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Letter to the Editor

Field and laboratory investigations of *Lumbricus badensis* ecology and behaviour

Dear Editor,

Deep burrowing and soil surface feeding earthworms, so-called strict-anecic and epi-anecic species (Hoeffner *et al*., 2019), are a highly influential soil faunal group in many natural and manmade environments. Typically, they are relatively large and contribute substantially to the formation and processes of mull-type soils through effective foraging and incorporation of plant litter (Edwards and Bohlen, 1996). The wide, vertical, and deep burrows of anecic earthworms produce a unique influence on soil physical properties (Lamparski, 1985; Shipitalo and Butt, 1999; Nuutinen *et al*., 2001). *Lumbricus badensis* (Michaelsen 1907) is an intriguing example of this type of earthworm.

Lumbricus badensis is one of the largest earthworm species in Europe reaching a mass of 35–40 g (Lamparski *et al*., 1987). It is neo-endemic to the Schwarzwald (Black Forest) area of SW Germany and thought to have recently evolved to the local soil conditions (Kobel-Lamparski and Lamparski, 1987). The distribution of *L. badensis* is restricted to a relatively small geographical region as it requires highly humid climatic conditions but can tolerate soils with low pH (Lamparski and Zöttl, 1981). The soil that *L. badensis* inhabits is a brown earth (Mullbraunerde), poor in bases with a mull-type humus (Kobel-Lamparski and Lamparski, 1987). As with well-known anecic species, such as *Lumbricus terrestris* L., *L. badensis* visits the soil surface to feed and to gather materials from around its burrow to form a midden (Lamparski *et al*., 1987) and its burrows are known to reach a depth of 2.5 m (Kobel-Lamparski and Lamparski, 1987).

There is a great deal of information on the basic biology of *L. badensis* from field investigations in the Schwarzwald in the 1980s. Specifically, Lamparski (1985) described the effects of this species on soil physical properties; Lamparski and Zöttl (1981) examined population distributions, while burrows and burrow construction were explored and described by Kobel-Lamparski and Lamparski (1987) and Lamparski *et al*. (1987). However, many fundamental aspects of *L. badensis* ecology still remain poorly known. Our objectives were to supplement the previous findings by gathering more information on the population density of *L. badensis*, to describe the broader earthworm community of its living environment, and for the first time, to record *L. badensis* foraging and possible reproductive behaviour at the soil surface. We also aimed to collect live specimens for elucidation of life history traits in the laboratory.

In June 2015, an appropriate *Fagus sylvatica* (beech)/ *Picea abies* (spruce)-dominated forest site was chosen, which was located close to Belchen (47°49′45′′ N, 7°51′36′′ E; altitude 1 160 m above sea level) in the Schwarzwald area, with understory vegetation of *Sorbus aucuparia* (rowan) and a ground layer with *Vaccinium myrtillus* (blueberry) and pteridophytes. The site was on an east-facing slope (20°). Survey of the soil surface, of a largely vegetation-free ground layer, indicated the presence of *L. badensis* middens, which comprised up to seven gently ascending side branches from the main burrow and were often surrounded by castings and loosely scattered stones at the soil surface. Standard earthworm sampling (*e.g*., Butt and Grigoropoulou, 2010) was undertaken at the site with randomly placed quadrats of 0.1 m^2 ($n = 10$) dug with a spade to 25–30 cm depth. The soil was hand-sorted on a plastic sheet and any earthworms recovered were preserved in 0.4% formaldehyde. All *L. badensis* individuals were collected alive and stored in moist soil from the site. The basal area of the excavation was examined for any large burrows, which were then measured. A vermifuge of mustard powder in water (5 g L^{-1}) was applied to the base of the sampling pit. All earthworms emerging were added to those previously collected.

Two juvenile *L. badensis*, determined in the field by colour, behaviour, and size, were sampled with hand-sorting. No other locally found, red-coloured earthworms could be mistaken for this species. The other species recovered were *Aporrectodea icterica* (Sav.), *Lumbricus rubellus* (Hoff.), and *Octolasion tyrtaeum* (Sav.), which accounted for 65%, 9%, and 26% of the earthworm community, respectively. *L. rubellus* at this site were small, with adults recorded with masses of less than 1 g. The overall mean density of earthworms was 90 individuals m^{-2} and their mean total mass was 28.1 g m^{-2}. The mean population density of adult *L. badensis* was estimated at 7 individuals m^{-2} based on the location of individual, vertical burrows below active middens. The *L. badensis* burrows had a mean (± standard error) diameter of 10.4 ± 0.9 mm ($n = 7$; maximum 13 mm). One burrow had two very large *L. badensis* cocoons associated with it. These had a size of 13 mm × 9 mm and, with an additional cocoon located from further excavations, had a mean mass of 462 ± 43 mg.

Sampling for adult *L. badensis* with a mustard vermifuge (5 g L^{-1}), octet apparatus (electro-sampling) (Thielemann, 1986), or foot trembling (Simmons, 1961) was unsuccessful. To capture adult *L. badensis* a “grab and wait” technique was necessary. This entailed snatching partially emerged individuals from their burrows at the soil surface at night under red light conditions. Using this technique, 14 partially emerged adults were successfully extracted from the soil. These had a mean mass of 25.1 ± 2.2 g (minimum 15 g, maximum 41 g).

To record behaviour at the soil surface, night vision webcams (F19803EP, Foscam, Houston, USA) were set up before dusk on five consecutive nights (June 2–6) and set to record beyond sunrise (21:30–05:30). Six webcams were used, each powered by its own 6 V battery and supported on a tripod (viewing distance 65 cm from the soil surface) to monitor an area of *ca*. 0.25 m^2 (Fig. 1) with the same areas filmed on all nights. After the first night, the relatively dry soils were irrigated prior to recording and larger pieces of woody debris were removed to assist observations. Irrigation was not necessary on two nights after heavy rain. Air temperature was monitored with a max-min thermometer placed at ground level in the forest.

Fig. 1 Set-up for webcam recording of *Lumbricus badensis* behaviour at the soil surface in a beech/spruce-dominated forest at Belchen in the Schwarzwald area, Germany.

Recording of behaviour showed that *L. badensis* was active at the soil surface during hours of darkness but always maintained tail-contact with the burrow. Soil surface behaviour was mainly foraging and movement of materials (such as spruce cones) close to the burrow entrance (Fig. 2). Subdividing the night into hourly sections, peak activity was recorded from 22:00 through to 02:00 from where it decreased steadily until dawn. During filming, air temperatures recorded at ground level were in the range of 12 to 18 °C.

Fig. 2 Adult *Lumbricus badensis* (bottom of image) foraging in the forest (0.1 m^2 shown is part of a frame captured from webcam recording).

Activity occurred throughout the night but was seen to be deterred by heavy rain. Nevertheless, immediately after rain ceased, a large number of *L. badensis* emerged, and one mating event was recorded. This interaction lasted for 3 h (00:30–03:29) with an indication (partially obscured) that reciprocal anterior insertions into the partner’s burrow occurred during a pre-mating behavioural sequence.

The field-collected, lemon-shaped cocoons of *L. badensis* (Fig. 3) each produced a single hatchling (mean mass 364 ± 21 mg). They were individually maintained in 750-mL pots, provided with Boughton loam and birch leaves/horse manure as food (Butt, 2011), and incubated at 15 °C. Growth was monitored and when a mass of 5 g was reached, animals were transferred to 2-L pots under the same conditions and monitored until mature. Growth to maturity (at 20–25 g) took 10–12 months at a steady rate of increase, with tubercular pubertatis present after 9 months at a mass of 12–14 g.

Fig. 3 Lemon-shaped cocoon of *Lumbricus badensis* with a size of 13 mm × 9 mm and a mean mass of 462 ± 43 mg ($n = 3$).

Surviving, field-collected mature *L. badensis* and juveniles grown to maturity in the laboratory ($n = 6$ in total) were transferred to soil-filled plastic tubes (16 cm in diameter, 50 cm in depth) for webcam recording of behaviour at the soil surface (as used in the field) but under more controlled conditions. The Boughton loam used had a moisture content of 25% and a pH of 7.7. The base of each tube was sealed and the upper surfaces of three tubes were aligned within a soil-covered arena (55 cm × 45 cm), mimicking a forest floor, to permit interactions on emergence from burrows. Thirty leaves and 5 g of horse manure were provided as feed at the soil surface. The soil surfaces of the two units with three individuals were then monitored from above overnight (18:00–06:00) for 11 nights, under ambient conditions in July 2016 (temperatures from 10 to 24 °C). A timer-activated light was turned on at 06:00 and remained on until 18:00 to prevent surface activity outside of the webcam recording period.

In the laboratory, recorded activity of *L. badensis* consisted of foraging on the leaves and horse manure and casting at the soil surface. Bouts of foraging showed no discernible temporal pattern and occurred equally throughout the hours of darkness, with only a slight lag period at the outset of the recording session. A single mating attempt was recorded which in total lasted more than 7 h (20:02–03:37). Here, the two individuals (ill-matched in size: 35 *vs*. 20 g), showed a range of behaviours. These included reciprocal insertions of anterior segments into each other's burrows, mutual anterior touching, lining up of the bodies, and clitellar contact but with a pronounced gap between the two (Fig. 4). Despite repeated attempts, the pair obviously did not manage to reach the position required for the start of mutual sperm transfer and no mating occurred. During the last 5 d of the experiment, ambient temperature reached 24 °C and no surface activity was recorded.

Fig. 4 Mating attempt of differently sized *Lumbricus badensis* individuals in the laboratory with the frame captured from webcam recording.

These preliminary investigations in the field and laboratory allowed us to address most of our objectives. At the field site, species richness of earthworms was low but not atypical of such soils (Judas, 1992). The density of adult *L. badensis* (7 individuals m^{-2}), estimated by main burrows associated with active middens, was within the range of 5 to 10 individuals m^{-2} described by Lamparski (1985). Such low density is understandable for a population of very large, sedentary individuals sharing a two-dimensional foraging space. As shown for *L. terrestris*, the natural densities permit mating with neighbours without loss of burrow contact, but also reduce competition for food (Grigoropoulou and Butt, 2010). Burrow diameters of *L. badensis* were slightly smaller than the 14–16 mm previously described by Lamparski *et al*. (1987). Discovery of only a small number of cocoons close to the surface of the soil accords with the information that they are typically deposited in side burrows (cocoon chambers) at greater depth (0.4–1.5 m) (Kobel-Lamparski and Lamparski, 1987). Construction of such chambers is not dissimilar to the behaviour described by Grigoropoulou *et al*. (2009) for *L. terrestris*.

In the laboratory, hatchlings and immatures generally fared well under conditions that have been shown suitable for many temperate earthworm species (Lowe and Butt, 2005). For such a large earthworm as *L. badensis*, growth to maturity at a mass of *ca*. 20 g was relatively rapid (10–12 months) compared with *L. terrestris*, which takes around 6 months to reach 4–6 g (Butt, 2011). Laboratory growth of *L. badensis* was more rapid compared with 3–5 years estimated for attainment of maturity in the high mountain location of the Schwarzwald (unpublished data), where high soil temperature of 10 °C at 10 cm depth is recorded for only a few months each year. Linked with less favourable food material such as beech and spruce litter with high C:N ratios, this will naturally prevent the rapid growth observed in the laboratory. The high growth rate of *L. badensis* may, however, indicate potential for a relatively high assimilation efficiency as previously shown for *L. terrestris* (Daniel, 1991; Curry and Schmidt, 2007).

With the first records of this behaviour, it could be confirmed that *L. badensis* mates at the soil surface, with indications that mating involved a pre-mating sequence as described for *L. terrestris* (Nuutinen and Butt, 1997). The laboratory observations further suggested that large size difference of partners can be detrimental for successful mating, as earlier indicated for *L. terrestris* by their preference of equal-sized partners (Michiels *et al*., 2001).

This study on *L. badensis* shows that much can be learned about the behaviour and ecology of this remarkable species from combining conventional field and laboratory methods of earthworm ecology with behavioural observations. Further investigations of a similar nature are warranted to confirm and broaden the findings of this preliminary study. It will be of particular interest to understand how the strong modification of the soil environment by *L. badensis* is reflected in above- and belowground ecological communities.

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