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Social thermoregulation in the subterranean Mashona mole-rat (*Fukomys darlingi*): the role of socio-physiological effect

Master thesis

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Annotation:
Life underground is one of the most challenging tasks for animals. The subterranean environment is seasonally and diurnally stable and provides shelter from predators. On the other hand food is scarce, the cost of digging is very high and closed burrows create hypoxic and hypercapnic conditions. Thus the physiological and behavioral adaptations to save energy are very important for underground dwellers. Social thermoregulation is such behavioral adaptation. Additionally, it has been suggested that social animals suffer from "isolation stress" and that they decrease their metabolic rates when other family members are present = socio-physiological effect. In this study I measured the resting metabolic rates (RMR) of social Mashona mole-rats (*Fukomys darlingi*) in isolated individuals, pairs and groups of three to eight individuals. Measurements were carried out at two ambient temperatures, in the thermoneutral zone (TNZ; 30°C) to test the presence of a socio-physiological effect and below their TNZ (20°C) to test the effect of social thermoregulation. A socio-physiological effect was distinctive neither in pairs nor in larger groups. At temperature below the TNZ the Mashona mole-rat saved 21% of its energetic expense in pairs due to social thermoregulation. With an increase in group size, energetic savings rose up to four animals. In larger groups, social thermoregulation did not influence the energetic expenditure, possibly because Mashona mole-rat´s families naturally contain around four to five adults.

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Content:

1. Introduction

Social thermoregulation is the process of body heat conservation via active and close aggregation of animals (= huddling) (Prychodko 1958, Gilbert et al. 2010). Huddling leads to a smaller bodily surface area being exposed to the external environment through which heat is lost. As a result, huddling is responsible for lowering the total metabolic expenditure (Canals et al. 1989). In addition, this heat-conserving behavior raises the temperature of the ambient microclimate in confined spaces, such as nests (Andrews et al. 1987), thereby increasing the chances of survival in cold environments (Batchelder et al. 1983, Gilbert et al. 2008) and limiting the water loss (Russell 1972, Yahav and Buffenstein 1991).

Social thermoregulation is especially important for small mammals with less favorable (high) surface-to-volume ratios (Speakman 1997). There are many studies describing a decline in energetic expenditure in groups of small mammals (e.g. Pearson 1960, Górecki 1968, Fedyk 1971, Tertil 1972, Gilbert et al. 2010, Merritt and Zegers in press). For example, the African Four-Striped Grass Mouse (*Rhabdomys pumilio*) spent 20% less energy in larger groups (six to eight individuals) than in smaller groups (one to three individuals) at an ambient temperature (T_a) of 5°C, which is below its thermoneutral zone (TNZ). The authors also found that in free-living individuals, the daily energy expenditure (DEE) values were lower in natural group sizes (nine mice) than in groups reduced by half (Scantlebury et al. 2006). Similarly, white-tailed antelope squirrels (*Ammospermophilus leucurus*) consumed 34% less oxygen in groups of five individuals than solitary squirrels at T_a = 15°C (Karasov 1983). White-footed mice (*Peromyscus leucopus*) lowered their oxygen consumption by 27% in pairs at T_a = 5°C (Glaser and Lustick 1975). Effects related to energetic savings were also studied. Siberian hamsters (*Phodopus sungorus*) were shown to decrease their body mass during the winter, however, when the hamsters were grouped into fours, their body mass reduction was lower than when compared to isolated individuals (Jefimow et al. 2011).

Huddling is also essential for developing pups. Maternal contact or huddling with siblings enables them to maintain a stable body temperature (T_b) before they fully develop their thermoregulatory mechanisms (Cutrera et al. 2003, Zemanová 2010). For example, rabbit pups expended 40% less energy when huddled in groups of eight compared to isolated pups (Gilbert et al. 2007).

*Abbreviations: TNZ = Thermoneutral zone, T_a = Ambient temperature, RMR = Resting metabolic rate, DEE = Daily energy expenditure, $\dot{V}O_2$ = Oxygen consumption, $\dot{V}CO_2$ = Carbon dioxide production

Martin et al. (1980) suggested that there might be another factor involved in social thermoregulation in addition to decreasing surface-to-volume ratio. This factor is called a "socio-physiological effect" (Speakman and Rossi 1999) or "group effect" (Schlenker et al. 1981) or "psycho-physiological effect" (Martin et al. 1980). This phenomenon is related to the presence of other conspecific (mostly related) individuals in social species, such that they have a calming effect on the individual, resulting in less stress and therefore lower energy expenditure. Martin et al. (1980) tested this hypothesis on laboratory mice and Mongolian gerbils (*Meriones unguiculatus*). The authors found that individuals in spatially separated trios (the animals were tested simultaneously but prevented from physical contact by a wire mesh) had lower metabolic rates than isolated individuals, but not different from huddled trios in temperatures below TNZ. The highest energetic savings in separated trios were at T_a = 10°C. Mice conserved 16% of their energy and gerbils 52% (Tab. 5). In 1984 Luis C. Contreras repeated this experiment using the same species (Contreras 1984), but his results did not support the socio-physiological theory, nor did another study carried out on laboratory mice by Speakman and Rossi (1999).

Interestingly, Herreid and Schlenker (1980) observed a decline in the metabolism of stable pairs (pairs housed together for relatively long periods) compared to unstable pairs (individuals housed with a different partner every day), as well as isolated individuals of laboratory mice. The authors thus pointed out the importance of familiarity among huddled individuals. Additionally, in a setting with two interconnected metabolic chambers each with an isolated individual, the authors observed a significant decline in the metabolism of recipient mice – those mice downwind of the air stream, that received their "air" via the other mouse (donor). The authors suggested that some airborne factor may also influence metabolic rates. To exclude the possibility that the metabolic decline observed in recipient mice was caused by an airborne factor such as pheromones, Schlenker et al. (1981) tested anosmic individuals. Anosmic recipient mice still had lower metabolic rates than anosmic donors in the same experimental setting. In addition, the authors demonstrated that an increase in CO_2 concentration (from 0.05% to 0.2%) induced the decline in metabolism by 34%, so they concluded that CO_2 could be involved in the group effect in addition to a socio-physiological effect. However Speakman and Rossi (1999) did not confirm these conclusions. In their study, mice tested in pairs had an even higher metabolic rate than isolated individuals (mice tested separately were exposed to 0.22% of CO_2 content and paired mice to 0.44% of CO_2 - in the same CO_2 concentration range in which Schlenker et al. (1981) observed the suppression of metabolism). In the second experiment of the same

study, the authors exposed mice to concentrations of 0.12% CO_2 for isolated mice and 0.24% for pairs, levels within the boundaries between which the evident suppression was observed by Schlenker et al. (1981). Under these conditions the relative metabolic rate of paired mice was still higher than in isolated mice. In a third experiment, mice were placed in two interconnected chambers. Here, the mice metabolic rate was higher when a CO_2 absorber between the chambers was absent than in the situation when the CO_2 absorber was present and thus preventing CO_2 produced by the first mouse from reaching the mouse in the second chamber. The final metabolic rate was also higher than the sum of the solitary measurements. Thus, in this study neither an effect of socio-physiological factor nor the physiological suppression of metabolism mediated via CO_2 was observed.

The question of whether or not the socio-physiological effect of partner presence exists is not trivial. Metabolic values like resting metabolic rate or daily energy expenditure could be remarkably overestimated in social species if this factor is not taken in account, because animals could be under "isolation stress". As a result, many comparative studies, especially those based on comparison with solitary species (Lovegrove 1986, 1987, Haim et al. 2005, Sobrero et al. 2011, Zelová et al. 2007), using metabolic rates could be biased to some extent. It should be noted that the interspecific comparison of BMR across many species is very popular in various studies (see Hayssen and Lacy 1985, Lovegrove 2000 or McNab 2008).

It is well known that subterranean rodents have a generally low RMR as a physiological adaptation against overheating in humid burrows (McNab 1966) and/or as an adaptation which compensates for the high energetic costs of burrowing (Buffenstein 2000). A low RMR also reduces gas exchange in the environment with low O_2 and high CO_2 concentration (Arieli 1979). The interesting question is whether the metabolic rates of these rodents is further reduced during social thermoregulation, especially in TNZ. There are three studies testing this topic in African mole-rats (Bathyergidae, Rodentia). The naked mole-rat (*Heterocephalus glaber*) was shown to reduce its oxygen consumption at low ambient temperature in groups of two, four and eight (Withers and Jarvis 1980, Yahav and Buffenstein 1991). At T_a of 20°C, which is well below their TNZ (31-34°C) the mole-rats saved 43% of their energy expenditure in groups of two and 52% in groups of four when compared to isolated individuals. Interestingly, they also saved 27% in groups of four individuals within their TNZ (34°C). Despite the fact that this finding indicated the influence of a socio-physiological effect, the authors did not discuss this interesting result (see Withers and Jarvis 1980). In another study, Kotze et al. (2008) compared the energy conserved due to

huddling in two mole-rat species, the Damaraland mole-rat (*Fukomys damarensis*) and the Natal mole-rat (*Cryptomys natalensis*). At a lower ambient temperature (22°C), mole-rats conserved 24% and 26% respectively of their energetic expense in pairs, but in larger groups the authors found very high energy savings of 88% in groups of nine or ten individuals for *F. damarensis* and 79% in groups of ten for *C. natalensis*. Surprisingly, their measurements that were carried out at a temperature of 30°C, which is within the TNZ of both species (Damaraland mole-rat: 27-31°C see Lovegrove (1986) and Natal mole-rat: 30-31.5°C see Bennett et al. (1993b)), again showed very high energetic savings. When the metabolic rates of isolated and grouped individuals were compared, the graphic results showed that *F. damarensis* saved 23% energy expenditure in pairs and 89% in groups of 10 individuals and *C. natalensis* 19% in pairs and 81% in groups of 10 individuals (see Tab. 2 and 4). The definition of TNZ implies that the effect of social thermoregulation should not be apparent in this temperature range (Willmer et al. 2005). This would mean that the socio-psychological effect is very apparent and represents a substantial portion of metabolic savings in these mole-rat species.

Subterranean rodents possess many physiological adaptations related to their particular lifestyle niche (Buffenstein 2000, Nevo 1999). The hypoxic and hypercapnic environment in burrows (Nevo 1999, Burda et al. 2007) presents one of the most important physiological challenges. The previously mentioned low metabolic rate is linked to low ventilation and should result in reduced levels of CO_2 produced by mole-rats. That and the high tolerance of elevated CO_2 concentrations (physiological adaptations enabling greater acid tolerance) by underground dwellers (Buffenstein 2000) makes strictly subterranean rodents ideal model organisms for testing social thermoregulation and socio-physiological effects, especially whether high CO_2 concentrations do indeed influence metabolic rates.

In this study I tested the magnitude of energetic savings due to social thermoregulation in the social Bathyergid Mashona mole-rat (*Fukomys darlingi*) to find out if socio-physiological effect plays an important role in the ecophysiology of mole-rats. Measurements were taken at two ambient temperatures. Firstly, I tested the socio-physiological factor at an ambient temperature within its TNZ (30°C; TNZ for *F. darlingi* is 27−34°C for Mashona mole-rat of study population, Zemanová et al. 2012). Possible energetic savings in TNZ will not be connected with heat conservation but more likely with the calming effect of the group. Thus, if mole-rats in pairs and/or in bigger groups decrease their RMR in TNZ, it would be due to the socio-physiological factor. The second experiment was carried out to establish the energetic savings due to social thermoregulation at T_a below

TNZ, with the expectation that mole-rats will decrease their RMR with increasing group size as result of huddling. I chose 20°C because there should be an apparent difference in RMR compared to RMR at 30°C, because the species probably face this temperature in burrows in its natural environment (Bennett et al. 1988) and this T_a does not pose any risk to mole-rats (see Zemanová et al. 2012). I measured the RMR of isolated and paired individuals at both ambient temperatures. To ensure the most natural design, pairs consisted of breeders only. In both experiments, I added also non-breeders to create larger groups (three to eight individuals) to test how other family members influence the socio-physiological and social thermoregulation effects.

2. Materials and methods

2.1 Study animals

The Mashona mole-rat (*Fukomys darlingi*), previously known as *Cryptomys darlingi* (Kock et al. 2006), is a subterranean social African mole-rat (Bathyergidae, Rodentia) occurring in shrub habitats and Miombo woodland in the northern and eastern parts of Zimbabwe, probably in western Mozambique (Happold 2013) and southern Malawi. (The population from southern Malawi was originally thought to be a population of *Cryptomys hottentotus*, but recent cytochrom b and karyological analyses demonstrated that it is the northernmost population of *F. darlingi*; Van Daele, unpublished results in Zemanová et al. 2012). Families of this species can contain up to nine individuals with reproduction restricted to a single breeding pair. Reproduction is aseasonal (Bennett and Faulkes 2000, Bennett et al. 1994). This well-furred mole-rat was long believed to have strong poikilothermic traits (Bennett et al. 1993a). Later, it was shown that at least the Malawian population is able to maintain a stable body temperature at T_as ranging from 10 to 25°C and thus real homeotherms (Zemanová et al. 2012). Nonetheless, there might be differences between the Malawi and Zimbabwe populations. Mashona mole-rats from Zimbabwe are considerably smaller (45–90g compared to 78–222g in the Malawian population). Mole-rats from this population even show lethargy when exposed to low temperatures (18°C) for a couple of hours (Boyles et al. 2012). This phenomenon has not been observed in individuals from the Malawi population.

Measurements of oxygen consumption were conducted at the University of South Bohemia, Czech Republic. In this experiment 44 animals from ten families were used. Six individuals were trapped in Nsanje 16º55´S, 35º16´E, Southern Malawi in August 2005 and the rest of the animals were born in captivity. Mole-rats are housed in terrariums (80 × 60 × 50 cm). Peat is used as a substrate. In the breeding room we keep a stable temperature of 24 ± 1°C, air humidity 45 ± 5% and 12L:12D light cycle (light from 7.30AM to 7.30PM). Mole-rats are fed three times a week with carrots, potatoes and commercial rodent pellets. Filter-paper is provided as a nesting material and flower-pots and plastic tubes for shelter.

2.2 Metabolic measurements

Oxygen consumption and carbon dioxide production was analyzed in an open-flow system with the FoxBox Portable Oxygen/Carbon Dioxide Analysis System (Sable Systems International). Fresh air was pushed through the carbon dioxide (sodalime, Sigma-Aldrich) and water (Drierite, W.A. Hammond Drierite Co.) absorber into the metabolic chamber. Excurrent air from the chamber was again dried and its flow measured by FoxBox´s integrated flowmeter (normal temperature and pressure corrected). The air flow was then subsampled at a rate of 100-150 ml/min and pushed to the oxygen and carbon dioxide analyzers (for scheme of apparatus see Fig. 2, Supplementum). The apparatus was calibrated to the ambient oxygen concentration (20.95%) at the beginning and end of each measurement. Calibration with 99.99% N_2 (Linde, České Budějovice) for 0% CO_2 content was made on a monthly basis. Because of relatively long measurement the oxygen readings sometimes drifted, we improved the measuring apparatus during the course of this study to enable recalibration of the oxygen sensor without affecting the air flow and pressure in the metabolic chamber and thus without disturbing the mole-rats. This setup enabled calibration of the apparatus just prior to RMR measurements in the later phase of this study. Nevertheless, because some of the early oxygen measurements were unreliable, I decided not to use oxygen consumption data for RMR calculations. RMR was calculated only from CO_2 readings using the equation:

$$VCO_2 = flow * \frac{F_eCO_2 * 60}{w}$$

where VCO_2 is carbon dioxide production [ml CO_2 $g^{-1}h^{-1}$], flow is a downstream air flow rate [ml/min], F_eCO_2 is the excurrent fractional concentration of CO_2 and w is body mass of the measured individual(s).

Resting metabolic rate was expressed in units of oxygen consumption using the equation:

$$VO_2 = \frac{VCO_2}{RQ}$$

where VO_2 is oxygen consumption [ml O_2 $g^{-1}h^{-1}$], VCO_2 is carbon dioxide production [ml CO_2 $g^{-1}h^{-1}$], RQ is respiratory quotient. The RQ was calculated from our measurements (those with reliable oxygen calibration and was equal 0.85 (data not shown).

Oxygen consumption was calculated by equation:

$$VO_2 = flow * \frac{(F_iO_2 - F_eO_2) + F_iO_2 * (F_iCO_2 - F_eCO_2)}{(1 - F_iO_2)}$$

where VO_2 is oxygen consumption [ml O_2 $g^{-1}h^{-1}$], flow is a downstream air flow rate [ml/min], F_iO_2 is an input fractional concentration of O_2, FeO_2 is the excurrent fractional concentration of O_2, F_iCO_2 is an input fractional concentration of CO_2, $FeCO_2$ is the excurrent fractional concentration of CO_2.

The flow rate of air for one individual was 300ml/min. I increased the flow rate with increasing number of tested individuals in order to sustain a relatively equal CO_2 level in the chamber. The metabolic chamber was submerged in a water bath to ensure a stable temperature (ThermoHaake C10 and K15, Haake, Germany). The temperature was monitored using the thermal probe of a digital thermometer (Thermometer, Solid 898) inserted in the chamber. The activity of mole-rats was observed through transparent perplex lid of the chamber during recording. For one to three individuals, for groups of four and for groups of five to eight individuals metabolic chambers of total volume of 2.74 l, 3.9 l and 11.8 l respectively were used (Fig. 1, Supplementum).

An average measurement took from 60 to 180 minutes. Mole-rats were placed in the metabolic chamber and left for 30 minutes to acclimate (30 min was supposed to be sufficient, animals usually settle down within 5 – 15 minutes; see Kotze et al. 2008, Martin et al. 1980). After that period, the VO_2 and VCO_2 were recorded and the activity of mole-rats observed at the same time. I waited till every individual tested was at rest and appeared to be asleep and the O_2 with CO_2 level was stable. For all analyses 5 minutes interval of the lowest O_2 consumption and/or CO_2 production was used.

I used six family groups of two animals, one family of six animals, one of eight and two of nine animals for the experiment. Only adult individuals older than two years were measured. The mean weight of tested mole-rats was 137 ± 29 g (78 – 222 g). The weights of the breeding females were checked in order not to measure them in advanced pregnancy. Mole-rats were food-deprived at least 3 h prior to measurement so as to ensure a post-absorptive state (Nespolo et al. 2003). Each animal was tested individually and then compared with measurements in pairs. To ensure a natural scenario, measurements of paired mole-rats were carried out using breeding males and females from the same family. Additionally, I tried to examine the influence of increasing group size on the socio-physiological effect and social thermoregulation, even though I had to use a number of individuals more than once (e.g. Kotze et al. 2008) because of the relative shortage of animals we have. To the breeding pair I added in random order non-breeding family members and created group sizes of three to eight individuals. There was no repetition of

non-breeding individuals in any particular group size. However, the same individual could have been used in bigger groups as well (Tab. 3 and 4, Supplementum). I am aware of the fact that this design is not ideal. The ideal method is to test different individuals in each group size. Unfortunately, to have at least 20 measurements in each group size and test each individual only once I would need 720 mole-rats. This is beyond the capabilities of any mole-rat facility. Thus I consider my results, especially those in larger group sizes, as tentative. However, because metabolic rate is influenced mainly by body mass (Hayssen and Lacy 1985, Zelová et al. 2007), I suppose that the observed values would be comparable if each mole-rat was used only once in the experiment. For my study I used all 44 adult individuals in our stock.

2.3 Statistical analyses

All statistical analyses were performed in STATISTICA 10 (Statsoft Inc.). Following tests were performed on data obtained at 30 and 20°C. For clearer and comparable representation of the differences in RMR of individuals and RMR of pairs or groups I used energetic savings parameter in % according to the formula:

$$\text{energetic savings}[\%] = 100 - \frac{\text{RMR of } AB_P}{(\text{RMR of } A_I + \text{RMR of } B_I)} * 100$$

where (AB_P) is the resting metabolic rate in pairs and (A_I) and (B_I) are the resting metabolic rates of those particular animals measured individually. These energetic savings data were not statistically evaluated.

2.3.1 Pairs

For the evaluation of socio-physiological or social thermoregulation effect in pairs of breeding mole-rats the dependent t-test for paired samples was used. In this test I compared the RMR of paired individuals measured together versus the sum of RMR of both animals measured individually. Because I have conducted this test together with six other tests on related dataset (see below), I corrected the single sided p-value by Holm-Bonferonni method for seven repeated tests.

2.3.2 Larger groups

For each family and group size I have calculated the mean RMR values of all metabolic measurements and only these means were statistically compared. Mean RMR values of groups > 2 were pair-wise compared by t-test for paired samples to mean RMR values of all isolated members of respective families. This resulted into balanced dataset not differing between breeders and non-breeders. I corrected single sided p-value by Holm-Bonferonni method for seven repeated tests (see previous section for explanation).

3. Results

3.1 Test of socio-physiological effect

3.1.1 Pairs

The average RMR in TNZ (T_a = 30°C) in isolated adult individuals (breeders and non-breeders) was 0.73 ± 0.2 ml O_2 $g^{-1}h^{-1}$ (Tab. 1, Supplementum). Mole-rats in pairs consisting of a breeding male and breeding female had per capita lower RMR (0.70 ± 0.11 ml O_2 $g^{-1}h^{-1}$) than the same individuals isolated (0.77 ± 0.25 ml O_2 $g^{-1}h^{-1}$) (Fig. 1). This difference was not significant (t-test, t = 1.3, p = 0.58, n = 10). The amount of saved energy in TNZ was 5.13 ± 18.38%.

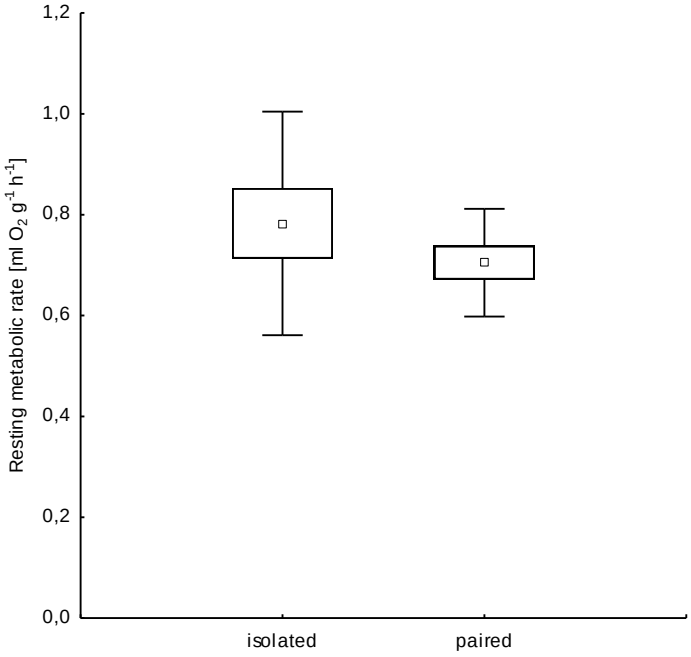

Fig. 1. Resting metabolic rate (RMR) [ml O_2 $g^{-1}h^{-1}$] of isolated breeding individuals (N=20) and pairs (N=10) measured in TNZ (30°C). ▫ Mean, □ SE, ⌶ SD.

3.1.2 Larger groups

There was no significant difference between the metabolic rates of isolated individuals and groups of different sizes (Tab. 1). It is possible to see that in groups of three or four the RMR is lower, but not significantly so (Fig. 3). Energetic savings in groups of three or four was low. Mole-rats in groups of five to eight individuals had even higher metabolic rates than isolated mole-rats (see Tab. 2).

Tab. 1. Differences in RMR of isolated individuals and groups of three to eight mole-rats in T_a = 30°C. $Mean_1$ is mean RMR value for isolated individual from the given family, $Mean_N$ is mean RMR value for particular group size, t is t-value, N is number of compared families for given group size, p-values are single sided and Holm–Bonferroni corrected.

Comparison	$Mean_1$ ± S.D.	$Mean_N$ ± S.D.	t	N	p
1 vs. 3	0.68 ± 0.08	0.65 ± 0.07	0.77	4	0.25
1 vs. 4	0.68 ± 0.08	0.63 ± 0.04	1.3	4	0.56
1 vs. 5	0.68 ± 0.08	0.78 ± 0.13	-2.88	4	0.22
1 vs. 6	0.68 ± 0.08	0.81 ± 0.13	-1.57	4	0.63
1 vs. 7	0.70 ± 0.09	0.79 ± 0.24	-1.07	3	0.39
1 vs. 8	0.70 ± 0.09	0.82 ± 0.22	-1.35	3	0.47

Tab. 2. Energetic savings [%] in TNZ of four African mole-rat species in groups of different sizes. Metabolic rate of isolated individual is 100%. References: [1]This study, [2]Withers and Jarvis (1980), [3]Kotze et al. (2008).

	Number of individuals in group										
Study species	2	3	4	5	6	7	8	9	10	15	T_a (°C)
F. darlingi[1]	5±18%	3±19%	5±19%	-19±28%	-19±22%	-14±26%	-15±17%	–	–	–	30
H. glaber[2]	-8%	–	27%	–	–	–	–	–	–	–	34
F. damarensis[3]	23%	36%	59%	57%	39%	89%	57%	84%	89%	–	30
C. natalensis[3]	19%	50%	52%	69%	–	71%	52%	–	81%	71%	30

3.2 Test of social thermoregulation effect

3.2.1 Pairs

The average RMR below the TNZ (T_a = 20°C) in isolated individuals (breeders and non-breeders) was 1.46 ± 0.34 ml O_2 $g^{-1}h^{-1}$ (Tab. 1, Supplementum). Resting metabolic rates below the TNZ for pairs of a breeding male and breeding female was 1.04 ± 0.24 ml O_2 $g^{-1}h^{-1}$, which was significantly lower than the RMR of 1.39 ± 0.35 ml O_2 $g^{-1}h^{-1}$ for isolated breeding mole-rats (t-test, t = 3.2, p = 0.03, n = 10) (Fig. 2). The value of energetic savings was 21.31 ± 24.59%.

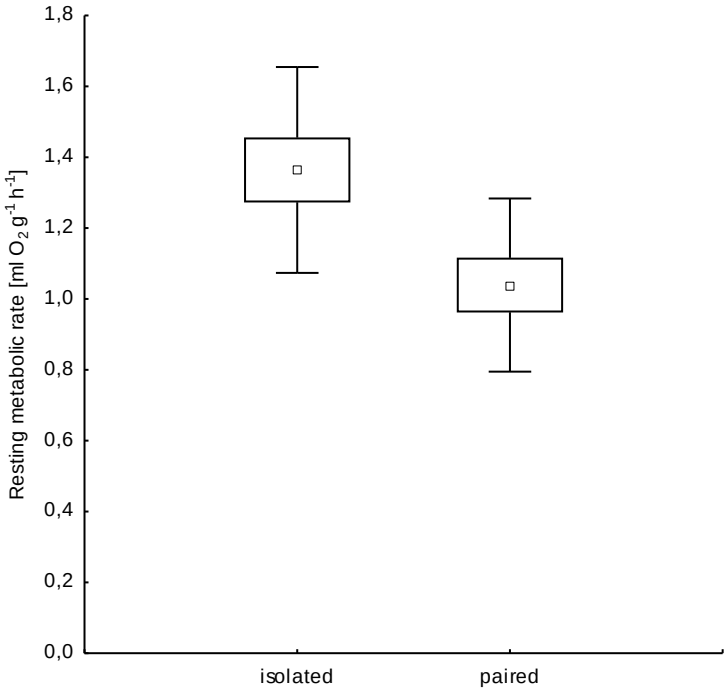

Fig. 2. Resting metabolic rate (RMR) [ml O_2 $g^{-1}h^{-1}$] of isolated individuals (N=20) and pairs (N=10) measured below TNZ (20°C). ▫ Mean, □ SE, ⌶SD. RMR of isolated mole-rats is statistically higher than that of isolated ones (p=0.03).

3.2.2 Larger groups

Metabolic rates of isolated mole-rats were significantly higher than the metabolic rates of groups of three or four mole-rats (see Tab. 3). Metabolic rates of groups of five to eight were not different from those of isolated individuals (Tab. 3, Fig. 3). Three mole-rats grouped together saved 26.69 ± 13.93% of energy expenditure, four individuals 32.27 ± 11.83% and six individuals 9.78 ± 28.74%. However, mole-rats in groups of five, seven and eight expended more energy than isolated individuals (see Tab. 4).

Tab. 3. Differences in RMR of isolated individuals and groups of three to eight mole-rats in T_a = 20°C. $Mean_1$ is mean RMR value for isolated individuals from the given family, $Mean_N$ is mean RMR value for particular group size, t is t-value, N is number of compared families for given group size, p-values are single sided and Holm–Bonferroni corrected.

Comparison	$Mean_1$ ± S.D.	$Mean_N$ ± S.D.	t	N	p
1 vs. 3	1.53 ± 0.11	1.06 ± 0.10	5.05	4	**0.038***
1 vs. 4	1.53 ± 0.11	0.99 ± 0.10	6.83	4	**0.022***
1 vs. 5	1.53 ± 0.11	1.54 ± 0.10	-0.29	4	0.39
1 vs. 6	1.53 ± 0.11	1.32 ± 0.30	1.14	4	0.68
1 vs. 7	1.49 ± 0.05	1.45 ± 0.07	0.68	3	0.86
1 vs. 8	1.49 ± 0.05	1.44 ± 0.18	0.39	3	0.73

Tab. 4. Energetic savings [%] below TNZ of four African mole-rat species in groups of different sizes. Metabolic rate of isolated individual is 100%. References: [1]This study, [2]Withers and Jarvis (1980), [3]Kotze et al. (2008).

	Number of individuals in group										
Study species	2	3	4	5	6	7	8	9	10	15	T_a (°C)
F. darlingi[1]	21±25%	27±14%	32±12%	-4±13%	10±29%	-0.6±10%	-0.3±14%	–	–	–	20
H. glaber[2]	43%	–	52%	–	–	–	–	–	–	–	20
F. damarensis[3]	24%	35%	68%	61%	54%	87%	72%	88%	88%	–	22
C. natalensis[3]	26%	40%	49%	72%	–	72%	60%	–	79%	75%	22

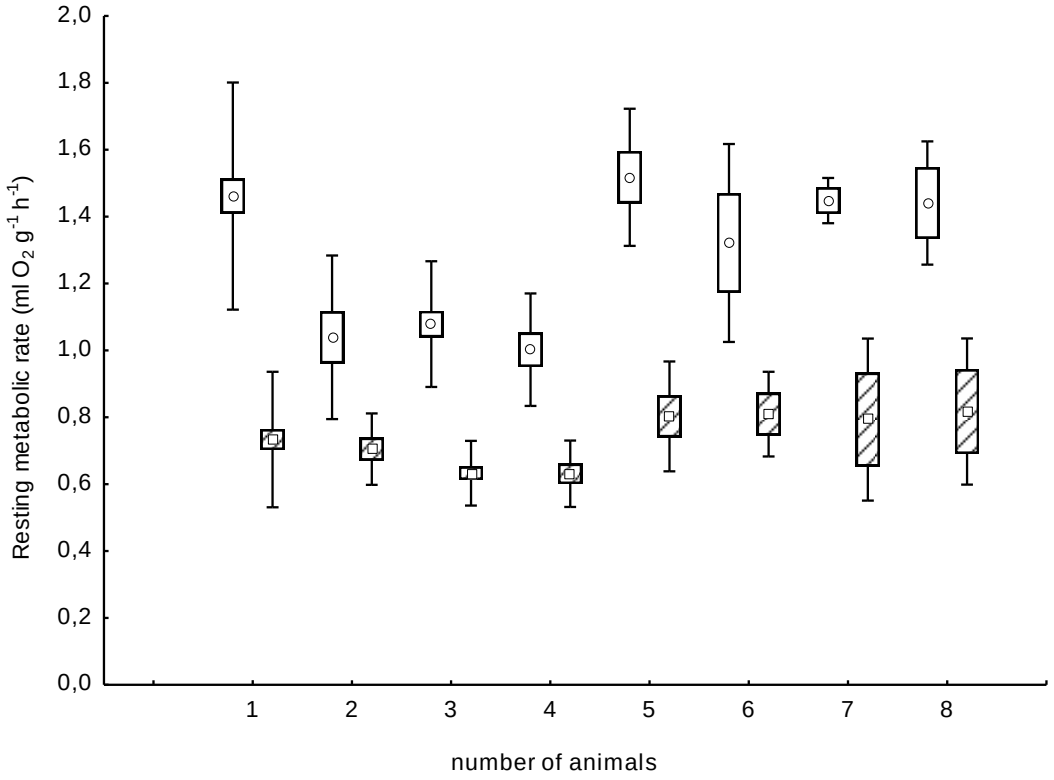

Fig. 3. Resting metabolic rate [ml O_2 $g^{-1}h^{-1}$] of isolated individuals (N=44), pairs (N=10), group of three (N=24), group of four (N=11), five (N=7), six (N=4), seven (N=3) and eight (N=3) individuals. Hatched (▨) is metabolic rates in TNZ (30°C) and blank (▯) below TNZ (20°C); ▫ Mean, □ SE, ⌶ SD. (This figure was not used in statistical analyses).

4. Discussion

In my study, tested individuals of the social bathyergid, the Mashona mole-rat, did not significantly reduce their metabolic rates either in pairs or larger groups at T_a within the TNZ. A socio-physiological effect was thus not remarkable in this subterranean rodent. At T_a below TNZ, the paired individuals had significantly lower metabolic rates than isolated ones. The energetic savings increased with increasing group size but only up to the group size of four individuals. It seems that social thermoregulation is effective only in small groups of the Mashona mole-rat.

As expected, the RMR of isolated Mashona mole-rats in their TNZ was 25.3% lower than predicted by the curve for rodents RMR = 4.98 $M^{-0.331}$ ml O_2 $g^{-1}h^{-1}$ (Hayssen and Lacy 1985). This result confirms that subterranean rodents have a lower RMR than most other rodents or mammals in general (Kleiber 1961). Mashona´s RMR was very similar (6.8% lower) to the value predicted by the subterranean rodent curve RMR = 3.79 $M^{-0.322}$ ml O_2 $g^{-1}h^{-1}$ (Lovegrove 1986). The same value of RMR in *F. darlingi* (0.76 ± 0.20 ml O_2 $g^{-1}h^{-1}$) was detected by Zemanová et al. (2012). However, Bennett et al. (1993a) observed a remarkably higher RMR in this species (0.98 ± 0.14 ml O_2 $g^{-1}h^{-1}$). This is another indication that there are some differences between the Malawi and Zimbabwe populations of the Mashona mole-rat (see above).

4.1 Socio-physiological effect

Generally, mammals do not necessarily have to belong to a social species to profit from social thermoregulation, because even solitary species or loosely social species may engage in communal nesting to reduce their thermoregulatory costs (Williams et al. 2013). On the other hand, the conditions of socio-physiological effect are stricter than simple aggregation. In my opinion, in order to provoke so-called "isolation stress", the tested species should be social or the tested individuals should be familiar with each other. Herreid and Schlenker (1980) showed that laboratory mice housed together over six days had reduced metabolic rates by 15% when compared with unstable pairs (new partners) (Tab. 5). Therefore, I studied a highly social species and measured groups that always consisted of individuals from the same family.

Mashona mole-rat in pairs consisting of a breeding male and female conserved only 5% of their energetic expenditure compared to isolated individuals in the TNZ. This result is in agreement with several other studies. For example, the bank voles (*Myodes glareolus*) had

the same metabolic rates in isolated individuals and in pairs in TNZ (30°C) (Gebczyński 1969) (Tab. 5). Neither Speakman and Rossi (1999) found any support for a socio-physiological effect on the metabolism of laboratory mice, because paired mice had higher metabolic rates than the sum of their solitary measurements in the TNZ. However, the mice in that experiment were housed separately before the experiment and were thus probably unfamiliar with each other, which could be the reason why the "calming effect" of the group was not observed. In addition, the rise in metabolic rate in pairs was caused by increased activity of the mice while in pairs as the authors suggested. Naked mole-rats (*Heterocephalus glaber*) also did not show metabolic decline in pairs compared to isolated individuals at 34°C, which is in the range of their TNZ (Withers and Jarvis 1980) (Tab. 2). On the contrary, Damaraland and Natal mole-rats saved a high amount of energy expenditure in pairs in the TNZ. Damaraland mole-rats saved 23% and Natal mole-rats 19% of energy expenditure in pairs compared to isolated individuals (Kotze et al. 2008) (Tab. 2). A similar amount of energetic savings in TNZ was described in the lesser mouse-lemur (*Microcebus murinus*), because pairs saved 17-20% of energy (Perret 1998) (Tab. 5).

It was surprising, that a socio-physiological effect was not apparent in larger groups of Mashona mole-rats. After adding non-breeders, three individuals saved only about 3% of metabolic expense and four individuals about 5%. Individuals in groups of five to eight had relatively higher metabolic rates compared to isolated individuals. It seems that no relevant socio-physiological effect exists in Mashona mole-rats (at least in the population studied in this thesis). The same conclusion came from the work of Contreras (1984). Laboratory mice and Mongolian gerbils (*Meriones unguiculatus*) did not alter their metabolic rates in groups of three individuals in their TNZ (30°C) compared to single individuals. Additionally, when mice and gerbils were measured in settings of three individuals separated by wire mesh, they had the same metabolic rates as animals measured individually, but higher than groups of three animals, which huddle together (Tab. 5). Unfortunately, the author used only eight male white mice and six male gerbils. In addition, tested groups consisted only of males is probably not the ideal setting for testing the socio-physiological effect, because such social units are not natural. Similarly, bank voles did not save any metabolic energy in groups of three and five compared to isolated individuals in TNZ (Gebczyńsky 1969). On the contrary, their metabolic rates were even higher. Nevertheless, the author admitted, that the measurements did not usually include periods of sleep or rest, so the increase in metabolism in larger groups could have been caused by increasing activity in relatively small chamber (Gebczyńsky 1969) (Tab. 5).

On the contrary, a socio-physiological factor was observed in laboratory mice and gerbils (*Meriones unguiculatus*) by Martin et al. (1980) (see above). Interestingly, at T_a = 20°C, trios of mice separated by mesh had higher metabolic rates than both huddled trios and single individuals. The authors attributed this result to a possible increase in activity at that ambient temperature. However, the tested mice were housed individually before the measurements and this could have influenced the results (the metabolism of mice could have been raised by social interactions between unfamiliar individuals). This explanation is supported by the fact that gerbils housed in trios prior to measuring - thus familiar with each other - showed lower metabolic rates compared to isolated individuals and similar metabolic rates compared to huddled trios at all ambient temperatures. Yahav and Buffenstein (1991) as well as Withers and Jarvis (1980) observed a metabolic decline in larger groups of naked mole-rats in TNZ. Damaraland mole-rats and Natal mole-rats also reduced their metabolic rates with increasing number of individuals in groups in TNZ (Kotze et al. 2008) (see Tab. 2).

Tab. 5. Energetic savings related to possible socio-physiological factor [%] in groups of several small mammal species. [1]100% is the metabolic rate of isolated individual measured at given temperature, [2]100% is the metabolism of individuals that were separated with wire, that prevent the huddling (separated trios), [3]100% is the metabolism of unstable pairs (animals housed separately before the experiment).

Species	Ambient temperature	N individuals in group	Energetic savings	References
Myodes glareolus	T_a = TNZ (30°C)	2	-5% [1]	*Gebczyński 1969*
		3	-1% [1]	
		5	-22% [1]	
Microcebus murinus	T_a = TNZ (25°C)	2	17% [1]	*Perret 1998*
		3 or 4	22% [1]	
laboratory mouse	T_a = 5, 12.5, 20, 30°C	3	30, 31, 21, 0% [2]	*Contreras 1984*
Meriones unguiculatus	T_a = 5, 12.5, 20, 30°C	3	29, 32, 23, 6% [2]	
laboratory mouse	T_a = 10, 15, 20°C	3	8, 10, 31% [2]	*Martin et al. 1980*
Meriones unguiculatus	T_a = 10, 15, 20, 25°C	3	-6, -6, -5, -3% [2]	
laboratory mouse	T_a = 25°C	stable pairs	15% [3]	*Herreid & Schlenker 1980*

I tried to meet all the requirements for the optimal testing of the socio-physiological effect. The experiments were run on familiar individuals from the same family and I measured groups where the breeding pair was present to ensure the most natural conditions. I also increased the flow rate when increasing number of individuals in the metabolic chamber, thus limiting the variability in CO_2 concentration. Finally, I also measured the metabolic rate of mole-rats at total rest. Nevertheless, I did not observe a significant decline

in metabolism due to a socio-physiological effect. There might be several reasons for this phenomenon. The family members can exhibit a desynchronized activity of the family as was observed in nature in Ansell´s Mole-rat (*Fukomys anselli*) (Šklíba et al. submitted). Mole-rats may have desynchronized activity in order to keep some members awake to patrol within the burrow system. A similar pattern has been observed in bats. When the colony of the great fruit-eating bat (*Artibeus lituratus*) was sleeping there was at least one bat keeping watch to potentially alert the colony (Muñoz-Romo 2006). Secondly, the variability of RMR measurements was too high and thus more trials and new families are needed to reveal the slight nuances of metabolism in groups and individuals.

The question of a potential influence of socio-physiological effect on RMR in social species is still open. As shown above, different studies have produced contradicting results. In addition, studies focused on this topic are very rare to provide any conclusive results. As mentioned above, the accuracy of determining the metabolic values (i.e. RMR) is very important as many comparative studies use this value for comparing a wide range of animal species, including social and solitary. Also, when I used a different statistical approach the socio-physiological effect was significant in groups of three individuals (Tab. 2, Supplementum). Different approaches to statistical evaluation of metabolic data for group sizes > 2 were considered including linear mixed models, but most statistical methods require independent data, which I was not able to obtain for reasons stated in the end of section 2.2. Modeling by linear mixed models was not used because there were no repeated measurements within one particular group and the number of random factor was too high. Thus I had to use a test where only one value for each family and group size was evaluated. This reduced the observations and significantly decreased the strength of the test. Clearly, further measurements are needed. Nevertheless, at this point it seems that Mashona mole-rats from Malawi do not use the socio-physiological factor in order to lower their metabolic rates when in groups.

4.2 Test of social thermoregulation effect

The expected increase in RMR at T_a = 20°C was caused by the increased energetic costs of thermoregulation. The resting metabolic rate at this temperature was double compared to RMR in TNZ. The subterranean environment is low in productivity and the energetic cost of digging in order to get food is very high (c.f. Zelová et al. 2010), therefore mole-rats cannot waste much energy when the temperature falls. Social thermoregulation is one of the most important mechanisms that enable mammals to conserve energy. Mashona mole-rats saved

21% of their energetic costs in pairs. That is comparable to savings in other small rodents in T_a below TNZ. For example, the striped field mouse (*Apodemus agrarius*) decreased its metabolism by 17% in pairs at 20°C (Górecki 1969), Townsend's Vole (*Microtus townsendii*) by 12% in pairs at 18.5°C (Andrews et al. 1987) and the bank vole (*Myodes glareolus*) reduced its metabolism by 22% in pairs at 20°C (Gebczyński 1969). The effect of social thermoregulation in pairs was also similar in two mole-rats species, *F. damarensis* and *C. natalensis*, as they reduced their metabolic rates by 24% and 26% respectively in groups of two at 22°C (Kotze et al. 2008) (Tab. 4). I can thus conclude that *F. darlingi* can reduce its energetic expenditure due to social thermoregulation in pairs as effectively as other small rodents and similar-sized hairy mole-rats below the TNZ. On the other hand, social thermoregulation in paired naked mole-rats was more effective than Mashona mole-rats, because *H. glaber* saved 43% of its energetic costs (Withers and Jarvis 1980) (Tab. 4). Naked mole-rats lack an isolation layer of fur and are non-endothermic. Social thermoregulation in this species not only saves energy, but also ensures that naked mole-rats are able to regulate their body temperature and thus increase their homeothermy (Yahav and Buffenstein 1991). It is thus understandable that in the naked mole-rat, social thermoregulation is more important in enabling the reduction of metabolism even more so than other small rodents.

After other family members were measured with the breeding pair in my study, the effectiveness of social thermoregulation rose in groups of up to four individuals (Fig. 3). Mole-rats in groups of three and four decreased their RMR by 27% and 32% respectively. This trend is in agreement with the theory that the effect of social thermoregulation increased with increased number of individuals in the group (e.g. Fedyk 1971, Trojan and Wojciechowska 1968). The same trend was observed in other mole-rat species (Withers and Jarvis 1980, Kotze et al. 2008). Nevertheless, social thermoregulation of other mole-rat species was far more effective when the group size increased (Tab. 4). The social thermoregulation of Mashona mole-rat in groups of five to eight did not influence individual metabolic rates.

At 20°C, social thermoregulation in Mashona mole-rat seems to be effective only in smaller groups (two to four), even though at this T_a the tested individuals were huddled in all measurements. Sometimes they created two huddling groups, but mostly just one. There might be several explanations why I did not observe RMR decrease in groups bigger than four. First of all, the more individuals are measured at once, the more difficult it is to get records of the “deepest” sleep of all individuals in one short period. Therefore, I might have

been simply unable to measure the real RMR value of the group. The second factor, which may complicate measurements of RMR in larger groups, is the desynchronized activity of the family as previously mentioned. Thirdly, mole-rats in larger groups could be more stressed by the presence of more family members. Mashona mole-rats in the wild have a family size about 5 – 9 individuals (Bennett et al. 1994). Nevertheless, such a family usually consists of about half adults and half adolescents including pups. For measurements of a large group size I used up to eight fully grown individuals, so it could mean that there was some tension, because such a family composition is not so natural (Bennett et al. 1994). A similar trend was observed in the European common vole (*Microtus arvalis*). Voles decreased their metabolic rates with increasing number of huddling individuals up to a group size of six. In larger groups metabolic rates increased. Nevertheless, in contrast to Mashona mole-rats, voles in bigger groups still saved a considerable amount of energy when compared with isolated individuals (Trojan and Wojciechowska 1968).

4.3 Damaraland and Natal mole-rats

It should be mentioned that there was a striking discrepancy between my study and the study on social thermoregulation in two mole-rat species (Kotze et al. 2008). After I compared the energetic savings of Mashona mole-rats with two species with similar body weights *F. damarensis* and *C. natalensis*, published by Kotze et al. 2008 (Tab. 2 and 4) it was possible to see that the metabolic savings in temperature below the TNZ (T_a = 22°C) in the two latter species were very high. The graphic results showed that *F. damarensis* spend 35% less energy in groups of three individuals and 68% less in groups of four, *C. natalensis* lowered its metabolic rate by 40% and 49% at groups of three and four. Moreover, the energetic savings increased with the group size reaching savings of 88% and 75% respectively! What is more surprising is the fact that the energetic savings in their TNZ (T_a = 30°C) are similarly high. Damaraland mole-rats in their TNZ saved 36% of metabolic energy at group of three, 59% at group of four and the savings went up to 89% in groups of seven and ten animals! Very similar values were found for Natal mole-rats. This result would mean that a socio-physiological factor is extremely effective in these two species. The authors increased the flow rate with increasing number of animals and therefore a reduction in metabolism due to a higher amount of CO_2 is not probable. It should be also mentioned that the metabolic rates in groups at two T_as decreased up to 0.09 ml O_2 $g^{-1}h^{-1}$ in T_a = 22°C and 0.05 ml O_2 $g^{-1}h^{-1}$ in T_a = 30°C. These values are not known for any mammals (only in mammals during hibernation see Tab. 1 in Geiser 2004). Further, these values are more typical for large insects (see Davis et al. 2000). It seems likely that values of energetic savings are overestimated and that authors made some mistake or computational error.

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6. Supplementum

Tab. 1S. Mean resting metabolic rate RMR [ml O_2 $g^{-1}h^{-1}$] and S.D. in and below the TNZ of individuals and groups of two to eight.

	N	RMR at 30°C	Range at 30°C	RMR at 20°C	Range at 20°C
isolated individual	44	0.73 ± 0.20	0.41-1.65	1.46 ± 0.34	0.75-2.16
pair	10	0.70 ± 0.11	0.57-0.90	1.04 ± 0.24	0.68-1.43
group of three	24	0.63 ± 0.10	0.47-0.86	1.08 ± 0.19	0.76-1.60
group of four	11	0.63 ± 0.10	0.47-0.81	1.00 ± 0.17	0.73-1.35
group of five	7	0.80 ± 0.16	0.54-0.99	1.52 ± 0.21	1.31-1.83
group of six	4	0.81 ± 0.13	0.65-0.96	1.32 ± 0.30	0.94-1.58
group of seven	3	0.79 ± 0.24	0.52-0.97	1.45 ± 0.07	1.40-1.53
group of eight	3	0.82 ± 0.22	0.59-1.02	1.44 ± 0.18	1.23-1.58

Tab. 2S. Mann-Whitney U test for groups of *F. darlingi* at TNZ. For this statistical analysis all measurements were used (not only mean values for the family). Socio-physiological effect is significant in group of three mole-rats, p-values are Bonferroni corrected. Please note that one individual enters the test repeatedly which violates the presumptions of the test.

	Z	N(1)	N(X)	p
1 vs. 2	0,17	44	10	3.04
1 vs. 3	2,52	44	24	0,041*
1 vs. 4	2,07	44	11	0,13
1 vs. 5	-1,30	44	7	0.68
1 vs. 6	-1,21	44	4	0.79
1 vs. 7	-0,63	44	3	1.85
1 vs. 8	-0,72	44	3	1.65

Fig. 1S. Metabolic chamber (11.8 l) submerged in the water bath.

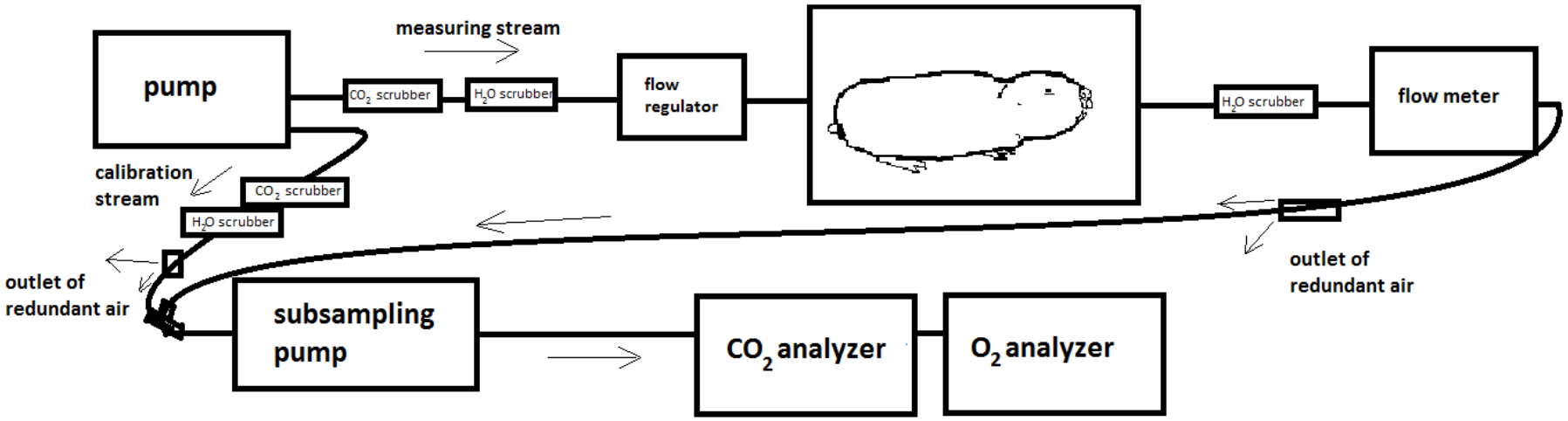

Fig. 2S. Schematic projection of the metabolic measurement apparatus.

Tab. 3S. Oxygen consumption (VO_2) [ml O_2 $g^{-1}h^{-1}$] at 30°C for isolated individuals VO_2 (1) and grouped animals of group size 2 to 8 (VO_2 (2 - 8)). Animals were from ten families (F1 - F10), A is a breeding male, B is a breeding female, C-I are non-breeders.

Family	Animal	VO_2 (1)
F1	A	1,65
	B	0,78
F2	A	0,66
	B	0,67
	C	0,72
	D	0,62
	E	0,42
	F	0,71
	G	0,41
	H	0,61
	I	0,55
F3	A	0,48
	B	0,71
	C	0,58
	D	0,71
	E	0,66
	F	0,70
F4	A	0,54
	B	0,82
	C	0,84
	D	0,54
	E	0,67
	F	0,61
	G	0,99
	H	0,87
	I	0,76
F5	A	0,62
	B	0,61
	C	0,74
	D	0,80
	E	0,82
	F	0,63
	G	0,85
	H	1,03
F6	A	0,64
	B	0,63
F7	A	0,89
	B	0,61
F8	A	0,73
	B	0,93
F9	A	0,85
	B	0,95
F10	A	0,93
	B	0,73

Family	Animal	VO_2 (2)
F1	AB	0,78
F2	AB	0,74
F3	AB	0,57
F4	AB	0,59
F5	AB	0,74
F6	AB	0,58
F7	AB	0,67
F8	AB	0,68
F9	AB	0,78
F10	AB	0,90

Family	Animal	VO_2 (3)
F2	ABC	0,66
	ABD	0,61
	ABE	0,58
	ABF	0,69
	ABG	0,47
	ABH	0,52
	ABI	0,52
F3	ABC	0,67
	ABD	0,76
	ABE	0,86
	ABF	0,66
F4	ABC	0,47
	ABD	0,62
	ABE	0,64
	ABF	0,65
	ABG	0,61
	ABH	0,61
	ABI	0,59
F5	ABC	0,58
	ABD	0,75
	ABE	0,60
	ABF	0,60
	ABG	0,81
	ABH	0,67

Family	Animal	VO_2 (4)
F2	ABHI	0,47
	ABGF	0,60
	ABDE	0,73
F3	ABDF	0,75
	ABCE	0,55
F4	ABDH	0,81
	ABGC	0,57
	ABEF	0,66
F5	ABHC	0,60
	ABGE	0,60
	ABDF	0,60

Family	Animal	VO_2 (5)
F2	ABECF	0,54
	ABHGD	0,89
F3	ABCDE	0,64
F4	ABHID	0,99
	ABEGC	0,76
F5	ABFDC	0,93
	ABEGH	0,87

Family	Animal	VO_2 (6)
F2	ABDFCI	0,80
F3	ABCDEF	0,65
F4	ABCEGF	0,96
F5	ABEGDF	0,82

Family	Animal	VO_2 (7)
F2	ABDCHGI	0,52
F4	ABDFCIH	0,89
F5	ABCFGDH	0,97

Family	Animal	VO_2 (8)
F2	ABGFECHD	0,59
F4	ABCFGIEH	1,02
F5	ABCDEFGH	0,84

Tab. 4S. Oxygen consumption (VO_2) [ml O_2 $g^{-1}h^{-1}$] at 20°C for isolated individuals VO_2 (1) and grouped animals of group size 2 to 8 (VO_2 (2 - 8)). Animals were from ten families (F1 - F10), A is a breeding male, B is a breeding female, C-I are non-breeders.

Family	Animal	VO_2 (1)
F1	A	1,55
	B	1,50
F2	A	1,27
	B	2,04
	C	1,48
	D	1,76
	E	1,71
	F	1,27
	G	1,43
	H	1,25
	I	1,63
F3	A	1,16
	B	1,90
	C	1,22
	D	1,84
	E	2,16
	F	1,78
F4	A	1,55
	B	1,63
	C	0,95
	D	1,28
	E	1,81
	F	2,11
	G	1,15
	H	1,35
F5	A	1,42
	B	1,26
	C	1,56
	D	1,39
	E	1,66
	F	1,37
	G	1,82
	H	1,02
F6	A	1,84
	B	1,93
F7	A	0,75
	B	1,35
F8	A	0,92
	B	1,24
F9	A	1,05
	B	1,15
F10	A	1,05
	B	1,25

Family	Animal	VO_2 (2)
F1	AB	0,78
F2	AB	1,43
F3	AB	1,07
F4	AB	1,17
F5	AB	1,06
F6	AB	1,11
F7	AB	1,35
F8	AB	0,77
F9	AB	0,68
F10	AB	0,98

Family	Animal	VO_2 (3)
F2	ABC	0,92
	ABD	1,19
	ABE	1,12
	ABF	1,25
	ABG	0,87
	ABH	1,19
	ABI	1,60
F3	ABC	0,95
	ABD	0,76
	ABE	0,95
	ABF	1,07
F4	ABC	1,09
	ABD	0,92
	ABE	1,03
	ABF	1,42
	ABG	1,01
	ABH	0,85
F5	ABC	1,22
	ABD	1,06
	ABE	1,05
	ABF	1,00
	ABG	1,14
	ABH	1,13

Family	Animal	VO_2 (4)
F2	ABHI	0,88
	ABGF	1,15
	ABDE	1,35
F3	ABDF	0,88
	ABCE	1,00
F4	ABDG	1,00
	ABFC	0,96
	ABEH	0,73
F5	ABHC	1,00
	ABGE	1,16
	ABDF	0,93

Family	Animal	VO_2 (5)
F2	ABECF	1,31
	ABHGD	1,83
F3	ABCDE	1,67
F4	ABGHD	1,32
	ABEFC	1,59
F5	ABFDC	1,58
	ABEGH	1,32

Family	Animal	VO_2 (6)
F2	ABDFCI	1,53
F3	ABCDEF	0,94
F4	ABDGHC	1,58
F5	ABEGDF	1,24

Family	Animal	VO_2 (7)
F2	ABDCHGI	1,40
F4	ABDFCHG	1,53
F5	ABCFGDH	1,42

Family	Animal	VO_2 (8)
F2	ABGFECHD	1,51
F4	ABCDEFGH	1,23
F5	ABCDEFGH	1,58