Herpetology Notes, volume 14: 1203-1205 (2021) (published online on 13 September 2021)

Direct observation of subterranean refugium use by *Epidalea calamita* (Natterjack Toad) in a dehesa ecosystem in Extremadura, Spain

Andrew M. Durso[1,2,*], Kendalynn A. Morris[1,2], and Richard K. F. Nair[1]

Epidalea calamita (Laurenti, 1768) (formerly *Bufo calamita*) is a medium-sized bufonid toad (maximum size 95 mm) found in southwestern and central Europe (Speybroeck et al., 2016). These toads use underground shelter sites during the day and throughout the winter and summer non-breeding seasons. Depending on soil moisture and composition, toads either create primary burrows in the soil (which lack connections to the ground surface because the channels collapse when the toad digs deeper) or exploit existing burrows or cavities between soil and rocks or other objects (Sinsch, 1989, 1998; Leskovar and Sinsch, 2001). Individuals move within and among shelters in order to maintain optimal temperature and moisture conditions (Oromí et al., 2010), but shelter depth and seasonal fidelity are not easy to measure. We were able to directly observe hibernating toads using minirhizotrons, which are clear plastic tubes inserted into the soil measure root growth.

Between December 2016 and January 2020, we directly observed four *E. calamita* (Fig. 1, Table 1) in burrows beneath the ground adjacent to minirhizotrons (10 cm in diameter, 30 cm long, installed on 25 May 2016 at 20° to the horizontal) in a dehesa ecosystem at Majadas de Tiétar, Extremadura, Spain (39.94°N, 5.776°W; WGS84). Photographs of the toads were taken using a custom built minirhizotron camera system based around a commercially available digital, visible-light camera, which was lowered into the tube to capture images of root presence in the soil close to the tube surface (Nair et al., 2019). Soil temperature and soil water content were measured in the week before the observations by three probes at each site.

All toads were initially immobile at depths ranging from 7–27 cm. Measured depths of *E. calamita* cavities range from a few centimetres to almost 2 m (Sinsch, 1989; Lindenthal et al., 1991; Golay, 1994) and vary seasonally and with soil type. The seasonally moist, sandy soil at this site is underlain by a clay-rich layer at 30 cm depth, such that water forms ponds during periods of high precipitation in the spring and fall. Water-saturated soil limits the ability of *E. calamita* to burrow (Lindenthal et al., 1991). The *E. calamita* we observed in December was partially submerged in groundwater (Fig. 1A). One of the two *E. calamita* observed in November was sufficiently mobile that it was able to leave the visibility range of the camera within a few minutes.

At the corresponding depth, mean soil temperatures in the week before the observations ranged from 9.6 ± 2.8 °C to 13.6 ± 1.9 °C (maximum = 21.4 °C; minimum = 2.2 °C) and were warmer than the range of 1.1–9.8 °C reported from Germany (Sinsch, 1989). Soil water content at the corresponding depth ranged from 17–30%. Although Iberian spadefoot toads (*Pelobates cultripes*) made shallower burrows in dry sand than in damp sand (Zamora-Camacho et al., 2019), there is no evidence in our limited data that *E. calamita* do so as well.

Toads were observed during surveys in November, December, and March. Some populations of *E. calamita* frequently leave their hibernacula during mild winter periods to forage (Miaud et al., 2000; Miaud and Sanuy, 2005; Sinsch et al., 2007), but we did not sample often enough to assess whether this was the case at our site. No toads were observed during surveys in May and September, although their burrows were still visible. Lindenthal et al. (1991) stated that *E. calamita* remain underground for 26 days at most during summer aestivation, but we never detected a toad during summer. These observations might suggest that *E. calamita* does

[1] Max Planck Institute for Biogeochemistry, Hans-Knöll-Straße 10, 07745 Jena, Germany.
[2] Present address: Florida Gulf Coast University, Ft. Myers, 33965 Florida, USA.
[*] Corresponding author. E-mail: amdurso@gmail.com

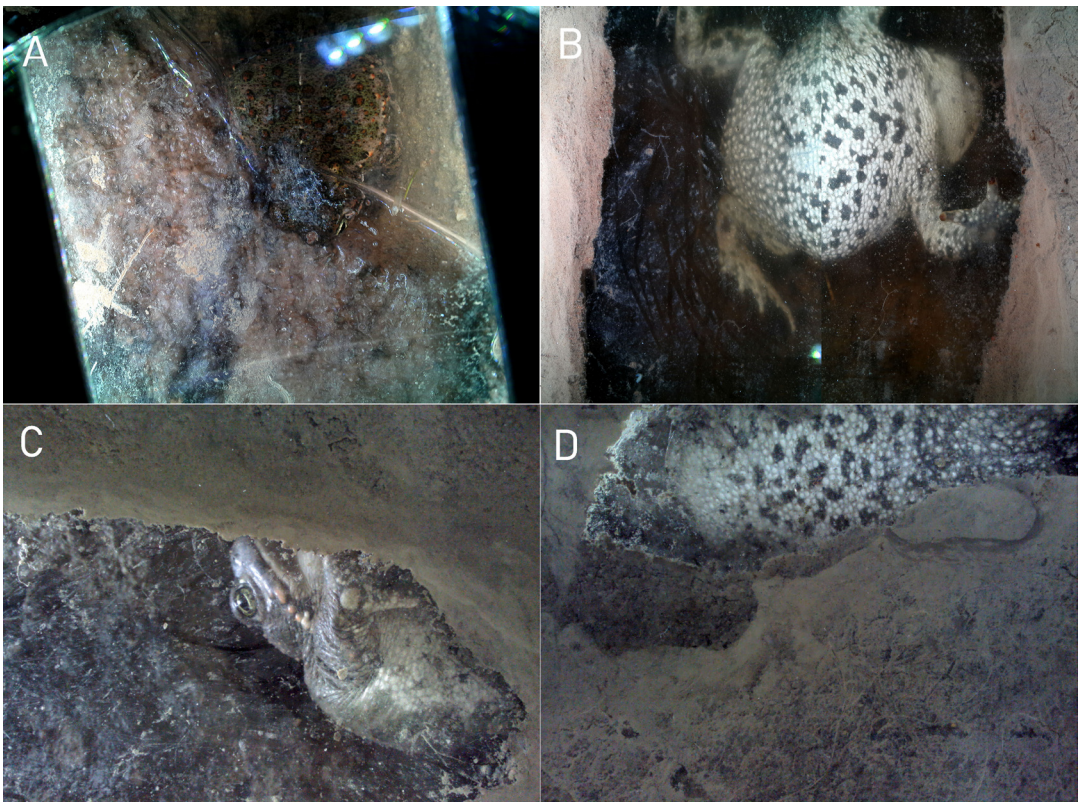

Figure 1. Four dated photographs of *Epidalea calamita* in subterranean refugia. (A) 3 December 2016. Dorsal aspect, taken from above. (B) 17 March 2018 (composite of two photos). Ventral aspect, taken from the side. (C) 28 November 2018. Left side, taken from below. (D) 30 November 2018. Ventral aspect, taken from below. Photos taken by Richard Nair using a custom-built minirhizotron camera (FCB-MA 130-FG, JenCamGmBH, Germany). See Table 1 for additional details of each observation.

not aestivate for prolonged periods of time in dehesa, despite the prevalence of seasonally dry conditions, although additional observations are needed.

Although an equal number of minirhizotrons were installed under *Quercus ilex* (Holm oak) and *Q. suber* (Cork oak) canopies and in open grassy areas (18 in each habitat, checked 12 times each for a total of 432 toad observation opportunities), all *E. calamita* were observed exclusively at minirhizotrons near trees. Access to landscape structures such as mammal burrows, fissures, crevices, root channels and lacunas between soil and natural or man-made objects are important for protection against temperature and dehydration stress during any time of the year when the soil surface is

Table 1. Abiotic characteristics of *Epidalea calamita* subterranean refugia. An asterisk (*) indicates that the toad moved just after the image was taken, while a dagger (†) indicates that the toad was partially submerged in water.

Date	Time of day	Camera depth (cm)	Toad depth (cm)	Soil water content (%) at toad depth	Soil temperature (°C) at toad depth, week before (mean ± S.D.) [min – max]
03-Dec-2016	13:00	5	7	26†	12 ± 2 [7.7 – 18.1]
17-Mar-2018	17:00	10	10	30	9.6 ± 2.8 [2.2 – 17.4]
28-Nov-2018	17:00*	24	20.2	17	13.6 ± 1.9 [8.9 – 21.4]
30-Nov-2018	14:00	12	8.2	28	13.4 ± 2 [7.2 – 21.4]

impenetrable to a burrowing toad. Distance from the nearest permanent water bodies (natural ponds that have been impounded and vary in size from 0.2–2.9 ha throughout the year) ranged from 240–455 m, and that from the nearest ephemeral water bodies (slow-flowing drainages that can reach nearly 0.1 ha in size but are dry from April–October) ranged from 25–95 m.

These incidental observations suggest that comparable customised subterranean viewers or a similar but modified experimental setup could potentially serve to obtain repeated, non-invasive observations of aestivating *E. calamita* or other reptile and amphibian species in a repeatable way across seasons, habitats, and regions. Amphibians are known to use artificial refugia during both aestivation and hibernation (Denton and Beebee, 1992; Balogová et al., 2017), enabling a more detailed understanding of otherwise intractable aspects of their ecology.

Acknowledgments. We thank the Max Planck Institute for Biogeochemistry and the University of Plasencia (especially Gerardo Moreno) for financial and logistical support in the field. The MaNiP project is supported by the Alexander von Humboldt Stiftung through the Max Planck Research Prize to Markus Reichstein. Martin Hertel assisted with design and construction of the custom camera.

References

Balogová, M., Jelić, D., Kyselová, M., Uhrin, M. (2017): Subterranean systems provide a suitable overwintering habitat for *Salamandra salamandra*. International Journal of Speleology. **46**: 321–329.

Denton, J.S., Beebee, T.J.C. (1992): An evaluation of methods for studying natterjack toads (Bufo calamita) outside the breeding season. Amphibia-Reptilia **13**: 365–374.

Golay, N. (1994): Eine Methode zur äußerlichen Befestigung von Telemetriesendern an Kreuzkröten sowie erste Ergebnisse bei der Anwendung. Berichte des Landesamtes für Umweltschutz Sachsen-Anhalt **14**: 44–48.

Leskovar, C., Sinsch, U. (2001): Hibernation behaviour of radiotracked natterjack toads *Bufo calamita* and green toads *Bufo viridis*. Biota (Suppl.) **2**: 33–34.

Lindenthal, E., Sinsch, U., Schneider, H. (1991): The behavioural and physiological ecology of estivation in natterjack toads (*Bufo calamita*). Verhandlungen der deutschen Zoologischen Gesellschaft (Tübingen) **84**: 318.

Miaud, C., Sanuy, D. (2005): Terrestrial habitat preferences of the natterjack toad during and after the breeding season in a landscape of intensive agricultural activity. Amphibia-Reptilia **26**: 359–366.

Miaud, C., Sanuy, D., Avrillier, J.-N. (2000): Terrestrial movements of the natterjack toad *Bufo calamita* (Amphibia, Anura) in a semi-arid, agricultural landscape. Amphibia-Reptilia **21**: 357–369.

Nair, R.K.F., Morris, K.A., Hertel, M., Luo, Y., Moreno, G., Reichstein, M., Schrumpf, M., Migliavacca, M. (2019): N & P stoichiometry and habitat effects on Mediterranean savanna seasonal root dynamics. Biogeosciences **16**: 1883–1901.

Oromí, N., Sanuy, D., Sinsch, U. (2010): Thermal ecology of natterjack toads (*Bufo calamita*) in a semiarid landscape. Journal of Thermal Biology **35**: 34–40.

Sinsch, U. (1989): Sommer- und Winterquartiere der Herpetofauna in Auskiesungen. Salamandra **25**: 104–108.

Sinsch, U. (1998): Biologie und Ökologie der Kreuzkröte. Bielefeld, Germany, Laurenti Verlag.

Sinsch, U., Oromi, N., Sanuy, D. (2007): Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. The Herpetological Journal **17**: 129–137.

Speybroeck, J., Beukema, W., Bok, B., van der Voort, J. (2016): Field Guide to the Amphibians and Reptiles of Britain and Europe. London, United Kingdom, Bloomsbury Natural History.

Zamora-Camacho, F.J., Medina-Gálvez, L., Zambrano-Fernández, S. (2019): The roles of sex and morphology in burrowing depth of Iberian spadefoot toads in different biotic and abiotic environments. Journal of Zoology **309**: 224–230.

Accepted by Jesse Erens