Journal Pre-proof

Gas composition and its daily changes within burrows and nests of an Afroalpine fossorial rodent, the giant root-rat *Tachyoryctes macrocephalus*

Radim Šumbera, Matěj Lövy, Jorgelina Marino, Miloslav Šimek, Jan Šklíba

PII: S0944-2006(20)30078-7
DOI: <https://doi.org/10.1016/j.zool.2020.125819>
Reference: ZOOL 125819
To appear in: *Zoology*
Received Date: 1 December 2019
Revised Date: 27 June 2020
Accepted Date: 2 July 2020

Please cite this article as: Šumbera R, Lövy M, Marino J, Šimek M, Šklíba J, Gas composition and its daily changes within burrows and nests of an Afroalpine fossorial rodent, the giant root-rat *Tachyoryctes macrocephalus*, *Zoology* (2020), doi: <https://doi.org/10.1016/j.zool.2020.125819>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier.

**Gas composition and its daily changes within burrows and nests of
an Afroalpine fossorial rodent, the giant root-rat *Tachyoryctes
macrocephalus***

Radim Šumbera¹, Matěj Lövy¹, Jorgelina Marino², Miloslav Šimek³, Jan Šklíba¹

¹Faculty of Science, University of South Bohemia, Czech Republic

²Wildlife Conservation Research Unit, Zoology Department, University of Oxford, Oxford,
UK

³Biology Centre CAS, Institute of Soil Biology, České Budějovice, Czech Republic

Correspondence: Radim Šumbera, University of South Bohemia, Faculty of Science,
Branišovská 1760, 370 05 Czech Republic.

E-mail: sumbera@prf.jcu.cz

Tel: +420 387 772 240

Highlights

- Low oxygen and high carbon dioxide concentrations are expected in underground burrows of mammals
- Burrow and nest atmospheres were surveyed in burrow systems of an Afroalpine endemic, the giant root-rat

- Radio-tracking allowed to detect and sample active nest chambers for the first time in a fossorial rodent
- Elevated, but still harmless concentration of CO₂ was recorded in nests and sometimes in burrows
- Relatively large chambers and light soils may prevent hypoxia and hypercapnia in the nests of the giant root-rats

Abstract

Fossorial mammals are supposed to face hypoxic and hypercarpnic conditions, but such conditions have been rarely encountered in their natural burrow systems. Gas composition in burrows after heavy rains, deeper burrows and especially nest chambers, where animals usually spend most of the day, could be even more challenging than in shallow burrows. Such situations, however, have been rarely surveyed in the wild. In our study, we determined concentrations of O₂, CO₂ and CH₄ in active burrows and nests of the giant root-rat *Tachyoryctes macrocephalus*, a large fossorial rodent endemic to the Afroalpine zone of the Bale Mountains in Ethiopia. We were able to determine the precise location of nests by tracking individuals equipped with radio-collars. To the best of our knowledge, this is the first study that analyses air samples taken directly from the nests of actually occupied burrow systems in any free-living fossorial mammal. We found no evidence for environmental hypoxia in the examined burrows and nests (range 19.7–21.6% O₂). Concentrations of CO₂ in the burrows increased after the burrows were plugged in the evening, but did not reach physiologically problematic levels. The highest CO₂ concentrations in burrows were detected in the evening during a wet period (up to 0.44%). In root-rat nest chambers, the highest (but still harmless) CO₂ concentrations (up to 1.31%) were detected in the morning (measured in

the late dry season only) together with an elevated concentration (up to 13.5 ppm) of CH₄.

Regular surface activity of giant root-rats, combined with the relatively large dimensions of their nest chambers and tunnels, and the absence of heavy soils, may contribute to harmless atmospheres within their burrow systems.

Keywords: burrow atmosphere, fossorial rodent, afroalpine, *Tachyoryctes*

1. Introduction

Defining the environmental conditions to which animals are exposed helps us to understand the species' adaptations allowing survival under such conditions. This is especially true for animals adapted to colonize hostile environments, such as fossorial mammals. Fossorial mammals spend most of the time, or their entire lives in the case of strictly subterranean species, within burrow systems, which are often temporarily or permanently sealed. It is generally supposed that the burrow systems are characterized by limited gas ventilation resulting thus in hypoxic (low O₂ level) and hypercapnic (high amount of CO₂) atmospheres (McNab, 1966; Arieli, 1979; Contreras and McNab, 1990; Nevo, 1999).

Most attempts to measure gas concentrations within burrows, dens and nests of mammalian species confirmed hypoxic and hypercapnic atmospheres, but the majority of these studies were conducted in captive or semi-captive unnatural settings with limited gas exchange (see Roper et al., 2001 for critical overview). Analogous studies conducted under natural conditions brought little evidence for environmental hypoxia and hypercapnia of the natural mammalian burrows, or at least their shallow parts (Roper et al., 2001; Šumbera et al., 2004; Holtze et al., 2018), with only a few exceptions, such as burrows of the blind mole rat *Spalax ehrenbergi* in heavy soils during rainy periods (Shams et al., 2005) and the Mandarin vole *Lasiopodomys mandarinus* (Hong et al., 2019). This is surprising because many fossorial mammals possess physiological adaptations for hypoxic and hypercapnic conditions (e.g. Shams et al., 2004; Larson and Park, 2009; Avivi et al., 2010; Park et al., 2017). It is therefore possible that the lack of evidence for hostile burrow atmospheres reflects the scarcity of studies conducted during rainy periods in areas of heavy soils.

It is also expected that environmental hypoxia and hypercapnia increase with burrow depth (Burda et al., 2007). Burrow systems of some fossorial rodents are stratified into shallow foraging burrows, deeper nests and axial tunnels, and even deeper boltholes

(Hickman, 1990). Especially nests are of crucial importance because they are less ventilated than shallow burrows (Nevo, 1999) and animals stay there usually for more than half of the day (c.f. Cameron, 1988; Benedix, 1994; Šklíba et al., 2007; Vlasatá et al., 2017).

Unfortunately, there are no data about gas composition in the nests under natural conditions, which is the result mainly of the difficulty in locating the underground nest in the field without excavating the burrows leading to the nests and destroying at least part of the burrow system. To the best of our knowledge, this is the first time that information about gas composition has been systematically collected in nests of any fossorial mammals in occupied natural burrow systems.

We studied the gas composition in the nests and burrows of the giant root-rat *Tachyoryctes macrocephalus* (Ruppell, 1842), a large (up to 1 kg) fossorial rodent endemic to the Bale Mountains of Ethiopia, where it lives in the Afroalpine zone at elevations between 3000–4200 m a.s.l. Giant root-rats occupy burrow systems of several tens of meters (Yalden, 1985) with home-range areas ranging from 56 to 132 m² (Šklíba et al., 2020). They forage on the surface at daytime only, usually close to temporary burrow openings (Yalden, 1985; Sillero-Zubiri et al., 1995) spending 7% of the daytime aboveground (Vlasatá et al., 2017). Life at high elevations, which are characterised by low partial oxygen pressure and very low temperatures at night contributes to stressful living conditions within their burrow systems. Giant root-rats keep the entrances to their burrow systems blocked with soil between approximately 5.00 p.m. and 8.00 a.m. (Sillero-Zubiri et al., 1995) and spend most of the day (about 80%) in their nests (Vlasatá et al., 2017). Root-rats are also known to deposit faeces in the nests. It has been proposed that decaying plant material mixed with faeces can serve as a “nest heating” mechanism in another species of the genus *Tachyoryctes* (c.f. Jarvis and Sale, 1971) increasing nest temperatures by about 5–7 °C (Rahm, 1980).

We determined the concentrations of O₂ and CO₂ in the root-rat burrow atmosphere in three periods of the year (wet, moist and dry) combined with three daytimes: afternoon, evening (after the burrow entrances were plugged), and morning (before the burrow entrances were open again). In addition, we determined concentrations of O₂, CO₂ and CH₄ in root-rat nest chambers in the morning (after the nest occupant spent a whole night in it) and at midday (when the nest occupants were usually active away from the nest) in the dry period. We did not sample the air from nests during wet and moist periods to avoid disturbing root-rats involved in parallel behavioural and ecological studies (see Vlasatá et al., 2017; Šklíba et al., 2020). We predicted that the root-rat burrow atmosphere would be similar as the aboveground atmosphere in the afternoon and become hypoxic and hypercapnic in the evening and especially in the morning. We also predicted that environmental hypoxia and hypercapnia are more severe during the wet period due to lower air/gas permeability of the wet soil. Furthermore, we predicted that environmental hypoxia and hypercapnia are most severe inside the nest chambers in the morning, after the root-rats spent the whole night there (Vlasatá et al., 2017). The same pattern was predicted for CH₄ as a metabolic product of intestinal symbionts. Finally, we mapped the nests of several root-rat individuals in order to measure the dimensions of the nest chambers and the surrounding tunnels. We also collected data about soil characteristics such as hardness, moisture, bulk density and temperature, since all these characteristics may influence gas exchange and the burrow microenvironment.

For the purpose of the present study, we consider atmosphere to be hypoxic or hypercapnic when the concentrations of the respective gases reach levels potentially harmful for humans at sea level under 24h exposure (<17% O₂ and >2.5% CO₂, National Research Council, 2008). In concordance with (to our knowledge) all published studies on burrow atmosphere composition, we present % (or ppm) concentrations of the gases and do not

consider the hypobaric hypoxia, assuming that the animals are adapted to the elevations they live at.

2. Materials and methods

2.1. Study sites

The study was conducted in the Bale Mountains, Ethiopia. Most field works were carried out on a site located in the Web Valley (N6°59.6' E39°42.0'; 3500 m a.s.l corresponding to mean atmospheric pressure about 0.65 atm and mean partial pressure of O₂ 65.7 kPa) on the shore of a periodic wetland and adjacent to a gently sloping afroalpine pasture. Additional data were collected in a similar habitat on the Sanetti Plateau (N6°51.0' E39°52.9'; 4100 m a.s.l. corresponding to mean atmospheric pressure about 0.60 atm and mean partial pressure of O₂ 60.6 kPa). Climatic conditions in the Bale Mountains are characterized by large fluctuations in daily temperatures, with an amplitude of up to 30 °C. Annual rainfall is 1157 mm and climatic conditions are characterized by a colder April–October rainy season (897 mm) and a November–March dry season (260 mm) with relatively warm days and sub-zero night temperatures. March, April and May are the warmest months of the year (www.worldclim.org, accessed April 2014).

2.2. Fieldwork

Air samples were collected from burrows leading to the surface and from nests of radio-collared individuals subjected to a parallel radio-tracking study (Vlasatá et al., 2017; Šklíba et al., 2020). The samples from the burrows were collected in the Web Valley site at two periods of the early dry season separated by one month (wet period 4–5 November, 2013 and moist period 7–8 December, 2013) and in the late dry season (dry period 1–2 April, 2014).

Additional samples were taken on the Sanetti Plateau site in the wet period (6–7 November,

2013). Nests in the Web Valley site were examined only in the late dry season (1–4 April, 2014). In the Web Valley site, the temperature on the soil surface during all periods of the study was measured using a R0110E temperature logger (Comet system, s.r.o., Rožnov pod Radhoštěm, Czech Republic).

Active entrances of ten burrow systems were localized on a given study site before start of the air sampling (10 different burrow systems were selected during each period). We sampled each tunnel three times in a sequence: in the afternoon (2.00–4.00 p.m.), before the burrow entrances were plugged; in the evening (9.00–10.00 p.m.), approximately five hours after the burrow entrances were plugged; and in the morning (6.00–7.00 a.m.), before the giant root-rats re-opened the burrow entrances or made new ones. In the afternoon sampling, a probe was inserted into the open entrance of a burrow, as far as 50 cm and two to three 7 ml air samples were collected from there using a gas-tight syringe. Since the probe was a straight metal pipe of a defined length, we precisely estimated the place where the air sample was taken (on a horizontal plane). We marked this place with a small stone or a cross carved on the soil surface. Each air sample was immediately injected through a rubber septum into an air-proof tube by means of a hypodermic needle. We used Venoject 5ml (Terumo Europe, Belgium) and Labco 5 ml (Labco, Lampeter, UK) pre-evacuated glass tubes for temporary gas storage. Control air samples (one to three) were collected from just above the soil surface. During the evening sampling, we pushed the probe through the soil at the marked place until the point of the probe entered the tunnel. This was indicated by a sudden decrease of the soil resistance. After the evening samples were taken, the hole was plugged by soil until next morning when the sampling took place again at the same place.

Nests of 19 root-rats were localized using radio-telemetry. The root-rats had been captured by snare traps and fitted with radio-collars (Brass collar, Pip transmitter; Biotrack Ltd, Dorset, UK) as a part of a parallel study (for details see Vlasatá et al., 2017 and Šklíba et

al., 2020). Since the animals spend most of the time in the nest and have a clear daily activity pattern, it was easy to locate a nest when the animal was inside by the triangulation technique. Since the observer could walk directly above the animal, and fix its position repeatedly from many directions, the precision of the nest location was very high. We used an IC-R20 receiver (Icom America Inc.) and a 3-element handheld Yagi antenna to localize the tagged root-rats. The air samples from the nests were collected in the morning (6.00 a.m.–8.00 a.m.) and then again around midday (11.00 a.m.–1.00 p.m.) when most of the individuals were usually active away from their nests. The probe was driven into the nest chamber using a pre-drilled 5mm-diameter hole. Six 7ml air samples were taken from about 10 cm above the nest's chamber floor. After that a temperature sensor probe was inserted and the temperature of the nest chamber was measured using a S0111 temperature-logger (Comet system s.r.o., Rožnov pod Radhoštěm, Czech Republic). Additional air samples and temperature measurements were taken from undisturbed soil at the same depth but 2–4 m away from the nest. In 10 out of the 19 nests, the place of air sampling was roughly in the middle of the nest chamber, which was subsequently confirmed by its excavation. The remaining nine cases were excluded from the analyses.

After the air sampling, 18 of the 19 nests were excavated (one was not excavated because of its position below a road) and mapped onto graph paper. We measured nest chamber diameter, height and depth (from the chamber floor to the soil surface), and the depth (from the floor to the soil surface), height and diameter of three tunnels located at least 1 meter from the nest chamber.

Throughout the study, we measured soil temperature at various depths by a set of temperature loggers placed on the soil surface and at depths of 10, 20, 30, and 40 cm at the Web Valley study site. We also measured soil hardness (cone resistance, $N\ cm^{-2}$) and soil moisture and bulk density in the Web Valley site (see Šklíba et al., 2017 for details).

2.3. Analysis of air samples

CO₂ and O₂ concentrations were quantified in 0.5 ml samples taken from the Venoject tubes, using an HP 5890 GC gas chromatograph (Hewlett-Packard, USA) equipped with a thermal conductivity detector and fitted with two parallel steel columns with Porapak Q and MS 5A respectively, operated at 70 °C. CH₄ levels were quantified in 0.5 ml samples taken from the Labco tubes, using an HP 5890 Series II gas chromatograph (Hewlett Packard, USA) equipped with a flame ionization detector and fitted with a steel column with Porapak N operated at 80°C. Peak areas were estimated using an HP integrator and results computed from detector responses of gas standards. Results were corrected for gas losses in connection with the transfer of air samples to and from the glass vials. The barometric pressure was not measured in the site of sample collection. We report concentrations of the particular gases without any type of elevational correction.

2.4. Statistical analyses

Concentration of a particular gas in a particular burrow or nest at a particular day and time was computed as the arithmetic mean of all values where more than one sample was successfully analysed. If the afternoon, evening or morning value was not obtained for a particular burrow, or if the burrow remained open in the evening (indicating that it might not have been part of an active burrow system anymore), the burrow was omitted from the analysis.

Concentrations of O₂ and CO₂ detected in the air samples collected from open burrows in the afternoon, the nest chambers and from the soil at the same depth away from the nests in the morning were compared against the values reported for the Earth's atmosphere, i.e. 20.95% O₂ and 0.042% CO₂. The difference was considered not significant when the Earth

atmospheric value was within the 0.025 and 0.975 quantile of a normal distribution approximating log-transformed concentrations of a particular gas in the collected air samples.

Differences between gas concentrations at different times of the day (afternoon, evening and morning) for a particular study site and season, were tested using a repeated measurements ANOVA (in variables with homogeneous variances between the groups as revealed by the Bartlett's test) or with the non-parametric Friedman's ANOVA for repeated measurements (in variables with heterogeneous variances between the groups). Tukey HSD tests were used for multiple comparisons, where appropriate.

Differences in the concentrations of O₂, CO₂ and CH₄ between nest chambers and control samples were tested with either paired t-tests or paired non-parametric Wilcoxon tests, separately for morning and midday samples. The same test was used to compare morning and midday control samples. Spearman correlation was used to investigate the relationships between CO₂ and CH₄ concentrations in the nests, and the difference between the temperature inside and away from the nests. All tests were performed with the R statistical software (R Core Team, 2019). Throughout the text, means are presented $\pm$ SD.

3. Results

3.1. Environmental parameters

Soil hardness increased substantially during the dry season in the Web Valley site (Table 1). Daily temperatures measured at the soil surface were characterized by a relatively low amplitude in the wet period and high amplitude in the dry period, whereas the mean temperature increased only moderately (Table 1). The soil moisture was 21% in the moist period.

3.2. Gas concentrations in giant root-rat burrows

Across sites and periods, afternoon concentrations of O₂ in open burrows were similar to that of the Earth's atmosphere (the Earth mean atmospheric value 20.95% corresponded to the 44–85% quantile of normal distributions approximating log-transformed values of O₂ concentrations in the samples). In all periods and both localities, the concentration of O₂ in burrows decreased after the burrow openings were plugged in the evening, but the decrease was relatively small and insignificant (Friedman's repeated measurement ANOVA: $\chi^2_{2, 14} = 0.1$, $p = 0.687$ for Sanetti in the wet period, repeated measurements ANOVA: $F_{2, 12} = 2.4$, $p = 0.131$ for Web Valley in the wet period, $F_{2, 6} = 3.2$, $p = 0.111$ for Web Valley in the moist period and $F_{2, 18} = 2.0$, $p = 0.162$ for Web Valley in the dry period) and did not continue until the next morning even though the burrows had been sealed for more than 12 hours at this time (Table 2). The minimum O₂ concentration detected in a plugged root-rat tunnel was 19.7% during the wet period in Sanetti (~57 kPa).

Afternoon concentrations of CO₂ in the burrows at both localities and all periods were similar to the CO₂ concentration in the Earth's atmosphere (the mean atmospheric value 0.042% corresponded to the 19–41% quantile of normal distributions approximating log-transformed values measured). The mean concentration of CO₂ in burrows increased in the evening after the burrow entrances were plugged, which was significant in the wet and moist periods in the Web Valley site (Friedman's repeated measurement ANOVA: Sanetti – wet period: $\chi^2_{2, 14} = 3.3$, $p = 0.197$; Web Valley – wet period: $\chi^2_{2, 12} = 6$, $p = 0.05$; Tukey test: $p = 0.043$; repeated measurements ANOVA: Web Valley – moist period: $F_{2, 6} = 5.3$, $p = 0.046$; Tukey test: $p = 0.066$; Friedman's repeated measurement ANOVA: Web Valley – dry period: $\chi^2_{2, 18} = 5.6$, $p = 0.061$), but the increase did not continue until the morning (Table 2). There was a high variability in CO₂ concentrations between different tunnels in the evening and morning hours, especially in the wet period in the Web Valley. The maximum CO₂

concentration detected in a plugged tunnel was 0.44% (in the evening during the wet period in the Web Valley).

3.3. Gas concentrations and temperatures in giant root-rat nests

Morning oxygen concentrations in the nest chambers were similar as in the control samples taken from the soil of the same depths (Paired t-test, $t = 1.2$, $n = 10$, $p = 0.249$; Table 3), with neither being significantly different from the mean atmospheric value. There was no significant difference between O_2 concentrations in the morning and the afternoon both in the nests and control samples (Paired t-tests, $t = -1.0$ and 0.8 , $n = 10$ and 10 , $p = 0.344$ and 0.439 respectively). The lowest concentration of O_2 detected in a nest chamber was 19.8% (~62 kPa).

The concentration of CO_2 in the nests in the morning was significantly higher than in the control samples (Wilcoxon's matched-pair test, $V = 55$, $n = 10$, $p = 0.002$) and to the mean atmospheric value (0.045% corresponds to 0.05% quantile of the normal distribution approximating CO_2 concentrations in individual nests following log transformation). The CO_2 concentrations both in the nests and in the control samples decreased significantly in the afternoon (Wilcoxon's matched-pair tests, nests: $V = 49$, $n = 10$, $p = 0.027$; control samples: $V = 41$, $n = 10$, $p = 0.033$; Tab. 3). The highest concentration of CO_2 detected in a nest chamber was 1.3%.

The concentration of CH_4 in the nest chambers in the morning was higher than in the control samples (Wilcoxon's matched-pair test, $V = 54$, $p = 0.004$) and it decreased, although not significantly, in the afternoon (Wilcoxon's matched-pair test, $V = 43$, $p = 0.131$). CH_4 concentrations in the control samples did not change until the afternoon (Wilcoxon's matched-pair test, $V = 24$, $p = 0.770$). The highest value detected in the nest chamber was

13.5 ppm CH₄. The concentrations of CO₂ and CH₄ in the nests in the morning were correlated (Spearman correlation, $p = 0.01$, $r_s = 0.754$).

Nests temperatures were $2.4 \pm 0.8^\circ\text{C}$ and $2.2 \pm 0.8^\circ\text{C}$ higher than temperatures in the control sites, in the morning and afternoon respectively, and varied little between morning and afternoon (Table 3).

3.4. Nest and tunnel dimensions

Nests chambers were 28 ± 4 cm wide (horizontal diameter), 22 ± 4 cm high (vertical diameter) and were located 40 ± 5 (range 30–47, $n = 20$) cm under the soil surface (measured from the nest chamber floor). Nest chambers were filled with 390 ± 180 (160–600) g of bedding (mostly dry plant material) and large amounts of partly decomposed old nest bedding (the weight was not determined). Small amounts of food (i.e. pieces of fresh plant biomass, altogether 7–145 g) were usually located either in a niche of the nest chamber or mixed with the nest bedding. Although dung pellets were sometimes encountered among the nest bedding, they were more frequently mixed with the soil and plant debris blocking the tunnels close to the nest.

Tunnels close to the nests were 32 ± 5 cm deep, 13 ± 1 cm high (vertical diameter) and 11 ± 1 cm wide (horizontal diameter). Tunnels deeper than 50 cm found close to some nest chambers had a smaller diameter (9 ± 1 cm).

4. Discussion

Our study provides microenvironmental characteristics of burrows and nests, including their daily and seasonal variations, of a fossorial rodent, the giant root-rat *Tachyoryctes macrocephalus*, living at elevations above 3000 m. Radio-tracking allowed us to precisely localise nest chambers without destroying any part of the burrow systems and to collect gas

samples from occupied nests. This is a unique approach, never employed before in any fossorial mammal. Against expectations, we found no evidence for environmental hypoxia and hypercapnia; CO₂ levels in burrows increased after burrows were plugged, and were the highest in nests in the morning, but never harmful.

Our main finding is that root-rats are not being normally exposed to challenging atmospheres as expected in fossorial mammals that spend most of the day in a complex of underground burrows (McNab, 1966; Buffenstein, 2000) (of course apart from a mild hypobaric hypoxia due to high altitude). In comparison with similar studies on fossorial and subterranean rodents, the root-rat's burrow and nest gas composition is among the most convenient (Table 4). Even the relatively high O₂ and CO₂ values, recorded during a period of rains in the study area, were similar to those reported for the naked mole-rat during the dry season in Ethiopia (Holtze et al., 2018) and for several *Spalax* species living in light soils during the wet season in Israel (Shams et al., 2005). Among these studies, the most challenging gas composition, i.e. with the lowest concentration of O₂ and/or the highest concentration of CO₂, was found in burrows and aboveground nesting mounds of blind mole rats living in heavy soils and during the wet season (see below). Interestingly, burrows of the Damaraland mole-rat *Fukomys damarensis* had relatively high CO₂ concentrations despite the species lives in sandy soil (Roper et al., 2001). This might be possibly explained by deeper burrows. Hong et al. (2019) also found hypoxic and hypercapnic conditions in burrows of the mandarin vole *Lasiopodomys mandarinus*, and these were more severe during the rainy season (minimum O₂ concentration of 16.04% and maximum CO₂ concentration of 2.55%); they also found that a O₂ had decreased and CO₂ increased by the morning (8.00) compared to other measurements carried out during the day in the dry season. In contrast, our study did not find marked differences between wet and dry periods. The relatively stable and suitable gas composition detected in our study could be explained by better ventilation within the burrow

systems of giant root-rats due to their regular excursions to gather food on the surface (see below).

There are several factors, which may contribute to the suitable gas composition in the root-rat burrow systems. Research has demonstrated that soil characteristics, including soil type and permeability (Roper et al., 2001; Šumbera et al., 2004), water content (Shams et al., 2005) as well as ventilation enhanced by wind (Olszewski and Skoczen, 1965), diffusion along a thermal gradient at different soil depths (Kennerly, 1964) or animals moving through the tunnels (Arieli, 1979), could be responsible for the lack of remarkable differences between aboveground and burrow atmospheres. We did not examine soil texture in the study sites, but at least in the Web Valley, the soil is relatively light and with a low humus content (Šklíba et al., 2017), which indicates good permeability for both water and gases. The differences found in the burrow atmosphere and its daily dynamics between the Web Valley and Sanetti Plateau sites could be explained by the different properties of the plateau and valley soils.

It has been speculated that fossorial mammals select the depth of their nests to ensure enough O₂. Kennerly (1964) proposed that, in burrows located to a depth of 30 cm, the levels of O₂ and CO₂ equilibrate with the surface atmosphere simply by diffusion. In accord with this, the burrows and nests of giant root-rats are relatively shallow, with the highest point of nest chamber ceilings only about 20 cm below the soil surface. Similar nest depths were reported also in other subterranean rodents, including *Heliophobius argenteocinereus* (15–40 cm, Šumbera et al., 2004), *Spalax galili* (29–70 cm measured from the nest bottom, approximate depth of the nest ceiling was 25 cm, Lövy et al. 2015) and *Tachyoryctes splendens* (30–60 cm, Jarvis and Sale, 1971). In these cases, gas could be easily exchanged between the burrows and aboveground atmosphere through mesocavernous spaces. In addition, the relatively large diameter of giant root-rat burrows probably makes exchange of air easier. The windy conditions in Afroalpine zones could also create underpressure above

the mounds, “sucking” air through the mounds, as described in European moles (Olszewski and Skoczen, 1965). Increased air movement due to the animals moving through burrows could be also relevant because giant root-rats frequently and repeatedly resurface and retreat into the burrow system during the day, and also usually open more than one burrow entrance during mornings (own unpubl. observation; Yalden, 1975), possibly improving ventilation. Finally, marked differences in temperature between the soil layers and surface can drive air exchange in burrows as has been speculated for naked mole-rats (Holtze et al., 2018), whose shallow burrows are warmer than deeper ones during the warm part of the day, and colder during the remaining time. In giant root-rats, this could be an effective way of air convection, especially during the dry season when the daily temperature amplitudes on the surface are very high (20–30 °C). An interesting strategy is that adopted by blind mole rats, who build aboveground breeding mounds that serve as shelters for females and their pups in heavy soils experiencing repeated floods (Nevo, 1961). Aboveground chambers of blind mole rats had the lowest concentration of O₂ (7.2%) and the highest concentration of CO₂ (6.1%) in any fossorial rodent recorded so far and these correlated with peaks in the soil-water content (Shams et al., 2005).

We also measured levels of CH₄ in giant root-rat nests. As these correlated with CO₂ levels, we hypothesized that both were primarily produced by the animal, rather than decomposition of plant material, as the latter would require anaerobic conditions. Still, the large amounts of dead plant material deposited in unused parts of burrow systems and in the nest chamber could also decompose under aerobic conditions (being composted), contributing to elevated CO₂ levels in the nests and surrounding tunnels, and even in the surrounding soil which had been likely subject to root-rat disturbance (c.f. Šklíba et al., 2017).

Higher nest temperatures in comparison with the temperature of soil away from the nests corroborate the findings obtained in another species of *Tachyoryctes* (Jarvis and Sale,

1971; Rahm, 1980). In our case, however, higher temperatures were also recorded in empty nests, indicating that the difference cannot be explained by the presence of the animal in the nest chamber. It has also been suggested that decomposition of vegetation mixed with soil and faeces can improve thermal conditions in nests by decaying organic material, particularly in cold and wet seasons (Jarvis and Sale, 1971; Rahm, 1980). We observed, however, relatively few faeces in nest bedding compared with that on blocked tunnels close to the nest in the dry season.

Giant root-rats leave their burrow systems open to the aboveground environment during the day, which makes them different from other congenics. This reflects differences in their foraging ecology, however it can have a thermoregulatory purpose. Giant root-rats usually open their burrow entrances around 8 a.m. and seal them around 5 p.m. The part of the day the burrow systems are open roughly corresponds with the time during which aboveground temperatures are higher than soil temperatures at all depths (Vlasatá et al., 2017). We can therefore suggest that the large spaces of the nest chamber and especially the surrounding tunnels may act like a heating system holding warm air accumulated during the day for at least a part of the night. Further study of this phenomenon would be highly interesting.

Although we cannot exclude more severe situations to occur for example after very heavy long rains, the results of our study demonstrate that the giant root-rat normally does not face stresses related to hypoxic and hypercapnic burrow and nest atmospheres. Using radio tracking to locate nests before sampling the nest air by a probe driven through the ceiling proved to be a very suitable – if not the only – way to obtain information on gas composition from nests of free living fossorial mammals without destroying their burrow systems. The next step would be to implement this approach to study strictly subterranean mammals such as African mole-rats (Bathyergidae), which spend all their lives within sealed systems of

burrows and are known to have developed adaptations to hypoxia. Special attention should also be paid to social species, where several individuals share a nest simultaneously, such as the large-bodied giant mole-rat *Fukomys mechowii*, or small-bodied naked mole-rat who live in families of several tens or hundreds of individuals. Probably the most challenging for future studies would be to place a measuring device to the nest ceiling to continuously measure gas composition in nests and its dynamics without forcing animals to abandon their nests.

5. Conclusions

We found no evidence for environmental hypoxia in the examined burrows and nests. Concentrations of CO₂ in the burrows increased after the burrows were plugged in the evening, but did not reach physiologically problematic levels. The highest CO₂ concentrations in the burrows were detected in a wet period. In root-rat nest chambers, the highest (but still harmless) CO₂ concentrations (up to 1.31%) were detected in the morning (measured in late dry season only) together with an elevated concentration (up to 13.5 ppm) of CH₄. Apart from the shallow depths, the relatively suitable gas composition in the root-rat burrows and nests could be caused by the relatively large dimensions of the nest chamber and surrounding tunnels, its regular surface activity as well as by the absence of heavy soil in the study sites surveyed.

Funding

The study was funded by the Czech Science Foundation GAČR (P506/11/1512 and 17-19896S) and ERC (669609).

Acknowledgements

We are grateful to the Ethiopian Wildlife Conservation Authority for permission to conduct research in Ethiopia. We thank the Bale Mountains National Park and the Ethiopian Wolf Conservation Programme for access to the Sodota and Sanetti research camps and for their help with logistics. We thank Sena Gashe and Abbas Geda for assistance during fieldwork, Jan Okrouhlík and two anonymous reviewers for comments on an earlier version of the manuscript and Keith Edwards for language correction.

References

- Arieli, R., 1979. The atmospheric environment of fossorial mole rat (*Spalax ehrenbergi*): Effect of season, soil texture, rain, temperature and activity. *Comp. Biochem. Physiol.* 63A: 569–575.
- Avivi, A., Gerlach, F., Joel, A., Reuss, S., Burmester, T., Nevo, E., & Hankeln, T., 2010. Neuroglobin, cytoglobin, and myoglobin contribute to hypoxia adaptation of the subterranean mole rat *Spalax*. *P. Natl. Acad. Sci-Biol.*, 107: 21570–21575.
- Benedix Jr, J. H., 1994. A predictable pattern of daily activity by the pocket gopher *Geomys bursarius*. *Animal Behav.* 48: 501–509.
- Buffenstein, R., 2000. Ecophysiological responses of subterranean rodents to underground habitats, In: Lacey, E.A., Patton, J.L. (Eds.), *Life Underground: the Biology of Subterranean Rodents*. The University of Chicago Press, Chicago and London, pp. 62–110.
- Burda, H., Šumbera, R., Begall, S., 2007. Microclimate in urrows of subterranean rodents - revisited, In: Begall, S., Burda, H., Schleich, C.E., (Eds.). *Subterranean Rodents: News from Underground*. Springer, Berlin, Heidelberg, New York pp. 20–33.

- Cameron, G.N., Spencer, S.R., Eshelman, B.D., Williams, L.R., Gregory, M.J., 1988. Activity and burrow structure of Attwater's pocket gopher (*Geomys attwateri*). J. Mammal, 69: 667–677.
- Contreras, L. C., McNab, B.K., 1990. Thermoregulation and energetics in subterranean mammals. Prog. Clin. Biol. Res., 335: 231.
- Hickman, G.C., 1990. Adaptiveness of tunnel system features in subterranean mammal burrows. Prog. Clin. Biol. Res. 335: 185–210.
- Holtze, S., Braude, S., Lemma, A., Koch, R., Morhart, M., Szafranski, K., Hildebrandt, T.B., 2018. The microenvironment of naked mole- rat burrows in East Africa. Afr. J. Ecol. 56: 279–289.
- Hong, S., Yifeng, Z., Baishi, W., Yangwei, L., Wenbo, X., Runkun, M., Zhenglong, W., 2019. Investigation on oxygen and carbon dioxide fluctuations in *Lasiopodomys mandarinus* burrows. Pakistan J. Zool. 51: 1519–1526.
- Jarvis, J.U.M., Sale, J.B., 1971. Burrowing and burrow patterns of East African mole-rats *Tachyoryctes*, *Heliophobius* and *Heterocephalus*. J. Zool. 163: 451–479.
- Kennerly Jr., T.E., 1964. Microenvironmental conditions of the pocket gopher burrow. Tex. J. Sci. 16: 395–441.
- Larson, J., Park, T.J., 2009. Extreme hypoxia tolerance of naked mole-rat brain. Neuroreport, 20: 1634–1637.
- Lövy, M., Šklíba, J., Hrouzková, E., Dvořáková, V., Nevo, E., Šumbera, R., 2015. Habitat and burrow system characteristics of the blind mole rat *Spalax galili* in an area of supposed sympatric speciation. PLoS one, 10(7), e0133157.
- McNab, B.K., 1966. The metabolism of fossorial rodents: A study of convergence. Ecology 47: 712–733.

- National Research Council, 2008. Emergency and continuous exposure guidance levels for selected submarine contaminants (Vol. I). National Academies Press.
<https://www.nap.edu/read/11170>.
- Nevo, E., 1961. Observations on Israeli populations of the mole rat *Spalax e. ehrenbergi* Nehring 1898. *Mammalia* 25: 127–144.
- Nevo, E., 1999. Mosaic Evolution of Subterranean Mammals: Regression, Progression and Global Convergence. Oxford University Press Inc., New York.
- Olszewski, J. L., Skoczen, S., 1965. The airing of burrows of the mole, *Talpa europaea* Linnaeus, 1758. *Acta Theriol.* 10: 10–17.
- Park, T.J., Reznick, J., Peterson, B.L., Blass, G., Omerbašić, D., Bennett, N.C., ..., Applegate, D.T., 2017. Fructose-driven glycolysis supports anoxia resistance in the naked mole-rat. *Science* 356: 307–311.
- R Core Team, 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, <https://www.R-project.org/>.
- Rahm, U., 1980. Die afrikanische Wurzelratte: *Tachyoryctes* (Vol. 528). Ziemsen.
- Roper, T.J., Bennett, N.C., Conradt, L., Molteno, A.J., 2001. Environmental conditions in burrows of two species of African mole-rat, *Georychus capensis* and *Cryptomys damarensis*. *J. Zool.* 254: 101–107.
- Shams, I., Avivi, A., Nevo, E., 2004. Hypoxic stress tolerance of the blind subterranean mole rat: expression of erythropoietin and hypoxia-inducible factor 1 α . *Proc. Nat. Acad. Sci.* 101: 9698–9703.
- Shams, I., Avivi, A., Nevo, E., 2005. Oxygen and carbon dioxide fluctuations in burrows of subterranean blind mole rats indicate tolerance to hypoxic-hypercapnic stresses. *Comp. Biochem. Physiol. - Mol. Integr. Physiol.* 142: 376–382.

- Sillero-Zubiri, C., Tattersall, F.H., Macdonald, D.W., 1995. Habitat selection and daily activity of giant molerats *Tachyoryctes macrocephalus*: significance to the Ethiopian wolf *Canis simensis* in the Afroalpine ecosystem. *Biol. Conserv.* 72: 77–84.
- Šklíba, J., Šumbera, R., Chitaukali, W.N., Burda, H., 2007. Determinants of daily activity patterns in a free-living afrotropical solitary subterranean rodent. *J. Mammal.* 88: 1009–1016.
- Šklíba, J., Vlasatá, T., Lövy, M., Hrouzková, E., Meheretu, Y., Sillero- Zubiri, C., Šumbera, R., 2017. Ecological role of the giant root- rat (*Tachyoryctes macrocephalus*) in the Afroalpine ecosystem. *Integr. Zool.* 12: 333–344.
- Šklíba, J., Vlasatá, T., Lövy, M., Hrouzková, E., Meheretu, Y., Sillero- Zubiri, C., Šumbera, R., 2020. The giant that makes do with little: small and easy- to- leave home ranges found in the giant root- rat. *J. Zool.* 310: 64–70.
- Šumbera, R., Chitaukali, W.N., Elichová, M., Kubová, J., Burda, H., 2004. Microclimatic stability in burrows of an Afrotropical solitary bathyergid rodent, the silvery mole-rat (*Heliophobius argenteocinereus*). *J. Zool.* 263: 409–416.
- Vlasatá, T., Šklíba, J., Lövy, M., Meheretu, Y., Sillero-Zubiri, C., Šumbera, R., 2017. Daily activity patterns in the giant root rat (*Tachyoryctes macrocephalus*), a fossorial rodent from the Afro-alpine zone of the Bale Mountains, Ethiopia. *J. Zool.* 302: 157–163.
- Yalden, D.W., 1975. Some observations on the giant mole-rat *Tachyoryctes macrocephalus* (Rüppell, 1842) (mammalia rhizomyidae) of Ethiopia: Pubblicazioni del centro di studio per la faunistica ed ecologia tropicali del cnr: ci. *Monitore Zoologico Italiano* Supplemento 6: 275–303.
- Yalden D.W., 1985. *Tachyoryctes macrocephalus*. *Mammal. Spec.* 237: 1–3.

Figure 1. A schematic view of the nest chambers (plan and lateral section) and surrounding tunnels of three adult *T. macrocephalus*. Hatching marks nest bedding, black spots represent food and grey represents old nest bedding mixed with soil and faeces pushed into some parts of the burrow system and into mounds.

Table 1. Physical soil parameters and mean surface temperature at the Web Valley site.

Temperature measured over a 7-day period. Data are arithmetic means $\pm$ SD.

Period	Soil hardness (N cm ⁻²)	Soil moisture (%)	Soil density (g cm ⁻³ of the dry mass)	Mean daily surface temperature (°C)	Mean daily surface temperature amplitude (°C)
Wet	n/a	n/a	n/a	8.6 $\pm$ 0.6	5.8 $\pm$ 1.1
Moist	191 $\pm$ 25	21 $\pm$ 0.1	1.1 $\pm$ 0.1	8.0 $\pm$ 0.6	20.0 $\pm$ 1.7
Dry	473 $\pm$ 119	n/a	n/a	12.0 $\pm$ 1.0	29.0 $\pm$ 4.2

Table 2. Concentrations of O₂ and CO₂ in burrows of *T. macrocephalus* (n = number of individuals in which burrows were examined). Data are arithmetic means $\pm$ SD (range). Gas concentrations are not corrected to sea level.

Locality and season	O ₂ concentration (%)			CO ₂ concentration (%)		
	Afternoon	Evening	Morning	Afternoon	Evening	Morning
Sanetti wet (n = 8)	20.9 $\pm$ 0.1 (20.7 – 21.0)	20.8 $\pm$ 0.6 (19.7 – 21.6)	20.8 $\pm$ 0.3 (20.3 – 21.2)	0.05 $\pm$ 0.03 (0.03 – 0.14)	0.09 $\pm$ 0.07 (0.04 – 0.20)	0.09 $\pm$ 0.07 (0.03 – 0.21)
Web V. wet (n = 7)	20.7 $\pm$ 0.2 (20.4 – 21.0)	20.4 $\pm$ 0.3 (20.0 – 20.8)	20.6 $\pm$ 0.2 (20.4 – 21.0)	0.06 $\pm$ 0.02 (0.04 – 0.08)	0.26 $\pm$ 0.17 (0.07 – 0.44)	0.17 $\pm$ 0.12 (0.04 – 0.37)
Web V. early dry (n = 4)	21.0 $\pm$ 0.4 (20.5 – 21.5)	20.7 $\pm$ 0.12 (20.6 – 20.8)	20.5 $\pm$ 0.3 (20.2 – 21.0)	0.07 $\pm$ 0.04 (0.04 – 0.12)	0.19 $\pm$ 0.04 (0.15 – 0.23)	0.19 $\pm$ 0.10 (0.11 – 0.33)
Web V. late dry (n = 10)	20.9 $\pm$ 0.2 (20.6 – 21.3)	20.7 $\pm$ 0.2 (20.3 – 21.0)	20.7 $\pm$ 0.3 (20.3 – 21.5)	0.05 $\pm$ 0.02 (0.03 – 0.09)	0.10 $\pm$ 0.05 (0.06 – 0.20)	0.10 $\pm$ 0.05 (0.06 – 0.21)

Table 3. Concentrations of O₂, CO₂ and CH₄ and temperature (T) inside nests of *T. macrocephalus* and in control samples at the same depth away from the nest. Data are arithmetic means $\pm$ SD (range).

	Nests (n = 10)	Control samples (n = 10)
O ₂ in the morning (%)	20.5 $\pm$ 0.4 (19.8 – 21.1)	20.6 $\pm$ 0.4 (20.0 – 21.2)
O ₂ in the afternoon (%)	20.6 $\pm$ 0.3 (20.3 – 21.1)	20.5 $\pm$ 0.5 (19.4 – 21.0)
CO ₂ in the morning (%)	0.61 $\pm$ 0.38 (0.26 – 1.31)	0.16 $\pm$ 0.12 (0.07 – 0.48)
CO ₂ in the afternoon (%)	0.35 $\pm$ 0.36 (0.07 – 1.19)	0.11 $\pm$ 0.07 (0.04 – 0.28)
CH ₄ in the morning (ppm)	6.8 $\pm$ 3.5 (3.4 – 13.5)	2.7 $\pm$ 0.8 (2.0 – 4.2)
CH ₄ in the afternoon (ppm)	4.5 $\pm$ 2.5 (2.6 – 11.1)	2.7 $\pm$ 0.4 (2.2 – 3.3)
T in the morning (°C)	14.4 $\pm$ 1.1 (13.5 – 16.5)	12.0 $\pm$ 0.5 (11.3 – 12.8)
T in the afternoon (°C)	14.5 $\pm$ 1.0 (13.5 – 16.5)	12.3 $\pm$ 0.5 (11.6 – 13.2)

Table 4. The lowest concentration of O₂, and the highest concentration of CO₂ detected in natural burrows of fossorial and strictly subterranean rodents. For both gases, means $\pm$ SD and range is given if available. An asterix denotes conditions considered either hypoxic or hypercapnic.

Species	Fossoriality level	Season/period	Soil type	Site and depth	O ₂ (%)	CO ₂ (%)	Elevation (m a.s.l.)	Source
<i>T. macrocephalus</i>	fossorial	Wet		Plugged burrow (15 cm)	20.8 $\pm$ 0.6 (19.7–21.6)	0.26 $\pm$ 0.1 7 (0.07–0.44)	3500, 4100	this study
		Dry		Nest (40 cm)	20.5 $\pm$ 0.4 (19.8–21.1)	1.31	3500	this study
<i>Spalax galili</i>	subterranean	Wet	heavy basaltic	Plugged burrow (20–40cm)	12.7*	5.6*	ca. 820	Shams et al., 2005
		Wet	light rendzina	Plugged burrow (20–40cm)	19.6	0.6	ca. 760	Shams et al., 2005
<i>Spalax golani</i>	subterranean	Wet	heavy basaltic soil	Plugged burrow (20–40cm)	10.5*	2.9*	ca. 980	Shams et al., 2005
		Wet	light terra rossa	Plugged burrow (20–40cm)	18.9	0.43	ca. 1030	Shams et al., 2005
<i>Spalax carmeli</i>	subterranean	Wet	heavy clay	Chambers in the aboveground breeding mound	7.2*	6.1*	ca. 10	Shams et al., 2005
		Wet	light terra rossa	Plugged burrow (20–40cm)	18.5	0.68	ca. 450	Shams et al., 2005
<i>Spalax galili?</i>	subterranean	Likely wet	heavy	Above-ground breeding mound	14.0*	4.8*	NA	Arieli, 1979
		Likely wet	heavy soil	Fresh burrows, animals pushing soil	19.0 $\pm$ 1.5 (17.4–20.9)	1.9 $\pm$ 1.0 (0.6–2.8*)	NA	Arieli, 1979
		Wet	heavy soil	Plugged burrow	19.0 $\pm$ 0.6 (18.0–19.7)	0.9 $\pm$ 0.5 (0.3–1.5)	NA	Arieli, 1979
		Wet	light soil	Plugged burrow	20.0 $\pm$ 0.5 (19.4–20.6)	0.2 $\pm$ 0.2 (0.1–0.4)	NA	Arieli, 1979
<i>Heterocephalus glaber</i>	subterranean	Dry		Burrow	20.5 $\pm$ 0.3 (19.9–20.9)	0.17 $\pm$ 0.0 9 (0.05–0.40)	ca. 1363	Holtze et al., 2018

<i>Heliophobius argenteo-cinereus</i>	subterranean	Dry season	hard compact	Plugged burrow (10–20cm),	19.2±0.8 (17.8–20.0)	N.A.	1120	Šumbera et al., 2004
<i>Georychus capensis</i>	subterranean		Light loamy	Plugged burrow (8–14cm)	20.4 (19.8–20.8)	0.4 (0.1–1.2)	ca. 110	Roper et al., 2001
<i>Fukomys damarensis</i>	subterranean		Sandy soil	Plugged burrow (20–50cm)	20.4 (19.9–21.0)	0.4 (0.1–6.0*)	1075	Roper et al., 2001
<i>Geomys pinetis</i>	fossorial	Warm (October)		Burrow (30 cm)	19.5±0.3 (16.7*–21.0)	1.4±0.1 (0.8–2.6*)	ca. 50	McNab, 1966
<i>Thomomys bottae</i>	fossorial			Shallow burrows	15.5*–20.5	0.6–3.8*	NA	Darden 1970 in Darden, 1972
<i>Lasiopodomys mandarinus</i>	fossorial	Wet		Plugged burrow (40–50cm)?	16.0*	2.6*	ca. 110	Hong et al., 2019
		Dry		Plugged burrow?	18.4–20.0 (mean min and max values)	Around 0.3-0.6 (mean min and max values)	ca. 110	Hong et al., 2019

Journal Pre-proof