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Unexplored aspects of African mole-rat thermal biology: Daily energy expenditure and development of thermoregulation in *Fukomys darlingi* (Rodentia: Bathyergidae)

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Abstract. In our study, we analyzed two poorly known aspects of thermal biology in subterranean rodents, the African mole-rats, that being daily energy expenditure and development of thermoregulation in juvenile mole-rat using the social Mashona mole-rat, *Fukomys darlingi* from southern Malawi. We performed laboratory measurements over 24 h to assess the daily energy expenditure (DEE) in adults as well as the development of the thermoregulatory abilities of juveniles at different ages. To assess the effect of ambient temperature (T_a), we exposed mole-rats to either 30 °C or 20 °C, which represents a thermoneutral as well as a thermally challenging temperature, respectively. The DEE at a T_a of 20 °C was lower than expected based on the calculation from the resting metabolic rate (RMR) at the same T_a, this suggested that heat derived from physical activity can substitute the thermoregulatory heat and thus decrease the cost on thermoregulation. To assess the development of thermoregulation in juveniles and the effect of the presence of family members on it, we measured core body temperature (T_b) and resting metabolic rate (RMR) in juveniles under several social contexts: while alone, with littermates, with littermates and mother and with littermates and both parents. Only juveniles older than one month could generate heat to keep T_b higher than T_a. Thermoregulation appeared to be fully developed in three-month-old juveniles. The presence of adult(s), but not littermates, helped to increase T_b and to decrease the RMR of juveniles. Although the results are mostly preliminary and some interpretations are limited due to low sample size in some social contexts, we may conclude that in this mole-rat species, development of thermoregulation is slow even compared to other altricial mammals.

Key words. Bathyergidae, development of thermoregulation, daily energy expenditure, *Fukomys darlingi*.

INTRODUCTION

Subterranean mammals acquire food, reproduce, and disperse predominantly in the subterranean environment (Nevo 1999). The burrows of their underground systems are closed by mounds of soil, which contributes to microclimatic stability, especially in terms of temperature and humidity (Bennett et al. 1988, Burda et al. 2007). Although these mammals take advantage of relatively stable microclimatic conditions in burrows, their lifestyle is very challenging, mainly due to the high energy costs of burrowing (e.g., Luna et al. 2002, Zelová et al. 2010). Interestingly, unrelated taxa of subterranean mammals have convergently evolved physiological adaptations

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such as low basal metabolic rate (BMR), low body core temperature (T_b) and high conductance (McNab 1966, Contreras & McNab 1990, Nevo 1999, Buffenstein 2000).

African mole-rats (Bathyergidae) are some of the most studied subterranean mammals (Bennett & Faulkes 2000). One of their most remarkable features is a wide diversity of strategies to face the challenges of a low T_a. Among the relatively low number of species in this family, there are truly homeothermic species, such as the Cape dune mole-rat *Bathyergus suillus* (Lovegrove 1986, Okrouhlík et al. 2021), heterothermic species such as Mashona mole-rat *Fukomys darlingi* from Zimbabwe (Bennett et al. 1993, Jacobs et al. 2022), or the naked mole-rat *Heterocephalus glaber*, which is described as the only poikilothermic species (Buffenstein & Yahav 1991), but see Braude et al. (2021).

Among the many directions of African mole-rat metabolic research, studies have focused on two aspects. Firstly, a large number of studies define BMR or resting metabolic rate (RMR), T_b, thermal conductance and the region of the thermoneutral zone (TNZ) as well as evaporative water loss (see Šumbera 2019 for review). Nevertheless, this research provides little about the energetics of mole-rats over a longer period, because measurements are only short-term, mole-rats are measured at rest and usually in a post-absorptive state. Short-term measurements can give us information on the minimal energy for maintaining vital functions, but they cannot provide information on the daily energetic requirements of an animal under given conditions. In field, the method of choice for estimation of the long-term energy expenditure is the doubly labelled water technique (Speakman 1999, Hart et al. 2022, and studies cited therein), but it does not allow to assess temporary changes within the tested period and is rather expensive.

Secondly, because the majority of African mole-rats are highly social species, another well studied topic deals with energetic savings from huddling. Huddling reduces the relative body surface area, which represents an important way of reducing heat loss to the environment and thus energy needed for thermogenesis (Kauffman et al. 2003, Scantlebury et al. 2006, Hwang et al. 2007). This is relevant mainly in subterranean rodents, because of their relatively high thermal conductance (Contreras & McNab 1990). Although several studies on different social mole-rat species have demonstrated energetic savings from huddling (Yahav & Buffenstein 1991, Kotze et al. 2008, Wiedenová et al. 2018, Vavrušková et al. 2022), no study has attempted to look at the effect of huddling of adults together with developing pups or young. This is actually quite surprising because it is known, that social thermoregulation is of importance to young pups in small mammals (e.g. Gilbert et al. 2012) and we may speculate that it is extremely important in social mole-rats with generally very altricial pups as well (Bennett et al. 1991). In mammals with dominant subterranean activity, such a study was carried out only in the solitary Talas tuco-tuco *Ctenomys talarum* (Cutrera et al. 2003). It showed that the development of thermoregulatory abilities of pups is relatively fast and that the presence of the mother in the nest is important from a thermal and energetic point of view (Cutrera et al. 2003).

Here we addressed both of the little-known aspects of mole-rat physiology by undertaking laboratory measurements on the social Mashona mole-rat, *Fukomys darlingi* from southern Malawi. To estimate the daily energy requirements of this species and the subsequent cost of thermoregulation, we quantified the daily energy expenditure (DEE) in adults using respirometry. We then focused on the development of thermoregulation in juveniles and the effect of huddling with other juveniles as well as with mother, or both parents. All measurements in both tasks were done within the thermoneutral zone (30 °C) and also at a temperature below thermoneutrality (20 °C). The latter is regularly encountered by different free-living mole-rat species in their burrows (Bennett et al. 1988, Šumbera 2019), but poses a thermal challenge for the Mashona

mole-rats from this population (Zemanová et al. 2012). Although the interpretation of our results related to the postnatal development of thermoregulation is severely restricted due to the small sample size in some age categories, we believe that our study will stimulate future research on these physiological phenomena, which are surprisingly understudied in African mole-rats and subterranean mammals as a whole.

MATERIAL AND METHODS

Experimental animals

The Mashona mole-rat, *Fukomys darlingi* (Thomas, 1895) is a social African mole-rat that lives in savannah habitats in Zimbabwe, Mozambique and southern Malawi, where it inhabits underground systems of tunnels (Bennett & Faulkes 2000, Šumbera et al. in press). It forms families of five to nine individuals with non-seasonal reproduction restricted to the dominant pair (Bennett et al. 1994). Whereas representatives of the nominate *F. darlingi* from Zimbabwe do not generate enough heat to maintain a stable T_b at T_as below 25 °C (Bennett et al. 1993), those from southern Malawi are truly homeothermic (Zemanová et al. 2012) keeping their T_b even in 10 °C.

Our breeding stock originated from five adult pairs captured in the vicinity of Nsanje town in southern Malawi (16°55'S, 35°16'E, 53 m a. s. l.). Animals used were kept in the laboratory for more than four years before measurements or were born in captivity. The families were kept in 80×60×50 cm glass terraria with horticultural peat as bedding. The breeding room was maintained on a 12L:12D light cycle and a constant ambient temperature of 25±1 °C and relative humidity of approximately 40–60%. The animals were fed three times per week with carrots, potatoes, commercial rodent pellets and supplemented with apple, lettuce, apples, and sweet potatoes.

We are grateful to the Genetic Resources and Biotechnology Committee (GRBC) and the technical committee of the National Research Council of Malawi (NRCM) for permission to collect and export ancestors of our breeding stock. All experimental procedures conformed to the legal requirements of the Czech Republic and to institutional guidelines (accreditation no. CZ 2045/20041020).

Daily energy expenditure measurements

To estimate the whole day energy requirements of the species, we established DEE by respirometry at 30 and 20 °C in 12 adults, whose body mass was 140.8±25.8 g (100.2–194.5 g; N=12). The temperatures were chosen to allow for comparison between thermoneutral and thermally challenging conditions as well as with previously published data on the species (Zemanová et al. 2012, Wiedenová et al. 2018). The thermoneutral zone of the population ranges from 27 to 34 °C and resting metabolic rate (RMR) at 20 °C is approximately twice as high as within TNZ (Zemanová et al. 2012). Three mole-rats were breeding males, three breeding females, three non-breeding males and three non-breeding females.

Respirometry measurements of DEE (see below for a detailed setup) lasted for 24 h. Each individual was measured alone in the metabolic chamber, the measurement was repeated three times over three days and the average value used for statistical analysis. Prior to each measurement, animals were placed in the chamber for one hour to acclimate.

Development of thermoregulation and the effect of huddling

To assess the development of the thermoregulatory abilities of *F. darlingi*, core body temperature (T_b) and RMR of various age-groups in six social contexts at 30 and 20 °C were measured. There were nine age groups comprised of 3, 15, 32, 46, 63, 77, 94, 125 and 156 days old juveniles (±1 day tolerance). This timing was based on a preliminary trial and covers the development of thermoregulation with higher sample at the younger age cohorts. As a reference of adult thermoregulatory abilities, we used 13 non-breeding adults and measured them in the same social contexts as we measured the juveniles. The social contexts

were: (1) single juvenile, (2) whole litter, (3) whole litter with mother, (4) whole litter with both parents, (5) mother without litter, and (6) parents only. Social contexts (5) and (6) were used only for calculations of RMR of litter in contexts (3) and (4), respectively (see below). In social contexts (2), (3), (4) and (6) animals were allowed to huddle and huddling behavior was observed.

We started the experiments with 20 juveniles, which originated from five litters born to three mothers. Due to the high juvenile mortality, which is common in the species (R. Šumbera unpubl. data), complete data were obtained only for three juveniles and incomplete data for 17 juveniles.

Body temperature measurement

The core body temperature (T_b) of the animals was measured using a Thermalert TH-8 digital thermometer (Physitemp Instruments Inc, USA) with a RET-2 rectal thermocouple probe in adults and RET-3 in juveniles. The probe was inserted in the rectum (10 mm in adults and 4–10 mm in juveniles) within 30 seconds after the end of the experiment and stabile T_b readings were recorded.

Resting metabolic rate measurement

Resting metabolic rate of animal(s) was determined by indirect calorimetry as oxygen consumption (VO_2) in a flow-through respirometry setup (see below). In order not to jeopardize the lactation of the female and the development of the juveniles, mole-rats were not starved and 2 g of food per adult or per older juvenile was provided in the metabolic chamber when the metabolic measurement started and its oxygen consumption was neglected. To acclimate the animals to the metabolic chamber in order to facilitate their calming down during the trial, the whole family was housed in the metabolic chamber one night prior to the measurements.

Each RMR recording took at least 90 min (20 min in individual juveniles younger than 63 days). Ten-minute interval with the lowest average recorded VO_2 and when all animals were resting was averaged to obtain the resting metabolic rate.

The mass specific VO_2 of litter ($VO_{2\text{-juv}}$) measured with mother, or both parents (context 4 or 5, see above) was calculated as a difference of VO_2 for the whole group ($VO_{2\ group}$) and VO_2 of the mother or both parents ($VO_{2\ adult}$; context 2 or 6) divided by combined mass of offspring ($mass_{juv}$; Cutrera et al. 2003).

$$msVO_2 = (VO_{2\ group} – VO_{2\ adult}) / mass_{juv}$$

Because a difference between two metabolic measurements is used and this is further divided by animal mass, these results are loaded with large error and may thus result even in negative or unrealistically high RMR. Therefore, these results should be treated with caution.

Respirometry

The metabolic chamber was submerged in a water bath (ThermoHaake C10 and K15, Haake, Germany) to ensure a stable temperature throughout the measurements and the activity of the mole-rats was observed through a transparent lid of the chamber during the whole measurement period. The air flow was set with respect to the size of animal(s), the air volume of the chamber, number of individuals and measurement duration. Small and large chamber dimensions (volume) were 7×7×11 cm (539 cm^3) and 8×20×17 cm (2740 cm^3), respectively. The air flow was measured upstream by a rotameter flowmeter (S082-03, Aalborg, USA) and kept at 108 and 343 $mL \times min^{-1}$ in the small and large chamber, respectively.

Dry fresh air was pushed through a flowmeter (082-03 S, Aalborg, USA) to a metabolic chamber. The excurrent air was then pushed through a CO_2 trap (NaOH+CaO) and water trap (Drierite with color-indicator) to a heated paramagnetic oxygen sensor (PAROX 1000, MBE ElectronicAG, Switzerland). The analog output of the sensor was digitized and recorded each second by the DIAdem 8.00 computer program (GfS Aachen, Germany). The instrument was calibrated with 99.99% N_2 (Linde) for zero oxygen content on a monthly basis. The slope of the analyzer was calibrated prior to and after each metabolic

rate measurement by dry CO_2 free air outside air and in case of DEE at least three more times during the trial. Barometric pressure was measured during the experiments by a digital weather station (JVD Digi Time RH7) and corrected for altitude. The body mass of the animals was determined by electronic scales (Kern & Sohn GmbH 572-45).

The VO_2 (mL $O_2 \times h^{-1}$) was calculated from O_2 readings using the equation:

$$VO_2 = ([0.2095 – FeO_2] \times FR) / (1 – FeO_2)$$

where FeO_2 is the excurrent fractional concentration of O_2 and FR (mL$\times h^{-1}$) is upstream flow rate corrected to standard temperature and pressure. Mass specific VO_2 (msVO_2) was obtained by dividing of VO_2 by mass in grams (Cutrera et al. 2003).

Statistics and data visualization

Unless specified otherwise, data are presented as mean±s.d. (range, number of observations). Statistical program R (R Core Team, 2021) was used for statistical analyses and data visualization. Repeated measures ANOVA (RMANOVA) was used to assess the effect of sex, breeding status and T_a on DEE and RMR in adults (function aov, package stats). Packages dplyr (Wickham et al. 2021), ggplot2 (Wickham 2016), and ggpubr (Kassambara 2020), were used for calculations and data visualization of T_b and RMR during development. The T_b differences among social contexts and T_as were evaluated by ANOVA, followed by a Tukey test. Similar statistical evaluation of RMR among social contexts was not possible due to the low number of replications in social contexts (2), (3) and (4), which was a consequence of measurement of RMR on the litter rather than on individual level as in case of T_b. Therefore, we only statistically compared RMR of three-day juveniles at 30 °C and 20 °C by Welsch test.

RESULTS

Daily energy expenditure in adults at 30 and 20 °C

The DEE of adult *F. darlingi* at 30 °C was 1.84±0.25 mL $O_2 \times g^{-1} \times h^{-1}$ (1.47–2.19 mL $O_2 \times g^{-1} \times h^{-1}$; N=12). At 20 °C, the DEE was 2.99±0.34 mL $O_2 \times g^{-1} \times h^{-1}$ (2.73–3.65 mL $O_2 \times g^{-1} \times h^{-1}$; N=12; see Appendix) and was significantly higher than at 30 °C (RMANOVA, $F_{1,11}=179.77$, $p<10^{-7}$). No effects of sex (RMANOVA, $F_{1,9}=2.513$, $p=0.147$) or breeding status (RMANOVA, $F_{1,9}=1.545$, $p=0.245$) were observed.

Development of thermoregulation

The T_b of three-day old juveniles differed between both T_a (ANOVA, $F_{1,63}=851.28$, $p<10^{-15}$) and social contexts (ANOVA, $F_{3,63}=83.8$, $p<10^{-15}$); there was an interaction between social context and T_a (ANOVA, $F_{3,63}=10.52$, $p<10^{-4}$). Post-hoc tests revealed that in all social contexts T_b of three-day old juveniles at 30 °C was higher than at 20 °C (Tukey post hoc test, all $p<0.001$). Differences in T_b of three day old juveniles among social contexts at 30 °C and at 20 °C are given in Fig. 1A–D and Fig. 2A–D, respectively. In three-day old juveniles, RMRs at 30 °C (Fig. 1E) and at 20 °C (Fig. 2E) were not different (Welsch test, $t=1.47$, $df= 19.85$, $p=0.16$).

At 30 °C, single three-day juveniles had a T_b of 30.0±1.0 °C (28.8–31.4 °C; N=12). The T_b increased with age up to 46 days, when it reached 34.2 °C (N=1) and was similar to adult T_b, which was 34.5±0.8 °C (33.4–36.1 °C; N=12; Fig. 1A). In juveniles with littermates, the T_b values and the overall trend were very similar (Fig. 1B). The presence of a mother resulted in higher T_bs in litters of younger age and especially in the three and 15-day old juveniles, whose

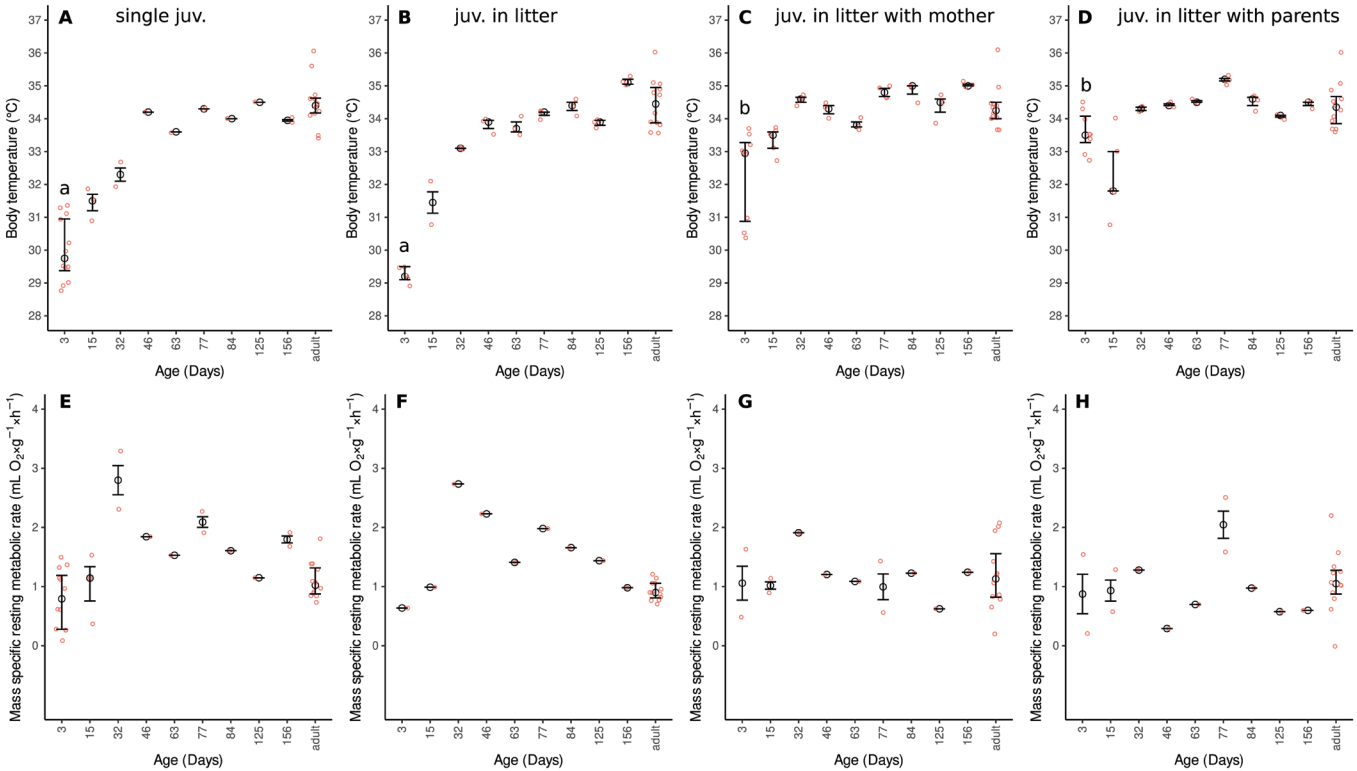

Fig. 1. Development of thermoregulation in juvenile (juv.) *Fukomys darlingi* at 30 °C. Body temperature (T_b) and resting metabolic rate (RMR) in juveniles were measured under different social contexts: A, E – single juvenile, B, F – juveniles in litter, C, G – juveniles in litter with mother and D, H – juveniles in litter with both parents. Red circles represent individual data-points, median is represented by a black circle and 2nd and 3rd quartile range by whiskers. Differences among T_bs among social contexts in three-day juveniles are indicated by letters at the top of the graphs. Contexts not sharing a letter differed from each other ($p<0.05$). No statistical tests were performed on RMR of the litter due to their low count.

T_bs were 32.3±1.4 °C (30.4–33.7 °C; N=8) and 33.3±0.4 °C (32.7–33.7 °C; N=5), respectively (Fig. 1C). In litters with both parents, the juveniles had very similar T_bs as to when they were measured with the mother only and the trend was also very comparable (Fig. 1D).

At 30 °C in single juveniles, RMR was the lowest in three-day juveniles in which it was 0.79±0.50 mL $O_2 \times g^{-1} \times h^{-1}$ (0.09–1.50 mL $O_2 \times g^{-1} \times h^{-1}$; N=12). In 15-day single juveniles, the RMR was higher 1.01±0.59 mL $O_2 \times g^{-1} \times h^{-1}$ (0.37–1.53 mL $O_2 \times g^{-1} \times h^{-1}$; N=3) and at the age of 32 days, the RMR reached its peak of 2.80±0.69 mL $O_2 \times g^{-1} \times h^{-1}$ (2.31–3.29 mL $O_2 \times g^{-1} \times h^{-1}$; N=2). In older single juveniles, RMR decreased towards the adult level of 1.10±0.31 mL $O_2 \times g^{-1} \times h^{-1}$ (0.73–1.81 mL $O_2 \times g^{-1} \times h^{-1}$; N=12; Fig. 1E). While in litters, the overall trend was very similar to that in single juveniles. Presence of a mother or both parents resulted in the disappearance of the relationship between RMR and juvenile age and RMRs of juveniles were very similar to those adults, in which RMR was 1.19±0.59 mL $O_2 \times g^{-1} \times h^{-1}$ (0.20–2.08 mL $O_2 \times g^{-1} \times h^{-1}$; N=12; Fig. 1F).

At 20 °C single three and 15-day old juveniles had a T_b of 20.7±0.6 °C (20.0–22.1 °C; N=13) and 21.4±0.5 °C (21.0–21.7 °C; N=2) respectively (Fig. 2A). The T_b rose gradually up to 84 days of age, when it was 33.1±0.1 °C (33.0–33.1 °C; N=2) and was very similar to adult T_b, which was 33.2±0.5 °C (32.6–34.1 °C; N=13; Fig. 2A). In litters, the overall trend of T_bs was very similar to single juveniles and the T_b values were higher. The T_b of adults was 33.1±0.6 °C (32.1–34.5 °C; N=13; Fig. 2B). The presence of a mother resulted in a higher T_b in up to 63 days old juveniles with an average increase of T_b of 3.7 °C. In older juveniles, the T_b was similar to that of adults, which had T_b of 33.2±0.4 °C (32.6–34.0 °C; N=13). When measuring the litter with parents, the three to 32 day old juveniles had a notably higher T_b (average increase of 2.8 °C) than when measured with mother only. The body temperature of older juveniles was similar to adult T_b, which was 33.1±0.5 °C (32.1–33.9 °C; N=13) and about the same as when the juveniles were measured with mother only (Fig. 2D).

At 20 °C RMR of single three and 15 days old juveniles were 0.54±0.36 °C (0.08–1.17 °C; N=13) and 0.73±0.39 mL $O_2 \times g^{-1} \times h^{-1}$ (0.45–1.00 mL $O_2 \times g^{-1} \times h^{-1}$; N=2), respectively (Fig. 2E). Metabolic rate increased with age reaching 5.49±0.67 mL $O_2 \times g^{-1} \times h^{-1}$ (5.44–5.53 mL $O_2 \times g^{-1} \times h^{-1}$; N=2) when the juveniles were 77 days old. In older juveniles, RMR decreased with age and approached the adult level, which was 2.15±0.29 mL $O_2 \times g^{-1} \times h^{-1}$ (1.56–2.68 mL $O_2 \times g^{-1} \times h^{-1}$; N=13; Fig. 2E). In litters, the RMR of three and 15 day old juveniles was 1.05±0.84 mL $O_2 \times g^{-1} \times h^{-1}$ (0.45–1.63 mL $O_2 \times g^{-1} \times h^{-1}$; N=2) and 0.78 mL $O_2 \times g^{-1} \times h^{-1}$ (N=1), respectively (Fig. 2F), whereas the trend in older juveniles was very similar to that for single juveniles, reaching a maximum of 4.31 mL $O_2 \times g^{-1} \times h^{-1}$ (N=1) in 77 days old juveniles (Fig. 2E, F). The presence of a mother resulted in a decreased RMR in 32 days old and older juveniles, while the overall trend between RMR and the age of juveniles was similar (Fig. 2G) to that in juveniles in litter. No such trend was, however, observed in juveniles measured with both parents (Fig. 2H), in which the RMR of all ages was like that in adults, in which the RMR was 1.09±1.33 mL $O_2 \times g^{-1} \times h^{-1}$ (–0.78–3.35 mL $O_2 \times g^{-1} \times h^{-1}$; N=13).

DISCUSSION

Although we started with a reasonable number of individuals for statistical evaluation when investigating development of thermoregulation in juveniles, most of the juveniles did attain adulthood. This was most probably result of natural mortality, but we cannot exclude that our experimental procedure may have also an impact, because of the temporal separation of the mole-rat juveniles and increased disturbance of the family in the first two weeks after their

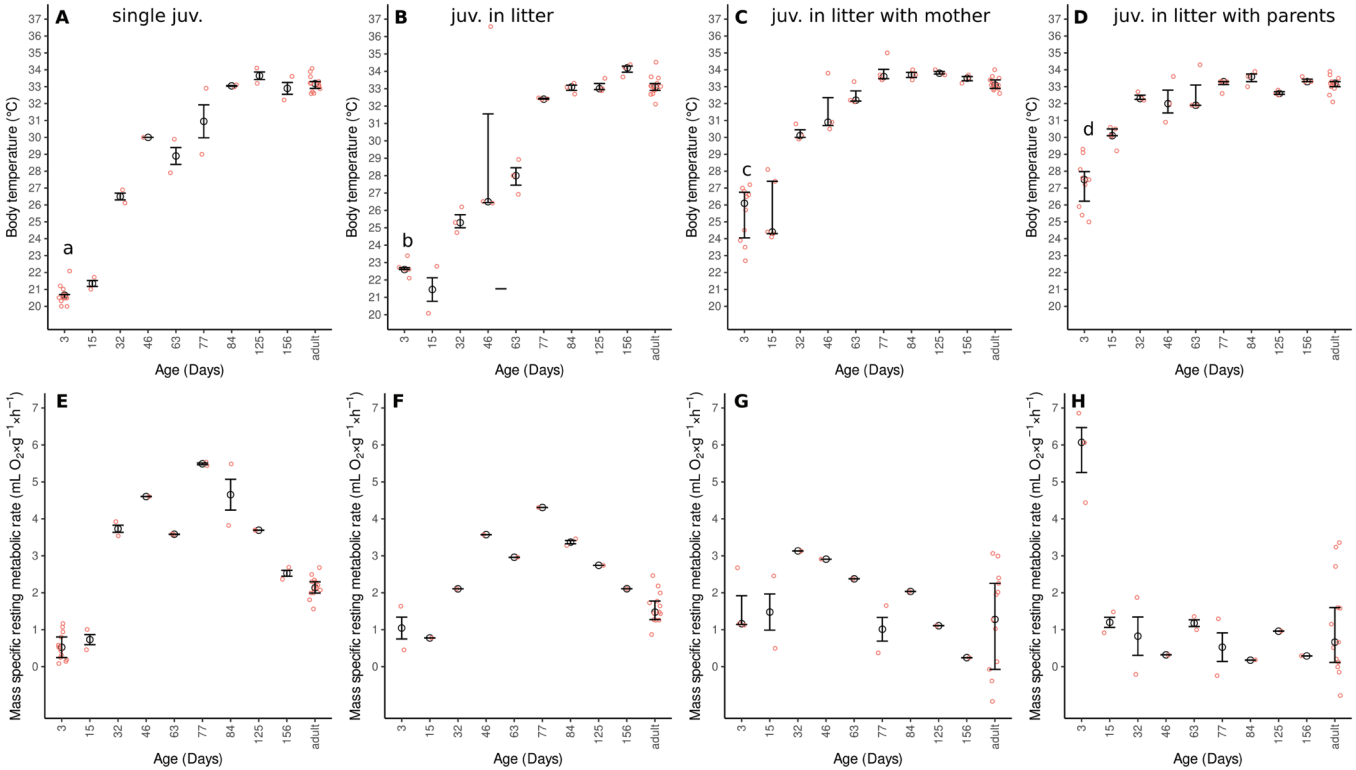

Fig. 2. Development of thermoregulation in juvenile *Fukomys darlingi* at 20 °C. Body temperature (T_b) and resting metabolic rate (RMR) in juveniles were measured under different social contexts: A, E – single juvenile, B, F – juveniles in litter, C, G – juveniles in litter with mother and D, H – juvenile in litter with both parents. Red circles represent individual data-points, median is represented by a black circle and 2nd and 3rd quartile range by whiskers. Differences among T_bs among social contexts in three-day juveniles are indicated by letters at the top of the graphs. Contexts not sharing a letter differed from each other ($p<0.05$). No statistical tests were performed on RMR of the litter due to their low count.

birth (N. C. Bennett, pers. comm.). Low sample sizes are notable for the RMR part of this study, because much of the data was collected for litters (social contexts 2, 3, and 4) and not for individuals as T_b measurements. In addition, the RMRs of the litters when measured with the parents may be loaded with large measurement errors which resulted in unrealistic RMRs in contexts (3) and (4). This is a consequence of the methodology employed which is the most apparent on RMRs of three-day juveniles measured in litter with both parents at 20 °C. These juveniles have very high RMRs (Fig. 2H). Thus, to obtain the RMR of a litter with the mother, we measured the RMR of the whole litter with the mother (A) and then we measured the RMR of the mother alone (B) and subtracted the latter from the former, i.e. A–B. Both A and B have very similar values because the majority of the measured mass is the mother's body mass and thus A–B is close to zero. Therefore, if the RMR of the mother changes between measurements A and B even by a little for any reason, this difference is then assigned to the litter, which may equate to an unrealistic litter RMR. Similar measurement errors are apparent in non-breeding adults measured with parents at 20 °C in which individual values are zero or very high in some cases, but the mean is realistic (Fig. 2G, H). It is therefore rather surprising that our results on the limited sample size on juvenile RMR are in general relatively internally coherent and corresponds with the T_b data. Despite the above-mentioned issues, we present T_b alongside RMR data as they complement one other and together report on thermoregulatory abilities of species under study.

Knowledge of the DEE gives a realistic estimate of energetic cost and on a longer timescale, but unlike the field metabolic rate, our approach does not involve energetically demanding activities as digging. On the other hand, this approach allows us to estimate DEE under controlled conditions, and in our case, under different thermal conditions. At 20 °C, the DEE was about 60% higher when compared to DEE at 30 °C. This is lower than the difference in RMR at the same T_as, which was found to be about 120% (Zemanová et al. 2012). Relatively smaller difference during the whole day measurement between two temperatures in our study is probably caused by the fact, that exercise heat may substitute thermoregulatory heat at the lower T_a (Chappell et al. 2004, Humphries & Careau 2011). Since all physical activities generate heat, this heat can be used to keep a stable T_b instead of being dissipated into the environment (Chappell et al. 2004). In this sense, activity of the animal fully or partially reduces the need to generate heat by thermogenesis, thus substituting thermoregulatory heat with exercise heat. Interestingly, Humphries & Careau (2011) modelled activity-thermoregulatory heat substitution in mammals and predicted that a running mammal of the size of *F. darlingi* is able to substitute a substantial part of its thermoregulatory heat by activity heat at 20 °C.

The proportion of DEE to BMR, known as the sustained metabolic scope (SusMS), is a measure of long-term energetic demands (Peterson et al. 1990). In our experiment, we observed that SusMS at 30 °C and 20 °C was 2.4 and 4.0, respectively, assuming a RMR of 0.76 mL $O_2 \times g^{-1} \times h^{-1}$ (Zemanová et al. 2012). This ranked SusMS of *F. darlingi* among the highest values observed in African mole-rats (range 1.4–3.2 kJ/day) (see Hart et al. 2022 for an overview). However, the referred values were obtained on free ranging mole-rats and thus cannot be compared to the results of our experiments carried out on isolated animals with *ad libitum* feeding.

In mammals, there are two classes of pups that can be inferred based on their development, which differ in the time when pups develop insulative fur, functional eyesight, locomotor abilities or ability to eat solid food, those being altricial or precocial (Vaughan et al. 2015). Precocial pups are usually born furred and keep high and stable T_b in a relatively wide range of T_a, with their mass specific metabolic capacity the highest during the first days after delivery.

Altricial pups are born sparsely furred, their postnatal development is slower, and the ability of self-sustained thermogenesis appears later and their mass specific metabolic rate reaches its peak later as well (Derrickson 1992, Vaughan et al. 2015). It is well known that pups of social African mole-rat including *F. darlingi* from Zimbabwe are altricial (Bennett et al. 1991, 1994, Bennett & Faulkes 2000) having slow rates of growth. Our findings confirm this also in *F. darlingi* from southern Malawi. Development of the thermoregulatory capacity of the Mashona mole-rat is slow and a presence of one or both parents is important for maintaining higher T_bs and reducing the cost for thermoregulation. Interestingly, low postnatal development is also confirmed by slow postnatal body mass increment of juveniles *F. darlingi* from Malawi, which is the lowest among bathyergids and ranks among the lowest in mammals (Šumbera et al. in press). Slow postnatal development and poor thermoregulatory capacity in *F. darlingi* from Zimbabwe is indicated also by the inability to keep stable T_b in T_a slightly below TNZ in adults (Bennett et al. 1993).

In both tested T_as, three and 15-days old juveniles performed similarly, having very low RMRs and being hypothermic. Contrary to the later stages, these juveniles were not able to thermoregulate by active thermogenesis at 20 °C and their RMR and T_b were lower at 20 °C than at 30 °C. In juveniles with extremely high surface to volume ratio, the endogenous heat production is clearly not sufficient to balance the thermal losses to the environment and to increase the T_b. The ability to increase T_b at 20 °C first appeared in the third or fourth week after birth, when the juveniles were around 20 g, well furred and consuming solid foods. The thermoregulatory ability of the juveniles appeared to improve with age. Based on our anecdotal data, juveniles were able to keep their normothermic T_b from the sixth week of their life at 30 °C and from the third month at 20 °C. The ability to keep a stable T_b developed in *F. darlingi* much later than in the solitary *C. talarum*, which thermoregulates well from the age of about one month (Zenuto et al. 2002), or in altricial mammals in general (Brück & Hinckel 1996). Interestingly, the highest MR at 30 °C was recorded in month old juveniles (about 2.7 mL $O_2 \times g^{-1} \times h^{-1}$; Fig. 2E, F). This is again much later than in *C. talarum*, in which the peak of RMR within TNZ in two weeksold juveniles. At 20 °C, the peak of RMR was observed in more than two month old juveniles (Fig. 2E), while in *C. talarum*, the peak was observed at the age of about one month (Zenuto et al. 2002, Cutrera et al. 2003). A high RMR is necessary to sustain a high heat generation capacity. When it is high, it should reflect a good thermoregulatory capability of the developing juvenile (Cutrera et al. 2003). For example, pups of laboratory mouse and rats are able to keep a stable T_b at 20 °C when older than two weeks (Lagerspetz 1966, Leon 1986). We can conclude that the onset and full development of thermogenetic capacity in *F. darlingi* is unusually delayed.

At 20 °C, juveniles in a litter did not increase their T_b (Fig. 2B) and neither increased their RMR (Fig. 2F). The inability of very young huddling juveniles to maintain high T_b at low T_a in the absence of adults has been previously described in *C. talarum* (Cutrera et al. 2003) and in laboratory rats (Alberts 1978, Sokoloff & Blumberg 2001). In these species, the ability to keep a high T_b at 20 °C is reported in six and five days old huddling juveniles, respectively (Alberts 1978, Cutrera et al. 2003).

The presence of a mother or both parents is important for thermoregulation and energy conservation in altricial pups in general (Newkirk et al. 1998, Cutrera et al. 2003, Luis et al. 2004, Schradin & Pillay 2005). We can confirm this beneficial function of adults for juveniles of *F. darlingi*. This is most apparent at 20 °C. At this T_a, RMR of juveniles older than one month measured with adults was much lower (Fig. 2G, H) when compared to single

juveniles (Fig. 2E, F). The presence of an adult in the nest prevents high energetic requirement and saves resources which may be allocated for other activities and processes such as growth. It also seems, that the presence of both parents makes social thermoregulation more effective than the presence of the mother alone (see Fig. 1G, H).

Slow development of juvenile thermoregulation may be an energetic conservation adaptation for energetically costly life in the subterranean environment. Limited nutritional resources are not invested in the development of thermoregulatory capacity since the subterranean environment is thermally very stable and predictable (Bennett et al. 1988, Burda et al. 2007) and growth and maturation are prioritized (Cutrera et al. 2003). Though mole-rat adults are capable of non-shivering thermogenesis (NST), such capacity is rather low ranging from 13 to about 60% of NST capacity predicted for a rodent of their size (Luna et al. 2021). Delayed ability to thermoregulate in juveniles may thus be a consequence of a relatively low NST capacity in adult mole-rats. This contrasts with precocial pups, which are able to generate heat by NST soon after birth (Derrickson 1992). Mole-rat pups thus depend mainly on their mother for heat. This probably saves their resources, which may then be allocated into growth, instead of thermoregulation during the presence of parents in the nest (Newkirk et al. 1998, Kauffman et al. 2003, Schradin & Pillay 2005, Scantlebury et al. 2006, Tauson et al. 2006). From a parental perspective, relatively low energetic investments into reproduction spread over a longer period of time may be especially important after establishing a new family, when usually only the founding pair is available to establish a burrow system which is an energetically very demanding activity (e.g. Luna et al. 2002, Zelová et al. 2010). In this sense, slow development of juveniles may thus allow energy reallocation into other processes (Braendle et al. 2011).

Our findings on the slow development of thermoregulation and the effects of the presence of other family members are, however, only preliminary and should really be confirmed on a larger sample size. Similar studies on other species of social mole-rats will be important to allow for generalization. Analogous studies on developmental thermoregulation in solitary African mole-rats would be very helpful in revealing benefits of sociality in African mole-rats. According to Bennett et al. (1991), there are some differences between juveniles of solitary and social species, such as the presence of fur in neonates and a faster body mass increment during the first months after the birth, although such difference may diminish if longer periods of postnatal development are considered (Šumbera et al. 2003). Unfortunately, solitary African mole-rats were rarely bred in captivity and this may heavily constrain commencement of such studies.

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APPENDIX

Daily energy expenditure (DEE) of *Fukomys darlingi* individuals at 30 and 20 °C. Individuals' ID, sex, breeding status (B=breeding, N=non-breeding) and body mass are given. Data are presented as mean±SD of three 24-hours measurement

ID	sex	breeding status	mass [g]	DEE 30 °C [mL $O_2 \times g^{-1} \times h^{-1}$]	DEE 20 °C [mL $O_2 \times g^{-1} \times h^{-1}$]	DEE 30 °C [kJ×day^{-1}]	DEE 20 °C [kJ×day^{-1}]
1673	♀	B	137.5	2.01±0.19	2.84±0.13	133.32±12.6	188.38±8.62
3565	♀	B	131.0	1.48±0.06	2.77±0.38	93.53±3.79	175.05±24.01
0000	♀	B	139.4	2.07±0.23	3.65±0.31	139.2±15.47	245.45±20.85
9516	♂	B	152.3	1.55±0.09	2.47±0.09	113.88±6.61	181.47±6.61
2884	♂	B	153.7	1.59±0.11	2.73±0.15	117.89±8.16	202.42±11.12
9953	♂	B	145.2	1.75±1.13	2.99±0.21	122.58±79.15	209.43±14.71
2332	♀	N	100.2	1.94±0.35	2.85±0.22	93.77±16.92	137.76±10.63
4036	♀	N	107.7	1.95±0.09	3.61±0.13	101.31±4.68	187.56±6.75
0815	♀	N	122.7	2.17±0.41	3.04±0.33	128.44±24.27	179.94±19.53
0190	♂	N	170.9	1.87±0.10	3.29±0.30	154.17±8.24	271.23±24.73
4396	♂	N	194.5	1.47±0.16	2.73±0.03	137.93±15.01	256.15±2.81
8653	♂	N	134.2	2.19±0.16	2.97±0.31	141.78±10.36	192.27±20.07
mean			140.8±25.8	1.84±0.25	2.99±0.34	124.98±16.98	203.09±23.09