SPATIAL AND TEMPORAL USE OF CAVES BY *ELEUTHERODACTYLUS*

MARNOCKII AND *CRAUGASTOR AUGUSTI* IN THE WESTERN EDWARDS

PLATEAU OF CENTRAL TEXAS

By

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Abbreviation – Description

ANOVA – Analysis of Variance

IGP – Inter-Guild Predation

NND – Nearest-Neighbor Distance

ABSTRACT

Understanding the spatial and temporal habitat associations of rarely encountered species is an important component of understanding their ecology. Barking frogs (*Craugastor augusti*) and Cliff-chirping frogs (*Eleutherodactylus marnockii*) are rarely encountered inhabitants of the rugged limestone terrain of the western Edwards Plateau of Texas. In order to explore the mechanisms of co-occurrence between these two species in a spatially restricted environment I examined the habitat-use and dispersion patterns within and between these species. Six caves varying in length from 6 m to 120 m were surveyed monthly from January through December 2017. Additionally, one cave was also surveyed at 6 h intervals across a 24 h period, quarterly. The location of individuals with respect to cave entrance were recorded during each survey. Caves were not used as daily or seasonal refugia as both species were present day and night throughout the year with peak numbers observed during spring-summer and summer-fall for *E. marnockii* and *C. augusti* respectively. Both species were found throughout the lengths of caves but differed in patterns of microhabitat use. Cave occupancy was not restricted temporally during the 24 h period or seasonally. As well, *E. marnockii* but not *C. augusti* exhibited seasonal patterns of aggregation. However, inspection of near-neighbor distances consistently failed to reveal evidence of interspecific repulsion with the curious exception being during peak abundance of *C. augusti*, *E. marnockii* displayed a repulsed pattern of dispersion towards this species. This study is a primer to further investigations into the ecological interactions between these anurans.

I. INTRODUCTION

Understanding the spatial and temporal habitat associations of rarely encountered species is a challenging but important component of understanding their ecology (Thompson et al., 2018). Rarely encountered species are by definition difficult to monitor due to the constraints and uncertainties of sampling (Gu and Swihart, 2004; Panahbehagh et al., 2011; Kristensen and Kovach, 2018). Thus, necessary first steps in laying the foundation for studies of the ecology or population dynamics of rarely encountered species include identifying the habitats where the species can be readily sampled and developing sampling methodologies for monitoring. Moreover, sampling methodologies for monitoring rare species are important for conservation planning and for consideration in the overall management of the areas occupied (Walls et al., 2011; Hunt et al., 2012). When rarely encountered species are found to congregate spatially and temporally, either seasonally or daily, sampling can be much more efficient (Hernandez et al., 2006) and yield samples of sufficient size to support thorough scientific investigations of the species' ecology.

For species that co-occur on spatially restricted habitats determining the nature of interspecific interactions is essential for understanding the mechanisms that facilitate coexistence (Tilman, 1987) and more broadly for understanding how ecological communities are structured (Vignoli et al., 2017). Therefore, a critical first step in understanding interspecific interactions is determining the spatial and temporal patterns of distribution and dispersion of co-occurring species (Waddle et al., 2010). Given adequate sampling, information on the spatial distribution of conspecifics and heterospecifics can yield valuable insights into the type of interactions potentially

involved within and between species (Reichling, 1999; Birkhofer et al., 2006).

Conspecific individuals may be randomly, aggregated, or regularly dispersed in space

within populations (Taylor et al.,1978). An aggregated pattern of spatial dispersion is

indicated by near-neighbor distances (NND) that on average are shorter than predicted by

a random distribution, while uniform spatial dispersion is indicated by relatively constant

NND. Nearest-neighbor distances in a population characterized by random dispersion of

conspecifics are typically described by a normal distribution. Many species that are

territorial exhibit patterns that match the normal distribution (Birkhofer et al., 2006). In

contrast, aggregated spatial patterns may be exhibited by those species whose clumped

distribution reflects a response to variation in habitat quality (Folt et al., 2018) or for

those species that engage in breeding choruses to attract mates (Angeli et al., 2015).

The spatial dispersion of conspecifics does not necessarily indicate the way in

which individuals of species (i) are dispersed relative to species (j). The dispersion of

heterospecific individuals may be randomly, positively (aggregated), or negatively

(segregated or repulsed) relative to one another. Two species are described as positively

associated when individuals of both species are spaced closer than expected by random,

while a negatively associated species pair are spaced further than random (Coomes et al.,

1999). Typically, ecological studies describe and evaluate intra- and inter-species

distributions within a two-dimensional framework (Veech et al., 2003). However, for

species occupying habitats such as forest canopies, pelagic systems, alpine environments,

and caves, assessment of intra- and interspecific spacing requires information on the

three-dimensional distribution of the species (Koeppl et al, 1977).

Herein I examine the spatial and temporal patterns in the use of cave environments by each of two anuran species that are rarely encountered above ground but co-occur in caves. I conducted daily, monthly, and seasonal surveys and monitored habitat use and environmental conditions throughout the lengths of caves to describe patterns of spatial and temporal dispersion, and interactions between, Barking frogs (*Craugastor augusti*) and Cliff-chirping frogs (*Eleutherodactylus marnockii*). These two species inhabit limestone caves in the western Edwards Plateau, of Central Texas. I tracked the spatial distribution and dispersion of both frog species for a one-year interval within a series of caves to test hypotheses regarding patterns of habitat use and patterns of associations within and between these species within caves. I address the following questions for each species: 1) are caves used as temporal refuges on a daily or seasonal basis or as continually occupied habitats throughout the year?; 2) does each species make use of the full length of caves or is cave use restricted to particular regions, for example the entrance zone?; 3) do patterns of distribution within caves reflect response to environmental zonation within caves?; 4) are patterns of dispersion within caves both within and between species random, aggregated, or repulsed?; and 5) do the species differ in their use of microhabitats? Addressing these questions provides insight into the ecology of, and interactions between these two infrequently encountered and understudied species in an overlooked environment while evaluating whether caves provide reliable sampling locations for further ecological and behavioral studies.

Study System. —Craugastor augusti and *Eleutherodactylus marnockii* represent the northernmost latitude representatives of the families Craugastoridae and Eleutherodactylidae respectively (Hedges et al., 2008; Streicher et al., 2014). Species in

these families exhibit direct development in which metamorphs emerge from eggs deposited in terrestrial habitats (Jameson, 1950). *C. augusti* is represented by a discontinuous group of nine lineages distributed throughout Mexico, Texas, New Mexico, and Arizona (Streicher et al., 2014). The subspecies *Craugastor augusti latrans* occurs in the Edwards Plateau of central Texas (Figure 1A). *E. marnockii* has a narrower range extending from the eastern edge of the Edwards Plateau west to the Stockton Plateau (Figure 1B). Both species inhabit environments that provide interstitial space within rubble, such as limestone outcrops, cliffs, crevices, and caves (McCalister, 1954; Jameson, 1955; Reddell, 1994; Goldberg & Schwalbe, 2004b). These environments along with these frogs' nocturnal activity and cryptic nature challenges researchers seeking to detect these species (Goldberg and Schwalbe, 2004a). To date, much of the life cycle and life history of these frogs is unknown and studies of their population ecology are restricted (Jameson 1955). Competitive interactions based on space or resource use are likely hypotheses based on evidence of dietary overlap (Jameson, 1950; Jameson, 1955) and the restricted productivity of cave environments. As well, given the disparity in body size (*E. marnockii* is much smaller than *C. augusti*, at only 18–35mm on average, while *C. augusti* have an average length of 47–94mm), intraguild predation (IGP), defined as when a competing species within an ecological guild, consumes heterospecifics of the same guild at some life-stage, (Holt & Polis, 1997) could be possible. Coexistence in communities where IGP is present can be mediated by the presence of spatial refuges used by IG prey to avoid potential IG predators (Wilson et al, 2010). Alternatively, spatial and or temporal patterns within and between the two species could also be driven proximately by each species' response to daily and seasonal abiotic environmental cues

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throughout cave systems. Base line information on intraspecific and interspecific patterns

of co-occurrence, seasonal and temporal patterns of space and habitat use of *E. marnockii*

and *C. augusti*, are needed as foundations to address these biotic and abiotic hypotheses.

II. MATERIALS AND METHODS

Study Sites.— Limestone outcrops, canyons, and karst features including sinkholes and caves associated with the regional hydrology characterize the Edwards Plateau region in central Texas where this study was conducted (Reddell, 1994). Karst features in this region range from small crevices and sinkholes to large caves spanning 300–9000m in length. I surveyed six caves in the western Edwards Plateau during 2017: two caves in Edwards and four caves in Real county, Texas. These sites are located on the western edge of the Balcones Escarpment in typical Ashe Juniper/Plateau Live Oak scrub of the region (Murray et al., 2013). While caves in this region are inventoried by the Texas Speleological Society, many karst features represent unregistered caves and sinkholes on private land with access limited by landowners. Access to the six caves was granted by private landowner agreements, a condition of which was to not disclose the physical locations.

These caves studied are representative of small to moderate size karst features in the region and ranged in size from 8–125m of navigable length (Figure 2). These caves were diverse in physiognomic features such as length, depth, aspect, surface vegetation cover, and size of entrance. The caves varied with respect to maintaining relatively constant temperature and humidity conditions with the shortest cave (Cave 4) and cave with largest opening (Cave 3) having yearly temperature and humidity profiles similar to the surface profiles due to the ease with which surface air could penetrate the entire length of each cave (Figure 3 and 4). My longest (Cave 1) and deepest (Cave 2) caves maintained constant temperature and humidity levels throughout the year (Figure 4).

Survey Methods.—Beginning January 2017 and continuing through December 2017 I conducted monthly surveys of both species within all caves. During each survey two or three observers systematically searched the length of each cave beginning at the entrance using headlamps to locate frogs. To avoid double counting observations were only recorded when the frogs were in front of surveyors as I searched towards the end of the cave and I did not record observations as I worked my way back towards the entrance. Surveys were conducted during daylight hours. Each cave was fitted with a transect rope staked from the entrance (0 m) to the furthest navigable end of the cave marked in 1m intervals. This reference line remained in place throughout the study and anchored all corresponding measurements of locations and abiotic measurements. Temperature and relative humidity were collected at designated stations from the cave entrance (surface or 0 m), every 20 m of transect, and at the end of the transect during every survey using a Kestrel Drop data logger. Only Cave 1 was surveyed in April due to private-land access limitations.

24-Hour Surveys. —Monthly surveys were restricted to daylight hours. However, at Cave 1 I were able to test whether patterns of abundance and space use observed during daytime surveys are indicative of patterns observed across the 24 h cycle. I conducted four surveys during a 24 h interval (600, 1200, 1800, and 2400) in Cave 1 once per season (winter, spring, summer and fall). For these 16 surveys, the transect was divided into four quartiles of equal length (34 m). During each survey the two to three observers conducted 10-minute searches of each quartile and recorded the number of each species encountered. This design enabled us to evaluate seasonal and time-of-day effects on observed frog locations and frequencies. I analyzed this data using a three-way

contingency table in conjunction with a log-linear analysis to test the effects of time-of-day and season on the frequency of occurrence and distribution among the four quartiles.

This survey design served a dual purpose as it also allowed us to test whether the patterns of seasonal variation that I observed for each species across the 12 monthly surveys in Cave 1 were better explained by sampling variation or more likely reflected actual seasonal changes in numbers. To test these alternatives, for each species, I first summed the number of frogs observed across the four quartiles during each of the 6 h surveys to yield four replicates of the total numbers observed during each quarterly–winter, spring, summer and fall survey.

I then used one-way ANOVA followed by means comparison to examine variation within and among quarterly surveys. Rejection of the null indicates that seasonal variation among quarterly surveys exceeds sampling variation within the quarterly surveys.

Spatial Distribution Sampling.—Monitoring intra and interspecific spatial dispersion of cave-inhabiting frogs involves circumstances uncommon to many studies of terrestrial species. Terrestrial landscapes are usually treated as two-dimensional when tracking the spatial distributions of anurans (Goldberg & Schwalbe, 2004b). But in the case of caves, these environments constitute a three-dimensional matrix of crevices, ledges, boulders, scree, and various rock formations and individual frogs at a given location in the cave may be located on the floor, walls, or roof. If the spatial dispersion of two frogs were compared when mapped two-dimensionally (x and y coordinates) individuals could appear to occupy the same point location when in fact, they are located at separate vertical points in the cave. Thus, a third term z is needed to denote the vertical

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position of each individual. During each survey each individual frog's three-dimensional

coordinates were recorded. Coordinate "x" denoted the location with respect to the cave

entrance ($x = 0$). The perpendicular measurement "y" denoted location to the right (+) or

left (-) of the central transect line while the z-coordinate recorded vertical distance from

the cave floor $z = 0$). This 3-dimensional mapping provided a spatially explicit point and

microhabitat (see below) for each observed frog during each survey and in conjunction

with the yearlong surveys provides the data to evaluate each species of frog's use of caves

over a yearly cycle.

The mapped locations also allowed us to evaluate intra and interspecific patterns of

dispersion using nearest-neighbor distances. Near-neighbor distances (NND) were

calculated by using the two-dimensional Euclidean distance between the two neighbors

summed to the difference of vertical positions, z. That is, $\text{NND}_{ij} = (x_i - x_j)^2 + (y_i - y_j)^2 + |z_i$

$- z_j|$, for individuals i and j. By using the mean NND for a particular survey period

(month) I were able to test observed patterns within and between each species in terms of

their spatial dispersion throughout the year. To determine whether the intraspecific spatial

dispersion pattern of each species was aggregated, random, or repulsed within each cave I

conducted a series of randomization tests. I conducted these tests only for Caves 1, 2, and

5 that had total annual observations > 30 sufficient to serve as a reference dataset for the

randomization procedure. In this procedure, statistically significant dispersion was tested

for by comparing the observed mean NND for a survey month against a distribution of

NND calculated by randomized sampling of a reference dataset for 1000 iterations. The

random samples maintained the same sample size (number of individual pairs) that the

observed mean NND was based on. The reference dataset included the pooled

observations of both species in each cave over all months as no habitat was excluded by either species and there were no strong seasonal effects. The reference dataset intentionally included only *xyz* points that were actually occupied by a frog at some time and thus such points were located on a surface (otherwise an unrestricted randomization of the *xyz* space might create some points that were floating in space). Thus, by null expectation a frog of either species would potentially be able to occupy any observed point (in the reference dataset) from the cave floor to ceiling.

Once the null (test) distribution of NND values was produced, a p-value testing for significant clumping or overdispersion was derived as the proportion of null values less than the mean observed NND or as the proportion greater than the mean observed NND. Deriving a p-value as a proportion of a null distribution is the most straightforward way to use a randomization test or null model as a test of significance (Veech, 2012). If neither significant clumping or overdispersion were revealed (at $\alpha = 0.05$) then I assumed the dispersion pattern was random. I applied this randomization test separately to the spatial dispersion of each species each month to assess intraspecific pattern. I then also applied it to assess interspecific dispersion using heterospecific NND values although this assessment was only possible for Cave 1 as the other caves did not have a sufficient number of heterospecific pairs. The test for interspecific dispersion was essentially an assessment of whether the two species were spatially associated with one another (significantly small mean observed heterospecific NND) or avoided each other (significantly large NND).

Microhabitat.—To describe the microenvironments used by each species within caves and to compare the distribution of the species among microhabitats I categorized

the microhabitat occupied by each frog during monthly surveys into one of seven categories based on perch substrate and perch height with respect to the cave floor. Four, floor ($z = 0$) categories and three elevated categories were used. Floor subcategories were crevices, face (bare limestone), scree (jumbles of rocks/pebbles), and soil (bare soil or guano). Elevated ($z > 0$) categories were crevice, face (bare limestone), or ledge. To test the hypothesis of nonrandom microhabitat for each species use I conducted chi-squared goodness-of-fit tests for the 3 caves with total yearly observations for a species of > 20. To test differences in microhabitat use between species I conducted a contingency table Chi-square test of independence using the data for Cave 1, which had robust numbers of observations for both species. To further explore perch height as a component of microhabitat I compared mean perch height (mean z) across all observations between the two species.

III. RESULTS

Cave use by C. augusti and E. marnockii. —Both *E. marnockii* and *C. augusti* were present and commonly encountered in caves 1–5 during monthly surveys (Figure 5). Neither species was detected in Cave 6 thru the first five surveys after which surveying stopped. Over the course of the one-year study, I recorded a total of 721 frog observations (155 *C. augusti*, 566 *E. marnockii)* within Caves 1–5. The observed frequencies represent an uncorrected index of abundance of each species throughout the year as well as uncorrected estimates of relative abundance of the two species (i.e., study wide ratio of 1 *C. augusti* per 3.65 *E. marnockii*). Both frog species were detected in one or more caves every month with the exception that *C. augusti* was not found in January (Figure 5). The number of observations per species varied across the monthly surveys in all caves. Generally, the caves where both species were present during most monthly surveys also had the highest number of observations. For *E. marnockii* observed numbers were usually highest during the spring and summer months while for *C. augusti*, seasonal variation was less pronounced with higher observed counts occurring in late summer–early fall months (Figure 5). This general pattern was supported by results of the one-way ANOVA applied to the quarterly 24 h survey data which demonstrated that seasonal variation among quarterly surveys was significantly greater than sampling variation within quarterly surveys for *E. marnockii* ($F = 5.322$, $P = 0.015$) but not *C. augusti* ($F = 1.386$, $P \geq 0.295$). For *E. marnockii* based on Tukey's multiple comparisons of means the pattern of seasonal abundance was spring = summer = > fall > winter. The two largest caves, defined on the basis of the combined survey length of the main and all secondary passages, had the highest number of observations for each species. However, for both

species, cave size was uncoupled from the number of frogs observed per meter of transect during surveys where the species was present. For example, Cave 1, the second largest cave, had the highest observed densities of both *E. marnockii* (0.44/meter) and *C. augusti* (0.22/m) while Cave 4 (< 10 m) also had relatively high observed densities of both species (0.37 *E. marnockii*/m and 0.12 *C. augusti*/m) due to its short length despite low raw counts.

Both *E. marnockii* and *C. augusti* made near full use of the length of each cave (Figure 6). In Caves 2–5 both species were observed throughout the first 60% of the caves' lengths measured from the entrance. In cave 1, however, both species were found throughout the entire 125 m survey transect length. For this cave the spatial distribution of both species of frogs with respect to the cave entrance was examined seasonally by compiling location data for each species quarterly and comparing the distributions. Location with respect to cave entrance did not vary among seasons.

Cave 1 maintained the most stable environmental conditions throughout its length and stayed cooler than summer extreme temperatures and warmer than winter extremes while maintaining consistent high humidity levels. The ability to buffer against surface conditions may explain the consistent use of this cave by both species throughout the year. Caves 3 and 4 were least efficient at insulating against environmental extremes due to the large entrance of Cave 3 and short length of Cave 4. These caves had the lowest frequencies of frogs encountered over usually nonconsecutive months. Neither of these caves were used during the peak winter months.

24-hour surveys.—Contingency table analysis of the distribution of *E. marnockii* along the length of Cave 1 (i.e., among the four quartiles of cave length) across the

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quarterly surveys that involved 4 surveys conducted at 6 h intervals during a 24 h period demonstrated a significant three-way interaction among time-of-day, season, and quartile ($G^2 = 102.3$, df = 54, $P < 0.0001$) (Table 1). However, the only significant two-way interaction was between time-of-day and season ($G^2 = 30.74$, df = 9, $P = 0.0003$). These results indicate that time of day and season significantly affected the frequency of encounters of *E. marnockii*. However, frequency of *E. marnockii* encounters was usually higher during daylight hours during each season . *C. augusti* also exhibited a significant three-way interaction among all three variables ($G^2 = 82.34$, df = 54, $P = 0.008$) (Table 2). The only significant two-way interaction was between quartile and season ($G^2 = 37.26$, df = 9, $P < 0.0001$). This result suggests that the distribution of *C. augusti* among quartiles varied with season.

Intra and Interspecific Patterns of Spatial Dispersion. Randomization tests applied to near-neighbor distances allowed us to characterize the pattern of spatial dispersion of each frog species for each month of the year in Caves 1, 2, and 5. *E. marnockii* was significantly aggregated in the majority of months tested across caves (all $P < 0.05$). For example, *E. marnockii* was aggregated in 10 of 12 months in Cave 1 (mean NND = 2.07m, mean randomized NND = 3.06m) where the species was most abundant and aggregated in four of eight months in Cave 2 (mean NND = 4.64m, mean randomized NND = 5.80m), which had the next highest abundance. In Cave 5 (mean NND = 3.50m, mean randomized NND = 5.87m) where, *E. marnockii* was less abundant, the species was aggregated in 4 of 11 surveys but was significantly over-dispersed in the November survey. In contrast, for *C. augusti* mean observed NND during most surveys was neither significantly small or large, thus indicating random dispersion. However, in Cave 1

(mean NND = 8.89m, mean randomized NND = 7.85m) for the September survey *C. augusti* found to be over-dispersed as indicated by a significantly ($P < 0.05$) large mean observed NND. In the only survey month tested in Cave 5 (NND = 0.23m, randomized NND = 6.75m) (September) *C. augusti* was significantly aggregated ($P = 0.001$).

I examined patterns of interspecific dispersion in Cave 1 during 9 of the 12 monthly surveys. *E. marnockii* generally showed random dispersion relative to *C. augusti* (mean NND = 5.71, mean randomized NND = 7.72), except for the August survey in which mean observed heterospecific NND for *E. marnockii* was significantly ($P < 0.05$) large indicating repulsion from (or avoidance of) *C. augusti*. Notably, I also observed the highest number of observations for *C. augusti*, in cave 1 in August. *C. augusti* displayed random dispersion relative to *E. marnockii* in every survey month.

Habitat Use. —*E. marnockii* exhibited nonrandom association with the seven micro classes in the three caves (Caves 1, 2, and 5) for which I examined the distribution of individuals among habitats. *E. marnockii* was observed in elevated-crevices and/or elevated-faces (> 25.0% of observations) at a higher frequency than expected in the three caves and usually less than expected for floor-face (≤ 1.0% of observations) (Figure 7). Habitat use by *C. augusti* was assessed in Caves 1 and 2 and in both caves *C. augusti* also showed nonrandom habitat use (Figure 7). In Cave 1 *C. augusti* was observed in elevated-crevices at a higher than expected frequency (35.7% of observations), while in Cave 2 the species was observed on the floor-soil (50.0% of observations) at a higher frequency than expected. Notably, both species were under-represented in open floor habitats and mean perch height (mean z) across all observations was higher for *E.*

marnockii (0.74±0.03m) in comparison to *C. augusti* (0.46±0.05m) in all four caves with sufficient data for comparison

The interspecific comparison of habitat associations showed that the species differed in their use of habitats in Cave 1 (χ^2 = 15.2, df = 6, *P* = 0.018). *C. augusti* used elevated open faces greater than expected, while *E. marnockii* used this microhabitat less than expected (Figure 7). *C. augusti* also used elevated crevices less than expected. The interspecific test of unequal distribution among the seven habitat categories is particularly revealing as the expectation per habitat per species is determined by the joint proportional distribution of both species among the habitat categories. Thus, unlike the intraspecific test of distribution among habitats the interspecific test is not predicated on the assumption of equal availability of the seven habitat types.

IV. DISCUSSION

Rarely encountered species are difficult to reliably sample and hence tend to be understudied (Taylor et al., 1978). The Cliff-chirping frog (*E. marnockii*) and the Barking frog (*C. augusti latrans*) are two such species. Aside from the studies of *E. marnockii* by Jameson (1955) in above ground rocky outcrop settings, very little is known regarding the population and spatial ecology of these species throughout their range in central Texas. Reddell (1994) reported *E. marnockii* and *C. augusti* as cave-inhabiting in his review of karst fauna of the western Edwards Plateau. However, these faunal surveys focused primarily on invertebrates with no systematic effort to record and report herpetofauna. Thus, despite the scattered notations of co-occurrence within caves, no studies to date have systematically assessed simple presence/absence of these species in caves throughout the Edwards Plateau of central Texas or assessed their co-occurrence. Yet in a sample of six caves in the western Edwards Plateau I was able to reliably find both species of frogs in five caves. Moreover, in a subset of these caves both species were present throughout the year at abundances that can facilitate cross-sectional or longitudinal studies of their population ecology, spatial ecology, demography, and life history. Given the large number of reported and unreported caves in this region my results suggest that both of these frog species are likely present in a considerable fraction of these habitats and thus great opportunities exist within cave systems in central Texas to study these species that are seldom encountered in terrestrial settings outside of the breeding season.

I observed both species year-round in caves and because these species display direct development, in theory, they are capable of completing their life cycles without leaving caves. Jameson (1950) noted a male *C. augusti* frog calling while tending eggs buried under a rock in moist soil and concluded that male *C. augusti* remain with the eggs. Given the high humidity that characterizes limestone caves and sinkholes in the Edwards Plateau these features likely contain suitable permanently moist sites for egg laying and juvenile development for both frog species. I observed both species engaging in breeding choruses within the caves during the spring and I noted juveniles of both species in summer and fall indicating temporal and spatial overlap of adults and juveniles. As well, during a parallel year-long study of vocalizations within these same caves using automated recording units (ARUs) I documented daily and seasonal patterns of calling activity for both species including chorusing during the breeding season (unpublished). Taken together these observations suggest that these continuously occupied caves are important centers of activity and figure prominently in the life cycle of both species.

Given the seasonal variation in temperature and humidity that characterizes the harsh landscape of the Edwards Plateau in Real and Edwards counties Texas, and the ability of caves to ameliorate these conditions, a natural hypothesis is that caves are used as seasonal refugia by both *E. marnockii* and *C. augusti*. However, I found no evidence of a sharp influx of animals during either the winter or summer to suggest that caves are used as seasonal refugia. Rather I observed both species to be present year-round with peak observations for both species in the spring and/or summer. Moreover, I ruled out sampling variation as an explanation for the variation in the number of observations

among monthly surveys in the case of *E. marnockii* for Cave 1. Finally, as shown in

Figures 3 and 4, while Cave 1 and Cave 2 maintained the greatest differential between

ambient and cave temperatures and humidity during peak summer months, only for Cave

1 was there a corresponding peak in the number of *E. marnockii* observed Figure 5. At

the same time, Cave 5 for which internal cave conditions closely tracked ambient

conditions also showed a summer increase in the number of *E. marnockii* observed

(Figure 5) Furthermore, multiple lines of evidence suggest that neither frog species used

caves strictly as daytime refugia. First, I did not observe *E. marnockii* or *C. augusti*

during the day or night when I systematically searched the outside perimeter of caves.

Second, within Cave 1 the average number of frogs observed during daytime and

nighttime (of the quarterly surveys) did not differ ($t = 0.26$, df = 7, $P = 0.80$). Third, I

observed frogs of both species in Cave 1 actively foraging during all hours of the day and

night and actively calling during the night. Finally, if frogs use caves as daily refuges

then evidence of evening aggregations of frogs near cave entrances could be expected.

However, individuals of each species were distributed throughout nearly the entire length

of each cave during both the regular monthly surveys and during the 24 h surveys. In

neither data set did I see clumping of frogs at the cave entrance.

The number of frogs observed per survey represent raw abundances uncorrected for

imperfect detection (Veech et al., 2016) and are thus minimum estimates of the number

of individuals of each species occupying caves during each survey (Johnson, 2008). My

simple enumeration of the number of individuals encountered during surveys leaves

unanswered two questions central to understanding both the composition of cave

populations and perceived changes in the abundance of cave populations of these two

species. First, I do not know if the individuals observed during each survey represent the same or different individuals, or a mix of previously observed and new individuals possibly drawn from terrestrial and/or cave sources. Second, I do not know the location of individuals when missing or when a population appears less abundant. Whether the missing individuals or population segments are available but simply undetected, hidden within caves where they are unavailable to be detected, or whether they have exited the cave is unknown. Two studies provide limited insight. Jameson (1955) conducted a mark-recapture study of a terrestrial population of *E. marnockii* in central Texas and estimated home range sizes of 267-622 m^2 and a mean dispersal distance for juveniles of 211 m. Similarly, Goldberg and Schwalbe (2004b) estimated home range sizes of 215 m^2 and fixed kernel home range of 1086 m^2 in a terrestrial population of *C. augusti cactorum* in Arizona. Movement distances were not reported for adults or juveniles, but no adult frogs were found outside of their initially occupied outcrop. However, if these estimates of home range size and vagility are applicable to *E. marnockii* and *C. augusti* occupying karst features in the Edwards Plateau then it is plausible to suggest that individuals of both species have the capacity to flux between caves and terrestrial environments among surveys and seasons and possibly even move between karst features.

The degree to which intra- and interspecific interactions structure patterns of aggregation, or repulsion, and patterns of microhabitat use within cave environments by these two species is presently unknown. In this study I provided the first descriptions and comparisons of the distribution of individuals among microhabitats used by these two species within caves as well as the first assessment of patterns of dispersion within, and co-association between, the species. Five aspects of the biology of *E. marnockii* and *C.*

augusti motivated my investigation of the spatial ecology of these species within caves with respect to habitat variability. First, the physical environment within the surveyed caves is highly diverse at multiple spatial scales with smooth rock surfaces of floors, walls, and ceilings with little three-dimensional variation in texture abruptly interspersed with highly corrugated and/or deeply-dissected three-dimensional surfaces or areas of scree and rubble with interstitial spaces ranging from mm to meters. Second, I observed the species to co-occur in all caves that were occupied and to co-occur temporally and spatially with heterospecifics often in close proximity. Third, differences in body size between the species suggests the possibility of differential use of microhabitats based on size-matching alone. Fourth, co-occurrence of the species in conjunction with the body size differences suggests the possibility of interguild predation (IGP) (Holt & Polis, (1997) with its associated prediction of coexistence in communities being mediated by the use of spatial refuges by the prey species (Wilson et al., 2010). Notably IGP has been reported by Jameson (1955) who observed *C. augusti* eating *E. marnockii* in captivity. (Observations of IGP have not been reported in nature.) Finally, competition between the two species with the associated prediction of differences in microhabitats occupied is possible given the reported dietary overlap between the species (McCalister 1954; Jameson (1955).

I found evidence of near continuous aggregation in *E. marnockii*, which may be due to their reproductive strategy of breeding choruses that irrupt throughout the year under suitable conditions (Jameson, 1954; Angeli et al., 2015). Another possibility is that *E. marnockii* are aggregating in spatial refuges in avoidance of the larger *C. augusti* which may be competitors and/or predators. In addition, I found evidence of nonrandom

microhabitat use within each species as well as evidence of differences between species. Perhaps the strongest signal of spatial partitioning among species was that *E. marnockii* were on average perched higher in the caves and were more likely to be found in elevated tight crevices while less likely to be found in more exposed microhabitats. However, inspection of near neighbor distances consistently failed to reveal evidence of interspecific repulsion with the curious exception being that during the month when *C. augusti* frogs peaked in abundance, *E. marnockii* were repulsed relative to *C. augusti*. Clearly the dispersion patterns within and between both species warrant further investigation. Future studies of the drivers of coexistence between these two species are likely to be richly rewarded if conducted in cave settings.
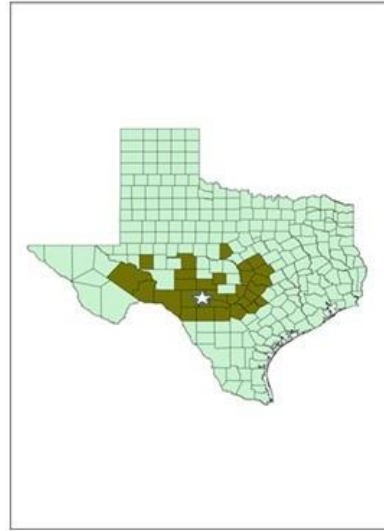
Table 1: Log-linear analysis of *E. marnockii* 24-hr habitat use over four seasons.
Surveys conducted seasonally (4 seasons) in Cave 1 throughout 2017 in four equal-length quartiles of cave length repeated every 6-hours from 6AM to 12AM.

Source	G^2	Df	P-value
Time X Quartile X Season	102.3	54	<.0001
Time X Quartile	14.86	9	0.09
Time X Season	30.74	9	0.0003
Quartile X Season	14.62	9	0.10

Table 2: Log-linear analysis of *C. augusti* 24-hr habitat use over four seasons.
Surveys conducted seasonally (4 seasons) in Cave 1 throughout 2017 in four equal-length quartiles of cave length repeated every 6-hours from 6AM to 12AM.

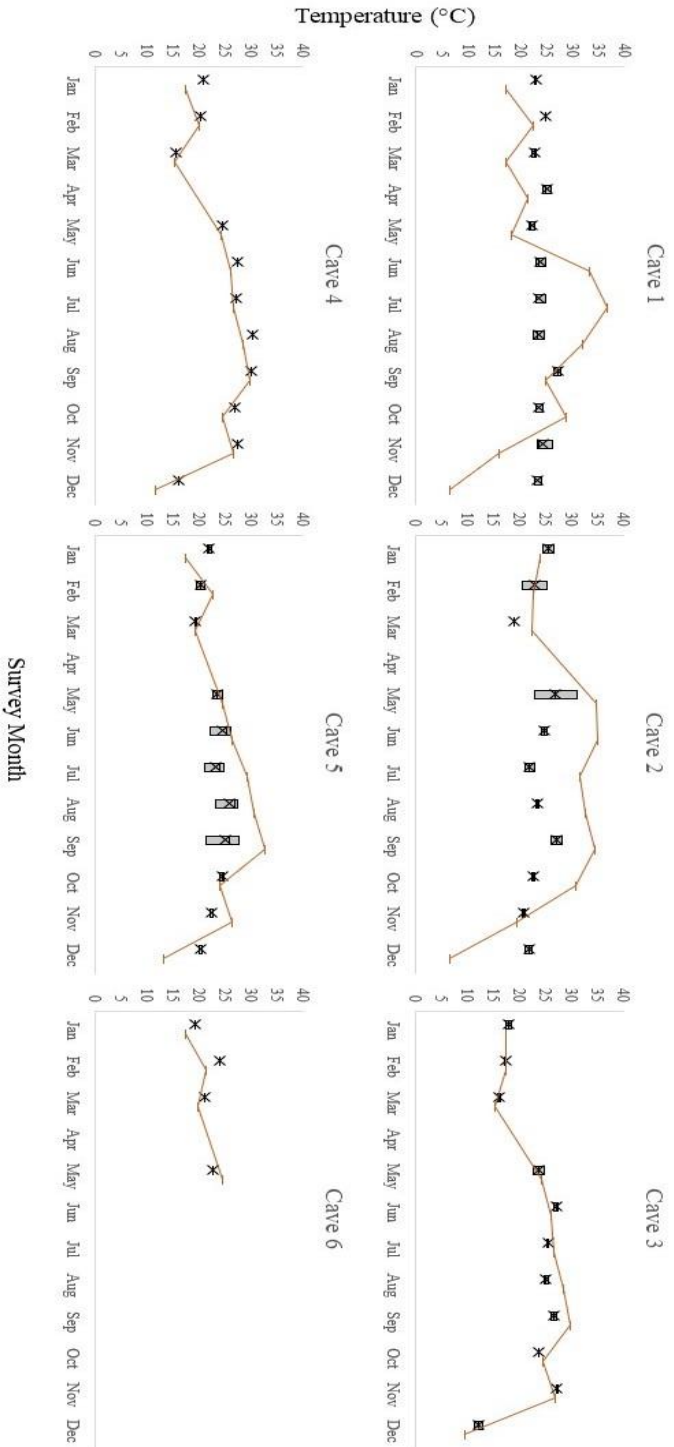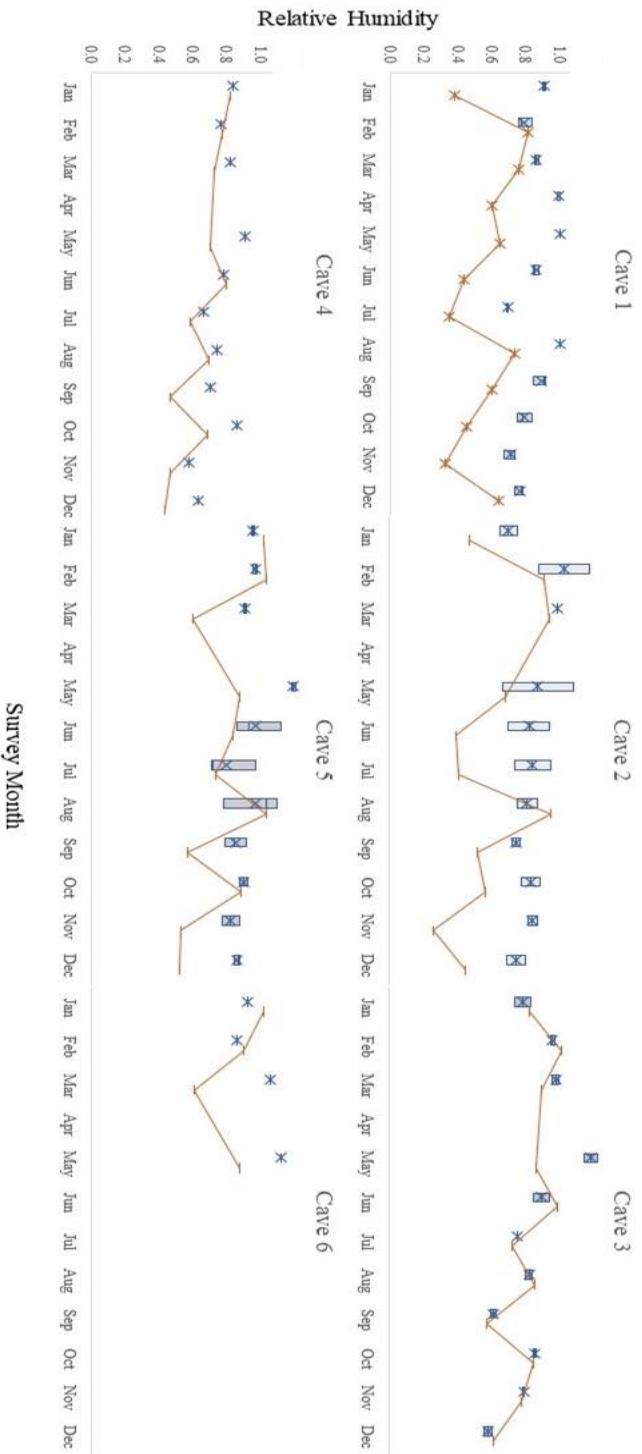| Source	G^2	Df	P-value
Time X Quartile X Season	82.34	54	0.0078
Time X Quartile	8.58	9	0.48
Time X Season	13.28	9	0.15
Quartile X Season	37.26	9	<0.0001

Figure 1: Geographic distributions of cave frogs. Barking frog (*Craugastor augusti latrans*) (1A) and the Cliff-chirping frog (*Eleutherodactylus marnockii*) (1B) in Texas, USA. Approximate location of study sites are shown with stars.
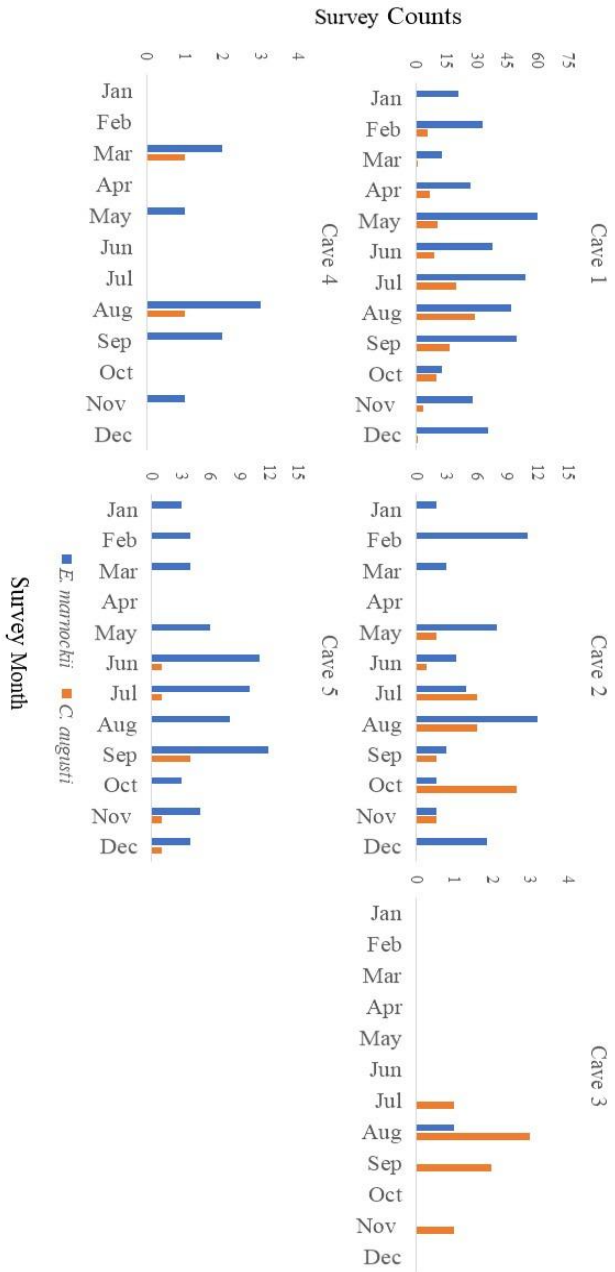
Figure 2: Diagrams of the physical dimensions of each surveyed cave. Two-dimensional aerial views of caves sampled monthly during 2017 for *E. marnockii* and *C. augusti*. Cave lengths varried from 8.2m to 140m.
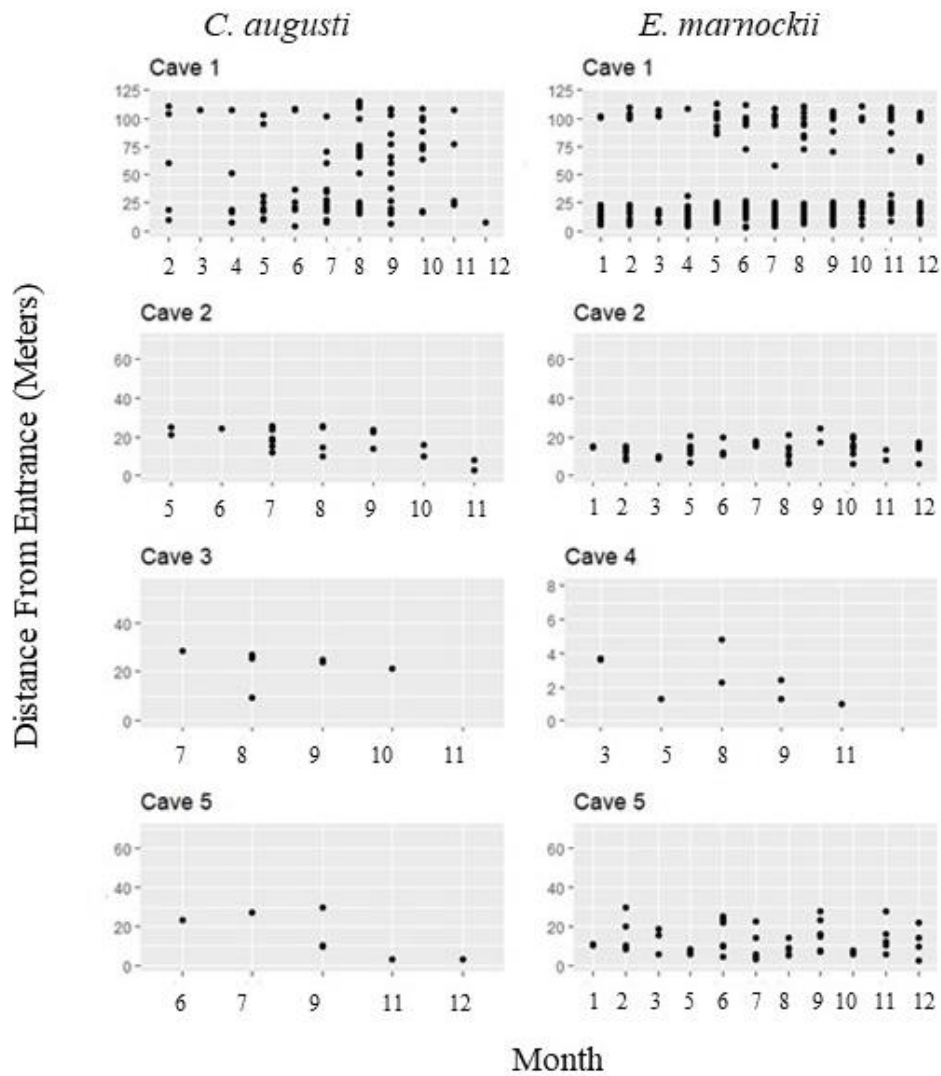
Figure 3: Average air temperature of cave (boxplots) and external surface above each cave (solid line). Average cave air temperatures were measured at middle lengths of caves. Surface air temperatures were recoded on surface surrounding cave immediately before surveys.
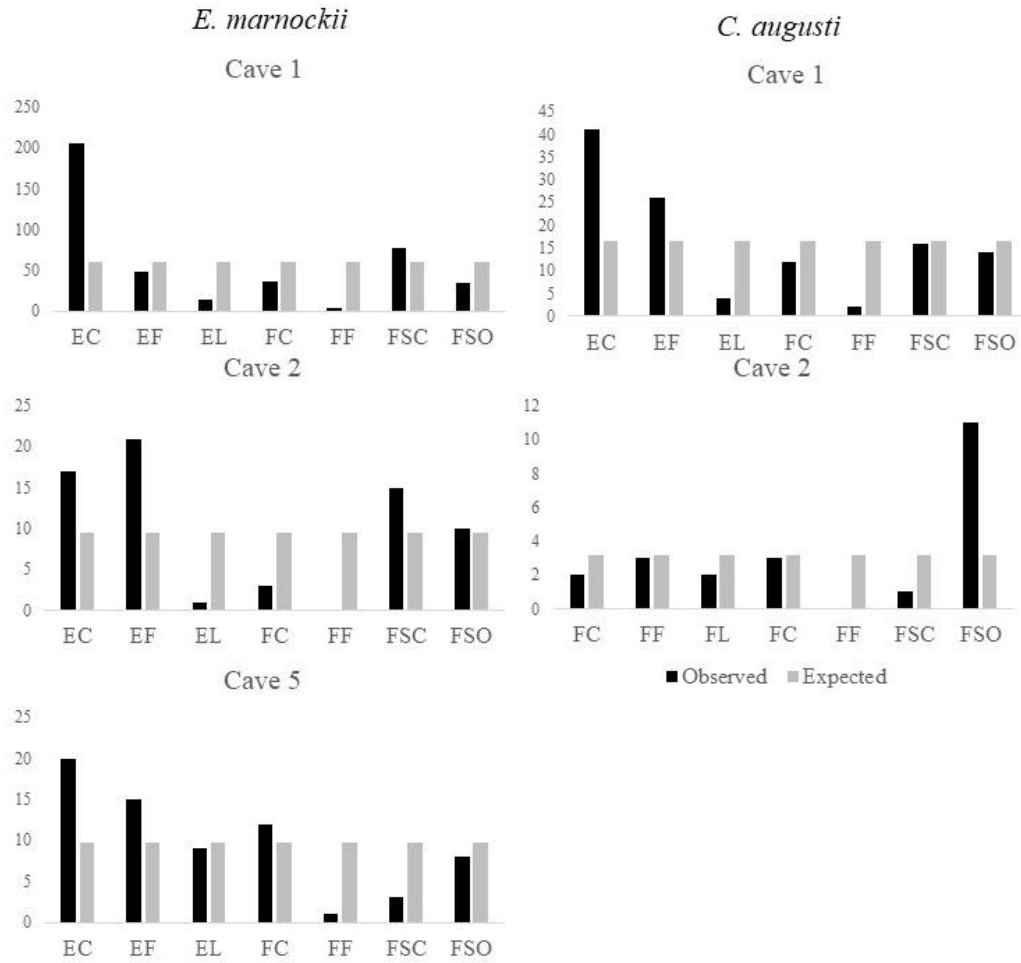
Figure 4: Average relative humidity of cave (boxplots) and external surface above each cave (solid line). Average cave relative humidity proportion was measured at middle lengths of caves. Surface relative humidity was recorded on surface surrounding cave immediately before surveys.

Figure 5: Raw abundances of *E. marnockii* (blue) and *C. augusti* (orange) monthly. Raw abundance calculated from survey totals from monthly spatial surveys in each occupied cave

Figure 6: Spatial distribution of *C. augusti* and *E. marnockii*. Length distributions were plotted for monthly surveys in which each respective species was encoutered in each cave based off of survey transect.

Figure 7: **Microhabitat use of *E. marnockii* and *C. augusti*.** Microhabitats of each frog observed during monthly surveys were recorded as one of seven microhabitat categories EC (Elevated Crevice), EF (Elevated Face), EL (Elevated Ledge), FC (Floor Crevice), FF (Floor Face), FSC (Floor Scree), and FSO (Floor Soil).

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