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Growth, Survival, Longevity, and Population Size of the Big Mouth Cave Salamander (*Gyrinophilus palleucus necturoides*) from the Type Locality in Grundy County, Tennessee, USA

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Salamander species that live entirely in subterranean habitats have evolved adaptations that allow them to cope with perpetual darkness and limited energy resources. We conducted a 26-month mark-recapture study to better understand the individual growth and demography of a population of the Big Mouth Cave Salamander (*Gyrinophilus palleucus necturoides*). We employed a growth model to estimate growth rates, age at sexual maturity, and longevity, and an open population model to estimate population size, density, detectability, and survival rates. Furthermore, we examined cover use and evidence of potential predation. Individuals probably reach sexual maturity in 3–5 years and live at least nine years. Survival rates were generally high (>75%) but declined during the study. More than 30% of captured salamanders had regenerating tails or tail damage, which presumably represent predation attempts by conspecifics or crayfishes. Most salamanders (>90%) were found under cover (e.g., rocks, trash, decaying plant material). Based on 11 surveys during the study, population size estimates ranged from 21 to 104 individuals in the ca. 710 m² study area. Previous surveys indicated that this population experienced a significant decline from the early 1970s through the 1990s, perhaps related to silvicultural and agricultural practices. However, our data suggest that this population has either recovered or stabilized during the past 20 years. Differences in relative abundance between early surveys and our survey could be associated with differences in survey methods or sampling conditions rather than an increase in population size. Regardless, our study demonstrates that this population is larger than previously thought and is in no immediate risk of extirpation, though it does appear to exhibit higher rates of predation than expected for a species believed to be an apex predator of subterranean food webs.

SALAMANDERS are the only tetrapod group to successfully invade and obligately live in subterranean habitats. At least ten species in two families, Plethodontidae and Proteidae (in North America and Europe, respectively), are restricted to caves and have evolved some degree of morphological, physiological, and behavioral adaptations to cope with generally limited food resources in perpetual darkness, within ecosystems regulated by bottom-up processes (Brandon, 1971; Huntsman et al., 2011a; Goricki et al., 2012). Energy limitation in subterranean ecosystems is thought to be an important selective pressure driving the evolution of troglomorphy, including physiological adaptations such as lower metabolic and growth rates, decreased reproductive output, slower development, and longer life spans, compared to surface relatives (Brandon, 1971; Hüppop, 2000; Culver and Pipan, 2009).

Although morphological adaptations in subterranean salamanders are well characterized (reviewed in Goricki et al., 2012), physiological and behavioral adaptations for most species are not well studied. The Tennessee Cave Salamander (*Gyrinophilus palleucus*) exhibits troglomorphic adaptations and is endemic to cave systems of the Interior Low Plateau of central Tennessee, northern Alabama, and northwestern Georgia (Beachy, 2005; Miller and Niemiller, 2008, 2011, 2012). Two subspecies are recognized, the Pale Salamander (*G. p. palleucus*) and the Big Mouth Cave Salamander (*G. p. necturoides*). Both subspecies are neotenic, with reduced eyes and an enhanced lateral line system compared to the epigean

(surface-dwelling) sister species, the Spring Salamander (*Gyrinophilus porphyriticus*; Brandon, 1966; Miller and Niemiller, 2012). *Gyrinophilus p. palleucus* is known from caves along the Eastern Escarpment of Cumberland Plateau within the Crow Creek watershed of the Tennessee River drainage in southern Franklin and Marion cos., Tennessee, and adjacent northeastern Jackson Co., Alabama. *Gyrinophilus p. necturoides* is known from caves in the Inner Nashville Basin, Eastern Highland Rim, and Western Escarpment of the Cumberland Plateau in central Tennessee, including the Collins, Duck, Elk, and Stones river watersheds (Miller and Niemiller, 2008, 2011, 2012). Populations in Walker Co., Georgia have not been assigned to either subspecies (Godwin, 2008; Miller and Niemiller, 2012). The life history and demography of this species has been little studied, with the most recent works comprising mark-recapture studies in two populations of the subspecies *G. p. palleucus* in Jackson Co., Alabama (Dent and Kirby-Smith, 1963; Simmons, 1975; Huntsman et al., 2011a). In particular, no studies have examined the demography and life history, particularly growth rates and longevity, for any population of *G. p. necturoides*.

In this study, we employed a mark-recapture approach during a 26-month period at the type locality of *G. p. necturoides* in Grundy Co., Tennessee, to estimate life history (growth rates, age at sexual maturity, and longevity) and demographic (population size, density, detectability, and survival rates) parameters important in the conservation

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and management of this species (Miller and Niemiller, 2008). Though *Gyrinophilus palleucus* is considered a top predator in the subterranean ecosystems it inhabits (Huntsman et al., 2011a), we quantify use of cover (refuge use) and present evidence of significant predation on this species.

MATERIALS AND METHODS

Study site.—Big Mouth Cave is located 5.5 km northeast of the town of Pelham in Grundy Co., Tennessee. The entrance is located on the north side of Payne Cove and is revealed by the collapse of a wall on the north side of a large sink. The cave is part of the Big Room Cave system. The entrance to Big Room Cave is located 270 m to the east, and the stream following through Big Room Cave is also the same stream that flows through Big Mouth Cave and ultimately issues on the surface at Sartain Spring. Both caves are located at the base of the Western Escarpment of the Cumberland Plateau in the Upper Elk River drainage and developed in the Mississippian-aged Monteagle Limestone. Big Mouth Cave consists of two main passages and exceeds 3 km in overall length. The eastern cave passage extends from the entrance for 115 m to the east before ending in breakdown. A west-oriented passage extends for 130 m to a low and wet crawlway. A wet-weather stream flows from the surface through this western fork and into this section of the cave. The crawlway passage continues for ca. 305 m, beginning as low crawl and gradually increasing in passage height. This section of the cave consists of a series of pools that are perched above the main cave stream, which is intersected at the end of the crawlway passage. The main stream can be traversed for several hundred meters before terminating at a sump. During wet weather, the main stream backs up into the perched crawlway passage.

The study area was confined to the final 50 m of the western fork passage and the entire crawlway passage for a total length of ca. 355 m. The study area passage averaged 2 m in height (range = 1–5 m) and 5 m in width (range = 2–8 m) and contained a series of pools with an average depth of 0.3 m, width of 2 m, but with depths up to 1.5 m depending on water levels. The substrate of pools was predominantly cobble and gravel with clay sediment and limestone bedrock also present. In some areas, large loose slabs were present where breakdown had occurred. Several pools contained large amounts of allochthonous organic matter (e.g., leaves, twigs) and garbage washed into the cave.

Sampling and data collection.—We surveyed the study area for *Gyrinophilus p. necturoides* on 11 occasions from June 2004 to July 2006: 29 June 2004, 21 July 2004, 6 February 2005, 10 March 2005, 17 April 2005, 16 June 2005, 25 August 2005, 10 November 2005, 26 January 2006, 16 April 2006, and 14 July 2006. Capture data from these 11 surveys were used in demographic analyses. We also surveyed the study area on 26 September 2008, but capture data collected during this latter survey were excluded from population size and survivability estimate analyses (see below). To locate salamanders, we wore wetsuits and slowly walked along, waded through, or crawled in the cave stream channel and searched for larvae and adults with headlamps and handheld flashlights. We also searched for salamanders beneath carefully lifted rocks and trash and within small cobble and detritus. We recorded where each salamander was observed (e.g., underneath a submerged rock, out in the open, etc.). At least two surveyors were

present per survey, and the duration of each survey lasted 7–10 hours.

We made a concerted effort to capture each salamander encountered with handheld bait nets and made note of any individuals that escaped capture. We placed each captured salamander into a clear plastic bag until processing, when each salamander was weighed to the nearest 0.1 g using a Pesola spring scale and measured, both total length (TL) and snout–vent length (SVL) from the tip of the snout to the anterior margin of the vent, to the nearest mm using a metric rule. We reported variation in body size and mass as the mean \pm SD. We noted any physical abnormalities, such as tail damage, tail regeneration, or missing limbs. Sex of *Gyrinophilus* sp. is difficult to determine without examination of cloacal anatomy, so we identified sex only of females when developing ova were visible through the abdominal wall. Based on dissections, Simmons (1975) found that males and females were sexually mature at ≥ 70 mm SVL; therefore, we classified each salamander we captured as either a juvenile (< 70 mm SVL) or an adult (≥ 70 mm SVL).

We marked each salamander by injecting a 1.2×2.7 mm visible implant (VI) alpha tag (Northwest Marine Technology Inc., Shaw Island, WA) into the dermis of the tail. Because of the size of the VI alpha tag injection needle and potential for harm to the animal, we did not mark salamanders < 40 mm SVL. Prior to the injection, we swabbed the needle with antiseptic to prevent infection. Each salamander was released at its point of capture after a short period (10–30 min) of recovery. Migration of VI alpha tags has been reported in other amphibians (Heard et al., 2008; Kaiser et al., 2009), and we also experienced low levels of local tag migration, and in some cases, inversion of tags. However, because tags were injected just underneath the translucent epidermis of the tail, the color and alphanumeric code of most tags could be easily determined. For those that were more problematic to accurately read, the tail of the salamander was manipulated until the alphanumeric code could be determined. On three occasions (2.1% of all marked individuals), inverted tags had to be extracted and reinserted. To maximize tag retention, we restrained salamanders in plastic bags during marking and tags were placed well away from entry wounds to minimize tag expulsion (Osborn et al., 2011).

Detectability, survival rates, and population size.—We used the package RCapture (Baillargeon and Rivest, 2007) implemented in the R statistical computing environment (v.3.0.2; R Core Team, 2013) to estimate population size, capture probabilities, and survival rates by fitting a Jolly-Seber open population model for the first 11 surveys (June 2004–July 2006). The September 2008 survey was excluded because of the long time interval since the previous survey compared to the interval between other survey events. An open population model was most appropriate for this mark–recapture dataset for several reasons. First, the duration of study and estimated growth rates (see Results) suggest that birth and death likely contribute to a lack of closure. Second, immigration and emigration by adults and larvae likely occurred in the study area, as we did not survey the entirety of available habitat in the cave system. In particular, salamanders have been observed in the main cave stream by MLN and BTM. We evaluated two open population models: one that allowed capture probabilities to vary between periods, and another that held capture probabilities equal across periods. We evaluated fit of each model via Akaike's Information Criterion (AIC). We estimated salamander

der density for the 355 m study area based on the population size estimates. Area of habitat was calculated assuming an average width of 2 m for aquatic habitat. However, note that available habitat is a coarse estimate as habitat width can vary dramatically depending on water levels. We reported variation in survivability, capture probabilities, and population size as \pm SE.

Growth rates and longevity.—We estimated growth rates for all recaptured individuals as the difference between SVL at the time of recapture and initial SVL at time of first capture divided by the number of days elapsed between capture events. We multiplied this rate by 30 to obtain a monthly growth increment. For individuals that were recaptured more than once, we calculated monthly growth increments using the recapture interval closest to 365 days. We regressed the monthly growth increments of recaptured individuals against their body sizes (i.e., the geometric mean of SVL over the recapture time interval per individual) to estimate a size-specific monthly growth rate. The regression equation was used to form the basis of a population-specific exponential growth model (Whitmore and Huryn, 1999) that allowed estimates of size-at-age. A bootstrap procedure (Efron and Tibshirani, 1993) was used to account for variation in growth observed among salamanders by randomly sampling the data with replacement 1,000 times. We seeded the size-specific growth rate model at 10 mm SVL, which represents the smallest size of hatchlings previously reported in the literature (Huntsman et al., 2011a), and iterated over monthly intervals until the body size of the largest individual in the population was encompassed. This approach generated a mean growth trajectory that allowed us to estimate the expected body size of different age-classes (Huntsman et al., 2011a; Venarsky et al., 2012; Fenolio et al., 2014). We generated 95% confidence intervals around the expected mean SVL of each month class using the frequency distribution of values. The values constrained by the 95% confidence intervals represent the estimated age range. The body size of the largest adult was plotted to estimate the minimum life span.

Cover use and predation.—We conducted a binomial test to determine if *G. p. necturoides* were more likely to be found out in the open or under cover (e.g., under a rock, log, or trash, or within allochthonous organic matter). We used a chi-square goodness of fit test to determine if adults and juveniles differed with respect to cover use, and chi-square tests to determine if (1) the proportion of individuals with tail damage differed between adults and juveniles, and (2) salamanders under cover were more or less likely to have tail damage than salamanders observed without cover. All statistical analyses were performed in R. For all statistical tests, $\alpha = 0.05$.

RESULTS

Between June 2004 and July 2006, we observed 301 individuals in the 710 m² study area (Table 1). A maximum of 34 salamanders was observed on two occasions: 6 February 2005 and 10 March 2005. A minimum of 12 salamanders was observed during the first survey on 29 June 2004. Water levels were substantially higher during this survey with significant turbidity limiting visibility. One hundred forty-four salamanders were captured and marked during the study period, of which 49 (34.0%) were recaptured. Ninety-five

Table 1. Estimates of survival probability, population size, and relative abundance for 11 surveys during the 26-month study of *G. p. necturoides* at Big Mouth Cave, Grundy Co., Tennessee, for an open population model with equal capture probabilities. Survival probabilities are reported as survival from the previous survey (\pm SE). Capture probability was estimated at $28.0 \pm 3.3\%$ across all surveys. The number of juveniles (<70 mm SVL) observed during each survey are indicated in parentheses.

Date	Survival probability	Population size	Relative abundance
29 June 2004	—	21 \pm 8	12 (8)
21 July 2004	0.88 \pm 0.26	91 \pm 18	31 (11)
06 February 2005	1.00 \pm 0.00	104 \pm 11	34 (12)
10 March 2005	0.83 \pm 0.13	87 \pm 13	34 (14)
17 April 2005	0.83 \pm 0.16	72 \pm 13	27 (9)
16 June 2005	0.78 \pm 0.18	80 \pm 14	30 (14)
25 August 2005	0.85 \pm 0.20	87 \pm 16	24 (10)
10 November 2005	0.57 \pm 0.16	61 \pm 12	26 (13)
26 January 2006	0.67 \pm 0.20	70 \pm 14	28 (12)
16 April 2006	0.79 \pm 0.24	67 \pm 14	23 (9)
14 July 2006	0.70 \pm 0.26	100 \pm 20	32 (17)

salamanders were captured only once, 28 salamanders twice, ten salamanders three times, six salamanders four times, one salamander five times, three salamanders six times, and one salamander was captured seven times. Furthermore, on 26 September 2008, 23 salamanders were observed, including four recaptures marked during the 2004–2006 study period.

Mean SVL at initial capture of marked individuals was 74.5 ± 18.2 mm (range: 40–105 mm); however, individuals as small as 17 mm SVL were captured. Mean mass of salamanders at the initial time of capture was 8.3 ± 5.1 g (range: 1.1–22.0 g). On 25 August 2005, we captured five adults with large, developing ova visible through the abdominal walls. The SVL of these females was 97–104 mm, and their mass was 14.5–21.5 g.

Detectability, survival rates, and population size.—An open model with equal capture probabilities among survey events was the best fit model (deviance = 182.0, df = 2028, AIC = 352.29) compared to the model with unequal capture probabilities (deviance = 175.2, df = 2022, AIC = 357.51). Capture probability was estimated at $28.0 \pm 3.3\%$ (\pm SE) among survey events under the best model. Individual survival probabilities for each survey event estimated under the best model were generally high (>75%) throughout much of the study period but declined during the study (Table 1). Estimates of population size for specific survey dates ranged from 21.4 to 104.0 (Table 1). Salamander density in the 710 m² study area reached a maximum of 0.15 ± 0.02 individuals per m² in February 2005 and a minimum of 0.03 ± 0.01 individuals per m² in June 2004. The mean population size during the study was 76.3 ± 22.8 individuals, corresponding to a density of 0.11 ± 0.03 individuals per m².

Growth rates, sexual maturity, and longevity.—Growth data for 49 recaptured salamanders were used in a regression of body size and monthly growth increment (Fig. 1), which formed the basis for a population-specific growth model to estimate size-at-age (Fig. 2). Based on the growth model, we estimated that sexual maturity was reached in 3.3–5 years. Based on the body size of the largest individual captured (105 mm SVL),

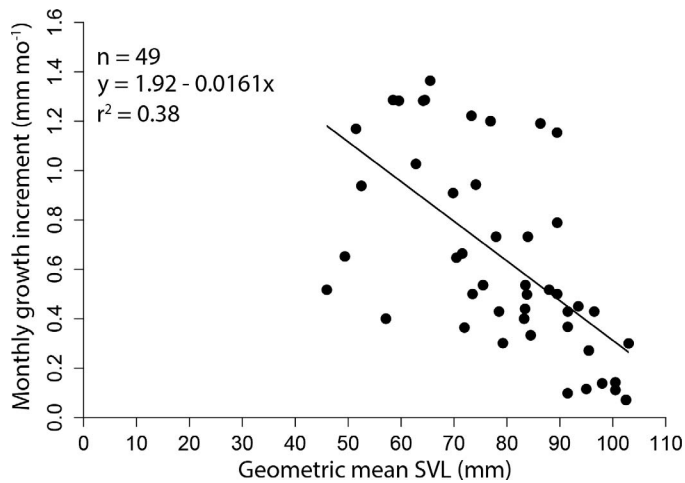


Fig. 1. Monthly growth increment vs. geometric mean snout-vent length (SVL) for *Gyrinophilus palleucus necturoides* at Big Mouth Cave, Grundy Co., Tennessee. The solid line is the result of least-squared regression.

we estimated life span to be 9–14 years. The mean time interval between recaptures was 157.9 ± 120.8 days. The shortest time interval was 22 days, while the longest time interval between captures was 723 days for two salamanders initially captured on 21 July 2004 and recaptured on 14 July 2006. Four salamanders were recaptured during the 26 September 2008 survey, including one individual that was first captured on 21 July 2004, representing 1528 days between captures.

Cover use and tail damage.—Adult and juvenile salamanders were found more frequently under cover than in the open (no cover), with 90.5% of all salamanders encountered first observed under cover ($P < 0.001$). Adults and juveniles did not differ with respect to cover use ($\chi^2 = 1.19$, $df = 3$, $P = 0.76$).

Evidence of tail damage was observed in 31.7% of salamanders captured. The proportion of adults with tail damage did not differ from the proportion of juveniles with tail damage ($\chi^2 = 0.29$, $df = 3$, $P = 0.96$). Salamanders found under cover were no more or less likely to have tail damage than salamanders found in the open (no cover; $\chi^2 = 1.89$, $df = 3$, $P = 0.60$). We captured five salamanders with regenerating limbs.

DISCUSSION

Caves and associated subterranean habitats represent not only some of the most challenging environments on the planet for organisms to inhabit, but also for humans to study. Consequently, life history and demographic data, important considerations for implementation of conservation or management practices, are lacking for many subterranean species, including most species of subterranean salamanders (Goricki et al., 2012; Fenolio et al., 2014). Our study provides such data for the Big Mouth Cave Salamander, *Gyrinophilus palleucus necturoides*, a taxon of conservation concern (Beachy, 2005; Miller and Niemiller, 2008, 2012) that is listed as “Vulnerable-Imperiled” (G2G3) by NatureServe (2014), “Vulnerable” on the IUCN Red List (Hammerson and Beachy, 2004), and “Threatened” by the state of Tennessee (Withers, 2009).

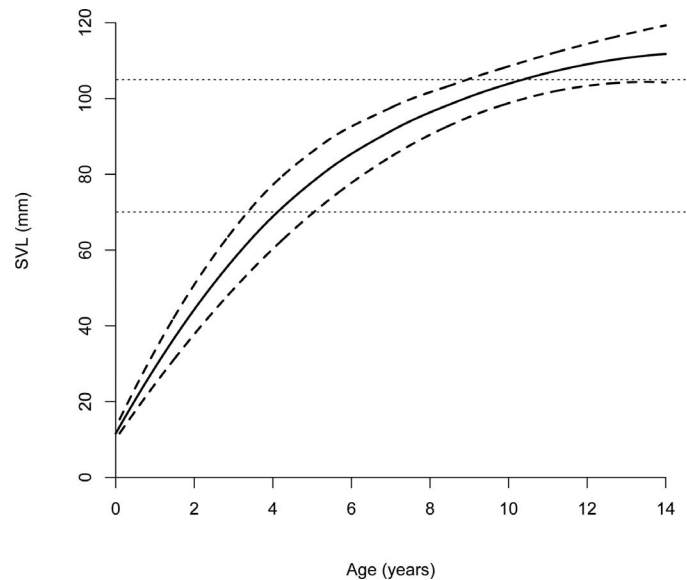


Fig. 2. Growth model for *Gyrinophilus palleucus necturoides* that estimates the relationship between body size (SVL in mm) and age (in years) at Big Mouth Cave, Grundy Co., Tennessee. Dashed lines are the lower and upper 95% confidence intervals. The lower and upper dotted horizontal lines represent the body size of the body size observed at sexual maturity (70 mm SVL), based on Simmons (1975) and the body size of the largest individual in the population (105 mm SVL), respectively.

Population size and survival.—Populations of the subspecies *G. p. palleucus* and *G. p. necturoides* are reportedly small, as few salamanders (<20 individuals) are observed during most cave surveys (Simmons, 1975; Caldwell and Copeland, 1992; Beachy, 2005; Miller and Niemiller, 2008). Previous estimates of population sizes at specific caves range from 25 to 88 salamanders (Simmons, 1975; Caldwell, pers. comm. in Petranksa, 1998). Nonetheless, recent studies, including our current study, suggest that some populations of *G. palleucus* are more robust than previously thought. For example, Huntsman et al. (2011a), using mark-recapture methods, estimated population sizes of 215 (95% CI: 128–302) and 109 (CI: 77–141) for two populations of *G. palleucus* (Tony Sinks Cave and Bluff River Cave) in Jackson Co., Alabama. Our maximum population size estimate of 104 individuals at Big Mouth Cave is comparable to the estimates of Huntsman et al. (2011a). Likewise, our estimates of salamander density (0.03–0.15 individuals per m^2) at Big Mouth Cave are comparable to estimates at Bluff River and Tony Sinks caves (0.03 and 0.10 individuals per m^2 , respectively; Huntsman et al., 2011a). Simmons (1975) reported a slightly higher density estimate (0.15 individuals per m^2) at Jess Elliot Cave, Jackson Co., Alabama. However, estimates of population size and density conducted by Huntsman et al. (2011a) are based on closed population models that do not allow birth, death, and migration to occur (but see their justification for using closed models). Consequently, comparisons between their studies and ours should be made with this caveat.

Survival probabilities for individual survey events were generally greater than 75% but gradually declined during the study (Table 1). Several factors may account for variability in survival probabilities among surveys other than reduced survival. These include heterogeneity in capture probability among surveys due to variation in abiotic search conditions, salamander activity, and search personnel methodologies, including tag loss. We noted that search conditions were not

optimal during our first survey on 29 June 2004, as recent rainfall resulted in higher water levels and increased turbidity. However, all other surveys were conducted when water levels and turbidity were low. Heterogeneity of survival rates might be related to tags being overlooked or lost over time. It is unlikely that tags were overlooked, as the same search techniques and methodologies were employed during each survey event. Furthermore, to ensure consistency among surveys, either MLN or BTM was present during each survey event. Tag loss can lead to an underestimation of survival rates and bias estimates of population size by decreasing the effective number of recaptures in the population. Tag loss has been identified as a potential limitation of VI alpha tags in amphibians (Heard et al., 2008; Osbourn et al., 2011). Sixteen percent of tags were dropped over 12 weeks in a study on juvenile *Ambystoma maculatum* (Osbourn et al., 2011), whereas Heard et al. (2008) observed a tag loss rate of 8% in anurans. In both studies, tag loss was attributed to expulsion of tags from slow-healing entry wounds. Our study was considerably longer in duration than that of Osbourn et al. (2011; 26 months versus 3 months), and we recognize that tag loss may occur over the course of longer duration mark-recapture studies. However, we made concerted efforts to minimize tag expulsion following recommendations of previous studies (e.g., Osbourn et al., 2011).

Longevity and growth.—Many cave-adapted organisms exhibit a more K-adapted strategy in life history traits (e.g., decreased growth rates, delayed sexual maturity, and increased lifespan) compared to related surface species. This is presumably in response to limited energy resources associated with the absence of light and consequent lack of primary production, as well as the environmental stability of cave habitats (Poulson, 1963; Hüppop, 2000; Culver and Pipan, 2009; Niemiller and Poulson, 2010; Venarsky et al., 2012). However, estimates of longevity based on direct study of natural populations of most troglomorphic salamanders are lacking. Exceptionally, using mark-recapture data, Fenolio et al. (2014) estimated that Grotto Salamanders (*Eurycea spelaea*) in a cave in the Ozark Highlands of Oklahoma live at least nine years, and Huntsman et al. (2011a) estimate that *Gyrinophilus palleucus* live at least six years in the two populations they studied in northeast Alabama. Our longevity estimates for *G. p. necturoides* at Big Mouth Cave were of slightly longer duration at 9–14 years. However, we likely are underestimating the potential life span of individuals in this population, as our growth model predicted a maximum body size of 113 mm SVL, 8 mm larger than the largest individual captured in the population, but within the range of body sizes reported for cave-dwelling *Gyrinophilus* (Miller and Niemiller, 2011, 2012). Furthermore, the oldest *G. palleucus* on record was collected in the wild at an unknown age and lived 18.5 years in captivity (Snider and Bowler, 1992).

Sexual maturity does not appear to occur at a greater body size in *G. palleucus* than in populations of epigeal Spring Salamanders (*G. porphyriticus*) studied (Bruce, 1972, 1980; Petranka, 1998). Simmons (1975) suggested that sexual maturity in *G. palleucus* occurs at about 70 mm SVL (although males as small as 66 mm SVL may also be mature). Based on a longevity growth model, and assuming maturity is reached at 70 mm SVL (Simmons, 1975), the two populations studied by Huntsman et al. (2011a) differ with respect to age at sexual maturity. Individuals inhabiting Tony Sinks Cave reached sexual maturity in 2–4 years, whereas

those in Bluff River attained sexual maturity in 3–6 (or more) years (Huntsman et al., 2011a: fig. 5). Similarly, Dent and Kirby-Smith (1963) estimated that male *G. palleucus* reach sexual maturity at two years, and that females are slightly older when they reach maturity. Our estimate that *G. p. necturoides* in Big Mouth Cave are sexually mature in 3–5 years is similar to these previous estimates for the species and does not differ appreciably from age estimates for sexual maturity in *G. porphyriticus*. Sexual maturity occurs in lowland populations of *G. porphyriticus* when individuals reach 55 mm SVL (Bruce, 1972), but individuals from highland populations may not mature until reaching 81 mm SVL. Sexual maturity in *G. porphyriticus* typically occurs immediately after metamorphosis in lowland populations, which may be in 3–4 years in lower elevation populations (Bruce, 1972; Petranka, 1998) or up to 6 years in high elevation populations (Bruce, 1972, 1980). Males and females in highland populations may delay sexual maturity up to a year after metamorphosing (Bruce, 1972). The similarity of life history traits between *G. palleucus* and *G. porphyriticus* (e.g., lack of cave-adapted life history traits observed in other troglomorphic species) supports the hypothesis that *G. palleucus* is a relatively young subterranean species recently derived from a *G. porphyriticus*-like ancestor that colonized caves recently in the mid-Pliocene to Pleistocene (Niemiller et al., 2008, 2009).

Predation.—*Gyrinophilus palleucus* is considered one of the top predators in aquatic subterranean food webs of the Interior Low Plateau (Huntsman et al., 2011a). Few predators of *G. palleucus* are known, with reports limited to American Bullfrogs (*Lithobates catesbeianus*) found near the entrances and twilight zone of caves (Lee, 1969) and conspecifics (Lazell and Brandon, 1962; Simmons, 1975). Antagonistic interactions with conspecifics are a probable explanation for many salamanders (particularly small individuals) observed with missing or regenerating tails or limbs (30% of captured individuals) but does not necessarily account for all reports. Petranka (1998) speculated that crayfishes might feed on small larvae. In Big Mouth Cave, two species of cambarid crayfishes co-occur with *G. palleucus*: the stygobiotic Southern Cave Crayfish (*Orconectes australis*) and the stygophilic and facultative cave-dwelling Cavespring Crayfish (*Cambarus tenebrosus*; Huntsman et al., 2011b). Crayfishes greatly outnumber *G. palleucus* in the study area by a 6:1 to 10:1 ratio based on direct census counts (Niemiller and Miller, unpubl. data). Although direct evidence for predation on *G. palleucus* is lacking, *C. tenebrosus* has been reported to capture and feed on other amphibians, including *Pseudotriton ruber* (Niemiller and Reeves, 2014) and *Lithobates clamitans* (Niemiller and Miller, 2005). Crayfishes have been reported to prey upon other troglomorphic salamander species (Fenolio et al., 2013). Thus, crayfishes may be significant predators of *G. palleucus*, including large adults.

Relative abundance and cover use.—Census data from previous surveys of Big Mouth Cave in the 1970s, 1980s, and 1990s have led several authors to speculate that this population, and *G. p. necturoides* in general, was experiencing a substantial decline (Simmons, 1975; Caldwell and Copeland, 1992; Petranka, 1998; Beachy, 2005). *Gyrinophilus p. necturoides* was reportedly abundant in Big Mouth Cave in the early 1960s (Lazell and Brandon, 1962) but experienced a dramatic decline by the early 1970s. Simmons (1975) found very few salamanders during the early 1970s, causing him to

speculate that the population could be extirpated within 25 years. Caldwell and Copeland (1992) observed only six salamanders in total during three surveys during the early 1990s. This perceived decline in population size was attributed to habitat disturbance associated with silvicultural and agricultural operations, which results in increased siltation and groundwater pollution from surface runoff into the sinkhole entrance (Simmons, 1975; Caldwell and Copeland, 1992).

However, we observed significantly more salamanders in Big Mouth Cave during our surveys (Miller and Niemiller, 2008; this study), suggesting that this population has either recovered during the past 20 years or has remained stable, and some other factor is responsible for the larger number of salamanders found more recently. For example, differences in relative abundance among surveys during the past five decades could result from differences in survey methods or sampling conditions. More than 90% of salamanders found during this study were first observed under cover, primarily rocks. If we had not searched underneath rocks and other cover, but searched only for salamanders visible in a pool, we would only have observed 2–7 salamanders during most surveys, and would have reached similar conclusions as past researchers on the status of this population. Unfortunately, Simmons (1975) and Caldwell and Copeland (1992) did not describe how they conducted their surveys, making comparisons with our surveys difficult. It is possible that our sampling effort was more intensive, that we used different sampling methods, or that sampling conditions were better during our surveys (e.g., water levels low and with low turbidity) compared to earlier surveys, thus explaining differences in abundance during the past 50 years. Alternatively, the population may have experienced a dramatic decline followed by a more recent recovery. Regardless, our study shows that the population of *G. p. necturoides* in Big Mouth Cave is larger than previously thought and is in no immediate risk of extirpation.

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