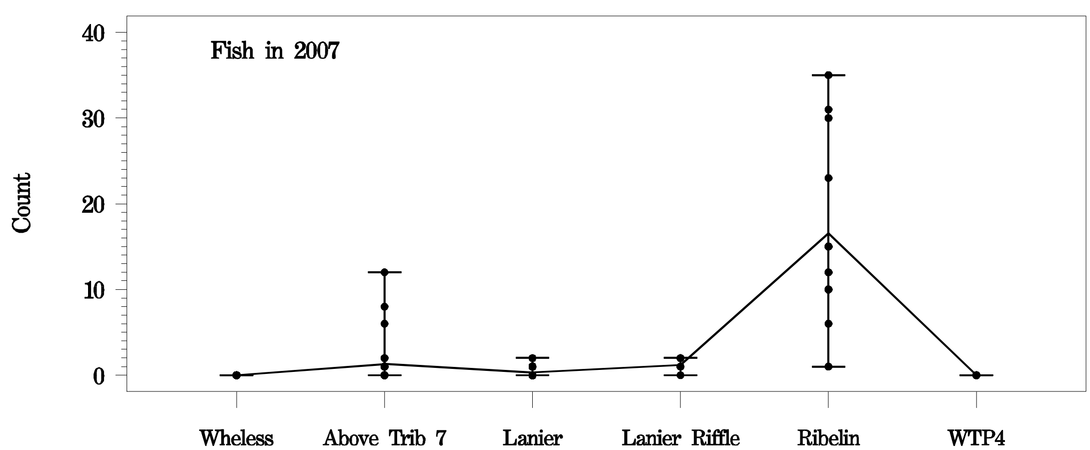
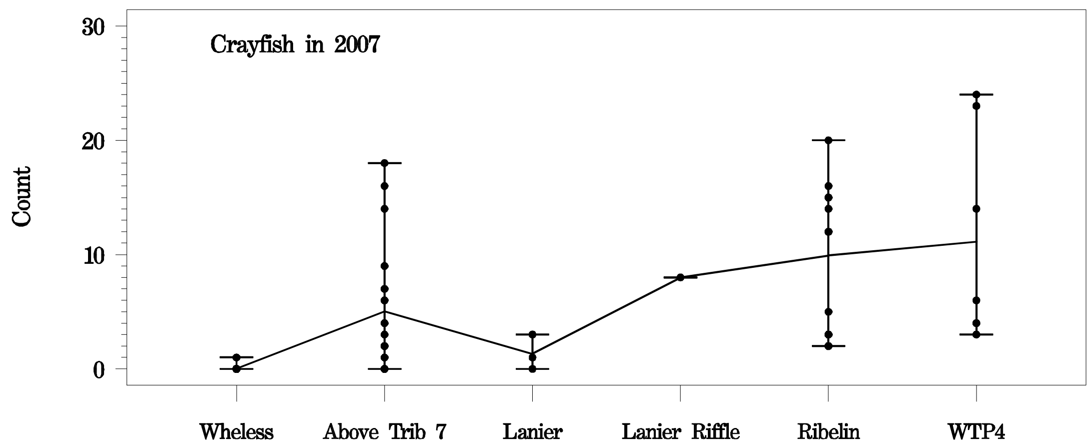


Figure B-7. Approximate numbers of large crayfish (>50 mm total length) at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.



Plant Cover – Overall, plant cover including algae, aquatic macrophytes, and leaf litter was highest at the Wheless site (Figures B-8, B-9, B-10). Leaf litter was high in all sections, but especially in the spring pool (Section 1) and sections 5-6. In these areas, JPS were commonly found in leaf litter. The species of algae were not identified during this study, but the predominant type was a green, silky, non-filamentous algae that has been observed at the Wheless site for many years. It was most abundant in the lower part of Section 3 and throughout Section 4, which are in full sun compared to the other sections. Macrophytes were also most abundant in Section 4.

For the Bull Creek sites, aquatic macrophytes were most abundant in the spring pool at Lanier Spring. Leaf litter was abundant in pools with low flows, including parts of the Lanier Spring pool and spring run, the upstream end of Bull Creek Above Tributary 7, and the downstream end of WTP4. Red algae (*Batrachospermum* sp.) were commonly observed in the creek channel at Bull Creek Above Tributary 7, Lower Ribelin, Lanier Spring, and Lanier Riffle. Unidentified species of algae were observed at the WTP4 site during a few surveys.

Figure B-8. Visual estimates of algae cover at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

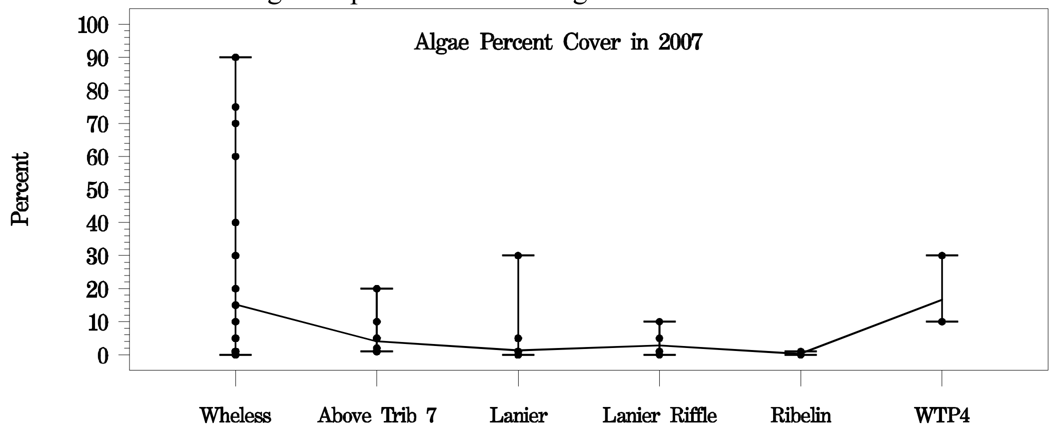


Figure B-9. Visual estimates of aquatic macrophytes at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.

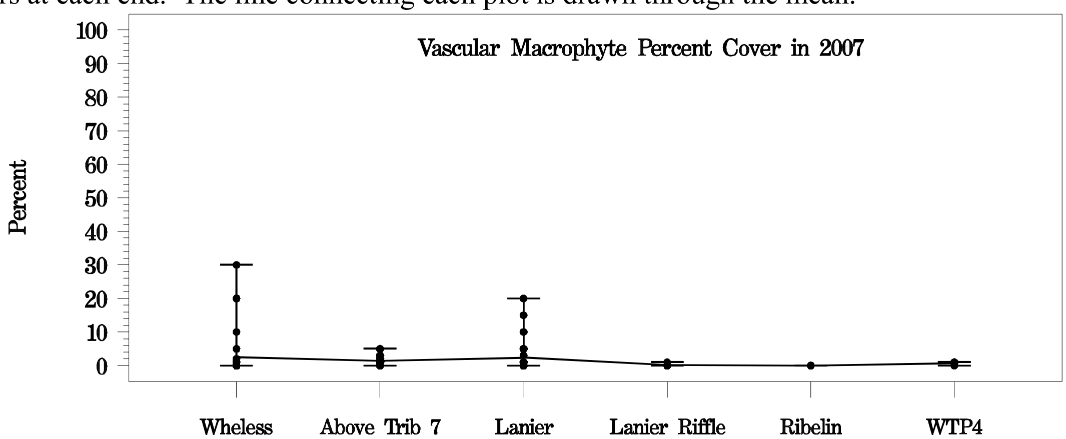
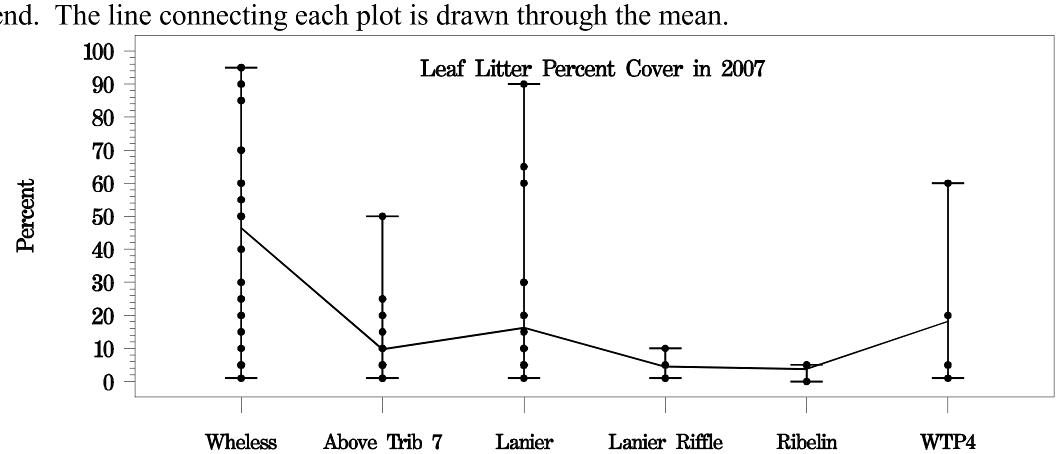


Figure B-10. Visual estimates of leaf litter at *Eurycea tonkawae* monitoring sites during 2007 surveys. Data are shown as points with a vertical line through their range, with minimum and maximum value bars at each end. The line connecting each plot is drawn through the mean.



Prey Items from Wild-Caught JPS

Four JPS were collected from Wheless Spring and Long Hollow Creek for the captive breeding program on January 31, 2007, about three weeks after the springs started flowing again. They were held for two days in small aerated containers to obtain fecal pellets prior to inclusion in the captive breeding pool. The contents of the fecal pellets were analyzed by Andrew Clamann (City of Austin, Watershed Protection and Development Review Department) and are presented in Table B-1. The predominant prey species were ostracods, chironomids, and copepods, which is consistent with previous fecal pellet and gut content analyses (Davis et al. 2001, O'Donnell et al. 2006). Based on these combined studies, JPS appear to be opportunistic predators of a variety of small invertebrates including chironomids, ostracods, copepods, mayflies, stoneflies, snails, limpets, diving beetles, and water mites. Observations in the captive breeding program also suggest they will feed on planaria (*Dugesia* sp.). The diet is likely more restricted within the aquifer, where stygobitic amphipods and isopods are commonly found.

Table B-1 Contents of fecal pellets from four wild-caught *Eurycea tonkawae* collected from Wheless Spring and Long Hollow on January 31, 2007. Order of the four collection sites is from upstream to downstream.

Invertebrate Taxon	**Location of Each JPS Collected From Wheless Spring and Long Hollow January 31, 2007**			
	Above Section 2	**Section 1 (spring pool)**	**Below low water crossing**	**Below first tributary downstream of lwc**
Chironominae (midge)			15	1
Hydracarina (Acari) (water mite)	1	1		
Ostracoda (crustacean)	9	13	56	10
Physella (snail)			2	1
Ferrissia (snail)			1	
Copepoda (crustacean)	11	8	6	4
Dytiscidae larvae (diving beetle)		1	2	
Unidentified arthropod	1		1	3
Zealeuctra (stonefly)	1	3	1	3

Eggs **–** Only two JPS eggs have been recorded in the wild, both of which were near spring outlets (O'Donnell et al. 2006; this study). During this study, one egg was found at Lanier Spring on March 21, 2007 (Figure B-11), in leaf litter just below the confluence of the spring run and creek. It was photographed and put back at the location where it was found. The absence of eggs in surface habitats suggests that egg deposition and development occur below the surface.

Figure B-11. Developing *Eurycea tonkawae* egg found at Lanier Spring on March 21, 2007.

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Appendix C. Observed Surface Movement of Jollyville Plateau Salamanders, 2007. See Appendix A for the location of individual sections within each monitoring site.

The majority of individuals captured at the Wheless and Lanier Spring sites were either recaptured within a few meters of where they were initially captured or were not recaptured at all. These observations were similar for the entire 8-month period as well as within and between primary periods. Since little mortality was observed during this study (<1% of the marked animals; Appendix D), most of the "disappearance" of individuals was attributed to vertical or horizontal movement beyond the study area.

Although most recaptures were found near the initial capture site, JPS are capable of moving long distances in a short period of time. One individual at Wheless Spring was observed over 45 m downstream from its previous capture site two days after it had been released. At Lanier Spring, two surveys conducted beyond the primary study area on May 14 and June 12, 2007 found seven marked individuals at distances ranging from over 20 to 37 meters upstream.

The pattern of increasing and decreasing numbers of JPS observed at all monitoring sites is believed to have been influenced in large part by dispersal of individuals based on surface flows, the size and number of spring outlets, and the amount of habitat at a given site. This pattern was observed in the dispersal of 38 individuals at Wheless Spring following their initial capture in the main spring pool shortly after it began flowing on March 12, 2007 (Table C-7). These individuals were presumably just moving to the surface from underground, which facilitated the tracking of a group of individuals from the time they emerged from the main spring outlet through the end of the 8-month study when the main spring was dry again. While overall movement appeared to be random, most of the individuals dispersed downstream of the main spring outlet.

Table C-1. Overall movement of marked *Eurycea tonkawae* at Lanier Spring, March-October 2007.

Section to and from	**Number**	**% of Observations**
1 to 1	140	47.3
1 to 2	5	1.7
1 to 3	9	3.0
1 to NULL	142	48.0
	296	
2 to 2	44	36.4
2 to 1	12	9.9
2 to 3	8	6.6
2 to NULL	57	47.1
	121	
3 to 3	252	45.1
3 to 1	10	1.8
3 to 2	14	2.5
3 to NULL	283	50.6
	559	
Total	976	
Stay Same	436	44.7
Move Up	36	3.7
Move Down	22	2.2
Disappear	482	49.4

Table C-2. Overall movement of marked *Eurycea tonkawae* at Wheless Spring, March-October 2007.

Section to and from	**Number**	**% of Observations**
1 to 1	22	11.5
1 to 3	18	9.4
1 to 4	12	6.3
1 to 5	6	3.1
1 to null	134	69.8
	192	
3 to 3	170	28.6
3 to 1	23	3.9
3 to 4	14	2.4
3 to 5	14	2.4
3 to null	373	62.8
	594	
4 to 4	224	27.8
4 to 1	17	2.1
4 to 3	52	6.4
4 to 5	35	4.3
4 to null	479	59.4
	807	
5 to 5	306	33.4
5 to 1	11	1.2
5 to 3	45	4.9
5 to 4	86	9.4
5 to null	468	51.1
	916	
Total	2509	
Stay Same	722	28.7
Move Up	234	9.3
Move Down	99	3.9
Disappear	1454	58

Table C-3. Movement of marked *Eurycea tonkawae* at Lanier Spring within primary sessions, March-October 2007.

% of Observations									
Movement	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Range**
1 to 1	10.9	13.3	22.2	0.0	0.0	17.6	36.7	21.2	0 – 36.7
1 to 2	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0 – 3.3
1 to 3	1.8	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0 – 6.7
1 to NULL	87.3	76.7	77.8	100.0	100.0	82.4	63.3	78.8	63.3 – 100
2 to 2	31.6	47.4	46.2	7.7	0.0	25.0	0.0	44.4	0 – 47.4
2 to 1	0.0	10.5	0.0	7.7	0.0	0.0	0.0	11.1	0 – 11.1
2 to 3	5.3	10.5	0.0	7.7	25.0	0.0	0.0	0.0	0 – 25
2 to NULL	63.2	31.6	53.8	76.9	75.0	75.0	100.0	44.4	31.6 – 100
3 to 3	48.2	36.9	23.5	20.0	39.3	37.5	39.3	32.1	20 – 48.2
3 to 1	0.0	1.2	0.0	0.0	0.0	6.3	0.0	1.9	0 – 6.3
3 to 2	0.0	0.0	4.4	2.5	3.6	6.3	0.0	1.9	0 – 6.3
3 to NULL	51.8	61.9	72.1	77.5	57.1	50.0	60.7	64.2	50 – 77.5
Stay Same	33.1	33.1	26.3	11.8	26.2	27.0	36.7	29.5	11.8 – 36.7
Move Up	0.0	2.3	3.0	2.6	2.4	5.4	0.0	3.2	0 – 5.4
Move Down	1.3	3.8	0.0	1.3	2.4	0.0	0.0	0.0	0 – 3.8
Disappear	65.6	60.9	70.7	84.2	69.0	67.6	63.3	67.4	60.9 – 84.2

Table C-4. Movement of marked *Eurycea tonkawae* at Wheless Spring within primary sessions, March-October 2007. Section 1 was dry and Section 3 was mostly dry on March 10 and 11, 2007; began flowing again on March 12. The spring run between Sections 1 and 3 was dry in September. Both sections were almost completely dry during the October primary period.

% of Observations									
Movement	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Range**
1 to 1	--	0.0	10.5	6.3	0.0	0.0	0.0	--	0 – 10.5
1 to 4	--	0.0	0.0	0.0	0.0	3.9	0.0	--	0 – 3.9
1 to 5	--	6.7	0.0	0.0	0.0	0.0	0.0	--	0 – 6.7
1 to null	--	93.3	89.5	93.8	100.0	96.1	100.0	--	93 - 100
3 to 3	--	15.5	23.1	12.5	7.5	9.1	0.0	--	0 – 23.1
3 to 1	--	0.0	0.7	0.0	2.5	3.0	0.0	--	0 – 3
3 to 4	--	0.9	0.0	0.0	0.0	0.0	0.0	--	0 – 0.9
3 to 5	--	0.0	1.5	0.0	2.5	0.0	6.3	--	0 – 6.3
3 to null	--	83.6	74.6	87.5	87.5	87.9	93.8	--	74.6 – 93.8
4 to 4	0.0	15.8	15.6	18.4	21.2	8.8	18.8	11.1	0 – 21.2
4 to 1	0.0	0.0	1.6	0.0	0.0	1.5	0.0	0.0	0 – 1.6
4 to 3	0.0	5.9	6.3	7.9	3.0	0.0	0.0	0.0	0 – 7.9
4 to 5	12.5	1.0	1.6	0.0	0.0	3.6	3.6	0.0	0 – 12.5
4 to null	87.5	77.2	75.0	73.7	75.8	86.1	77.7	88.9	73.7 – 88.9
5 to 5	6.8	18.6	19.7	33.3	34.9	31.3	32.9	28.2	6.8 – 34.9
5 to 3	0.0	1.7	2.6	3.3	0.8	0.0	0.0	0.0	0 – 2.6
5 to 4	0.0	6.8	1.3	0.0	0.0	0.0	7.6	0.0	0 – 7.6
5 to null	93.2	72.9	76.3	63.3	64.3	68.7	59.5	71.8	59.5 – 93.2
Stay Same	6.2	15.5	19.8	20.6	22.5	12.5	22.3	20.0	6.2 – 22.5
Move Up	0.0	3.8	3.1	2.9	1.8	1.0	2.8	0.0	0 – 3.8
Move Down	1.2	1.0	1.0	0.0	0.7	2.4	2.4	0.0	0 – 2.4
Disappear	92.6	79.7	76.1	76.5	75.1	84.0	72.5	80.0	72.5 – 92.6

Table C-5. Movement of marked *Eurycea tonkawae* at Lanier Spring between primary sessions, March-October 2007.

% of Observations								
Movement	**Mar to Apr**	**Apr to May**	**May to Jun**	**Jun to Jul**	**Jul to Aug**	**Aug to Sep**	**Sep to Oct**	**Range**
1 to 1	36.5	25.7	22.7	13.0	23.1	30.4	46.3	13 – 46.3
1 to 2	4.8	0.0	0.0	0.0	0.0	0.0	0.0	0 – 4.8
1 to 3	3.2	2.9	0.0	0.0	0.0	4.3	0.0	0 – 4.3
1 to NULL	55.6	71.4	77.3	87.0	76.9	65.2	53.7	53.7 – 87
2 to 2	33.3	50.0	8.7	0.0	0.0	0.0	0.0	0 – 50
2 to 1	6.7	14.3	4.3	0.0	0.0	50.0	0.0	0 – 50
2 to 3	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0 – 6.7
2 to NULL	53.3	35.7	87.0	100.0	100.0	50.0	100.0	35.7 – 100
3 to 3	26.0	19.2	6.5	11.3	23.1	31.3	42.4	6.5 – 42.4
3 to 1	2.6	0.0	0.0	0.0	3.8	0.0	0.0	0 – 3.8
3 to 2	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0 – 1.3
3 to NULL	70.1	79.5	93.5	88.7	73.1	68.8	57.6	57.6 – 93.5
Stay Same	31.0	24.4	10.3	10.5	21.4	26.7	41.3	10.3 – 41.3
Move Up	2.6	2.4	0.9	0.0	2.4	6.7	0.0	0 – 6.7
Move Down	3.9	0.8	0.0	0.0	0.0	2.2	0.0	0 – 3.9
Disappear	62.6	72.4	88.8	89.5	76.2	64.4	58.8	58.8 – 89.5

Table C-6. Movement of marked *Eurycea tonkawae* at Wheless Spring between primary sessions, March-October 2007. Null = not seen again. Section 1 was dry and Section 3 was mostly dry on March 10 and 11, 2007; began flowing again on March 12 (Section 3 not surveyed on March 12). The spring run between Sections 1 and 3 was dry in September. Both sections were almost completely dry during the October primary period.

% of Observations								
Movement	**Mar to Apr**	**Apr to May**	**May to Jun**	**Jun to Jul**	**Jul to Aug**	**Aug to Sep**	**Sep to Oct**	**Range**
1 to 1	13.2	11.1	12.9	8.3	0.0	0.0	--	0 – 13.2
1 to 3	21.1	0.0	6.5	4.2	0.0	0.0	--	0 – 21.1
1 to 4	10.5	0.0	0.0	0.0	0.0	0.0	--	0 – 10.5
1 to 5	5.3	0.0	0.0	0.0	0.0	0.0	--	0 – 5.3
1 to null	50.0	88.9	80.6	87.5	100.0	100.0	--	50 – 100
3 to 3	--	24.1	14.2	19.0	0.0	6.1	--	0 – 24.1
3 to 1	--	6.4	2.0	1.2	0.0	0.0	--	0 – 6.4
3 to 4	--	1.4	2.7	1.2	0.0	0.0	--	0 – 2.7
3 to 5	--	0.0	4.1	0.0	0.0	0.0	--	0 – 4.1
3 to null	--	68.1	77.0	78.6	100.0	93.9	--	68.1 – 100
4 to 4	47.8	17.8	16.7	23.3	11.5	9.4	24.8	9.4 – 47.8
4 to 1	0.0	1.9	1.5	0.0	5.7	0.7	0.0	0 – 5.7
4 to 3	0.0	15.9	9.1	10.0	1.1	2.0	0.0	0 – 15.9
4 to 5	0.0	3.7	4.5	3.3	3.4	2.0	1.7	0 – 4.5
4 to null	52.2	60.7	68.2	63.3	78.2	85.9	73.5	52.2 – 85.9
5 to 5	27.1	8.6	19.4	44.9	9.9	8.9	16.2	8.9 – 44.9
5 to 1	1.2	0.0	1.1	0.0	3.5	0.0	0.0	0 – 3.5
5 to 3	2.4	18.6	3.2	1.4	1.4	1.8	0.0	0 – 18.6
5 to 4	7.1	1.4	2.2	2.9	7.1	19.6	8.1	1.4 – 19.6
5 to null	62.4	71.4	74.2	50.7	78.0	69.6	75.7	50.7 – 78
Stay Same	26.7	18.2	16.0	26.6	7.0	7.5	19.2	7 – 26.7
Move Up	6.2	12.5	4.7	4.2	6.7	8.1	2.8	2.8 – 12.5
Move Down	9.6	1.8	4.4	1.7	0.9	0.9	0.9	0.9 – 9.6
Disappear	57.5	67.6	74.9	67.5	85.4	83.5	77.0	57.5 – 83.5

Table C-7. Movement of 38 *Eurycea tonkawae* captured and marked from the Section 1 springpool at Wheless Spring, March 12, 2007. The spring pool was dry March 10 and 11, 2007. Five (lavender) were found only in Section 1, and two (Yellow) were found in the pool the following month before being recaptured in another section. Salamander # refers to the database entry.

Sal #	3/12	4/23	4/24	4/25	5/21	5/22	5/23	6/18	6/19	6/20	7/16	7/17	7/18	8/20
503	1	3						3					3	
504	1	3			3									
505	1			3										
506	1								1					
507	1													
508	1													
509	1													
510	1	3					3							
511	1													
512	1				3					3				
513	1													
514	1	3	3			3								
515	1	1		5							6			
516	1								1					1
517	1		2	3										
518	1							5			5			
519	1													
520	1	3				3								
521	1	1												
522	1			5										
523	1													
524	1	1			1					1				
525	1													
526	1				4							5		
527	1						4		3	3				
528	1							4						
529	1	1												3
530	1													
531	1	4		4		5			4			3	3	
532	1	4	4		5						3			
533	1		3											
534	1													
535	1													
536	1													
538	1	1							1			1		
539	1		5			6			6					
540	1	4												
16807	1	4				3	3							

Appendix D. Summary of *Eurycea tonkawae* Mark-Recapture Survey Data.

Eurycea tonkawae Mark-Recapture Survey Data, Lanier Spring

Date	Total Collected for MR	# Marked	# Recaps	% Recaps	# Mortalities	# Too Small to Mark (<25mm)	Comments	Flow (cfs) Section 3	Flow (cfs) Section 2
02/16/07							Surface count (0 <1", 12 1-2", 27 >2"; 39 total)	0.67	0.04
3/13/2007*	18	18	--	--			*Discontinued due to rain. Does not include 14 released due to rain; surveyed sections 2, 3 only		
03/19/07	91	84	6	7%	1 unmarked*	2	*injury, missing most of tail	1.19	
03/20/07	70	41	29	41%		4	+4 batch marks (red)		
03/21/07	52	23	29	56%	(1 recap)*	2	+ 1 batch mark (red); found 1 egg; *injury		
04/16/07	62	40	22	35%		10	+ 4 batch marks (red)		
04/17/07	69	17	52	75%		12	+ 1 batch mark (red)		
04/18/07	45	7	38	84%	(1 recap)*	4	*injury due to processing/handling		
04/19/07								1.03	0.04
05/14/07	43	20	23	53%		3		unable to get flow due to rain	
05/15/07	56	28	28	50%		5			
05/16/07	37	11	26	70%		0	Heavy rain night before, overtopped nets		
06/11/07	33	16	17	52%		0			
06/12/07	42	23	19	45%		5			
06/13/07	22	12	10	45%		2			
06/14/07								1.65	0.04
07/09/07	28	14	14	50%		3		3.28	0.05
07/10/07	17	7	10	59%		0			
07/11/07	13	3	10	77%		0			
08/13/07	19	8	11	58%		9		unable to get flow due to rain	
08/14/07	18	8	10	56%		7			
08/15/07	20	8	12	60%		8			

09/10/07	39	22	17	44%		7		0.68	0.05
09/11/07	20	8	12	60%		0	Rained ~1.6" during am, nets pulled up from bottom		
09/12/07	42	17	25	60%		1			
09/13/07								1.36	0.06
10/08/07	49	21	28	57%		0			
10/09/07	46	15	31	67%	1 unmarked*	0	*appeared to be a mortality prior to survey		
10/10/07	27	10	17	63%		0			
10/11/07								0.13	0.03
2007 Total		**481**							
3/10/08	112	36	76	68%					
3/11/08	102	19	83	81%					
3/12/08	87	10	77	89%					

Eurycea tonkawae Mark-Recapture Survey Data, Lanier Riffle

Date	Total Collected for MR	# Marked	# Recaps	% Recaps	# Mortalities	# Too Small to Mark (<25mm)	Comments	Flow (cfs)
02/16/07							Surface count (0 <1", 2 1-2", 4 >2"; 6 total)	0.31
04/16/07	2	2	--	--				
04/17/07	--	--	--	--			Not enough time to survey this site	
04/18/07	8	8	0	0%				
04/19/07								0.68
05/14/07	7	3	4	57%				
05/15/07	5	3	2	40%				unable to measure flow due to rain
05/16/07	2	2	0	0%			Heavy rains night before, overtopped nets	
06/11/07	3	0	3	100%				
06/12/07	5	2	2	40%	1 unmarked*		*cannibalism by larger salamander	
06/13/07	0	0	0	--				
06/14/07								1.21
07/09/07	0	0	0	--				
07/10/07	1	0	1	100%				
07/11/07	0	0	0	--				
07/12/07								1.39
08/13/07								
08/14/07							Discontinued surveys due to time and low numbers	
08/15/07								
Total		**20**						

Eurycea tonkawae Mark-Recapture Survey Data, Lower Ribelin

Date	Total Collected for MR	# Marked	# Recaps	% Recaps	# Mortalities	# Too Small to Mark (<25mm)	Comments	Flow (cfs)
05/14/07	44	44	--	--		6		unable to get flow due to rain
05/15/07	30	21	9	30%		11		
05/16/07	6	6	0	0%		6	Heavy rains night before, overtopped nets	
06/11/07	24	11	13	54%		1		
06/12/07	24	10	14	58%		11		
06/13/07	32	17	15	47%		6		
06/14/07								0.40
07/09/07	23	12	11	48%		4		
07/10/07	27	10	17	63%		4		
07/11/07	20	7	13	65%		??	Missing summary data sheet	
07/13/07								0.26
08/13/07	49	32	17	35%		3		unable to get flow due to rain
08/14/07	37	16	21	57%		2		
08/15/07	34	9	25	74%		3		
09/10/07	46	23	23	50%		4		0.13
09/11/07	24	8	16	67%		2	Rained ~1.6" during am, nets pulled up from bottom	
09/12/07	30	9	21	70%		0		
10/08/07	69	30	39	57%		6		
10/09/07	46	10	36	78%		4		
10/10/07	39	6	33	85%		0		
2007 Total		**281**						
2/12/08	84	32	52	62%				

Eurycea tonkawae Mark-Recapture Survey Data, Wheless Spring

Date	Total Collected for MR	# Marked	# Recaps	% Recaps	# Mortalities	# Too Small to Mark (<25mm)	Comments	Flow (cfs)
01/11/07							Surface count (1 <1", 98 1-2", 17 >2"; 116 total)	0.10
01/31/07								0.39
02/15/07								0.11
3/10/2007*	42	42	--	--		0	*Section 1 dry, sections 2 and 3 almost dry. Also marked 18 from section 6	0.02
3/11/2007*	39	36	3	8%		0	*Surveyed sections and 6 only; section 1 dry, sections 2 and 3 almost dry. Also marked 16 from section 6	
3/12/2007*	70	63	7	10%		0	*Section 1 flowing following rain last night; surveyed sections 1, 4, 5, 6; also marked 6 from section 6	
04/23/07	177	140	37	21%	1 unmarked	33	+ 4 batch marks (blue)	0.16
04/24/07	115	74	41	36%		46		
04/25/07	104	55	49	47%		31		
05/21/07	175	103	72	41%		112		0.19
05/22/07	118	56	62	53%	(1 recap)	112		
05/23/07	115	47	67	58%	1 unmarked	92		
06/18/07	100	41	59	59%		57		0.21
06/19/07	72	24	48	67%		61		
06/20/07	107	43	64	60%	(1 recap)	81		
07/16/07	143	62	80	56%	1 unmarked (1 recap)*	42	*Injury	0.50
07/17/07	145	73	72	50%		40		
07/18/07	126	47	79	63%		24		

8/20/2007*	185	145	40	22%		15	*Does not include section 5, ran out of light before able to finish; had to release 29+ unmarked, 15+ recaptures	0.09
08/21/07	104	76	28	27%		9		
08/22/07	106	57	48	45%	1 unmarked (2 recaps)	11		
09/17/07	113	65	48	42%		4	water in deeper pools and trib below lwc has greenish tint from dye trace project	0.004
09/18/07	98	46	52	53%		2		
09/19/07	63	27	35	56%	1 unmarked (1 recap)*	7	*2 found dead in upper section 5 pool, believed to be due to Lethocerous predation	
10/15/07	115	64	51	44%		1	**believe flow is an error, barely flowing; no flow in sections 1-3	0.01**
10/16/07	35	18	19	54%		0		
10/17/07	51	17	34	67%	3*	0	*unable to tell if marked or not; believed to be due to Lethocerous predation	
2007 Total		**1421**						
3/24/08	102	28	74	73%				
3/25/08	65	24	41	63%				
3/26/08	41	4	37	90%				

Appendix E. Assumption of Closure Within Primary Periods - Statistical Analyses

The program CAPTURE was used to analyze the mark-recapture data to verify assumptions of closure within primary periods and to quantify potential behavioral or group responses to capture and marking. Data from each primary period was analyzed individually and by site (8 primary periods at Wheless, 8 primary periods at Lanier Spring, and 6 primary periods at Lower Ribelin). Data from the initial March 13 sampling at Lanier Spring was excluded from the analysis because this survey had to be terminated early due to weather and was not a complete (multi-day) sampling period (Table E-1).

Table E-1. Summary of primary period sampling months analyzed individually using CAPTURE. Cells with “x” indicate data collected at that site in the specified month.

Month	Primary Period	Lanier	Wheless	Ribelin
Mar	1	x*	x	
Apr	2	x	x	
May	3	x	x	x
Jun	4	x	x	x
Jul	5	x	x	x
Aug	6	x	x	x
Sep	7	x	x	x
Oct	8	x	x	x

*March 13 data collected at Lanier Spring excluded from analysis.

CAPTURE fits capture history data to a set of 8 closed population models in which capture probability may vary with trapping day (t), behavioral response to trapping (b), heterogeneity between groups of animals (h), and in all possible combinations of these effects along with a null model (Otis et al. 1978). Models and assumptions of CAPTURE are listed in Table E-2. The varying models estimated by CAPTURE were designed to relax the assumption of equal and constant probability of capture. Additionally, CAPTURE compares models and selects the best model(s) based on model comparisons and goodness-of-fit tests.

Table E-2. Models and assumptions of CAPTURE.

<table>
<tr><th></th><th>Models</th><th>Assumptions</th></tr>
<tr><td>M_o</td><td>Neither behavioral nor temporal variation nor capture heterogeneity</td><td rowspan="8">The population is closed within primary periods

Markings are not lost

Marks are correctly read and reported

Animals have equal and constant probability of capture</td></tr>
<tr><td>M_b</td><td>Behavioral response only</td></tr>
<tr><td>M_t</td><td>Temporal variation only</td></tr>
<tr><td>M_h</td><td>Individual heterogeneity only</td></tr>
<tr><td>M_{tb}</td><td>Behavioral and temporal variation only</td></tr>
<tr><td>M_{bh}</td><td>Behavioral response and capture heterogeneity only</td></tr>
<tr><td>M_{th}</td><td>Temporal variation and capture heterogeneity only</td></tr>
<tr><td>M_{tbh}</td><td>Behavioral response, temporal variation, and capture heterogeneity</td></tr>
</table>

Closure implies that the population is closed to recruitment (birth or immigration) and loss (mortality or emigration) within primary periods, i.e., across the three consecutive days of the secondary samples (White et al. 1982). The assumption of closure within primary periods is of critical importance to accurately estimate population size (Otis et al. 1978, Stanley and Burnham 1999). This may be of special concern in this study as the salamanders could not reasonably be restricted from vertical movement into or out of the subsurface aquifer through springs of subterranean crevices. In all of the study sites it was theoretically possible for animals to enter or leave the study site through vertical movement. The extent of such vertical movement during short periods of time is unknown. Horizontal closure (restriction of surface movement in and out of the study area) was enforced using temporary nets upstream and downstream of the study area. Secondary sampling periods occurred on consecutive days, reducing the probability of significant births, deaths, immigration or emigration. To verify horizontal closure, stream segments were searched both upstream and downstream of the survey area at the Lanier Spring and Wheless Spring sites, and occasional cursory checks were made above and below the Lower Ribelin site (little loose rock substrate or other protective cover occurred beyond the Lower Ribelin site).

CAPTURE includes a test of the validity of closure using the heterogeneous response to the capture (M_h) model as the null hypothesis. However, strong behavioral responses to capture (trap happy or trap shy) are not distinguishable from failure of closure (Otis et al. 1978, White et al. 1982, Stanley and Burnham 1999). Therefore, an additional test for closure was conducted using the CLOSETEST program. A subsequent analysis was also conducted in RDSURVIV, which has the advantage over CAPTURE because it provides parameter estimates for persistence, capture probability, and temporary emigration with confidence intervals and standard errors for a set of 12 defined models (Table E-3; Hines 1996a). Models may include behavioral effects, time effects and temporary emigration effects. RDSURVIV was developed to extend the likelihood-based approach for the estimation of demographic parameters from capture-recapture data (Kendall et al. 1995) using the SURVIV program (White 1983) to the robust design. Additionally, RDSURVIV provides a goodness-of-fit test for each model and likelihood ratio tests (LRT) for nested models (Hines 1996a).

Table E-3. Summary of models specified by program CNVRDSRV evaluated by RDSURVIV using capture-history data. S=survival (referred to in this study as persistence), p=probability of capture, c=probability of recapture, γ=temporary emigration, b=behavioral effects (p≠c).

Model notation	Brief Description
P(.,0)S(.)	constant S, p constant, p=c, no γ
P(t,0)S(t)	time-varying S, p constant within sessions but may vary between sessions, p=c, no γ
P(t,b)S(t)	time-varying S, p constant within sessions but may vary between sessions, p not equal c, c equal within sessions, no γ
P(t,t)S(t)	time-varying S, p and c vary between and within sessions, p=c, no γ
P(t,bt)S(t)	time-varying S, p varies between and within sessions, p not equal c, c varies between sessions, no γ
P(.,0)S(.)γ(.)	constant S, p constant, p=c, random constant γ
P(.,bt)S(.)γ(.)	constant S, p varies within sessions but not between sessions, p not equal c, random constant γ
P(.,bt)S(.)γ(.)γ2(.)	constant S, p varies within sessions but not between sessions, p not equal c, markovian constant γ
P(t,0)S(t)γ(.)	time-varying S, p constant within sessions but may vary between sessions, p=c, random constant γ
P(t,b),S(t),γ(.)	time-varying S, p constant within sessions but may vary between sessions, p not equal c, c equal within sessions, random constant γ
P(t,t)S(t)γ(.)	time-varying S, p and c vary between and within sessions, p=c, random constant γ
P(t,bt)S(t)γ(.)	time-varying S, p varies between and within sessions, p not equal c, c varies between sessions, constant random emigration

Capture history data in the standard 0/1 format (1=present, 0=absent) was converted to the input format required for RDSURVIV (Hines 1996a) using the program CNVRDSRV (Hines 1996b). Data from Lanier Spring, Lower Ribelin, and Wheless Spring were analyzed separately. The programs RDSURVIV and CNRDSRV were downloaded from the United States Geological Survey (USGS) Patuxent Wildlife Research Center software archive (mbr-pwrc.usgs.gov/software).

Data were analyzed in RDSURVIV by identifying candidate model(s) that best fit the data. Akaike's Information Criteria (AIC) scores were used to rank models (Anderson and Burnham 1999) with appropriate goodness-of-fit test scores (probability of larger χ^2>0.10, Bailey et al. 2004) to identify the best models. For models with low AIC scores, LRT were used to identify the most parsimonious models (least parameterized) among nested models that were not significantly different from each other. Additionally, model fit was evaluated using the χ^2 test for each cohort output by RDSURVIV expressed as the percentage of cohorts with non-significant χ^2 values (probability of larger χ^2>0.10).

An additional test for closure was performed using the CLOSETEST software (Stanley and Burnham 1999, Stanley and Richards 2005) downloaded from the USGS Fort Collins Science Center website (fort.usgs.gov/Products/Software). The Stanley and Burnham (1999) test is more robust for the detection of permanent emigration when a behavioral response to capture may be present (Stanley and Burnham 1999) than the closure test in the CAPTURE program (Otis et al. 1978). However, the Stanley and Burnham test is less sensitive to temporary emigration (Stanley and Burnham 1999).

Based on the output from CAPTURE (Table E-4), closure was potentially violated in 9 of 22 (41%) "populations". A population is defined by site and primary sampling period; there were two sites with eight primary periods, and one site with six primary periods. With the restriction of horizontal (surface) movement, the closure violations are assumed to have been due primarily to vertical (surface to subsurface) movement.

The test for a behavioral response after initial capture (null hypothesis of M_o versus the alternate hypothesis of M_b) indicated a potential behavioral response in 13 of 21 (62%) possible closed populations. A test could not be performed for the first period at Wheless due to insufficient data. Six of the 13 primary periods with a significant behavioral response were coincident with significant closure test failures. In the eight populations without evidence of a behavioral response to marking, closure was rejected three times, once in each site. These three primary samples occurred in June or July when surface counts were low and thus may have been prone to stochastic events. Time-varying capture probabilities are discussed in Model Selection below.

The CLOSETEST was applied to each of the individual primary periods at each sample site (Table E-5). Closure appears to have been maintained in the majority of primary periods. CLOSETEST output indicates a significant ($\alpha \leq 0.05$) failure of closure in only two primary sampling periods: the Lower Ribelin site in October and the Wheless Spring site in July. The component scores for the Ribelin October primary period suggest that the Lower Ribelin population was more affected by loss than recruitment. The failure of closure at Wheless is of particular interest since CAPTURE indicated the M_o model was the best fit for the data and the test for behavioral effects was non-significant. Component tests indicate that within primary periods, salamander populations may be equally likely to experience additions or losses. Based on the CLOSETEST output, assumption of closure within primary periods appears reasonable.

Model Selection - CAPTURE
Time-varying capture probabilities were indicated in 17 of 22 primary periods (or 77%) by program CAPTURE (Table E-4). The months in which the primary period data did not support time-varying capture probabilities occurred during the months of June, July and August. Constant capture probabilities were indicated more frequently at the Lower Ribelin site than at the Lanier Spring or Wheless Spring sites. Behavioral response to capture was significant in only one of the five sessions (August at Lower Ribelin) without time-constant capture probabilities.

The most frequently selected "best" model by CAPTURE for all sites was the M_{tb}, the model including time-varying capture probability and a behavioral response to capture, which was selected in 7 of 22 (32%) secondary sessions. The M_{tb} model was chosen only slightly higher than the null model (M_o), which was selected as best in 5 of 22 (23%) secondary sessions. The M_{tb} model was selected in 50% of the primary periods at the Wheless site (Table E-6). No other model was selected as frequently at any other site.

Based on the "best" model selected by CAPTURE, behavioral effects were indicated in 14 of 22 (64%) primary periods, while time-varying capture probabilities were indicated in 13 of 22 (59%) primary periods (Table E-6). Heterogeneity in capture probabilities was indicated in only 7 of 22 (32%) primary periods. *A posteriori* evaluation of the data by CAPTURE suggests that MARK models should account for unequal p and c values and time-varying p values.

Table E-4. CAPTURE output for closure test (Otis et al. 1978), behavioral response (null hypothesis of M_o versus alternate hypothesis of M_b) and time-varying capture probability (null hypothesis of M_o versus alternate hypothesis of M_t). Values where failure of closure is significant (<0.10) and the alternate hypothesis (M_b, M_t) is accepted (<0.10) are highlighted.

Site	Primary Period	Closure	Mo vs Mb	Mo vs Mt
		Pr<z	Pr>χ^2	Pr>χ^2
Lanier	March	0.55	<0.01	<0.01
Lanier	April	0.02	0.07	0.01
Lanier	May	0.34	0.36	0.07
Lanier	June	0.03	0.11	0.02
Lanier	July	0.08	0.01	0.06
Lanier	August	0.41	0.94	0.93
Lanier	September	0.93	0.62	<0.01
Lanier	October	0.02	0.01	<0.01
Ribelin	May	0.02	<0.01	<0.01
Ribelin	June	0.50	0.23	0.37
Ribelin	July	0.08	0.48	0.50
Ribelin	August	0.44	0.01	0.14
Ribelin	September	0.42	0.01	0.01
Ribelin	October	0.03	<0.01	<0.01
Wheless	March	0.81	test failed	<0.01
Wheless	April	0.97	<0.01	<0.01
Wheless	May	0.54	<0.01	<0.01
Wheless	June	0.45	0.43	0.01
Wheless	July	0.01	0.20	0.39
Wheless	August	0.85	<0.01	<0.01
Wheless	September	0.06	<0.01	<0.01
Wheless	October	0.99	<0.01	<0.01

Table E-5. Summary of CLOSETEST output, including overall closure test and components. JS = open-population Jolly-Seber model. NR = no recruitment model allowing for mortality. NM = no recruitment, no mortality model. M_t = temporal variation only model. Values where failure of closure is significant (≤0.05) and where additions or losses are occurring (≤0.05) are highlighted.

Site	**Primary Period**	**Closure**	**Additions**		**Losses**	
			NR vs JS	Mt vs NM	Mt vs NR	NM vs JS
Lanier	March	0.33	0.59	0.79	0.16	0.14
Lanier	April	0.11	0.57	0.43	0.04	0.05
Lanier	May	0.56	0.31	0.47	0.72	0.42
Lanier	June	0.41	n/a	n/a	0.41	0.25
Lanier	July	0.15	0.07	0.20	0.54	0.15
Lanier	August	0.80	0.54	0.50	0.79	0.96
Lanier	September	0.17	0.55	0.28	0.08	0.12
Lanier	October	0.08	0.05	0.29	0.25	0.04
Ribelin	May	0.27	n/a	n/a	0.27	n/a
Ribelin	June	0.32	0.14	0.21	0.83	0.40
Ribelin	July	0.50	0.61	0.73	0.28	0.26
Ribelin	August	0.72	0.93	0.67	0.42	0.50
Ribelin	September	0.07	0.15	0.47	0.08	0.03
Ribelin	October	0.00	0.05	0.14	0.00	0.00
Wheless	March	0.27	n/a	n/a	0.27	n/a
Wheless	April	0.17	0.09	0.06	0.42	0.93
Wheless	May	0.82	0.54	0.62	0.89	0.72
Wheless	June	0.11	0.04	0.05	0.77	0.57
Wheless	July	0.02	0.29	0.41	0.01	0.01
Wheless	August	0.23	0.78	0.32	0.09	0.16
Wheless	September	0.28	0.11	0.20	0.86	0.33
Wheless	October	0.41	0.55	0.36	0.23	0.33

Table E-6. Best model selected by CAPTURE with estimated surface population in comparison to number of unique individuals observed during each primary period by site.

Site	Primary Period	Best Model	N (estimated)	N (total unique captures)	# observed as % of estimated
Lanier	March	Mb	174	155	89.1
Lanier	April	Mth	238	129	54.2
Lanier	May	Mo	194	107	55.2
Lanier	June	Mth	234	86	36.8
Lanier	July	Mtbh or Mbh	71	42	59.2
Lanier	August	Mo	81	45	55.6
Lanier	September	Mt	138	80	58.0
Lanier	October	Mtb	115	91	79.1
Ribelin	May	Mtb	77	73	94.8
Ribelin	June	Mo	114	57	50
Ribelin	July	Mo	98	55	56.1
Ribelin	August	Mbh	116	92	79.3
Ribelin	September	Mtb	147	79	53.7
Ribelin	October	Mb	122	111	91.0
Wheless	March	Mtbh or Mh	285	146	51.2
Wheless	April	Mb	420	336	80.0
Wheless	May	Mtb	734	338	46.0
Wheless	June	Mt	601	238	39.6
Wheless	July	Mo	749	342	45.7
Wheless	August	Mtb	472	346	73.3
Wheless	September	Mtb	324	213	65.7
Wheless	October	Mtb	218	171	78.4

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Appendix F. Results of MARK Models for Lanier Spring, Wheless Spring, and Lower Ribelin Sites, Based on 2007 datasets.

Wheless Spring Analysis

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{S(s) p(s) c(s) random Huggins}	9938.423	0.00	0.56103	1.0000	29.000	15580.965
{S(s) p(s) c(s) random Huggins p1=0.05}	9939.194	0.77	0.38150	0.6800	28.000	15583.783
{S(s) p(s) c(s) markov Huggins}	9945.141	6.72	0.01951	0.0348	34.000	15577.423
{S(s) p(s) c(s) random cte Huggins}	9945.645	7.22	0.01516	0.0270	24.000	15598.406
{S(s) p(s) c(s) random cte Huggins p1=0.05}*	9946.427	8.00	0.01026	0.0183	23.000	15601.226
{S(s) p(s) c(s) markovian cte Huggins}	9947.058	8.63	0.00748	0.0133	25.000	15597.778
{S(s) p(s) c(s) markovian cte Huggins p1=0.05}	9947.838	9.41	0.00506	0.0090	24.000	15600.598
{S(s) p(s) c(s) no mov Huggins}	9988.224	49.80	0.00000	0.0000	23.000	15643.023
{S(s) p(s) c(s) no mov Huggins P1=0.05}	9989.007	50.58	0.00000	0.0000	22.000	15645.844
{S(.) p(s) c(s) random Huggins}	9995.289	56.87	0.00000	0.0000	23.000	15650.088
{S(s) p(.) c(.) random Huggins}	10020.375	81.95	0.00000	0.0000	15.000	15691.426
{S(s) p(s) = c(s) random Huggins}	10064.856	126.43	0.00000	0.0000	21.000	15723.727
{S(s) p(.) = c(.) random Huggins}	10095.004	156.58	0.00000	0.0000	14.000	15768.079

* Best model, where all parameters are defined

Wheless Spring analysis

Real Function Parameters of {S(s) p(s) c(s) random cte Huggins p1=0.05}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper	
1:S	0.8800699	0.0595359	0.7083767	0.9568379	
2:S	0.7589160	0.0504690	0.6470489	0.8438818	
3:S	0.6983698	0.0599570	0.5699564	0.8017743	
4:S	0.6991305	0.0730486	0.5405355	0.8211008	
5:S	0.2789912	0.0337586	0.2178218	0.3496594	
6:S	0.3582017	0.0389635	0.2859024	0.4375806	
7:S	0.4232745	0.0524548	0.3250832	0.5279254	
8:Gamma''	0.3752863	0.0389488	0.3025422	0.4541324	
9:p Session 1	0.0500000	0.0000000	0.0500000	0.0500000	Fixed
10:p Session 2	0.4038743	0.0396329	0.3291581	0.4833311	
11:p Session 3	0.3241020	0.0397202	0.2515479	0.4062247	
12:p Session 4	0.2269355	0.0295790	0.1741986	0.2900308	
13:p Session 5	0.3201400	0.0347641	0.2561287	0.3917246	
14:p Session 6	0.3693750	0.0425949	0.2904284	0.4559908	
15:p Session 7	0.4155400	0.0514782	0.3193951	0.5185749	
16:p Session 8	0.5631081	0.0479977	0.4678923	0.6538893	
17:c Session 1	0.0495868	0.0197354	0.0224459	0.1059877	
18:c Session 2	0.1314031	0.0159437	0.1031780	0.1659209	
19:c Session 3	0.1598173	0.0175090	0.1284018	0.1971802	
20:c Session 4	0.1581028	0.0229371	0.1181399	0.2083893	
21:c Session 5	0.1736973	0.0188718	0.1397509	0.2138398	
22:c Session 6	0.1008771	0.0141034	0.0764009	0.1320733	
23:c Session 7	0.1993126	0.0234181	0.1573326	0.2491817	
24:c Session 8	0.1171876	0.0201027	0.0831580	0.1626720	

Estimates of Derived Parameters

Population Estimates of {S(s) p(s) c(s) random cte Huggins p1=0.05}

Grp.	Sess.	N-hat	Standard Error	95% Confidence Interval Lower	Upper
1	1	1023.6634	78.445162	882.88189	1191.3413
1	2	426.31085	25.236765	388.76306	490.57878
1	3	488.98762	41.247398	427.24296	593.45165
1	4	442.38370	47.765466	368.06231	559.17450
1	5	497.25694	38.074465	438.59112	591.18908
1	6	461.82096	33.702329	412.23845	548.51826
1	7	266.13281	19.341454	239.61276	319.08051
1	8	186.55730	6.9471740	177.74469	206.88446

Lanier Spring Analysis

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{S(s) p(s) c(s) random cte Huggins}	3859.929	0.00	0.38109	1.0000	24.000	4632.750
{S(s) p(s) c(s) markov cte Huggins}	3860.301	0.37	0.31633	0.8301	25.000	4631.014
{S(s) p(s) c(s) random Huggins}	3860.743	0.81	0.25363	0.6655	29.000	4622.974
{S(s) p(s) c(s) markov Huggins}	3864.617	4.69	0.03656	0.0959	34.000	4616.143
{S(s) p(.) c(.) random cte Huggins}	3867.472	7.54	0.00877	0.0230	10.000	4669.350
{S(s) p(s) c(s) no mov Huggins}	3869.394	9.47	0.00335	0.0088	23.000	4644.320
{S(s) p(s) = c(s) random cte Huggins}	3874.383	14.45	0.00028	0.0007	16.000	4663.914
{S(s) p(.) = c(.) random cte Huggins}	3883.704	23.78	0.00000	0.0000	9.0000	4687.624
{S(.) p(.) c(.) no mov}	422828.810	418968	0.00000	0.0000	11.000	423628.640

Lanier Spring Analysis

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Real Function Parameters of {S(s) p(s) c(s) random cte Huggins}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:S	0.7703815	0.0738635	0.5967765	0.8837963
2:S	1.0000000	0.6492755E-05	0.9999873	1.0000127
3:S	0.3985912	0.0674718	0.2762758	0.5350260
4:S	0.5047146	0.1062846	0.3069353	0.7010294
5:S	0.7445124	0.1538414	0.3738683	0.9343043
6:S	0.7143949	0.1269385	0.4249291	0.8943743
7:S	0.7760029	0.1434659	0.4072898	0.9458450
8:Gamma''	0.4082046	0.0751180	0.2727212	0.5592393
9:p Session 1	0.5095196	0.0544140	0.4040358	0.6141626
10:p Session 2	0.4224070	0.0644810	0.3034535	0.5510975
11:p Session 3	0.1711379	0.0347537	0.1132674	0.2502317
12:p Session 4	0.2517244	0.0668501	0.1436801	0.4027986
13:p Session 5	0.1799700	0.0700324	0.0796845	0.3574473
14:p Session 6	0.1922110	0.0603008	0.1000279	0.3374900
15:p Session 7	0.3551236	0.0666175	0.2374443	0.4933895
16:p Session 8	0.4579087	0.0761548	0.3164618	0.6064825
17:c Session 1	0.2376682	0.0285040	0.1863569	0.2979341
18:c Session 2	0.3058823	0.0353402	0.2412787	0.3791399
19:c Session 3	0.2213739	0.0362737	0.1584044	0.3004392
20:c Session 4	0.1165047	0.0316122	0.0673637	0.1940353
21:c Session 5	0.2203388	0.0539601	0.1324647	0.3434300
22:c Session 6	0.2352942	0.0593974	0.1387543	0.3701358
23:c Session 7	0.2291664	0.0428963	0.1559103	0.3236450
24:c Session 8	0.2460318	0.0383696	0.1786819	0.3286106

Estimates of Derived Parameters

Population Estimates of {S(s) p(s) c(s) random cte Huggins}

Grp.	Sess.	N-hat	Standard Error	95% Confidence Interval Lower	Upper
1	1	175.73603	9.2048284	164.03074	202.61323
1	2	156.07444	13.889015	138.70360	197.19811
1	3	248.51273	45.143500	183.87824	367.48791
1	4	148.01345	30.413515	110.96328	240.05296
1	5	93.630583	31.380112	59.204396	196.94395
1	6	95.157748	25.889656	64.348357	175.02653
1	7	109.31679	13.935101	92.104033	151.00723
1	8	108.24320	9.7585737	97.136640	139.45128

Lower Ribelin Analysis

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	#Par	Deviance
{S(.) p(s) c(s) random cte Huggins}	2315.685	0.00	0.47883	1.0000	14.000	2963.736
{S(s) p(s) c(s) random cte Huggins}	2316.745	1.06	0.28186	0.5886	18.000	2956.333
{S(s) p(s) c(s) markov cte Huggins}	2318.023	2.34	0.14875	0.3106	19.000	2955.477
{S(s) p(s) c(s) no mov Huggins}	2320.858	5.17	0.03606	0.0753	17.000	2962.572
{S(.) p(s) c(s) random Huggins}	2320.966	5.28	0.03415	0.0713	17.000	2962.681
{S(s) p(s) c(s) random Huggins}	2322.303	6.62	0.01750	0.0365	21.000	2955.466
{S(s) p(s) c(s) markov Huggins}	2326.007	10.32	0.00275	0.0057	24.000	2952.677
{S(s) p(.) c(.) random cte Huggins}	2332.585	16.90	0.00010	0.0002	8.0000	2993.115
{S(.) p(.) c(.) random cte Huggins}	2340.520	24.83	0.00000	0.0000	4.0000	3009.227
{S(.) p(s) = c(s) random cte Huggins}	2349.440	33.75	0.00000	0.0000	8.0000	3009.969
{S(s) p(s) = c(s) random cte Huggins}	2351.453	35.77	0.00000	0.0000	12.000	3003.692
{S(s) p(.) = c(.) random cte Huggins}	2357.773	42.09	0.00000	0.0000	7.0000	3020.357
{S(.) p(.) = c(.) random cte Huggins}	2365.735	50.05	0.00000	0.0000	3.0000	3036.469

Lower Ribelin Analysis

Program MARK - Survival Rate Estimation with Capture-Recapture Data
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Real Function Parameters of {S(.) p(s) c(s) random cte Huggins}

Parameter	Estimate	Standard Error	95% Confidence Interval Lower	Upper
1:S	0.6946102	0.0303415	0.6321413	0.7506552
2:Gamma''	0.1900866	0.0911376	0.0685149	0.4282082
3:p Session 1	0.5962795	0.0699055	0.4553253	0.7229517
4:p Session 2	0.2215600	0.0502436	0.1385272	0.3350082
5:p Session 3	0.2263527	0.0529650	0.1392141	0.3461036
6:p Session 4	0.3513091	0.0670484	0.2332685	0.4908428
7:p Session 5	0.2863524	0.0577900	0.1873321	0.4112276
8:p Session 6	0.5569041	0.0553156	0.4475045	0.6610489
9:c Session 1	0.0803571	0.0256870	0.0423391	0.1472637
10:c Session 2	0.2419355	0.0543885	0.1514414	0.3633497
11:c Session 3	0.2238806	0.0509255	0.1397179	0.3387766
12:c Session 4	0.2148760	0.0373397	0.1506399	0.2969280
13:c Session 5	0.1962616	0.0383957	0.1315980	0.2823676
14:c Session 6	0.2576687	0.0342559	0.1963695	0.3302398

Estimates of Derived Parameters

Population Estimates of {S(.) p(s) c(s) random cte Huggins}

Grp.	Sess.	N-hat	Standard Error	95% Confidence Interval Lower	Upper
1	1	78.141931	3.6985333	74.450306	91.230264
1	2	107.89535	21.079652	80.333644	168.01296
1	3	102.43063	20.432162	76.128135	161.47722
1	4	126.54206	16.264355	106.36932	175.03482
1	5	124.10763	19.163308	99.300920	179.22688
1	6	121.57653	5.5144551	115.04509	138.65404

Time in seconds for last procedure was 0.44