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FACTORS INFLUENCING THE USE OF WATER-FILLED TREE CAVITIES BY EASTERN RATSNAKES (*PANTHEROPHIS ALLEGHANIENSIS*)

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Abstract.—**For some animals, specific microhabitats may be particularly important for certain behaviors and/or age or sex classes. Here we explore the use of previously unrecognized retreat sites (water-filled tree cavities) by Eastern Ratsnakes (*Pantherophis alleghaniensis*). During 4 y of radio telemetry, approximately half of the 45 ratsnakes monitored used water-filled cavities. Typically, water-filled cavities (phytotelmata) were in live Laurel Oaks (*Quercus laurifolia*) and Black Cherry (*Prunus serotina*) where limbs had broken off, internal wood had rotted, and water accumulated. Water-filled cavities were used by ratsnakes at about the same frequency as tree stumps but less frequently than snags, brushpiles, or downed logs. Snakes remained in water-filled cavities for an average of 10 d compared to only 2–4 d in other structures. Reproductive females (both pre- and post-egg laying) were four times more likely to use water-filled cavities than non-gravid or male ratsnakes, suggesting cavities are used to offset water loss associated with gestation. Ratsnakes used water-filled cavities far more in summer than spring even though thermal profiles of cavities were similar to those of other retreat structures, indicating their use was not for thermoregulation. Multiple snakes often used cavities simultaneously, suggesting that cavities are either limited or facilitate social interaction. Snakes did not use artificial water-filled cavities, suggesting that natural sites may provide snakes with some unknown benefit beyond hydration. Water-filled cavities appear to be important for ratsnakes, particularly reproductive females, and warrant further investigation.**

Key Words.—gestation; phytotelmata; retreat structure; thermoregulation; tree hollow

INTRODUCTION

Animals use habitats in complex ways and likely rely upon intrinsic features that may be difficult to detect at coarse scales (Beaudry et al. 2010). Snakes in particular spend extended time in refuges and may rely upon very specific types of shelters for thermoregulation, ecdysis, protection from predators, foraging, or digestion. Recognition of the roles that habitat structures play in the natural history of lives of snakes can be a crucial part of their conservation. For instance, the Broad-headed Snake (*Hoplocephalus bungaroides)* relies upon thermally suitable flat rocks for foraging and thermoregulation for much of the year (Webb and Shine 1998) and then moves to hollows of dead trees for the duration of the summer (Webb and Shine 1997). Both collection of rocks for landscaping and forest management practices (removal of dead trees) threaten the respective structures and hence the persistence of this already imperiled snake species (Webb et al. 2002). Similarly, the imperiled Indigo Snake (*Drymarchon couperi*) is reliant upon the burrows of the Gopher Tortoise (*Gopherus polyphemus*) and may spend up to 90% of its time in these burrows (Hyslop et al. 2009), which are used for shelter, foraging, thermoregulation, and nesting. Conservation of these snake species relies on protecting both suitable habitat at the landscape level as well as identifying and preserving the unique retreat sites upon which they rely.

Understanding how and why snakes use particular structures can provide insight for conservation and inform habitat improvement and restoration. Many snakes are quick to use artificial retreat sites (Leliévre et al. 2010). For instance, placement of artificial rocks (concrete pavers) may be an effective conservation technique to restore degraded rock outcrops for Broad-headed Snakes (Webb and Shine 2000). The restoration of artificial retreat sites can be improved by understanding why snakes rely on the structures and on the ability of the artificial structure to mimic the inherent properties (biotic and abiotic) of natural structures. Creation of artificial retreat sites, however, may have unintended consequences. The creation of brush piles near endangered bird nesting habitat may lead to increased predation by Western Ratsnakes (*Pantherophis obsoletus*) on nests of those birds (Sperry et al. 2010).

Ratsnakes (*Pantherophis* spp. formerly *Elaphe obsoleta*) frequently use natural and artificial retreat sites (Blouin-Demers and Weatherhead 2001). During the course of a 4-y radio telemetry study of Eastern Ratsnakes (*P. alleghaniensis*) and their interactions with nesting songbirds in South Carolina, we often located snakes within water-filled cavities of living trees (Fig 1). Such water-filled tree cavities (sometimes referred to as water-filled treeholes or phytotelmata) have long received attention for their importance in promoting biodiversity (Kitching 1971; Eric Walters and Jaime Kneitel, unpubl. report); however, few studies have gone beyond quantifying the species associated with water-filled tree cavities and explored patterns in the use of the structures by focal organisms. Water-filled tree cavities typically form when limbs break off the trunk of a tree and some natural process (disease, decay, fungus, or excavation) creates a hollow portion of the inner tree compartmentalized by living tissue that allows water to accumulate (Gibbons and Lindenmayer 2002). Although ratsnakes have anecdotally been documented in water-filled tree cavities (Eric Walters and Jaime Kneitel, unpubl. report), it is unknown why and how frequently snakes use these structures. Here, we quantify the extent and timing of water-filled cavity use by ratsnakes. Additionally, we explore four non-exclusive hypotheses that could explain why snakes use water-filled cavities: (1) snakes, particularly reproductive females, use cavities for hydration (reducing evaporative loss or drinking), (2) cavities provide a safe or effective place for shedding, (3), cavities provide a safe place and hydration to facilitate recovery from transmitter-implantation surgery, and/or (4) cavities allow snakes to avoid temperature extremes or extreme temperature fluctuations.

The first hypothesis that we explore is that ratsnakes are using cavities for access to drinking water. Because gravid ratsnakes have increased vulnerability due to their reduced locomotor ability and an increased demand for water due to gestation, they should more frequently be associated with water-filled cavities than other ratsnakes. Gravid females have greater water requirements than non-gravid females because of increased evaporative water loss associated with increased metabolism and transpiration through the skin due to prolonged body distension associated with gestation (Dupoué et al. 2015; Lourdais et al. 2015, 2017). Gravid snakes would be likely to benefit from staying submerged in water to reduce evaporative water loss and to have access to drinking water without needing to expose themselves by actively moving and searching for it. From this hypothesis we predict that water-filled cavities will be used more frequently by gravid female snakes than by males or non-gravid females. In addition, we predict that if snakes use cavities to avoid water loss or as a source of water to drink, they should remain in water-filled cavities longer than in other retreat sites because they do not need to go elsewhere to find water. Finally, we expect that if cavities are being used primarily for hydration and access to drinking water, snakes will use water-filled cavities most frequently during the warmest parts of the season when evaporative water loss is highest (Cohen 1975).

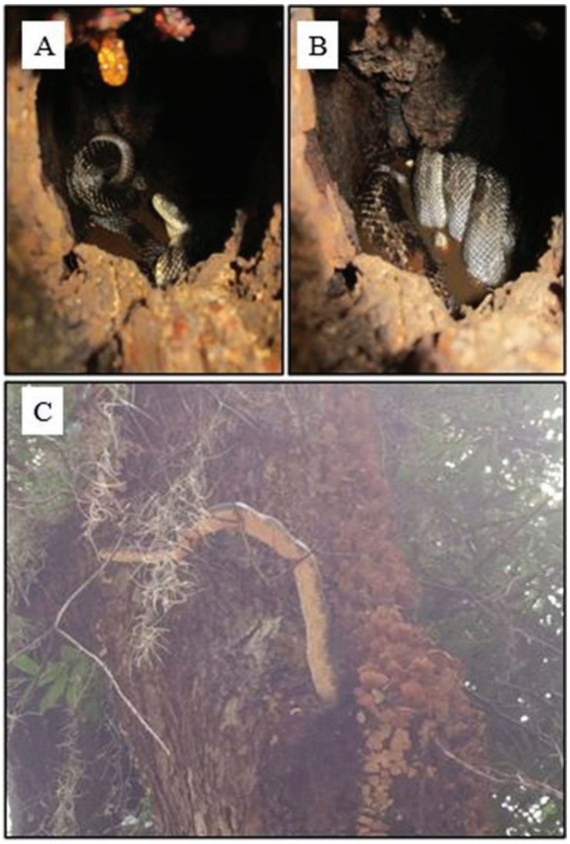

Figure 1. Eastern Ratsnakes (*Pantherophis alleghaniensis*) using water-filled tree cavities at a site in South Carolina, USA. (A) an adult male ratsnake uses a cavity while shedding. (B) Two adult ratsnakes (1 shedding, 1 not) occupy the same water-filled cavity. (C) A large adult male ratsnake exits a water-filled tree cavity. (Photographed by Patrick Roberts, used with permission).

The second hypothesis we explore is that ratsnakes preferentially use water-filled cavities while shedding. During ecdysis, snakes have reduced vision and are vulnerable to predators, necessitating that they find protected shelter sites for the days or weeks in which they are most vulnerable (Weatherhead and Charland 1985; Loughran et al. 2015). During this time, snakes often have a reduced appetite and the need for foraging is supplanted by the need to hide (King and Turmo 1997). Additionally, while shedding, snakes seek humid microenvironments to aid in sloughing off old skin and protecting their new skin (Murphy and Campbell 1987; Dupoué et al. 2015). If snakes are using water-filled cavities to aid in shedding, we simply predict that the observed use of cavities should be highly skewed towards visibly shedding snakes (those with opaque eyes and cloudy scales: Fig. 1). Similarly, reptiles are known to modify behavior to increase immune function (e.g., basking for thermal fever) and hydration can be important for immunity (Moeller et al. 2013). We test here whether snakes are more likely to use water-filled cavities when healing from transmitter-implantation surgery. If snakes use cavities to facilitate recovery from surgery, we expect that snakes will be most likely found in cavities shortly after being released from surgery.

Finally, we tested the hypothesis that, as ectotherms, snakes likely select retreat sites, potentially including water-filled cavities, for their thermal properties (Webb and Shine 1998; Kearney 2002; Leliévre et al. 2010). Because water in these cavities should increase their thermal inertia relative to other structures (i.e., less daily temperature fluctuation), we compared the temperature profiles of cavities with other retreat structures to assess their role in snake thermoregulation. If these water-filled cavities are primarily used by snakes to avoid high temperatures, we predicted that cavities should have fewer extreme temperatures than other available retreat structures and be used more during the hottest parts of the season. A final objective of this study was to evaluate whether artificial water-filled cavities could be created for use by snakes. Artificial retreat sites have been effectively deployed for numerous reptile species (Michael et al. 2004, Croak et al. 2010) and, if water-filled cavities are an important facet of snake habitat, conservation efforts may be enhanced by providing artificial cavities.

Materials and Methods

***Study site*.**—We conducted our research at the Ellenton Bay Set Aside Research Area on the U.S. Department of Energy Savannah River Site in Aiken County, South Carolina, USA. Ellenton Bay is an approximately 250 ha area that was once row-crop agriculture and pasture but has been reverting to forest since 1951. The site is primarily wooded with mixed forests of Laurel Oak (*Quercus laurifolia*), Loblolly Pine (*Pinus taeda*), and Slash Pine (*P. elliottii*) interspersed with open shrubland areas of Chicasaw Plum (*Prunus angustifolia*) and blackberry (*Rubus* spp.). The area has been further fragmented by several clear-cuts that have subsequently been planted with rows of Long-leaf Pine (*P. palustrus*) that were 10–15 y old at the time of the study. The site is bounded to the north by Upper Three Runs Creek and floodplain forest and to the south by a two-lane paved road with minimal daily traffic by site employees. The site is best known for the 10-ha ephemeral wetland, Ellenton Bay, located on the southern portion of the site that has been the subject of scores of ecological studies. Drinking water is available to snakes throughout the active season at Three Runs Creek and intermittently at Ellenton Bay, which seasonally holds water, but no reliable water is available between the two wetlands located at the southern and northern ends of the site (a straight-line distance of 1.7 km).

***Structure use*.**—We used radio telemetry to study ratsnake ecology and retreat site use at this site from 2011 to 2014 (e.g., DeGregorio et al. 2015, 2016). Because the original purpose of our study was to explore predator-prey interactions between snakes and birds, we primarily monitored snakes during the avian nesting seasons (March-July) of 2011–2014, with the exception of 2014 when snakes were tracked frequently until hibernation (November). We captured snakes opportunistically by hand throughout the nesting season and then transported them to a veterinarian who surgically implanted transmitters (model SI-2T, 9 g, 11 g, or 13 g; Holohil Systems Ltd, Carp, Ontario, Canada) following the technique by Reinert and Cundall (1982) as modified by Blouin-Demers and Weatherhead (2001). All transmitters weighed < 3% of the total mass of the snake. We released snakes at their capture locations 3–5 d following surgery. We then radio tracked snakes at various times of the day and night at approximately 48 h intervals and recorded each location using handheld GPS. At each location we recorded the behavior of the snake and the retreat structure (if any) with which it was associated. We categorized retreat structures as brushpiles, logs, stumps, snags (standing, dead trees), vine tangles, root systems, artificial structures (e.g., building foundation, pipes, tires), or water-filled tree cavities. If snakes were not clearly associated with a retreat structure, we recorded if it was using a tree, shrub, or traveling on the ground. Each time a snake was visible, we noted whether it was shedding, had a visible food bolus, or (if female) it was visibly gravid. Each time a snake was located in association with a structure, we determined the total number of consecutive days spent in the structure, the dates when use occurred, the sex and reproductive condition of the snake (if known), whether or not it was alone or with other snakes, and whether or not it was shedding (opaque eyes). We compared mean time spent in each structure type using a General Linear Model with the response variable the number of consecutive days spent in a structure and our fixed factor being the structure type (log, snag, brushpile, etc.).

To estimate and compare the proportion of time snakes used various retreat locations, we calculated the proportion of tracking relocations associated with each type of commonly used structure including water-filled cavities, brushpiles, stumps and logs, snags, elevated root systems, and dense vine tangles. We often tracked snakes to locations high up in trees (51% of relocations) and had to exclude these tracking events from analysis because snakes were too high up for us to determine their exact location and behavior. We also excluded observations where snakes were not associated with structures (e.g., traveling) and those associated with artificial structures (e.g., tires, tin coverboards, metal pipes) because these occasions were rare (< 2% of relocations) and these structures varied considerably in their physical and thermal properties.

To assess whether snake use of different structure types was correlated with individual factors, we used a Multinomial Generalized Linear Mixed Model. Our multinomial response variable was the type of structure a snake was found associated with at each tracking location (e.g., water-filled cavity, log, snag). We used snake ID as a random effect to control for individual variation. We explored the fixed factors of sex (male versus female), reproductive condition (gravid versus non-gravid female), time since transmitter surgery, month (categorical variable), presence of a conspecific (binary variable: yes or no), and shedding condition (yes or no).

We then performed a similar analysis to explore the factors influencing use of water-filled cavities. For this analysis, we used a Binary Generalized Linear Mixed Model. Our response variable for each tracking event was whether or not a snake was associated with a water-filled cavity (1 = yes, 0 = no). We again used the fixed factors of sex, reproductive condition (gravid versus non-gravid female), time since surgery, month, presence of a conspecific (binary variable: yes or no), and shedding condition (yes or no). We again used snake ID as a random effect to control for individual variation. We used SAS 9.0 (SAS Institute, Cary, North Carolina, USA) for all analyses and we determined significance at a P value of 0.05.

Temperature monitoring.—We monitored temperatures in cavities and other retreat structures to test the hypothesis that snakes used water-filled tree cavities to thermoregulate and to assess how daily temperatures within cavities may be different from other retreat structures. To continuously measure the temperatures available to snakes within different structure types, we used biophysical models that have been shown to gain and lose heat at the same rate as equivalently sized snakes (Blouin-Demers and Weatherhead 2001; DeGregorio et al. 2015). Each model consisted of a 40 cm length of 1.5 cm diameter copper pipe, filled with water, and painted glossy black and gray to approximate the reflectance of ratsnakes. We suspended a thermocouple in each model and capped the ends with rubber caps and silicone. The thermocouples were attached to miniature temperature loggers (HOBO Temp, ONSET Computer Corp., Pocasset, Massachusetts, USA). We programmed temperature loggers to record at 10 min intervals. Similar models calibrated with the carcasses of ratsnakes by Blouin-Demers and Weatherhead (2001) were found to accurately reflect the internal body temperatures experienced by snakes under a wide range of temperatures, humidity, wind, precipitation, and solar radiation conditions.

We deployed arrays of biophysical models three times during the 2014 field season and left them in place 20–40 d. One model was always placed within a different water-filled cavity that had been used by ratsnakes at some point during the study. We then chose 2–3 additional structures (brushpiles, shrubs, or logs) within 20 m of the water-filled cavity. We chose the structures to represent features similar to what ratsnakes were often found using and to span the range of available temperatures. For the first sampling period, we placed biophysical models in a water-filled tree cavity, under a brushpile, under a log, and in a shrub (1.5 m off the ground). For the second sampling period, we placed biophysical models in a water-filled cavity, under a brushpile, and in a shrub (1.5 m high). For the third sampling period, we placed models in a water-filled cavity, under a brushpile, and along the limb of a tree at the same height as the cavity (about 2.25 m high). For each of our three rounds of model deployment, we calculated mean daily temperature of each biophysical model and compared mean daily temperatures of each biophysical model using Kruskal-Wallis tests. To explore whether the daily temperature variation that a snake would experience within each structure was different, we calculated daily variance measurements for each structure and compared them using Generalized Linear Models with the logit link function and with Tukey's Post-hoc Tests. We performed three separate analyses for each group of structures to account for changing seasonal temperatures, such that comparisons between the structures were all recorded on concurrent days.

Artificial cavities.—To increase the occurrences of cavity use and to explore how different factors including height, orientation, and cavity size affected use by ratsnakes, we deployed 58 artificial cavities beginning in 2013, half containing water and half not. We created four types of cavities to increase our chances of attracting snakes and to mimic the range in sizes and shapes of natural cavities (Fig. 2). The first consisted of plastic gourds typically used to provide nesting sites for cavity-nesting birds. Each gourd was approximately 20 cm tall and had a 3 cm diameter hole in the front of it. The second type was modified Eastern Bluebird (*Sialia sialis*) houses. We built bird houses (22 cm tall × 13 cm wide × and 13 cm deep) out of rough-cut cedar planks

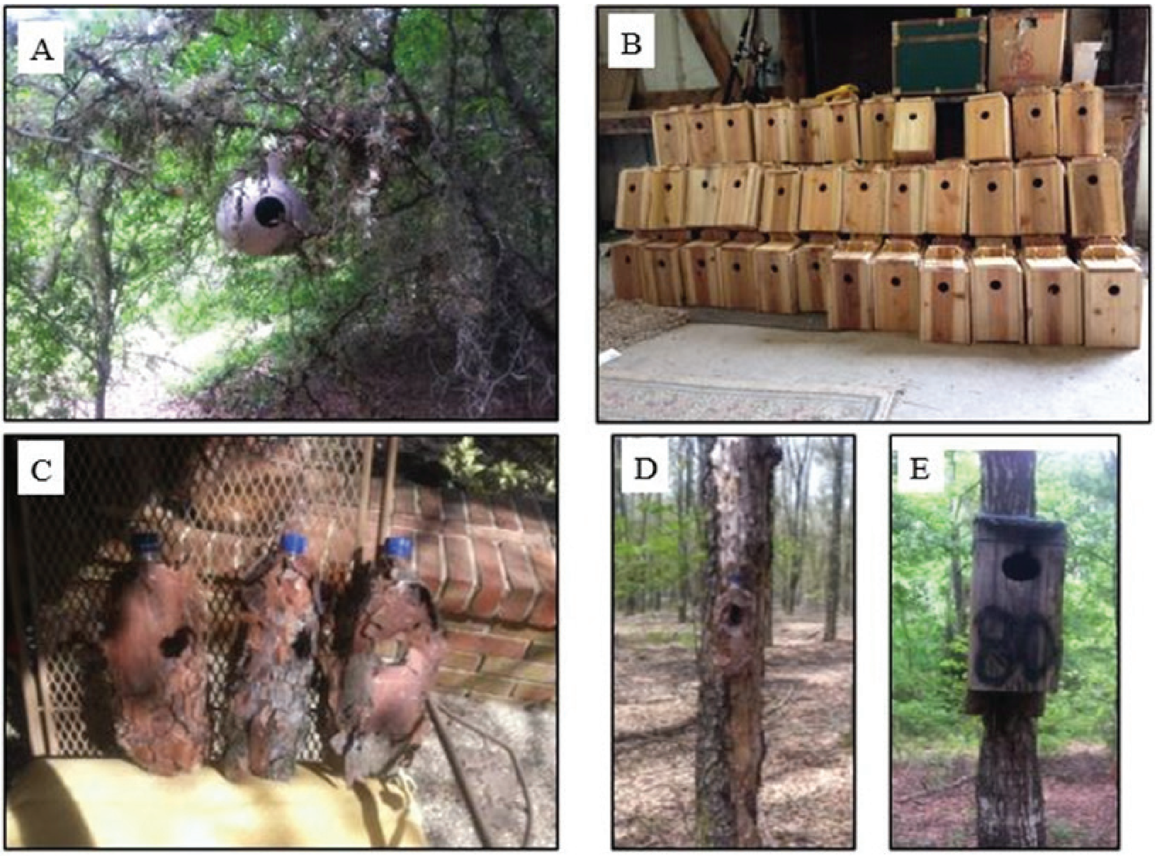

Figure 2. Artificial cavities used at a site in South Carolina, USA, to explore the factors behind Eastern Ratsnakes (*Pantherophis alleghaniensis*) use of water-filled cavities. Each type of artificial cavity was hung in pairs with one containing water and the other empty. (Photographed by Brett DeGregorio).

and we cut circular 2.5 cm entrance holes in each. We lined the inside of half of the houses with plastic to hold water. The third type of cavity consisted of cleaned, 2-L plastic soda bottles with panels of pine bark glued to the outside. We cut 2-cm circular holes in the front of each bottle to simulate cavities. The dry bottles had holes punched in the bottom of the container to prevent water from accumulating. The final type of cavity consisted of Wood Duck (*Aix sponsa*) boxes, half with plastic lining to hold water and half without. The wood duck boxes were large (60 cm tall × 30 cm wide × 30 cm deep) with a 10-cm circular entrance and made of rough-cut cedar.

We placed the cavities at varying heights (1–2.25 m) above the ground, orientations, and on different species of trees. For each cavity we recorded its height, orientation, tree species it was attached to, and macrohabitat type in which it was placed (forest, shrubland, or clear cut). We also measured the distance from each cavity to the nearest forest edge. We deployed all cavities in pairs (< 5 m apart) consisting of one with water (6–10 cm of standing water) and one without. We kept the height and orientation of paired cavities the same. We checked cavities a minimum of three times per week from 6 April to 31 July 2013 and 9 March to 31 July 2014. Each time we checked the cavities, we refilled any of the water-filled cavities that had < 6 cm of standing water. We captured any snake found in a cavity and measured its snout to vent length, determined its sex by cloacal probing, palpated females to determine their reproductive condition, and noted whether it was shedding and if it was with any conspecifics.

Results

From May 2011 until November 2014, we tracked 45 individual ratsnakes (27 males and 18 females) that produced 3,649 telemetry locations. We excluded 1,816 tracking events in which snakes were high in trees but not visible so that we were unable to determine their activity and structure association. We observed 22 individual ratsnakes (13 males and nine females: 49% of tracked snakes) using water-filled cavities on 42 separate occasions. We documented snakes using 20 different water-filled cavities across our site. Water-filled tree cavities were exclusively found in live hardwood trees, including Laurel Oak, Black Cherry, Tulip Poplar (*Liriodendron tulipifera*), and unidentified oak species (*Quercus* spp.). Most of these cavities (n = 18) consisted

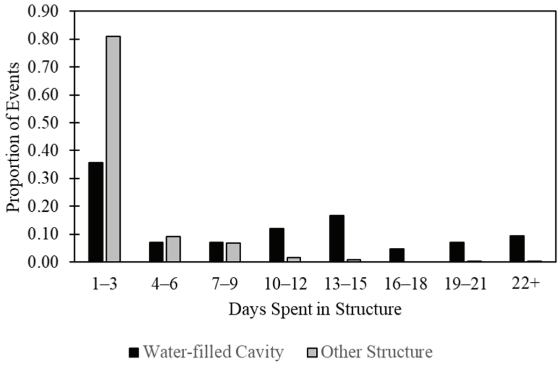

Figure 3. Length of time that Eastern Ratsnakes (*Pantherophis alleghaniensis*) from South Carolina, USA, spent in water-filled tree cavities compared to other retreat structures.

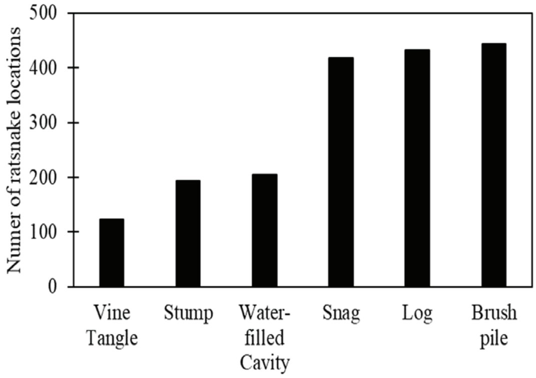

Figure 4. Number of radio telemetry locations at which Eastern Ratsnakes (*Pantherophis alleghaniensis*) from South Carolina, USA, were associated with different retreat structure types.

of small diameter openings (< 5 cm) where tree limbs had broken off and the wood had subsequently rotted or otherwise been excavated, although two cavities had much larger openings where the outside of the tree had decayed (Fig. 1).

Collectively, snakes spent 421 d in water-filled cavities. Time spent in cavities by individual snakes ranged from 1–34 continuous days (Fig. 3; mean = 10 ± 8 [standard deviation] d). Ratsnakes remained in water-filled cavities significantly longer than they did in other structures ($F_{6,3570} = 18.89$, $P < 0.001$). On average, ratsnakes spent between two and four consecutive days in all other structure types and on average spent 10 d in water-filled cavities. The most frequently used retreat structure types at our site were brush piles (24% of relocations), logs (24%), and snags (23%). Ratsnakes used stumps and water-filled tree cavities equally (11%) and were associated with shrubs and vine tangles only 7% of the time (Fig. 4). The type of retreat structure in which we located a snake during any given tracking event was not associated with sex, reproductive or shedding condition, time since surgery, month of the year, or the presence of a conspecific ($F_{84,1808} = 0.001$, $P = 0.999$).

When comparing the use of water-filled cavities versus all other retreat structure types, use of water-filled cavities was related to several factors ($F_{12,1278} = 11.88$, $P < 0.001$) including reproductive condition ($F_{2,1278} = 12.89$, $P < 0.001$), time since surgery ($F_{1,1278} = 16.48$, $P < 0.001$), month ($F_{6,1278} = 16.65$, $P < 0.001$), and the presence of conspecifics ($F_{1,1278} = 34.03$, $P < 0.001$). Females that were gravid or had been gravid during the current season were four times more likely to use water-filled cavities than non-gravid females (β = 1.459, 95% confidence interval [CI] = 0.921–2.018). Similarly, snakes were four times more likely to be found with another snake when using a cavity than they were to be alone (β = 1.408, 95% CI = 0.911–2.8011). On 20% of occasions when we located ratsnakes in water-filled cavities, we observed another snake with them, whereas we rarely saw snakes with conspecifics in other structures (ranging from 1% in tree stumps to 13% in snags). On eight occasions males and females were together in cavities, females with females on four occasions, and a male with an unmarked snake of undetermined sex on another occasion. Although two snakes used water-filled cavities shortly after being released from surgery, use of water-filled cavities occurred more frequently long after surgery (mean = 245 d; range, 6–848 d). Use of water-filled cavities was more common later in the year during the warmest months than it was earlier in the year (Fig. 5).

Temperature.—In general, temperatures inside water-filled cavities tended to be similar to those of other structure types located near them in forests but were cooler than other retreat structures located in more open areas. During the first model deployment period, we detected no differences in daily mean temperature experienced by biophysical models

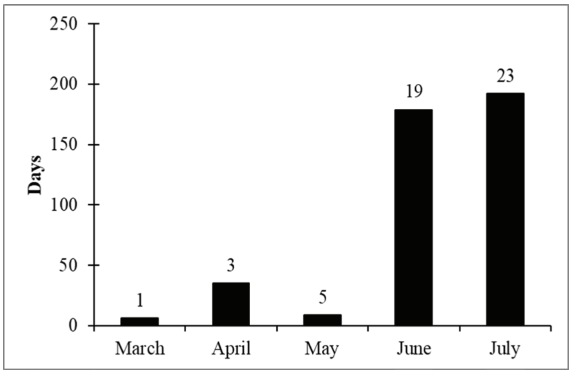

Figure 5. Number of days that telemetered Eastern Ratsnakes (*Pantherophis alleghaniensis*) from South Carolina, USA, spent in water-filled tree cavities 2011–2014. The number above each bar indicates the number of unique use events occurring during each month.

between the four structure types ($F_{3,47}$ = 0.35, P = 0.791). During the second model deployment period, there were differences between the mean temperatures a snake would experience within the three structures monitored ($F_{2,94}$ = 12.64, $P < 0.001$). There was no detectable difference in mean daily temperature within the brushpile and the water-filled cavity (Fisher's Exact Test, $P = 0.874$), but biophysical models in both structures experienced significantly cooler temperatures than the model deployed in a brushpile in the shrubland patch (Fisher's Exact Test, $P = 0.003$). During the third model deployment period, there were differences between the mean daily temperatures a snake would experience within the three structures monitored ($F_{2,60}$ = 7.81, $P = 0.001$). There was no detectable difference in mean temperature experienced within the brushpile and the water-filled cavity (Fisher's Exact Test, $P = 0.350$), but models in both structures were significantly cooler than the model deployed in a dense shrub (Fisher's Exact Test, $P < 0.041$). Thus, in general, snakes in water-filled cavities would experience temperatures similar to those in other retreat structures in forested areas.

To assess whether daily temperature varied more in some structures than others, we calculated daily temperature variance for each structure. During the first model deployment period, there was no significant difference in daily temperature variation between the four structures ($F_{3,52}$ = 0.421, $P = 0.742$). There were significant differences, however, in temperature variation detected during the second model deployment when comparing models placed in a water-filled cavity, a forest brushpile, and shrubland brushpile ($F_{2,96}$ = 53.20, $P < 0.001$). The temperature of the model in the shrubland brushpile varied significantly more each day than in either of the two forest structures (Fisher's Exact Test, $P < 0.001$). There was no detectable difference in daily temperature fluctuation between the water-filled cavity and forest brushpile (Fisher's Exact Test, $P = 0.997$). During the third model deployment period, we detected significantly greater daily temperature fluctuations between the three models ($F_{2,63}$ = 15.82, $P < 0.001$) with the model in a shrubland brushpile having greater daily temperature fluctuation than those in a forested brushpile or water-filled tree cavity (Fisher's Exact Test, $P > 0.003$). Thus, water-filled cavities fluctuated in temperature similarly to other retreat structure types in forested habitats. Over 2 y of weekly monitoring of 58 artificial cavities, we observed only one ratsnake (an adult female) using an artificial cavity: a water-filled Wood Duck box.

Discussion

Approximately half of the 45 individual ratsnakes tracked over 4 y in South Carolina were documented using water-filled tree cavities. All of these cavities were in live trees and contained enough water for snakes to at least partially submerge themselves. Unlike other snakes that may be entirely reliant upon particular retreat structures (e.g., Indigo Snakes and Gopher Tortoise burrows), ratsnakes did not rely exclusively on water-filled cavities. Instead, ratsnakes used a wide array of retreat structures, with water-filled cavities accounting for approximately 11% of snake retreat site use. When snakes used water-filled cavities, they remained inside them for an average of 10 d, which is far longer than they remained in other retreat structures (average of 2–4 d). We almost certainly underestimated the true extent of water-filled cavity use at our site because we excluded 51% of tracking events where snakes were too high in trees to confirm their exact behavior. It is likely that some of these relocations included snakes using water-filled cavities. We never found water-filled tree cavities on the landscape without tracking snakes to them, despite some searching effort, so we were unable to estimate their abundance. We never tracked snakes to cavities in living trees that did not contain water, although snakes frequently used hollow cavities without water in standing dead trees. Water-filled tree cavities are difficult to detect because the openings are often small (several cm diameter), their outside appearance is merely a small hole where a limb has broken off, and they are often above head-height. Ratsnakes have long been associated with tree cavities in both living and dead trees (Prior and Weatherhead 1996) and have been found in woodpecker holes containing standing water (Eric Walters and Jaime Kneitel, unpubl. report), but this is the first documentation of patterns in use of these retreat structures by ratsnakes.

We found support for our hypothesis related to reproductive state of snakes using water-filled cavities; reproductive females frequently made use of these features. While developing eggs and after laying them, snakes are particularly in need of water because of increased evaporative water loss associated with increased metabolism and transpiration through the skin due to prolonged body distension (Dupoué et al. 2015; Lourdais et al. 2015, 2017). Thus, reproductive females likely seek out retreat sites with high levels of humidity (Dupoué et al. 2015) and are more likely to need access to drinking water. Water-filled cavities are likely particularly attractive to reproductive females because they provide a safe refuge with both access to drinking water and extremely high humidity, and thus allow females to remain in the retreat site longer because they do not need to leave to find water. The combination of safety and access to drinking water without exposure to predators is an advantage conferred only by water-filled cavities among available retreat sites. While gravid females were most likely to use cavities, all reproductive classes of ratsnakes were found in cavities and the presence of drinking water in a safe location is likely beneficial for all ratsnakes. Evaporative water loss in most snakes is greatest when temperature is high (Cohen 1975) and thus our observation that snakes used cavities most frequently during the warmest months of the year could be indicative of ratsnakes seeking drinking water. Standing water at our site is seasonally available in Ellenton Bay and permanently available at Upper Three Runs Creek, but no reliable standing water can be found in the 1.7 km between the two wetlands. Most ratsnakes at this site would not have reliable access to surface water for drinking within their home ranges.

We also predicted that snakes that were shedding would preferentially use water-filled cavities, but we found little evidence that this was the case. Lack of humidity is the most frequent cause of incomplete shedding in captive snakes (White et al. 2011), and it has been suggested that humidity drives the timing of shedding in some tropical snakes (Daltry et al. 1998) because the risk of dehydration can be high. In fact, humidity has been linked to synchronized shedding events in ratsnakes (Carlson et al. 2014). Only two individuals, however, used cavities while visibly shedding. Also, despite one snake entering a water-filled cavity immediately after release from transmitter-implantation surgery, the average time since transmitter surgery for cavity use by snakes was 245 d, so snakes did not use water-filled cavities while recovering from surgery.

An unexpected outcome of this study was the frequency with which snakes were found with other ratsnakes when in water-filled cavities. The pairings consisted of males with females (n = 8), females with females (n = 4), and a male with an unmarked, unknown sex snake (n = 1). It is possible that males follow females into these cavities, an outcome made more likely by the tendency of snakes to remain in cavities for long periods of time, thus increasing the window of time for males to locate females. The fact that most females that used cavities had already mated, however, would mean few or no mating opportunities were available to males. Alternatively, these cavities may be limited on the landscape and thus snakes congregate in them by chance rather than by choice. We are unable to determine the relative abundance of water-filled cavities on the landscape because we never found them without tracking snakes to them and most are above head-height. Although we found snakes using 20 unique water-filled cavities across the study site, we documented five individuals using a single cavity over the course of the study and another four individuals separately using another cavity. Although it appeared that ratsnakes were more communal when using water-filled cavities than when in other retreat structures, it is likely that we underestimated the frequency with which ratsnakes were with other snakes in those other sites. We were able to document use of water-filled cavities by multiple snakes relatively easily, at least for those low enough to view, compared to structures such as brush piles where snakes often remain out of view.

We hypothesized that ratsnakes use water-filled cavities for thermoregulation and found mixed support for this hypothesis. Ratsnakes use different habitats and structures to attain and maintain their preferred body temperatures (Blouin-Demers and Weatherhead 2001). This hypothesis predicts that snakes would use water-filled tree cavities most frequently during the hottest months of the year and this was indeed the case. Use of water-filled cavities was relatively rare in March, April, and May but consistently high in June and July (we rarely tracked snakes past July). When comparing the thermal properties of water-filled cavities relative to other commonly used retreat structures, however, we found that cavities were similar in both temperature and daily temperature fluctuation to other retreat structures found in forested habitats. If ratsnakes are using water-filled cavities for thermoregulation, they are likely only one of the possible suitable options available. Our attempt to use artificial water-filled cavities to further elucidate patterns in the use of these structures was not successful. Either snakes did not discover enough of the structures during the 2 y in which they were deployed or the structures failed to mimic whatever characteristics snakes seek out in natural cavities.

In total, our study suggests that many ratsnakes use water-filled cavities during the active season but multiple explanations for this behavior are likely. It appears that these structures may be important for gravid females to maintain hydration during gestation or recover water balance after egg laying while males may use cavities to thermoregulate, or simply to seek refuge and hydrate. There are other reasons that ratsnakes may use these water-filled cavities that are more difficult to test, such as snakes using them to ambush other animals drawn to drinking water. Regardless of the reasons, the use of water-filled cavities highlights that micro-refuges within larger habitat patches may be important for individuals to meet their resource needs, but these refuges can be difficult to find and quantify. Our ability to recreate these important structures may be limited, emphasizing the need to protect intact habitat for species. Numerous studies have highlighted the importance of tree hollows (both water-filled and dry) to the maintenance of biodiversity (Kitching 1971; Gibbons and Lindenmayer 2002; Sebek et al. 2013) and raised concerns about the increasing rarity of these structures in managed forest. Our study highlights an additional species that relies, to some degree, on these structures.

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