Predicting surface abundance of federally threatened Jollyville Plateau Salamanders (*Eurycea tonkawae*) to inform management activities at a highly modified urban spring

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Abstract

Urban expansion has contributed to the loss of habitat for range restricted species across the globe. Managing wildlife populations within these urban settings presents the challenge of balancing human and wildlife needs. Jollyville Plateau Salamanders (*Eurycea tonkawae*) are a range restricted, federally threatened, species of neotenic lungless brook salamander endemic to central Texas, USA. Almost the entire geographic range of *E. tonkawae* is embedded in the Austin, Cedar Park, and Round Rock metropolitan areas of Travis and Williamson counties, Texas. Among *E. tonkawae* occupied sites, Brushy Creek Spring has experienced some of the most extensive anthropogenic disturbance. Today the site consists of small groundwater outlets that emerge in the seams within a concrete culvert underlying a highway. Salamanders persist within this system though they are rarely detected in the surface habitat. Here, we model the occurrence of salamanders within the surface habitat of Brushy Creek Spring using generalized linear models. In the absence of available data regarding the amount of water that is discharged from the spring, we use accumulated rainfall as a proxy for discharge to estimate salamander relative abundance. Using this approach, we determined rainfall that occurs 31-120 days prior to survey best predicts salamander occurrence in the surface habitat of Brushy Creek Spring. Additionally, we present evidence of reproduction, recruitment, and subterranean movement by *E. tonkawae* throughout this site. Infrastructure maintenance is inevitable at Brushy Creek Spring. We intend for our results to inform when maintenance should occur, i.e., during environmental conditions when salamanders are unlikely to occur in the surface habitat, to avoid unnecessary impacts to this federally threatened species.

Introduction

Urban expansion directly effects biodiversity in many ways, and one of the most concerning is the loss of habitat for range-restricted species (MacDonald et al. 2018). Managing wildlife populations in urban settings presents the challenge of balancing human and wildlife needs

43 (Aronson et al. 2017). This is especially difficult for threatened and endangered species
44 management, as new development, or the maintenance of existing infrastructure in or near
45 habitat, may result in "take" as defined by the U.S. Endangered Species Act of 1973 (as
46 amended; United States 1983). The U.S. Fish and Wildlife Service (USFWS) considers "take" as
47 actions that "harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or attempt to
48 engage in any such conduct" (United States 1983, USFWS 2013a). Incidental take permits are
49 commonly issued by the USFWS to allow take of a listed species from activities associated with
50 an otherwise lawful project, but incidental take is rarely directly informed by population models
51 (McGowan and Ryan 2010).
52
53 Almost the entire range of Jollyville Plateau Salamanders (*Eurycea tonkawae*) is embedded in
54 the Austin, Cedar Park, and Round Rock metropolitan areas of Travis and Williamson counties,
55 Texas, USA (USFWS 2013a, Devitt et al. 2019). Conservation concern for the taxon began
56 immediately upon its formal description due to its small geographic distribution in an urban
57 environment (Price et al. 1999, Chippindale et al. 2000). *Eurycea tonkawae* are neotenic,
58 permanently aquatic, plethodontid salamanders restricted to groundwater-fed aquatic habitats,
59 such as springs, spring-fed creeks, and caves, primarily in the northern segment of the Edwards
60 Aquifer (Chippindale et al. 2000, Chippindale 2005). Individuals are usually observed proximate
61 to a spring outlet or a stream segment gaining groundwater (Sweet 1982, Bowles et al. 2006), but
62 can also be observed downstream of springs and in second order creeks (Bendik et al. 2016,
63 Adcock et al. 2020). Typical surface habitat consists of shallow, flowing water with ample cover
64 objects (e.g., rocks, leaf litter), substrate that provides interstitial spaces and access to
65 subterranean water, and water chemistry associated with karst aquifers (Chippindale 2005,
66 Bowles et al. 2006, USFWS 2013a,b).
67
68 The USFWS listed *E. tonkawae* as threatened in 2013 because of concerns regarding water
69 quantity reduction, water quality degradation, and habitat loss due to urbanization (USFWS
70 2013a) and subsequently designated 32 critical habitat units (CHUs) for the taxon (USFWS
71 2013b). *Eurycea tonkawae* CHUs consist of both surface and subterranean components that are
72 defined as 80 m and 300 m radius circles, respectively, around the spring outlet (USFWS 2013b).
73 The surface CHUs are restricted to aquatic areas up to the ordinary high-water lines (USFWS
74 2013b). An aerial review of the CHUs demonstrates that approximately 67% of the surface and
75 86% of the subsurface circles contain anthropogenic structures (e.g., buildings, roads),
76 substantiating the urban nature of the taxon's distribution. These structures will inevitably
77 require maintenance for public safety, which may trigger federal consultations and evaluations of
78 incidental take. Although the USFWS excluded anthropogenic structures from the surface CHUs,
79 the subsurface CHUs extend below these structures, and construction or maintenance activities
80 adjacent to or above the CHUs may have adverse indirect effects through runoff into the surface
81 or subsurface aquatic environment (USFWS 2013b). These concerns are supported by previous
82 work that determined *E. tonkawae* counts and density are negatively correlated with impervious
83 cover, a metric of development and urbanization (Bowles et al. 2006, Bendik et al. 2014).
84
85 Brushy Creek Spring, aka Round Rock Spring, constitutes CHU 2 for *E. tonkawae* (USFWS
86 2013b). In a recent study of select *Eurycea*-occupied springs in the northern segment of the
87 Edwards Aquifer, this site had the second highest amount of impervious cover in its watershed
88 (Diaz et al. 2020). An office building, apartment buildings, and US 79 all occur directly adjacent

89 to, above, and upstream of the spring (Chippindale et al. 2000, Chippindale 2005). Additionally,
90 the site was drastically altered in the early 2000's when a large concrete culvert and gabion were
91 installed over the original spring outlet to convey stormwater runoff from upgradient urban
92 development. As such, Brushy Creek Spring is an excellent example of a location that will
93 require perpetual infrastructure maintenance within an *E. tonkawae* CHU.
94
95 *Eurycea* salamanders were first documented from Brushy Creek Spring in 1948 and were
96 considered Texas Salamanders (*E. neotenes*) until the formal description of *E. tonkawae* (Baker
97 1961, Sweet 1982, Chippindale et al. 2000). At this time, the only other known population of
98 what are now considered *E. tonkawae* was from the nearby Kreinke Spring. It is possible that *E.*
99 *tonkawae* were scarce within the surface habitat of Brushy Creek Spring prior to anthropogenic
100 alterations. At the time of their discovery, only one voucher specimen was collected within
101 Brushy Creek Spring, compared to 21 vouchered specimens at Kreinke Spring by the same
102 researcher (Baker 1961, Sweet 1982, VertNet.org). Sweet (1978) conducted two surveys at this
103 site between 1969 and 1974 without detecting salamanders, and only eight vouchers were
104 collected between 1990 and 1994 (Chippindale et al. 2000, VertNet.org). The number of
105 vouchered specimens may not necessarily reflect abundance; however, no alternative sources of
106 information are available from Brushy Creek Spring prior to anthropogenic disturbance. At the
107 time of federal listing, this site was considered the only known locality where *E. tonkawae* had
108 been extirpated (SWCA Environmental Consultants, unpubl. report), and prior to this study, the
109 status of *E. tonkawae* at Brushy Creek Spring was unclear.
110
111 Other central Texas *Eurycea* salamanders are known to occur or increase in density and
112 abundance in the surface habitat after periods of rainfall and/or increases in groundwater
113 discharge (Gillespie 2011, Tovar and Solis 2013, Bendik and Dries 2018). Urbanization may
114 result in the development of a shallow pseudo-karst (e.g., tunnels, conduits, utility networks),
115 which can evolve rapidly and dominate water flow and transport (Garcia-Fresca 2007). Slade et
116 al. (1986) demonstrates that within the Austin area, surface recharge (e.g., rainfall) influences
117 discharge, sometimes rapidly, with discharge increasing only days after rain events. In Austin-
118 area watersheds, rainfall and the associated runoff decreases the specific conductance (SC) of
119 water within spring runs. When runoff is absent and groundwater dominates flow, SC typically
120 rises (Johns 2006). However, increased groundwater discharge may decrease SC when it reduces
121 aquifer residence time (Gillespie 2011). Dissolved oxygen (DO) typically decreases as
122 groundwater discharge decreases (Gillespie 2011). Rainfall, SC, and DO may serve as proxies
123 for groundwater discharge volume when this metric is unknown. To the best of our knowledge,
124 no study of the dynamic relationship between aquifer recharge from rainfall and groundwater
125 discharge has been conducted at Brushy Creek Spring.
126
127 Here, we report the findings of seven years of surveys at Brushy Creek Spring. We first sought to
128 determine if *E. tonkawae* still occur at this site, and if so, where do they occur within the CHU.
129 Then, enabled by the detection of salamanders, we estimated capture probability and survival
130 rate. Next, we tested for differences in water chemistry (i.e., temperature, pH, DO, and SC)
131 across habitat components. Finally, we modeled environmental covariates (i.e., season, rainfall)
132 that predict *E. tonkawae* relative abundance in the surface habitat in order to identify periods
133 when maintenance activities are unlikely to disturb salamanders. We hypothesized that pulses in
134 rainfall are correlated to salamander abundance at Brushy Creek Spring, while considering that

135   these pulses may be delayed in their influence due to the unknown size of the underground karst
136   (or pseudo-karst) system which provides this site with groundwater.
137

Materials & Methods

139   ***Study Site.*** — Brushy Creek Spring is located 1.94 km northeast of downtown Round Rock,
140   Texas (Fig. 1). The site is characterized by two major components, a large, concrete culvert
141   system and a spring run, which we define here as a body of flowing water that is primarily fed by
142   a spring or group of springs. The culvert system consists of three tunnels that run under US 79
143   and empty into a two-sided box culvert. The culvert is owned by the City of Round Rock but
144   occurs entirely within a Texas Department of Transportation right of way, who have provided us
145   access and approval to conduct research at this site. During construction, polyvinyl chloride
146   (PVC) pipes were installed to divert groundwater from the original spring location into the box
147   culvert (C. Newnam, pers. comm.). Currently, groundwater emerges from the PVC pipes, seams
148   in the concrete culvert tunnel junctions under US 79, and at seams and cracks at the concrete
149   culvert tunnel and box culvert interface. The number of seams and cracks discharging
150   groundwater changes with fluctuating aquifer levels. Spring water flows in a shallow sheet, often
151   less than 1 cm deep, for 20 m inside the box culvert, over and through a gabion structure, and
152   into a deep pool before constricting into a spring run that travels approximately 30 m before
153   waterfalling into Brushy Creek (Fig. 2). The spring run exemplifies typical *E. tonkawae* habitat,
154   containing shallow, flowing water with abundant rocks and gravel substrate (Bowles et al. 2006,
155   USFWS 2013 a,b). In contrast, potential cover objects within the culvert system change in
156   response to flash floods. The system receives considerable stormwater from the upgradient
157   development, and these floods both deposit and flush potential salamander cover objects from
158   the culvert. Cover objects can include rocks but also litter (e.g., shopping carts, bottles, cans,
159   clothing) and landscaping debris (e.g., branches, grass clippings). Litter and debris frequently get
160   caught between the baffle walls in the box culvert (see Fig. 2C) forming temporary dams with
161   small pools.
162

163   ***Salamander Surveys.*** — We conducted monthly to bi-monthly *E. tonkawae* survey events at
164   Brushy Creek Spring from May 2014 to July 2021. On a few occasions, we were unable to
165   survey during a scheduled month because of high stormwater flow. We searched for *E. tonkawae*
166   under and in available cover objects and recorded the number of objects searched and the time
167   spent surveying. We surveyed just the spring run portion of the site from May 2014 to March
168   2015, as this appeared to be the most appropriate habitat for *E. tonkawae*. From April 2015 to
169   January 2018, we surveyed both the spring run and box culvert during each survey event. Right
170   of entry to the spring run was unavailable after January 2018, but we maintained culvert surveys
171   throughout the remainder of the study. We surveyed the spring run 28 times and the culvert 39
172   times over the course of this study.
173

174   We attempted capture of each observed *E. tonkawae* using aquarium nets, and we recorded body
175   and head photographs on a standardized grid background with the salamander in a water-filled
176   dish. Pigmentation patterns on the head were used to identify recaptured salamanders using
177   Wild-ID photographic recognition software (Bolger et al. 2012, Bendik et al. 2013). We
178   determined gravidity of all captured *E. tonkawae* by visually checking for oocytes through the
179   salamander's translucent venter (Fig. 3; Gillette and Peterson 2001; Pierce et al. 2014). We
180   measured snout-vent length (SVL) and total length (TL) of all captures. Salamanders were either

181 measured by hand using a metric dial caliper, or digitally using the software ImageJ, where size
182 standardization is taken from the gridded background of all salamander photos. We conducted
183 this study in compliance with Federal Fish and Wildlife Permits TE039544-1 and TE37416B-0,
184 Texas Parks and Wildlife Scientific Permits SPR-0102-191 and SPR-0319-056, and Texas State
185 University Institutional Animal Care and Use Committee (IACUC) permits 1202_0123_02 and
186 0417_0513_07.
187
188 We measured water temperature (° C), pH, DO (mg/L), and SC (μS/cm) during each survey.
189 These parameters were collected using the following instruments, based on their availability to
190 surveyors: HI 9828 multiparameter probe (Hannah Instruments, Smithfield, Rhode Island, USA),
191 Manta2® multiprobe (Eureka Water Probes, Austin, Texas, USA), Com-100 (HM Digital,
192 Culver City, California, USA), EcoTestr pH2 (Oakton Instruments, Vernon Hills, Illinois, USA),
193 or HI 9147 (Hannah Instruments). We downloaded daily precipitation accumulation from the
194 National Oceanic and Atmospheric Administration (NOAA) stations US1TXWM0039,
195 US1TXWM0178, US1TXWM0195, and US1TXWM0219 in Round Rock, Texas. We averaged
196 the daily values among stations when data overlapped.
197
198 ***Capture-Mark-Recapture Analyses.*** — We constructed all capture-mark-recapture models in
199 program R (R Version 3.6.1; R Development Core Team 2018) using the package 'Rmark'
200 (Laake and Rexstad 2008; Laake 2013) which calls program MARK (White and Burnham 1999).
201 We used a Cormack-Jolly-Seber (CJS) model (Cormack 1964, Jolly 1965, Seber 1965) to
202 estimate capture probability and survival rate of all captures (n=27). Under this formulation,
203 capture probability (p_i) is defined as the probability that a marked animal in the study population
204 at sampling period i (n=39) is captured or observed during period i (Williams et al. 2002).
205 Survival rate, also referred to as apparent survival, (φ_i) is defined as the probability that a
206 marked animal in the study population at sampling period i survives until period $i + 1$ and does
207 not permanently emigrate (Williams et al. 2002). Because of a limited sample size, and rare
208 occurrence of recaptures, we estimated a single CJS model with constant detection probability
209 and survival, the null model. Throughout our study two individuals were observed but evaded
210 capture. It is important to note that these individuals are included in our models predicting
211 abundance in response to rainfall accumulation but cannot be included in our CJS model. We
212 confirmed goodness-of-fit using package 'R2ucare' (Gimenez et al. 2018).
213
214 ***Environmental variable selection.*** — We sought to test the influence of rainfall accumulation,
215 as a proxy for groundwater discharge, on salamander observations. To do so, we calculated 30,
216 60, and 90 days of accumulated rainfall prior to each survey event. The amount of time that
217 occurs between a rainfall vent and increased groundwater discharge depends upon the amount of
218 rainfall, current aquifer levels, the size of the local karst system, and retention time (groundwater
219 age) in the karst system. This has been measured in other portions of the Edwards Aquifer of
220 Texas and can range from less than one year up to several decades (Hunt et al. 2012). Properties
221 of the local karst system are not known at Brushy Creek Spring. To account for this unknown,
222 we calculated 30, 60, and 90-day accumulations one, two, and three months previous to each
223 survey event. For example, 90 days of accumulation, occurring three months previous to surveys
224 provides the total rainfall accumulated 91-180 days prior to each survey event. In total, 12 ranges
225 of rainfall accumulation were tested.
226

227 Central Texas *Eurycea* salamander surface abundance is known to increase throughout the
228 spring, peak in the summer, and decrease in autumn and winter (Bowles et al. 2006, Pierce et al.
229 2010, Bendik 2017). To account for this phenology, we included the quadratic effect of day-of-
230 year (day^2) as a predictor within each model (Kéry and Royle 2016, Edwards and Crone 2021).
231 We included the lower-order term of "day" to adhere to the rules of marginality (McCullagh and
232 Nelder 1998, Kéry and Royle 2016).
233
234 Additionally, we sought to investigate if changes in water conditions (i.e., temperature, pH, DO,
235 and SC) caused by increasing groundwater discharge, rather than base flow or overland runoff,
236 influenced salamander relative abundance. However, we were concerned with overfitting our
237 models due to our limited sample size. Prior to utilizing water condition parameters as additional
238 predictors within our generalized linear models, we tested for differences among these metrics
239 with respect to salamander occurrence (present vs. absent) within the culvert, using Welch's two
240 sample t-tests conducted in Program R (package 'stats'). We also tested for differences in overall
241 water conditions between the culvert and the spring run portions of the site (Table 1). We
242 removed two surveys with outliers: one DO spike caused by a burst municipal water main which
243 contributed overland runoff during a survey event and one event where equipment failure yielded
244 untrustworthy estimates of SC (see supplemental R code).
245
246 Water temperature is subject to the influence of ambient air temperature, which peaks mid-year,
247 mirroring the influence of day-of-year in our analysis. Although water temperature and
248 salamander abundance are both reported to peak simultaneously, reproductive phenology is
249 thought to be the cause for increase in surface salamander counts (Bendik 2017), rather than
250 coincidental increases in water temperatures. Further, water conditions within the culvert showed
251 little variation within the duration of our study period (Tables 1 & 2, Fig. 4). Invariance can
252 cause issues with model fit, especially in examples using small sample sizes (Warton et al.
253 2016). For these reasons, and on the basis of the results of our t-tests, we chose to remove all
254 water chemistry parameters as predictors in our GLMs.
255
256 We additionally excluded data collected from the spring run because only a single salamander
257 was captured within this portion of the site during our study. Different variables may restrict
258 salamander occurrence in this portion of the site (see Discussion) compared to those that
259 influence salamander relative abundance in the culvert, thus making the spring run data not
260 suitable to test our general hypothesis.
261
262 ***Environmental model analyses.*** — We sought to model the environmental parameters that
263 predict *E. tonkawae* relative abundance in the surface habitat. Count data such as these are
264 generally analyzed using Poisson regression (O'Hara and Kotze 2010). We performed a chi-
265 squared goodness of fit test (package 'vcd') to test whether our data conform to a Poisson
266 distribution (χ^2= 2.61, p=0.27). We then fit a suite of Poisson regressions via generalized linear
267 models in Program R (GLMs; package 'stats'). Ecological processes can often result in
268 overdispersion, where variation is greater than is expected given a pure Poisson process (Linden
269 and Mäntyniemi 2011). Because our initial goodness-of-fit test demonstrated a lack of fit, we
270 tested for overdispersion (package 'msme'). For all models the dispersion parameter (i.e.,
271 Pearson-χ^2/ Residual degrees of freedom) was greater than 1 and less than 2, indicating
272 overdispersion (Kéry and Royle 2016). Thus, we proceeded by refitting models using the

273   negative binomial error distribution (Ver Hoef and Boveng 2007). We fit all models using *E.*
274   *tonkawae* observations (i.e., counts) as the response variable, including individuals observed but
275   not captured.
276
277   After reducing the number of predictors, we fit a model for each rainfall accumulation range,
278   with the additional predictors of day-of-year, and day-of-year^2, resulting in 12 total models. Each
279   model included a log offset of effort to control for count variations that may be due to
280   differential survey effort among location or events (Kéry and Royle 2016). We considered the
281   model with the lowest Akaike's Information Criterion value adjusted for small sample sizes
282   (AIC_c) and the greatest model weight (ω_i) as the best supported model, and we determined the
283   importance of covariates with a Wald Z-test (Burnham and Anderson 2002, Bolker et al. 2009).
284
285   We predicted salamander counts over a range of 0–60 cm of accumulated rainfall for the best fit
286   model. For predictions, we held the log offset of effort constant at the mean number of objects
287   searched (n = 172.3) and day-of-year constant at the mean value (194.74).
288

Results

290   We surveyed the spring run 28 times, for a total 725 person-minutes and 6,066 searched cover
291   objects. We surveyed the culvert 39 times, for a total 1,438 person-minutes and 6,720 searched
292   cover objects. We detected 24 individual *E. tonkawae* a total of 27 times (three recaptures) from
293   2014 to 2021. Captures ranged from 0 to 3 individuals per survey. Two individual salamanders
294   avoided capture by retreating into groundwater outlets in the cracks in the culvert. We treat these
295   individuals as unique, that is, not a recapture, because their identity cannot be verified. Two
296   recaptures occurred in the survey event immediately following initial capture (i.e., the following
297   month), one individual was recaptured a full year after first being detected, and no animals were
298   recaptured more than once. Salamander SVL ranged from 12.0–41.3 mm with a mean of 29.5 ±
299   7.7 mm, and TL ranged from 20.5–84.5 mm with a mean of 56.6 ± 16.7 mm. We captured three
300   gravid females (Fig. 3), and two juvenile-sized individuals (< 15.0 mm SVL). The three gravid
301   females were observed with 8, 17, and 24 oocytes visible through their venter.
302
303   We observed a single individual *E. tonkawae* on one occasion (no recaptures) downstream of the
304   culvert system and gabion. This salamander was approximately 37.5 m downstream of the
305   nearest spring outlet in rocks and gravel on the downstream edge of the pond before it constricts
306   into the spring run. All other observations occurred on the concrete culvert apron (n = 1), in gaps
307   between culvert tunnel junctions (n = 5), or inside of the culvert system under rocks, litter, or
308   debris on the concrete floor (n = 21). Most *E. tonkawae* (n = 20) were observed within 1.5 m of a
309   spring discharge outlet at the PVC diversion pipes or cracks and seams in the concrete (Fig. 5).
310
311   ***Capture-Mark-Recapture Model.*** — Our CJS model estimated capture probability (p_i) to be
312   0.063 ± 0.054 (lcl = 0.011, ucl = 0.289) and survival rate (φ_i) = 0.68 ± 0.159 (lcl = 0.337, ucl =
313   0.899). Goodness-of-fit tests demonstrated no lack-of-fit ($p = 1$). These estimates are highly
314   influenced by our discovery of one individual which survived a full year before being recaptured.
315   With this individual excluded, estimates converge upon their boundaries of $p_i = 1$ and $\varphi_i = 0$ (see
316   supplemental R code).
317

318   ***Environmental Covariate Models.*** — The AIC_c model selection resulted in two competing
319   models, that is, the difference between their AIC_c values is less than 2 (Table 3). In each of these
320   models, rainfall accumulation was the only significant predictor of salamander abundance (Table
321   4). The two most favored models both reflect a 90-day accumulation of rainfall; 31-120, and 1-
322   90 days of cumulative rainfall, respectively. The top model reflects rainfall delayed by a single
323   month (31-120 days), compared to the closest competing model which reflects recent rainfall (1-
324   90 days). Under both models, salamander counts increase as rainfall accumulation increases
325   (Table 4). Although seasonal shifts in surface abundance are well documented (Bowles et al.
326   2006, Pierce et al. 2010, Bendik 2017), the quadratic effect of day-of-year was not significant in
327   our top two models (Table 4). However, these predictors appear near significance, and it may be
328   that our limited sample size was simply not sufficient to realize this well-known seasonal
329   phenology.
330
331   The mean predictions made using our top model estimate that approximately 29 cm of
332   accumulated rainfall 31-120 days prior to survey are needed to observe one *E. tonkawae* inside
333   of the culvert at Brushy Creek Spring (Fig 6). The competing model's mean prediction was
334   approximately 25 cm of accumulated rainfall 1-90 days prior to survey. It is worth noting that
335   these predictions are probabilistic, and that it is possible to observe one *E. tonkawae* with less
336   rainfall accumulation, as indicated by the confidence intervals illustrated in Fig. 6.
337

Discussion

339   This study provides the first regular detections of *E. tonkawae* in the surface habitat at Brushy
340   Creek Spring. Additionally, we document the first salamander observation in 21 years (1994-
341   2015; Chippindale et al. 2000, VertNet.org) and the first since it was suggested that local
342   extirpation had likely occurred (SWCA Environmental Consultants, unpubl. report). We
343   conducted surveys across six reproductive seasons (Bendik 2017), observing three gravid
344   females and two juvenile individuals, indicating successful reproduction at this site. These gravid
345   females were observed during winter months (November, December, and January) and juvenile
346   individuals during summer months (June and July) in general accordance with the reported
347   reproductive phenology for this species (Bendik 2017).
348
349   Our analysis of environmental predictors of salamander counts demonstrates that the
350   accumulation of rainfall 31-120 days prior to survey best predicts *E. tonkawae* relative
351   abundance. This is congruent with reports that abundance is correlated to lagged rainfall for
352   other central Texas *Eurycea* taxa (Gillespie 2011, Krejca et al. 2017). We used rainfall as a
353   substitute for spring discharge because measuring discharge is not practical at Brushy Creek
354   Spring due to multiple small discharge points scattered throughout the wide culvert. We
355   acknowledge that an interaction between rainfall and aquifer water level would likely improve
356   the fit of our models and reduce the estimated confidence intervals, but aquifer data are not
357   available on a monthly scale for the duration of this project.
358
359   Ecological studies, and more specifically herpetological studies, often fail to account for
360   imperfect detection (Ficetola 2015, Kellner and Swihart 2014). For monitoring efforts that are
361   not designed around this explicit goal, accounting for imperfect detection can be challenging
362   (Kéry and Schmidt 2008). We attempted to model the probability of detecting marked
363   individuals, but we lacked sufficient recaptures to do so. We do not present our capture-mark-

364 recapture results as defensible estimates, given that our sample size limited us to fitting an overly
365 simplistic model. We recaptured only three individuals throughout the duration of our seven-year
366 study. Fitting capture-mark-recapture models to limited detections leads to uncertain parameter
367 estimates (Durso et al. 2011; Mazzerolle et al 2007). The inclusion of a single recapture after a
368 one-year period reversed our detection and survival estimates, indicating they are likely spurious.
369 We hypothesize that survival may be low for salamanders in the surface habitat unless they are
370 able to locate conduits that provide access back into the subsurface environment, as we observed
371 in our study. It is important to note that our estimate of apparent survival cannot be disentangled
372 from death (e.g., trapped in the surface habitat) or permanent emigration (e.g., retreating to the
373 subterrain), and that these events would equally reduce estimated apparent survival.
374 Additionally, we would expect detection to be high in the surface habitat of the culvert because
375 there are few cover objects and the concrete floor limits subsurface escape. We note at other
376 *Eurycea* occupied sites with similar concrete streambeds capture probability has been estimated
377 as high as 0.82 (Bendik et al. 2021).
378
379 For the salamander with the one-year recapture timeframe, we initially captured the individual in
380 a pool formed behind a debris pile in the box culvert within 1 m of a spring outlet. We recaptured
381 the individual in a seam between the concrete culvert tunnels approximately 20 m upstream of
382 the original capture location. The floor of the culvert tunnel and box culvert are disjunct (Fig.
383 2B), and this individual almost certainly had to travel through the subterranean environment to
384 this upstream location. This implies that, in spite of anthropogenic modifications, salamanders
385 are able to seek refuge and survive in the subterranean karst or pseudo-karst at Brushy Creek
386 Spring as in natural systems (Bendik and Gluesenkamp 2013). Further, a recent study also found
387 that Brushy Creek Spring has an exceptional aquatic invertebrate community score (Diaz et al.
388 2020), which is a measure of aquatic life use, and indicates a stable food source for salamanders
389 at this site.
390
391 During our study we only observed a single individual within the spring run downstream of the
392 box culvert. All other salamanders occurred in the culvert system within 22 m of a spring outlet.
393 On average salamanders were found 2.5 m from a spring outlet, and most captures occurred
394 within 1 m of an outlet. The spring run downstream of the box culvert possesses the shallow,
395 flowing water and abundant rocks with interstitial gaps that is typical of central Texas *Eurycea*
396 occupied springs (Sweet 1982, Chippindale 2005, Bowles et al. 2006), but it lacks any apparent
397 groundwater gaining sections. In contrast, the cracks and openings in the culvert system provides
398 spring discharge outlets but lacks the typical habitat structure, and often yard waste and litter are
399 the only available cover objects. Other studies document that salamanders are more likely to
400 utilize culverts if continuous rock substrate and cover objects are present because these features
401 mimic natural streambeds (Ward et al. 2008, Anderson et al. 2014), but at Brushy Creek Spring,
402 proximity and access to the subterranean habitat appears more important. Downstream
403 salamander distribution may be restricted by the pool between the gabion and spring run (Fig.
404 2D) which usually contains predatory fish thought to exclude *E. tonkawae* (Bendik et al. 2016).
405
406 It is highly unlikely that salamanders migrate to Brushy Creek Spring through surface water. The
407 two closest known surface populations are Kreinke Spring (CHU 1) and PC Spring (CHU 7)
408 which are approximately 7.5 km upstream in Brushy Creek and 14 km upstream in Lake Creek,
409 respectively (USFWS 2013b). These portions of Brushy and Lake Creeks contain deep water,

410 predatory fish, and large stretches without suitable *E. tonkawae* habitat. Further, we surveyed
411 portions of Brushy Creek between Kreinke Spring and Brushy Creek Spring 38 times from 2013
412 to 2019 without observing salamanders, and we conducted over 50 surveys of portions of Lake
413 Creek between PC Spring and Brushy Creek Spring from 2013 to 2019 without observing
414 salamanders. Regular monthly to bi-monthly salamander monitoring has occurred at PC Spring
415 from 2013 to 2021 and a salamander has never been observed more than 100 m from the spring
416 outlets (Adcock et al., unpubl. data). Therefore, the most plausible source of *E. tonkawae* is
417 subterranean animals immigrating into surface habitat. The entirety of our results indicates that
418 rainfall causes increased spring discharge at Brushy Creek Spring which either expels resident
419 subterranean salamanders (see Tovar and Solis 2013) into the surface habitat or allows
420 subsurface migration from another location.
421
422 Salamanders at this site predominantly occur within a structure that is property of, and
423 maintained by, the City of Round Rock, although it occurs within Texas Department of
424 Transportation Right of Way. The culvert system requires routine maintenance including debris
425 removal and painting over graffiti. The findings of this study indicate that by carefully
426 considering recent rainfall patterns in the area, maintenance could be scheduled to avoid
427 instances when the presence of *E. tonkawae* within surface habitat is more likely. Similar
428 findings have been highlighted for other amphibian groups, recommending road construction and
429 maintenance be timed to avoid periods of high activity and movement (Hamer et al. 2015).
430 Improved knowledge of the site-specific hydrogeology will help inform management of *Eurycea*
431 occupied locations throughout the urban landscape in which they occur. One limitation to our
432 study is the small sample size. Small sample sizes present difficulties in modeling/predicting the
433 influence of parameters of interest. The question of how to properly manage a site occupied by
434 threatened or endangered species for which only limited data exist deserves further investigation
435 and discussion. Management of urban populations is necessary to prevent local extirpation of *E.*
436 *tonkawae*, and improvement projects within this species' CHUs are inevitable. We encourage
437 additional studies aimed to inform anthropogenic activities to reduce incidental take of this listed
438 species.
439

Conclusions

440

441 Brushy Creek Spring is one of the most anthropogenically modified sites occupied by *E.*
442 *tonkawae* and is also a CHU for this federally threatened species (USFWS 2013b). Using
443 exhaustive surveys of the most heavily modified portion of the site, we documented salamanders
444 at this site for the first time in 21 years, negating the suggestion that salamanders had been
445 extirpated from this locality (SWCA Environmental Consultants, unpubl. report). The current
446 physical structure of Brushy Creek Spring prevents accurate measures of groundwater discharge,
447 but we overcame this issue by using local rainfall data to estimate recharge and to predict
448 salamander relative abundance within surface habitat. Rainfall occurring 31-120 days prior to
449 survey events best predicts salamander occurrence in the surface habitat, and on average 29 cm
450 of rainfall within that time period is likely to lead to salamander detections. Potential incidental
451 take can be reduced by scheduling maintenance activities during periods in which salamanders
452 are unlikely to occur in the surface habitat.
453

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454

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Water conditions are Brushy Creek Spring

Water conditions (μ ± σ) at the spring outlets inside of the culvert system (culvert) or in the spring run downstream of the spring outlets and culvert (run). Sample sizes calculated after missing values and outliers (n=2) were removed.

Location	Culvert		Spring Run	
Salamander Occurrence	Present (n=16)	Absent (n=18)	Present (n=1)	Absent (n=17)
Temperature (°C)	21.92 ± 1.89	22.15 ± 2.94	20.56 ± 0.0	21.74 ± 2.66
pH	7.04 ± 0.44	7.21 ± 0.35	7.84 ± 0.0	7.86 ± 0.30
Dissolved Oxygen (mg/L)	4.99 ± 1.29	6.52 ± 3.76	8.47 ± 0.0	6.57 ± 1.37
Conductivity (µS/cm)	917.08 ± 47.43	877.37 ± 90.67	916.4 ± 0.0	819.98 ± 71.36

Results for tests of water condition differences by location and occurrence

Degrees of freedom (DF), test statistic value (*t*), and p-value (*P*) for each water condition parameter measured. Data used to compare between salamander presence and absence was restricted to those collected from the culvert only. All data were used to test for differences between the culvert and spring run at Brushy Creek Spring. **Bold text** indicates statistical significance at $P \leq 0.05$.

Test Description	Present (n=16) vs. Absent (n=18)			Culvert (n=34) vs. Spring Run (n=18)		
Statistic	DF	t	P	DF	t	P
Temperature (°C)	29.31	-0.279	0.783	33.33	-0.491	0.627
pH	28.71	-1.281	0.210	44.85	7.625	**<0.001**
Dissolved Oxygen (mg/L)	27.14	-1.277	0.212	45.99	1.526	0.134
Conductivity (µS/cm)	29.52	1.329	0.194	35.75	-3.292	**0.002**

Results of model selection

Top generalized linear models (GLMs) assessing the response variable of Jollyville Plateau Salamander (*Eurycea tonkawae*) counts at Brushy Creek Spring as predicted by the accumulation of rainfall over the specified days. The model, number of parameters (*K*), Akaike's Information Criterion value adjusted for small sample sizes (AIC$_c$), difference in AIC$_c$ from the top model (ΔAIC$_c$), model weight (ω_i), and the Cragg-Uhler-Nagelkerke pseudo R^2 are presented.

Model	Rainfall Accumulation	K	AIC_c	ΔAIC_c	ω_i	Pseudo R^2
6	31-120	5	93.046	0.000	0.327	0.252
3	1-90	5	94.695	1.649	0.143	0.215
2	1-60	5	95.179	2.133	0.112	0.204
5	31-90	5	95.283	2.237	0.107	0.201
4	31-60	5	95.612	2.566	0.091	0.193
8	61-120	5	96.379	3.333	0.062	0.175
7	61-90	5	96.722	3.675	0.052	0.167
9	61-150	5	98.212	5.166	0.025	0.131
10	91-120	5	98.418	5.372	0.022	0.125
12	91-180	5	98.457	5.411	0.022	0.124
1	1-30	5	98.656	5.61	0.02	0.119
11	91-150	5	98.885	5.839	0.018	0.114

Summary and main effects of top competing models

Model summaries of all competing (Δ AIC$_c$ <2) generalized linear models assessing the response variable of Jollyville Plateau Salamander (*Eurycea tonkawae*) counts to environmental predictors at Brushy Creek Spring. **Bold text** indicates statistical significance at $P \leq 0.05$.

Parameter	Estimate	Standard Error	Z-value	P
Model 6				
Intercept	-7.036	0.735	- 9.579	**<0.001**
Day	0.011	0.008	1.532	0.126
Day^2	-3.995E-5	2.136E-5	-1.871	0.061
Rain Accumulation 31-120	0.038	0.015	2.551	**0.011**
Model 3				
Intercept	-6.777	0.716	-2.2620	**<0.001**
Day	0.012	0.008	1.4170	0.160
Day^2	-3.880E-5	2.171E-5	-1.6840	0.074
Rain Accumulation 1-90	0.033	0.016	2.0160	**0.032**

Figure 1

Location of Brushy Creek Spring.

Brushy Creek Spring (red star) in the context of the urban matrix of Round Rock, Williamson County, Texas, USA.
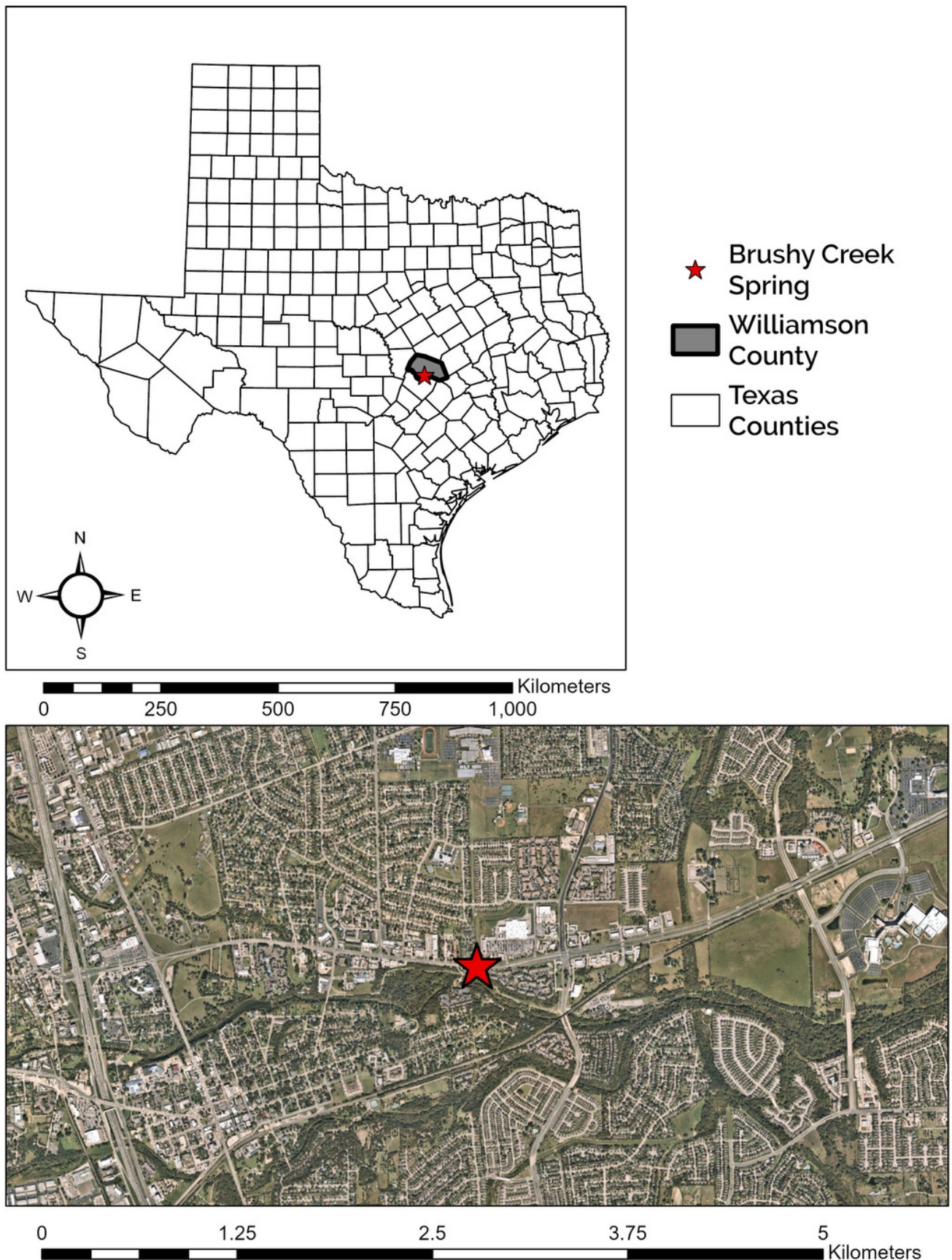
Figure 2

Descriptive site images of Brushy Creek Spring

(A) Aerial image (Google Earth imagery) of Brushy Creek Spring in Round Rock, Williamson County, Texas. Stars designate the locations of photographs B–E. All photographs face upstream. (B) Junction of culvert tunnel under US79 and box culvert with groundwater discharging from the PVC pipe, seams in the culvert tunnel junctions, and at seams and cracks at the culvert tunnel and box culvert interface. (C) Right side of the box culvert upstream of the gabion with shallow, spring water sheet flowing across concrete bottom. (D) Downstream entrance of box culvert, gabion, and pond. (E) Spring run downstream of gabion (Photographed by Zach Adcock).

Figure 3

Gravid female Jollyville Plateau Salamander

(A) Jollyville Plateau Salamander (*Eurycea tonkawae*) from Brushy Creek Spring. (B) Oocytes
are visible through the salamander's translucent venter (Photographed by Ryan Jones).

Figure 4

Water chemistry by occurrence and location

Mean and 95% confidence interval for water chemistry metrics (measured at Brushy Creek Spring with respect to salamander occurrence (presence or absence) and location (culvert or spring run).
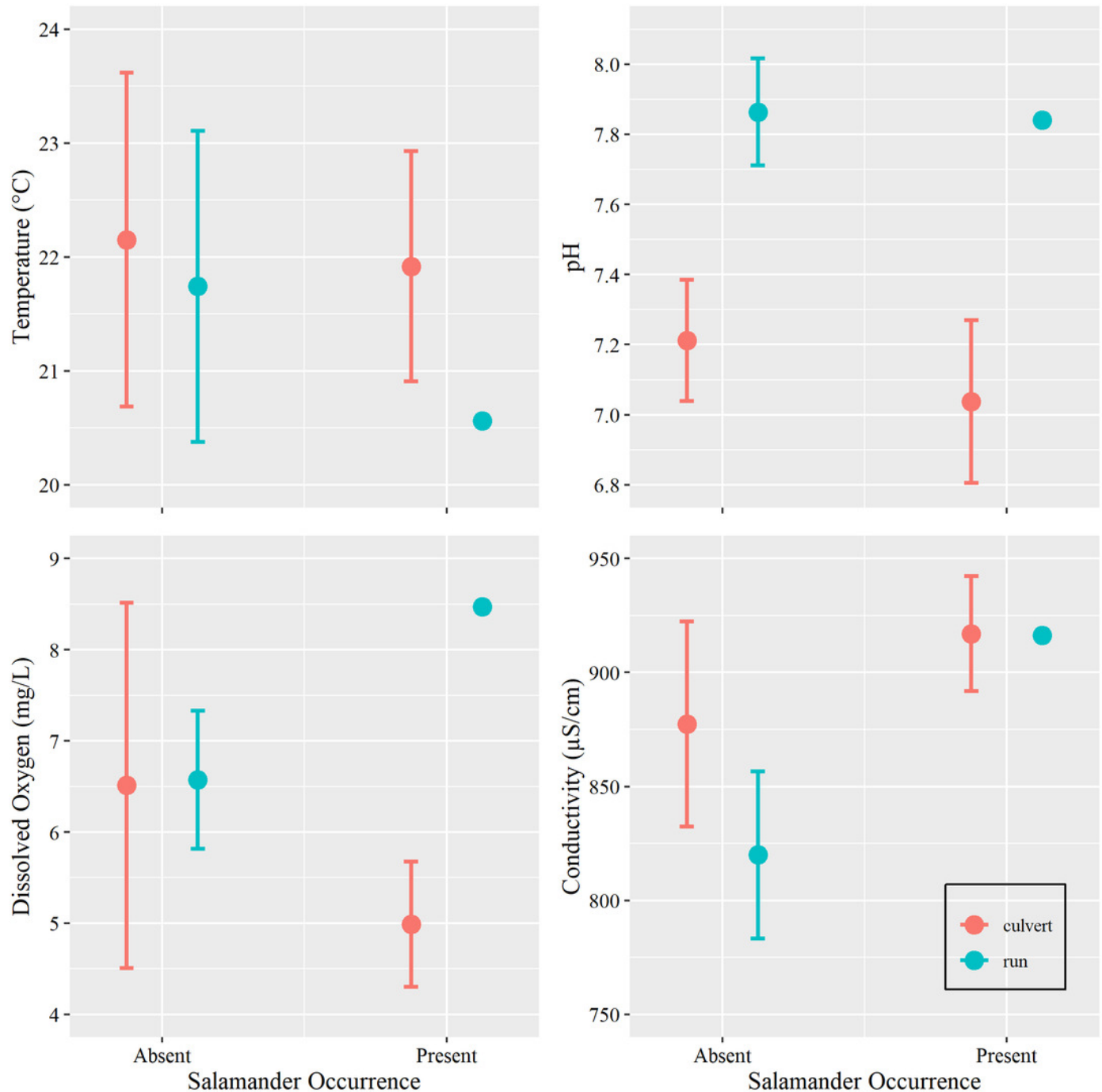
Figure 5

Jollyville Plateau Salamander (*Eurycea tonkawae*) capture location at Brushy Creek Spring

(A) Groundwater discharges from cracks in the concrete along the culvert wall and floor junction and from the nearby spring diversion PVC pipe. White square outlines the area in photo (B). One *E. tonkawae* partially covered by a large cobble (Photographed by Zach Adcock).

Figure 6

Number of salamander detections predicted by rainfall accumulation

(Top) Jollyville Plateau Salamander (*Eurycea tonkawae*) detections (circles) and predicted curve (black line) over a range of 0–60 cm of accumulated rainfall at Brushy Creek Spring for the best fit generalized linear model (Tables 3 & 4). The red line demarcates one salamander detection. The log offset of effort was held constant at the mean number of objects searched (172.3) and day-of-year as held constant at the mean day-of-year surveyed (194.74) within the culvert throughout the 39 surveys conducted for this study. (Bottom) Red bars represent the number of Jollyville Plateau Salamanders observed by date (secondary y-axis), and the black line represents rain accumulation 31-120 days prior to each date (primary y-axis).